Production Practices and Quality Assessment of Food Crops

Volume 1

Preharvest Practice

Edited by
Ramdane Dris and S. Mohan Jain

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PREFACE

Today, in a world with abundant food, more than 700 million people are chronically undernourished. Over the next 20 years, the world's population will probably double. The global food supply would need to double or to triple for the larger population to be fed adequately. Agriculture is closely linked to environmental quality in a variety of ways, and the challenge of our generation is how to feed a growing planet while maintaining the integrity of our ecological life-support system.The responsibility of governments for ensuring food security will grow proportionately with the growth of populations, and governments bear a special responsibility for promoting agricultural inputs. Agriculture in the 21st century, will certainly focus increasingly on adapting modern technologies to local farming systems, needs and environments.

Worldwide climatic changes have been raising concerns about potential changes to crop yields and production systems. Such concerns include the ability to accommodate these uncertain effects in order to ensure an adequate food supply for an increasing population. What can be done concretely to use agriculture to address some of the fundamental issues of today's world? We must recognize that agriculture is part of the solution and not just a problem. Agricultural development is a key to social stability and equity in many parts of the world. It can help to alleviate the subtle and unspoken fears of modernization and the space of change if innovation is handled transparently. The World Food Summit held at FAO-Rome in November 1996 gave priority to the development of urban and peri-urban agriculture as well as improving the efficiency of food supply and distribution systems and linkages between growth, production, quality handling, postharvest aspects and consumption areas, with the aim of facilitating access to food by low-income households and hence improving food security especially in developing countries and countries in transition. To increase food security and alleviate poverty there is a need to introduce improved crop-production technologies to farmers or growers and by promoting appropriate policies that help them to adopt new technologies. Responsible agriculture must be viable yet sustainable – economically, environmentally and socially. More information can be found in www.world-food.net or www.isfae.org or to take contact with World Food Ltd. Meri-Rastilantie 3 C, FIN-00980 Helsinki, Finland (info@world-food.net)

This book focuses on the preharvest practices on the production and quality of food crops. Nine chapters are included in this book, which are: Effect of Preharvest Factors on the Quality of Vegetables Produced in the Tropics – Vegetables: Growing Environment and the Quality of Produce; Effects of Agronomic Practices and Processing Conditions on Tomato Ingredients; Modelling Fruit Quality: Ecophysiological, Agronomical and Ecological Perspectives; Sprays Technology in Perennial Tree Crops; Chestnut, an Ancient Crop With Future; Improvement of Grain Legume Production in Semi-Arid Kenya Through Biological Nitrogen Fixation: The Experience With Tepary Bean (*Phaseolus Acutifolius a Gray* var. Latifolius); Impact of Ozone on Crops; Saffron Quality: Effect of Agricultural Practices, Processing and Storage; Fruit and vegetables Harvesting Systems.

This book provides recent and up-dated information on preharvest field practice. The book will stimulate guidance material on key constraints, which can be useful in the field of agriculture or horticulture. Readers will get acquainted with a wide range of information, technologies and methodologies.

The editors wish to express their sincere gratitude to all authors for their valuable contributions. We are grateful to Kluwer Academic Publishers for giving us an opportunity to compile this book.

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EFFECT OF PREHARVEST FACTORS ON THE QUALITY OF VEGETABLES PRODUCED IN THE TROPICS

Vegetables: Growing Environment and the Quality of Produce

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1. INTRODUCTION

Vegetables are plants whose parts are consumed in relatively small quantities as a side dish or relish with the staple food or as a snack. They are mainly cultivated, but some are collected from the wild. They form an important part of food supply in all countries and contribute to balanced nutrition, health, stability and productivity. Vegetables including roots and tubers occupy less than 10% of the whole area under cultivation for all crops in the world (Yamaguchi, 1983). Consumption of vegetables is correlated with per capita income, countries with low and middle incomes use more grain products (beans, peas, cowpeas and maize) or starchy root crops (sweet potato and cassava) in their diets compared to countries with higher per capita income. The demand for vegetables results in an increase in incomes, effecting better incomes for progressive farmers. Vegetables are important as sources of different mineral nutrients and for food security from household level to national concerns. A proper balance of human nutrition can be obtained from vegetables alone. Vegetables can provide the following nutrients carbohydrates e.g. Irish potato, sweet potato, cassava, dry beans and yams, oils e.g. legume seeds and mature vegetable seeds such as *Brassica napus* proteins and amino acids e.g. beans, peas, sweet corn, leafy green vegetables, vitamin A e.g. most yellow or orange coloured vegetables such as sweet potato, squash, peppers and green leafy vegetables, green beans and peas, vitamin C e.g. crucifers, peppers, tomatoes, most leafy vegetables and bean sprouts and minerals e.g. most leafy vegetables and root crops.

Generally, among horticulturists quality is often expressed only in quantitative terms, like yield and size. Postharvest quality changes are measured only as the percent of waste (Martens and Baardseth, 1987). Kramer and Twiggs (1970) defined quality as the composite of attributes that differentiate among units of a product and have significance in determining the degree of acceptability of the unit by the buyer. The quality of a product may be expressed by means of quality criteria which includes nutritional properties (vitamins, proteins, carbohydrates), hygienic properties (microbiological condition, content of toxic components) technological properties (appearance flavour, texture). There is a need for measurement methods (ranging from advanced instrumental techniques to sensory analysis) and establishment of specifications (with tolerance limits) referring to standards or grades

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for classification of fresh vegetables for market consumption or specifications for vegetables for the processing companies (Martens and Baardseth, 1987).

The establishment of specifications for a product of good quality is a complicated matter that requires the cooperation of producers, dealers, authorities and consumers. A consumer survey conducted to gain information about consumer satisfaction with food products showed that they were strongly critical of taste and appearance (Handy and Harger, 1977). Although vegetables are important from a nutritional point of view (Goddard and Mathews, 1979), the food has no nutritional value until it is eaten. Sensory quality properties are therefore of primary importance to the consumer in the decision to buy (and eat) vegetables (Stevens, 1974).

Quality can be further divided into external and internal quality (Martens and Baardseth, 1987). External quality includes attributes concerning appearance (e.g. colour, size and shape). Internal quality includes attributes concerning flavour and texture. Flavour is influenced by chemical properties and perceived mainly by the senses of taste and smell. The chemical substances may include carbohydrates, fat, protein, oils, organic acids and water. Texture is caused by a combination of physical properties and perceived by the senses of kinaesthesis, touch (including mouthfeel), sight and hearing. The physical properties may include number, nature and conformation of constituent structural elements (e.g. proteins, starch and cellulose). Instrumental, physical and chemical methods used for quality evaluation ought to be calibrated with respect to sensory response. A chemically measured change of a product may not be perceived as a change by the human sense. Most of the postharvest quality changes reported in the literature are measured by instruments or by chemical analyses with no reference to sensory criteria, or with incomplete description of how the sensory analysis is performed (Martens and Baardseth, 1987).

Several constraints are encountered in the production of quality vegetables in the tropics. These include land availability, pests, diseases and marketing. Pest control is hampered by lack of knowledge and high costs of inputs especially chemicals. Most producers have little choice in selecting land for cropping since there are no alternatives. Some of these factors also affect crop growth, development and quality of the harvested produce. Other factors that affect growth and development of the vegetables include the climatic conditions and the environment as a whole. During site selection factors such as rainfall, temperature, topography, soil, and day length must be considered, as they directly affect the quality of vegetables. These factors including water availability, water quality and general farm practice all integrate to give the required quality.

The environment is composed of elements such as land, climate and other plants. Some plants may adapt to the climate but fail to adapt to the soil conditions, this then affects the quality of the harvested produce. Vegetables can either be grown in protected environments or in the open field. Regardless of this fact, they still require specific temperature, water, light, soil and all the other conditions in order to grow and yield quality produce. This chapter aims to highlight the growing environmental conditions for vegetables and how they affect the quality of specific vegetables namely tomatoes, cabbages, sweet potatoes and squash.

2. GROWING ENVIRONMENT

2.1. Temperature

Temperature is a major factor controlling quality in vegetables and it is very difficult to modify especially for resource poor smallholder farmers. The extreme temperature range of plants is between killing frost at 0 °C and death by heat and desiccation at 40 °C with most plants permanently immobilized at 10oC and most plants ceasing to photosynthesize efficiently above 30 °C (Nonnecke, 1989). Seasonal temperature variation can change as little as 3 °C all year round in the tropics and as much as 45 °C in the polar zone. Temperature decrease with increases in altitude, a drop of about 6 °C occurs with each 1000 m in elevation. Cardinal temperatures, minimum and maximum (where plant growth ceases) and optimum (where growth occurs most rapidly) vary with different families, genera and species. For every 10 °C rise in temperature, growth doubles at the temperature range of 5 to 35 °C depending on the vegetable (Van't Hoff's Law).

Most vegetables are sensitive either to high or low temperatures. Tropical vegetables are mainly sensitive to low temperatures. Most vegetables are either injured at temperatures at or slightly below freezing (freezing injury). Tropical and subtropical plants may also be killed or damaged by the cold at temperatures below 10 °C but above freezing (chilling injury). These are discussed in Sections 3.1 and 3.2 respectively. Susceptibility to cold damage varies with different species and there may be differences among varieties in the same species. Susceptibility to cold damage also varies with stage of development, with most plants more sensitive to cold temperature shortly before flowering through a few weeks after anthesis. Chilling and frost damaged produce is not accepted by the consumers at the markets.

High temperature in many semi-arid and arid regions may be a limiting factor in the production of vegetables. Leaf temperatures can reach 8 °C above air temperature under high insulation and high humidity. Heat destruction of the protoplasm results in the death of the cells and this occurs at 45 to 50 °C. High temperatures are detrimental to the growth and development of many vegetables. High temperatures are implicated in flower fall in many tropical and subtropical vegetables for example tomatoes, peppers and paprika. The Cruciferae leaf vegetables such as kale (*Brassica oleraceae*) and mustard rape (*Brassica juncea*) have a bitter taste after exposure to high temperatures.

Temperature does not only affect growth but also development and in some cases it interacts with photoperiodism to affect growth and development. Some bean cultivars behave as day neutral plants at low temperatures and as short day plants at higher temperatures. Thermoperiodism (diurnal temperature range) also affects development, growth and quality of plants. Generally a large diurnal range is favourable for net photosynthesis. High night temperatures are not beneficial as respiration rates increase using up photosynthates produced during the day. In tomatoes, adult plant growth is most rapid and flowering more profuse with 10–12 °C difference between night and day temperatures. Some vegetables need to be vernalised (exposed to low temperatures to induce or accelerate flowering)

to develop from vegetative stage to flower initiation. Examples of such vegetables include most *Brassica* sp, *Allium* sp and *Daucus carota*.

2.2. Rainfall and water availability

Adequate soil moisture is essential to the production of vegetables. Many vegetables are about 80% water in terms of thesis composition. Vegetables require a lot of water to grow and develop normally. Water dissolves plant nutrients in the soil, plays an important role in biological activities, keeps the plant cool and transports food nutrients in the plant. Less than 1% of the water that passes through the plants is used in the photosynthetic process, however, in plants under water stress, photosynthesis and growth are reduced. Transpiration has a cooling effect on leaves. A rapidly transpiring leaf can lower the leaf temperature as much as 8 °C.

The total amount of rainfall received annually and its distribution is very important. For most vegetable crops little or no moisture stress during the entire growth period generally gives high yields and good quality. Some vegetables require water at particular critical stages in their growth and development (examples given in Table 1 below) while others consistently require water at all stages (e.g. tomatoes). In most places rainfall distribution is not even and so irrigation during dry periods is required for successful crop growth. The quality of the water must be acceptable as irrigation water has been implicated for several diseases, toxicity and some insect pests outbreaks. Saline or brackish water leads to death and retards growth of many vegetables. Toxicity of several salts can lead to leaf scorch and also retards growth. Usually water can be from wells, dams, streams and rivers. The irrigation system used by the farmer is usually determined by the crop requirements, resources available for irrigation equipment, the terrain and the source and quality of water available. Both excessive water and drought can affect the quality of vegetables. Too much water leads to water-logging of which many vegetables are susceptible. Lack of efficient water in the vegetables will affect taste, appearance and increase incidences of certain pests (insects and diseases).

Relative humidity is the amount of water present in air as a percentage of what could be held at saturation at the same temperature and pressure. High humidity increases the incidence of many diseases and increases insect population. It can

Table 1. Moisture sensitive stages for some selected vegetables.

Crop	Moisture sensitive stage
Cabbage	Head formation to harvest
Cauliflower	All stages of growth
Radish	Root enlargement
Turnip	Root enlargement to harvest
Lettuce	Heading stage to harvest
Onion	Bulb formation
Peas	Flowering through pod formation to harvest
Irish potato	Tuber initiation to enlargement
Sweet corn	Silking and ear development

be beneficial to the water economy as transpiration is decreased. Pollen viability increases in certain crops as humidity increases.

2.3. Soil

Generally vegetables grow in a wide range of soil types. It is however, necessary that these soils be fertile, well drained, and have a high water holding capacity. The soil must be fertile enough to provide the essential nutrients (N, P, K, Ca, S, Mg, Fe, Cl, Mn, Cl, Mn, B, Zn, Cu and Mo) required by the plants in the right amounts or fertilizers must be added to supplement the soil content. The critical mineral elements affecting quality of vegetables are covered in more detail in Section 3.3.

Heavy clay soils are very difficult to work, especially in the wet season. Organic matter must therefore be added to such soils. Sandy soils are also not good as they contain very little nutrients and have poor water holding capacity. Organic matter must therefore be added as well to improve humus content and water holding capacity. The ideal soil for most vegetables is the medium clay loams supplied with organic matter or other nutrients.

The acidity and/or alkalinity of the soils is very important in production of all vegetables. A pH range of between 5.5 and 7.5 is generally accepted by most vegetables although tomatoes and peppers can tolerate slightly more acid soils. At this pH range most important nutrients are available to plants as long as they have been applied to the soil. Table 2 below shows optimum pHs for a few selected vegetables. Calcium, P and Mg maybe unavailable at an acid pH below 6, and Mn, B, Zn, Fe and Al may become so available to the plant that they become toxic. Sandy soils are host to nematodes that hinder growth of many vegetables and are very difficult and expensive to control. Soil depth in the production of vegetables must be at least 40 cm as most vegetable roots can reach that depth.

2.4. Topography

Topography is very important in relation to temperature and temperature fluctuation. The diurnal or daily night temperature variations which occur at elevations over

Table 2. Optimum pH values for selected vegetable crops.

pH	Crops
6–8	Asparagus
6–7.5	Beet, cabbage, muskmelon, peas, spinach, summer squash
6–7.0	Celery, chives, endive, lettuce, onion, radish, cauliflower
5.5–7.5	Sweet corn, pumpkin, tomato
5.5–7.0	Snap beans, lima beans, carrots, cucumbers, peppers
5.5–6.5	Eggplant, watermelon
4.5–6.5	Potato

Adapted from Splittstoesser (1990).

800–1000 m above sea level is beneficial to many crops especially onions and leaf crops in the Cruciferae family in the tropics. Where there is a steep sloping or uneven land soil conservation measures are needed to save the soil and water from erosion. If the land is steep, erosion could be a problem and so ridging or terracing may be necessary. High elevations are linked to frost therefore freezing and chilling injury of tropical and sub-tropical crops are more common in such areas. In hilly areas the rainfall on the west-facing or leeward slopes is much less especially on coming down to sea level. If streams are coming from the mountain-tops the leeward side of hilly areas, are suitable for horticultural crops as long as irrigation is carried out.

2.5. Light

Light is necessary for photosynthesis. Daylight is also responsible for certain morphological induction's, and the quality of light can significantly affect growth patterns. Plants grown in full light develop several thicknesses of palisade tissue compared to those grown in low light indicating improved photosynthetic activity (Nonnecke, 1989). Leaves of salad vegetables such as celery and lettuce are generally considered to be of higher quality and more tender if they are grown under partially overcast skies. Photosynthesis is practically stopped at 4.31 lux, the compensation point (at which photosynthesis equals respiration). Plants generally respond to 350 to 780 nm wavelength light. Different wavelengths can affect some physiological processes in plant such as stimulation of bulbing in onions at 1000 to 720 nm light and suppression of bulbing in onions and red pigmentation formation in tomato at 690 to 650 nm light.

The flowering response of plants to relative length of day or night is called photoperiodism. Plants that develop and reproduce normally only when the photoperiod is less than a critical maximum are called short day plants and those that flower only when the photoperiod is greater than the critical minimum are long day plants (Yamaguchi, 1983). For short day plants the duration of the dark period is the critical condition, rather than the duration of the light period. Phytochromes which absorb red and far red lights, is the pigment necessary for the response in photoperiodims. In day neutral plants flowering is not affected by photoperiods. A few specific examples of vegetables fitting into the three categories of photoperiods are listed below:

a. Long-day vegetables e.g. spinach, radish and Chinese cabbage;
b. Short-day vegetables e.g. soyabean, sweet potato, whinged bean and amaranth;
c. Day-neutral vegetables e.g. tomatoes, squashes, beans, peppers, eggplant and most cucurbits.

Exotic vegetables in the tropics are the ones mostly affected by day length as they generally originate mostly from temperate regions. These regions experience day lengths longer than 12–13 hours per day. Cultivar selection is therefore necessary in such crops so that the farmer does not produce poor quality produce due to unsuitable day lengths. This information is also useful for timing production of specific vegetables.

Other responses that can also be caused by different daylenghts beside flowering in vegetables are listed below:

a. Long days for bulbing in onions;
b. Short days for tuber initiation in Irish potato, yams and Jerusalem artichoce;
c. Short days for root enlargement in cassava and sweet potato.

2.6. Winds

Winds are caused by differences in air pressure. Temperature differences produce pressure gradients which give rise to air movements. Winds affect the humidity in the atmosphere. They can remove the humid air adjacent to leaf surfaces, increasing transpiration rates and decreasing temperature. Winds can replenish the carbon dioxide supply to the leaves deep within the canopy thereby improving photosynthesis and vegetable growth. Winds can also injure or break the above ground portions of the plant thereby affecting quality of the product.

All the above must be considered as they do not only affect growth and development of vegetable but the quality of the produce. It is therefore necessary to check before introducing any vegetable into the tropics or any area, if its optimal growth temperature and photoperiodic requirements are compatible with local climatic conditions. Even for the quality of local varieties, it is important not to ignore the environmental conditions as they also affect quality of the produce. Where local conditions are marginal, artificial means can be used to promote the growth of the crop for better quality. These can be windbreaks, shades and green houses to protect the crops from wind and high solar radiation respectively.

3. MAJOR PRE-HARVEST DISORDERS IN VEGETABLES

3.1. Chilling injury

Chilling injury is the permanent or irreversible physiological damage to mostly warm season plant tissues, cells or organs, which results from the exposure of chilling sensitive plants to temperatures below some critical threshold for that species or tissue. A chilling temperature is any temperature (above freezing) below the critical threshold temperature that causes injury. The critical temperature for chilling injury varies with commodity, but it occurs when produce is kept at below 10–12 °C. All stages of growth and development of the entire plant (except perhaps dry seed) are susceptible to chilling injury in warm season crops. Factors that determine the extent of this injury include the temperature, duration of exposure to that low temperature, whether exposure is continuous or intermittent, physiological age or condition of the plant, part exposed and the relative responsiveness (sensitivity) of the crop to chilling. Chilling sensitive species except asparagus and potatoes are mostly warm season crops of tropical and subtropical origin. Prolonged exposure to temperature below the chilling threshold in these species results in death. Low temperature limits the geographical areas and time of year for production of chilling – sensitive crops.

3.1.1. *Causes of chilling injury*

The primary cause of chilling injury is thought to be damage to plant cell membranes. Changes in molecular species composition of phospholipids during cold storage and after rewarming were determined in mature green tomatoes (L'-Heureux et al., 1993) the minor molecular species shifted significantly towards unsaturation during cold storage. The presence of these polyunsaturated species and their breakdown products may induce membrane damage and dysfunction. The membrane damage sets off a cascade of secondary reactions, which may include ethylene production, increased respiration, reduced photosynthesis, interference with energy production, accumulation of toxic compounds such as ethanol and acetaldehyde, electrolyte leakage and altered cellular structure (Skog, 1998).

Some physiological parameters of chilling injury include electrolyte leakage, carbon dioxide and ethylene production in squash and cucumber (McCollum and McDonald, 1993). These parameters increase with increasing severity of chilling injury (Lee and Yang, 1999). Electrolyte leakage was shown to increase during cold storage (1 °C for 2 days) of mature green tomato fruits (L'-Heureux et al., 1993). In bell peppers chilling injury was also accompanied by high internal carbon dioxide and ethylene production (Lin et al., 1993). Ethylene – forming enzyme activity can also be used as an indice for thermotolerance of crops (McCollum and McDonald, 1993).

Chilling injury can be measured non-destructively in fruits using pulse amplitude modulated fluorometry before tissue damage is visible (Lurie et al., 1994). Three photosynthetic characteristics could be measured by this method; quantum yield (F_m/F_o), photochemical quenching (Q_p) and non-photochemical quenching (Q_{np}). F_m/F_o decreased by 90% during the first week of storage at 2 °C and remained low thereafter, while Q_{np} decreased after 2 weeks at 2 °C just before the fruits began to develop chilling injury. Q_p was similar at both chilling and non-chilling storage temperature. Whether this method can be used in a field situation is not known.

3.1.2. *Symptoms of chilling injury*

Symptoms of chilling injury as summarized by Morris (1982) and Skog (1998) include the following:

1. Surface lesions – pitting, large sunken areas, and discolouration. These symptoms occur most frequently in products with firm thick peel such as citrus or cucumber.
2. Water soaking of tissues – this disruption of cell structure and accompanying release of substrates favours the growth of microorganism. Water soaking commonly occurs in vegetables with thin or soft peels such as peppers and asparagus.
3. Water loss/desiccation/shrivelling
4. Internal discolouration (browning) of pulp, vascular strands, and seeds.
5. Breakdown of tissues.

6. Failure of fruits to ripen in the expected pattern following removal to ripening conditions.
7. Accelerated rate of senescence, but with an otherwise normal appearance.
8. Increased susceptibility to decay due to leakage of plant metabolites that encourage growth of microorganisms.
9. A shortened storage shelf life due to one or more of the above responses.
10. Composition changes – especially in relation to flavour and taste.
11. Loss of growth (sprouting capacity – important with stored propagules).

These symptoms are not unique to chilling injury and many can also be induced by water stress and anoxia. The symptoms may not be evident while the commodity is held at the chilling temperature and develop rapidly when removed from cool storage. In most studies directed toward determining the extent of chilling injury, a treatment of varying times and temperature is applied and then the test material is placed at 20–25 °C for 3 days for observation.

The factors that influence the relative susceptibility of vegetables to chilling injury are, genetic diversity, stage of physiological development and the conditions under which the commodities are grown. Cultivars within a sensitive species can differ in symptom expression. King and Ludford (1983) showed that differences in chilling sensitivity among 5 tomato cultivars as measured by electrolyte leakage could be discerned prior to the development of visible symptoms. Tomato fruit are quite susceptible to chilling in their mature green stage (Autio and Bramlage, 1986). Chilling sensitivity decline, as ripening progress and then increase again during the late stages of ripening. Fully mature honeydew melons were less susceptible to chilling injury than less mature melons (Lipton, 1978). Mature green fruits of bell peppers (*Capsicum* sp) showed surface pitting after storage at 1 °C for 3 days, while full colour of fruits showed no chilling injury after 2 weeks at 1 °C (Lin et al., 1993). The conditions under which the commodities are grown may influence the extent and development of chilling injury (Kader, Lyons and Morris, 1974). Some winter-grown tomatoes were more susceptible to chilling injury than summer grown fruit of the same cultivar (Abdel-Madsoud et al., 1974). Fully ripe, firm fruit can be held at 0–2.5 °C for short periods if they are to be consumed immediately upon removal from refrigeration. Some of the important vegetables susceptible to chilling injury and the potential symptoms are listed in Table 3.

3.1.3. *Alleviation of chilling injury*

Chilling injury can be avoided by limiting growing, handling and storage of sensitive crops above the critical threshold temperatures. Growing cultivars that are less sensitive or tolerant of chilling injury is probably the only way of effectively reducing chilling injury in the field. Several other temperature treatments have been tried with various degrees of success to control mostly chilling injury in storage. These will be discussed briefly below.

High temperature conditioning treatments have been reported to enhance the chilling tolerance of a number of chilling sensitive tissues (McCollum and McDonald, 1993). Cucumber fruits immersed in 42 °C water for 30, 45 or 60 minutes

Table 3. Vegetables susceptible to chilling injury.

Commodity	Time/temperature °C conditions for symptoms	Potential chilling injury symptoms
Asparagus	10 days at 0	Darkened and water-soaked areas at the tips followed by bacterial-soft rot.
Beans, snap	3 days below 4.5	Russeting and surface pitting
Cucumbers	2 days below 5 °C	Surface pitting starting at lenticel area followed by secondary pathogen rots.
Eggplant	3–4 days below 5 °C	Scald-like browning, surface pitting, flesh browning and secondary pathogen rots.
Melons		
Muskmelons	15 days at 0–2.5 °C	Water-soaking of rind, softening and surface becomes soft and sticky resulting in increased decay. Surface pitting, loss of flavour and fading of red colour.
Honey Dew	15 days at 0–2.5 °C	
Watermelons	7 days at 0 °C	
Okra	3 days at 0 °C	
Peppers, bell	3–4 days at 7.5 °C	Water-soaked appearance, sheet pitting, darkening and pre-disposition to rots.
Potatoes	20 weeks at 0–1.5 °C	Mahogany browning and sweetening.
Summer squash	4 days at 5 °C	Severe surface pitting and slight decay.
Pumpkins and winter squash		Rots
Sweet potatoes	4–7 days at 7.5–10 °C	Flesh discolouration, internal breakdown, increased decay, off-flavours, hard-core when cooked.
Tomatoes	6 days at 0 °C 9 days at 5 °C	Rubbery texture, watery flesh, irregular ripening and seed browning.

Adapted from: Lutz and Hardenburg (1986) and Skog (1998).

prior to storage at 2.5 °C for 1, 2 or 3 weeks had less electrolyte leakage than did untreated fruits indicating an increase in chilling tolerance in the fruits. In sweet pepper, however, treatment at 50 °C for 45 minutes resulted in severe membrane damage (Mencarelli et al., 1993). Alternating temperature can prevent symptom development under certain time/temperature regimes. The beneficial effects of warming after a period of exposure to chilling may be related to either, the restoration of normal metabolism so that potentially toxic compounds accumulated during chilling can be removed or the availability of some essential factor that became deficient can be re-supplied (Lyons and Breidenback, 1987) or to repair of damage incurred to membranes, organelles or metabolic pathways before degenerative changes occur.

Intermittent warming is warming the commodity to room temperature at intervals during storage before permanent injury has occurred and will allow the product to recover and prevent chilling injury symptom development. Intermittent warming plays a role in the control of chilling injury that occurs in the field prior to the harvest of fall grown tomatoes. Lyons and Breidenback (1987) showed that accumulation

of more than 13 hours of night temperature below 15.6 °C during the week preceding harvest would cause irreversible damage resulting in visible symptoms during marketing. This treatment may, however, cause undesirable softening and increase decay and may cause condensation to form on the product especially in storage (Skog, 1998). Preconditioning can also be used. It is the stepwise cooling of the commodity and can allow the vegetable to adapt to the cooler temperature and minimize chilling injury development in storage.

Controlled or modified atmospheres (generally $O_2 < 5\%$, $CO_2 > 2\%$) can slow plant metabolism and slow chilling injury development in certain crops. Controlled atmospheres can also allow longer storage of chilling sensitive crops when stored above their critical temperature (Skog, 1998). Modifying the atmosphere surrounding certain fruits subject to a number of low temperature disorders has been reported in some instances to alleviate or delay the symptoms usually associated with chilling injury (Lyons and Breidenback, 1987). Reduced O_2 and elevated CO_2 can overcome the impact of low temperature injury on the ripening process (Wade, 1979; Ilker and Morris, 1975). Controlled atmospheres may in some cases further stress crops and increase chilling injury susceptibility for crops such as cucumbers, tomatoes and asparagus (Skog, 1998). Maintaining a high RH around the commodity during storage, as occurs with film wraps and modified atmosphere storage, reduces the severity of chilling injury symptoms by minimizing desiccation.

Proper pre-harvest nutrition can minimize chilling susceptibility. Calcium treatments may stabilize cellular membranes and reduce chilling injury in certain commodities. Ilker and Morris (1975) found that treatment with solutions of calcium and potassium salts could afford some protection against chilling injury in okra. The severity of chilling injury in squash (*Cucurbita moschata*) fruits, stored at 4 °C was reduced by dipping in 1% $CaCl_2$ or 10 mM sodium benzoate at 20 °C for 30 minutes (Lee and Yang, 1999). Fruits treated with sodium benzoate showed a low incidence of chilling injury (< 10 °C) following 30 days of storage at 4 °C.

Reports examining possible approaches to alleviating chilling injury conclude that the most likely solution occurs through genetic modification (Graham and Patterson, 1982; Couey, 1982; Bramlage 1982). A better understanding of the primary causes of chilling injury will provide information for the development of rapid methods for screening potential germ-plasm and possibly control of this disorder. The debate as to whether there is a single primary response and whether the membrane plays a central role as the primary temperature sensor remains unresolved.

3.2. Freezing injury

Commodities may be frozen in the field before or during harvest, in transit and storage. Vegetables vary in their susceptibility to freezing injury. Some may be able to go through freezing and thawing several times with little or no apparent damage, while others are damaged by slight freezing. The relative susceptibility of fresh vegetables to freezing injury is shown in Table 4. The inherent susceptibility of tissues to freezing stress may be partially responsible for this variation in sensitivity. Ice nucleating bacteria may also play an important role. Whether or not ice formation in the tissues is accompanied by permanent injury and death

Table 4. Relative susceptibility of fresh vegetables to freezing injury.

Most susceptible	Moderately susceptible	Least susceptible
Asparagus	Broccoli	Beets
Snap beans	Cabbage	Brussel sprouts
Cucumbers	Carrots	Cabbage (savoy)
Eggplant	Cauliflower	Kale
Lettuce	Celery	Kohlrabi
Okra	Onions (dry)	Parsnips
Sweet peppers	Parsley	Rutabagas
Summer squash	Peas	Turnips
Sweet potatoes	Radishes	
Tomatoes	Spinach	
	Winter squash	

Adapted from Lutz and Hardenburg (1977).

depends upon how low the temperature and, the rate at which it falls, the duration of the freezing temperature, and the condition of the plant tissues prior to freezing (Flurry et al., 1977).

Frozen leafy tissues and storage tissues such as turnips and rutabagas, lose their natural luster and appear glossy. Immediately upon thawing, they become water soaked. Water-soaked areas of leafy green tissues also appear dirty or a muddy green colour. In colourless or fleshy tissues like cauliflower heads, there is no initial discoloration, later, the more sensitive tissues such as the fibro-vascular bundles may turn yellowish – brown to black. Fleshy roots, such as turnips, radishes, rutabagas, and horseradish, often show no discoloration except for the vascular tissues.

Vegetables may arrive at the market bearing symptoms of frost damage incurred during some earlier stage of their growth. Glove artichoke (*Lynara scalymus* L.) is an exceptionally long-season crop that can become frozen in the field when the temperature drops below –1.7 °C. Severe freezing kills the immature flower heads and causes them to turn black. Slight freezing results in breaking, cracking, and blistering of the epidermis on exposed outer bracts. The damaged epidermis becomes whitish and buds may become brown. This detracts from the market appearance of the buds (Ramsey et al., 1967).

When freezing injury occurs prior to harvest in Irish potatoes (*Solanum tuberosum*) it is often referred to as field frost. This condition can usually be diagnosed by the presence of bluish-gray blotches beneath the skin. Tissues at the stem end of tubers are more sensitive than those at the bud end, and the differentiated vascular cells, such as tracheae, sieve tubes, and tracheids, are more susceptible than are the starch-filled parenchymatous cells. Any freezing during storage and transit may manifest as or all the necrotic patterns known as ring necrosis, blotch, or net necrosis. Generally the symptoms of freezing progresses from the ring to net to blotch type as the freezing progress and as the freezing interval lengthens. Often the different types of symptoms will overlap. The various internal symptoms of freezing injury may not occur unless potato tubers are bumped, jarred, or dropped during the freezing period. Varieties of potatoes differ in their reaction to low

temperatures. Some show serious internal discoloration after prolonged storage at several degrees above their actual freezing point, while others may not show injury at –1.7 °C, the average freezing point of most potato varieties.

Freezing injury of celery can be readily recognized at harvest time by the flabby water-soaked condition of the leaves and leaf stalks. Frozen leaves, if not attacked by bacteria, dry out and become papery. A second type of freezing symptom is the appearance of isolated sunken lesions on the leafstalks. These two types of injury are most often apparent at harvest but are of little importance on the market.

3.3. Mineral nutrient disorders

In this section the key nutrients causing physiological disorders and their role in vegetables will be discussed. A mineral nutrient can function as a constituent of an organic structure, as an activator of enzyme reactions or as a charge carrier and osmo-regulator.

3.3.1. *Calcium*

Calcium is a relatively large, divalent cation. The Calcium content of plants varies between 0.1 and > 0.5% of dry weight depending on the growing conditions, plant species, and plant organ. Genotypically differences in Ca^{2+} requirements are closely related to the Ca^{2+} binding sites in the cell walls. Ca deficiency in plant tissues causes many physiological disorders which lead to significant losses in plant production. Calcium shortage in plants is related to poor Ca uptake, its limited movement to above – ground plant parts and strong competition for Ca between leaves and generative plant parts (fruits, seeds) (Wojcik, 1998).

Calcium readily enters the apoplasm and is bound in an exchangeable form to cell walls and at the exterior surface of the plasma membrane. Its rate of uptake into the cytoplasm is severely restricted and seems to be only loosely coupled to metabolic processes. The mobility of Ca from cell to cell and in the phloem is very low. It is the only mineral nutrient other than Bo which functions mainly outside the cytoplasm in the apoplasm. Most of its activity is related to its capacity for co-ordination by which it provides stable but reversible intermolecular linkages; predominantly in the cell walls and the plasma membrane. These Ca^{2+} mediated linkages respond to local changes in environmental conditions and are part of the control mechanism of growth and developmental processes. Calcium is a non-toxic mineral nutrient, even in high concentrations, and is very effective in detoxifying high concentrations of other mineral elements in plants.

There are two distinct areas in the cell wall with high Ca^{2+} concentrations, the middle lamella and the extension surface of the plasma membrane. In both areas Ca^{2+} has essential structural functions, namely, the regulation of the membrane permeability and related processes and the strengthening of the cell walls.

The fundamental role of Ca^{2+} in membrane stability and cell integrity is reflected in various ways. It can be demonstrated most readily by the increased leakage of low molecular weight solutes (e.g. in tomato fruits; Goor, 1968) and in severely deficient plants, by a general disintegration of membrane structures (Hecht-Buchholz,

1979) and a loss of cell compartmentalization. Calcium stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids (Caldwell and Hang, 1981) and proteins, preferentially at membrane surfaces (Legge et al., 1982). There can be an exchange between Ca^{2+} at these binding sites and other cations (e.g. K^+, Na^+, or H^+) although the latter cannot replace Ca^{2+} in its membrane stabilization role. To fulfill its functions at the plasma membrane, Ca^{2+} must always be present in the external solution where it regulates the selectivity of ion uptake and prevents solute leakage from the cytoplasm. The membrane protecting effect of Ca^{2+} is most prominent under various stress conditions such low temperature (Zsold and Karvalry, 1978) and anaerobiosis (Cristiansen et al., 1970).

The degradation of pectates in cell walls is mediated by polygalacturonase, which is drastically inhibited by high Ca^{2+} concentrations. In Calcium – deficient tissue polygalacturonase activity is increased (Konno et al., 1984), and a typical symptom of Calcium deficiency is the disintegration of cell walls and the collapse of the affected tissues, such as the petioles and upper parts of the stems (Bussler, 1963). The proportion of Ca in the cell walls is of importance for determining the susceptibility of the tissue o fungal infections and for the ripening of fruits. In tomato fruit the Ca^{2+} content of the cell walls increases to the fully-grown immature stage but this is followed by a drop in the content just before the onset of ripening (softening of the tissue) (Rigney and Wills, 1981). A shift in the binding stage of Ca^{2+} occurs in which water soluble Ca^{2+} is favoured over wall bound Ca^{2+}. This shift is associated with a sharp increase in ethylene formation in the fruit tissue.

High growth rates of low transpiring organs, increases the risk that the tissue content of Ca^{2+} falls below the critical level required for the maintenance of membrane integrity, leading to typical Calcium deficiency – related disorders such as tip burn in lettuce, blackheart in celery, blossom-end rot in tomato or watermelon, and bitter pit in apple (Shear, 1975; Bangerth, 1979). Low tissue levels of Ca^{2+} in fleshy fruits also increase the losses caused by enhanced senescence of the tissue and by fungal infections. Vitrescence, a physiological disorder in melons has been reported as being caused by Ca deficiency (Jean Baptiste et al., 1997). The symptoms are an intense colour, with a vitreous aspect and a delinquescent texture).

3.3.2. *Potassium*

Potassium is a univalent cation. It is characterised by high mobility in plants at all levels, within individual cells, within tissues, as well as in long-distance transport via the xylem and phloem. Potassium is the most abundant cation in the cytoplasm, and potassium salts make a major contribution to the osmotic potential of cells and tissues of the glycolytic plant species. Cytoplasmic K^+ concentrations are maintained in the relatively narrow range of 100 to 120 mM. The various functions of K^+ in cell extension and other turgor regulated processes are related to the K^+ concentration in vacuoles. Potassium ions act as a charge carriers of high mobility that form weak complexes in which they are readily exchangeable (Wyn Jones et al., 1979). The high concentrations of K^+ in the cytoplasm and the chloroplasts are required to neutralize the soluble (e.g. organic acid anions and inorganic anions) and insoluble macromolecular anions and to stabilize the pH

between 7 and 8 in these compartments, the optimum for most enzyme reactions. Potassium is required for enzyme activation and membrane transport processes. There are mechanisms (pumps) at the plasma membrane and probably also at the tonoplast for concentrating K^+ in the cytoplasm. Although several other univalent cations can partially replace K^+, they are toxic to intact cells at high concentrations (e.g. NH_4^+) or are not abundant in nature (e.g. Rb^+).

Potassium requirement for optimal plant growth is about 2–5% of the dry weight of the vegetative parts, fleshy fruits and tubers. Potassium deficiency causes chlorosis and necrosis in mature leaves and stems wilting and logging. The lower tolerance of K^+ deficient plants to drought is related to a) the role of K^+ in stomatal regulation which is a major mechanism controlling the water regime of higher plants and b) the role of K^+ as the predominant osmotic solute in the vacuole, maintaining a high tissue water level even under drought conditions. Plants receiving inadequate K^+ are often more susceptible to frost damage, which at cellular level, is related in some respect to water deficiency.

Frost damage is inversely related to the K^+ content of leaves (Marschner, 1989) (Table 5). The changes in enzyme activity and organic compounds that take place during K^+ deficiency are in part responsible for the higher susceptibility of plants to fungal attack. They also affect the nutritional and technological (processing) quality of harvested products. This is most obvious in fleshy fruits and tubers with their high K^+ requirement for growth. In tomato fruits, the incidence of the ripening disorder 'greenback' or 'green shoulders' increases with inadequate K^+ supply (Lune and Goor, 1977), and in potato tubers a whole range of quality criteria are affected by the K^+ level in the tuber tissue (Table 6). Too high K^+ supply affects plant composition and interferes with the uptake and physiological availability of Mg^{2+} and Ca^{2+}.

There are more than 50 enzymes which either depend on or are stimulated by potassium (Suelter, 1970). Potassium and other univalent cations activate enzymes by inducing conformational changes in enzyme protein. In general, potassium induced conformational changes of enzymes increase the rate of catalytic reactions, V_{max}, and in some cases also the affinity for substrate, K_m (Evans and Wildes, 1971). In K^+ deficient plants some gross chemical changes occur, including an accumulation of soluble carbohydrates, a decrease in the levels of starch, and an accumulation of soluble N compounds.

Potassium activates the following enzymes pyruvate kinase, phospofructokinase

Table 5. Relationship of potassium supply to potassium content in leaves, percentage of leaves damaged by frost and tuber yield in potatoes.

Potassium supply (kg/ha)	Potassium content of leaves (mg/g dry wt)	Percentage foliage damaged frost	Tuber yield (tons/ha)
0	24.4	30	2.39
42	27.6	16	2.72
84	30	7	2.87

Based on Grewal and Singh (1980) and Marschner (1989).

Table 6. Effect of increasing potassium concentrations in potato tubers, on the composition and quality of the tubers.

Type of change	Effect of increasing Potassium	Responsible mechanism
Water content	Increase	Osmoregulation
Reducing sugars	Decrease	Osmoregulation
Citric acid	Increase	Cation-anion balance
Starch	Decrease	
Black spot disorder	Decrease	Lower polyphenol oxidase activity
Darkening of press sap	Decrease	High citric acid/low polyphenol oxidase
Discoloration after cooking	Decrease	High citric acid/low chlorogenic acid
Storage loss	Decrease	Lower respiration and fungal diseases

Adapted from Marschner (1989).

(Lauchli and Pfluger, 1978), starch synthase (Nitsos and Evans, 1969), and membrane bound ATPases (which requires Mg^{2+} but are further stimulated by K^+). Potassium is required for protein synthesis in higher plants. The role of K^+ in protein synthesis is reflected in the accumulation of soluble nitrogen compounds, (e.g. amino acids, amides and nitrate) in K-deficient plants (Mengel and Halal, 1968).

In higher plants K^+ affects photosynthesis at various levels. K^+ is the dominant counter ion to light induced H^+ flux across the thylakoid membranes and the establishment of the transmembrane pH gradient necessary for the synthesis of ATP (photophosphorylation) (Lauchli and Pfluger, 1978). An increase in the leaf potassium content is accompanied by increased rates of photosynthesis, photorespiration and RuBP carboxylase activity, but a decrease in dark respiration. In most plant species K^+ also has the major responsibility for turgor changes in the guard cells during stomatal movement. An increase in the K^+ concentration in the guard cells results in the uptake of water from the adjacent cells and thus stomatal opening (Humble and Raschke, 1971).

3.3.3. *Boron*

Boron occurs mainly as boric acid, H_3BO_3 a very weak acid in aqueous solution. At physiological pH (< 8) mainly the undissociated boric acid is present in the soil or nutrient solution. This is also the preferred form taken up by the roots. Boron is strongly complexed to cell wall constituents even in the roots (Thelier et al., 1979). Long distance transport of boron from the roots to the shoot is confined to the xylem, and uptake and translocation are closely related not only to the mass flow of water to the root surface but also to the xylem water flow. There is no indication that boron is an enzyme component and there is only little evidence (Birnbaum et al., 1977) that the activity of any enzyme is enhanced or inhibited by boron. Interactions between boron and other mineral nutrients during uptake and in the plant itself seem to be of minor importance (Marschner, 1989).

Bo deficiency is a widespread nutritional disorder in vegetables. Boron deficiency usually occurs under:

1) High rainfall conditions where Bo is readily leached from soils as $B(OH)_3$.
2) At increasing soil pH especially in calcareous soils and soils with a high clay content presumably as a result of the formation of $B(OH)_4$ and anion absorption.
3) Under drought conditions, probably because of both a decrease in boron mobility by mass flow into the roots (Kluge, 1971) and polymerization to boric acid: $3B(OH)_3$ $B_3O_3(OH)_4 + H_3O + H_2O$.

Plant species differ characteristically in their capacity for Bo uptake when grown in the same soil. This is shown in Table 7 below.

Table 7. Boron content of the leaf tissue of plant species from the same location.

Plant species	Boron content (mg/kg dry weight)
Brussel sprouts	50.2
Carrots	75.4
Sugar beet	102.3

Based on Gupta (1979) adapted from Marschner (1989).

The critical deficiency range, expressed as milligrams boron per kg dry weight is about 5–10 in monocots, 25–60 in red clover, 30–80 in carrots and 40–100 in sugar beet (Bergmann, 1983). Differences in boron requirements are most likely related to differences in cell wall composition. High light intensities seems to increase sensitivity to boron deficiency, by raising the requirements for boron in the tissue.

Symptoms of B deficiency in the shoots are noticeable at the terminal buds or youngest leaves, which become discoloured and may die. Internodes are shorter, giving the plants a bushy or rosette appearance (witches broom). Interveinal chlorosis on mature leaves may occur and misshaped leaf blades are also symptoms of B deficiency in leaves. An increase in the diameter of petioles and stems is common and may lead to symptoms such as 'stem crack' in celery. Buds, flowers and developing fruits drop. In heads of vegetable crops (e.g. lettuce), water soaked areas, tipburn and brown or blackheart may occur.

In storage roots of celery or sugar beet, necrosis of the growing areas lead to heart rot. With severe deficiency the young leaves also turn brown and die, subsequent rotting and microbial infections of the damaged tissue being common. Nodule number was lower in B – deprived plants of *Vigna subterranean* Reduction or even failure of seed and fruit set are also common B deficiency symptoms. There was also reduced levels of crude fat and protein in soybean seed grown in B deficient soils (Eguchi, 1999).

The application of boron either to the soil or as a foliar spray, different sodium borates, including borax or sodium tetraborate can be used. Boric acid can also be used as foliar sprays. The amount of boron applied varies from 0.3 to 3.0 kg/ha depending on the requirement and sensitivity of the crop to Bo toxicity.

Boron toxicity may occur when large amounts of municipal compost are applied (Purves and Mackenzie, 1974), and it is of much concern in semi-arid and arid

areas where irrigation water contains high levels of boron. The critical boron concentration in irrigation water varies between 1 and 10 mg/L for sensitive and tolerant crops respectively. It is between 2 and 3 mg/L for wheat and 4 and 6 mg/L for peas (Chauhan and Powar, 1978). The critical toxicity levels expressed as mg boron per kg dry weight of leaves is 400 for cucumber and 1000 for squash (El-Sheik et al., 1971). Typical boron toxicity symptoms on mature leaves are marginal or tip chlorosis or both. They reflect the distribution of boron in shoots, following the transpiration stream.

3.3.4. *Phosphorus*

The phosphorus requirement for optimal growth of vegetables is in the range of 0.3 to 0.5% of the plant dry weight during the vegetative stages of growth. Plants suffering from P deficiency exhibit retarded growth, and often a reddish colouration occurs because of enhanced anthocyanin formation. P-deficient plants also often have a darker green colour than do normal plants. Deficiency leads to a general reduction of most metabolic processes including cell division and expansion, respiration and photosynthesis because of the functions of P in the growth and metabolism of plants (Terry and Ulrich, 1973). The regulatory function of inorganic phosphate P_i in photosynthesis and carbohydrate metabolism of leaves can be considered to be one of the major factors limiting growth particularly during the reproductive stage.

After uptake at physiological phosphate either remains as inorganic phosphate (P_i) or is esterified to a simple phosphate esteri or attached to another phosphate by the energy – rich pryophosphate bond e.g. in ATP. Phosphate forms a bridging group connecting units to more complex or macromolecular structures using another type of phosphate bond (C-P-C). In both RNA and DNA, phosphate forms a bridge between ribonuclesiode units to form macromolecules. Phosphorus is responsible for the strongly acidic nature of nucleic acids and thus for exceptionally high cation concentration in DNA and RNA structures. The bridging form of P diester is also abundant in the phospholipids of biomembranes where it forms a bridge between a diglyceride and another molecule (amino acid, amine or alcohol). In biomembranes the amine choline is often the dominant partner, forming phosphatidyl choline (lecithin). Most phosphate esters are intermediates in metabolic pathways of biosynthesis and degradation. Their function and formation is directly related to the energy metabolism of the cells and to energy rich phosphates. The energy required, for example, for biosynthesis or for ion uptake is supplied by an energy-rich intermediate or coenzyme, principally ATP. Energy liberated during glycolysis, respiration, or photosynthesis is utilized for the synthesis of the energy-rich pyrophosphate bond. ATP is the principal energy-rich phosphate required for starch synthesis.

Inorganic phosphate is also either a substrate or an end-product (e.g. ATP – ADP + P_i). Inorganic phosphate controls some key enzyme reactions. In fruit tissue of tomato, P_i released from the vacuoles into the cytoplasm can stimulate phosphofructokinase activity (Woodrow and Rowan, 1979). Phosphofructokinase is the key enzyme in the regulation of substrate flux into the glycolytic pathway. An

increase in the release of P_i from vacuoles can therefore initiate the respiratory burst correlated with fruit ripening (Woodrow and Rowan, 1979). Delayed fruit ripening in phosphorus deficient tomato plants (Pandita and Andrew, 1976) may be related to this function of P_i. ADP-glucose pyrophosphorylase is allosterically inhibited by P_i and stimulated by triose phosphates. The ratio of P_i to triose phosphates therefore determines the rate of starch synthesis in chloroplasts (Herdt et al., 1977). The enzymes being 'switched off' at high ratios.

3.3.5. *Nitrogen*

Elevated nitrate levels are uneconomical in relation to nitrogen utilization and are also undesirable nutritionally. Sometimes nitrite is formed from nitrate during either the storage or processing of vegetables. Infants fed on nitrite-containing foods run the serious risk of developing methemoglobemia.

4. EFFECT OF THE ENVIRONMENTAL CONDITIONS ON QUALITY OF SPECIFIC VEGETABLES

In this section specific examples of disorders caused by environmental factors are covered in a few chosen vegetables. The vegetable examples presented include tomato and squash which are two very different fruits, cabbage, a leaf vegetable and sweet potato a root vegetable crop.

4.1. Tomato

Tomato is a warm season vegetable belonging to the Solanaceae family. The tomato is adapted to a wide range of climatic and soil conditions. It is produced throughout the whole world from near the Arctic Circle under protected environments to the equator. The optimum range of temperature for tomato is 21–24 °C. Mean temperatures above 27 °C are not desirable while temperatures below 12 °C cause chilling injury. Tomatoes are a major source of vitamins and minerals to human nutrition. Production of tomatoes can be either in the field or in the greenhouse. The tomato crop has several disorders that are caused by environmental factors that ultimately reduce yield and quality. These are discussed below.

4.1.1. *Chilling injury*

This disorder shows surface pitting, poor colour development upon ripening, premature loss of firmness and increased susceptibility to decay. This is a result of very low above freezing temperatures especially night temperatures. Chilling injury in mature green tomato fruits could be alleviated by pre-harvest heat treatment for 20 days in greenhouses (maximum of 39 °C, average day temperature of 32 °C) compared to 27 °C. This could be achieved by reducing ventilation in the greenhouse (Kang and Park, 1999). Recent studies have indicated that heat treatment administered prior to chilling reduces the incidence and severity of chilling injury

in tomato fruits and other organs. Pre-storage heating of tomatoes at 38 °C for 3 days can extend their storage life and shelf life (Lurie et al., 1993). There was a correlation between maintenance of heat – shock protein mRNA during storage and the inhibition of chilling injury in heated tomatoes. Under certain conditions, however, heat treatment of tomato fruits may not reduce chilling injury as effectively as partial ripening (Whitaker, 1994).

4.1.2. *Sun scald*

Tomatoes at the mature green stage or beginning to show the pink colouration (breaker stage) are most susceptible to sun scald. The fruits develop a white necrotic tissue surrounded by a yellow halo. The patch often becomes sunken and wrinkled. The damage to the fruit occurs on the side or top half exposed to sunlight. The area may remain white or yellow or become dry and papery and may later become infected by fungal infection when the fruit ripens. It is caused by a sudden exposure of the fruit to direct sunlight during hot dry weather and can be worsened by practices such as pruning or harvesting or other operations involving working moving through the field and exposing the fruits. Sudden defoliation leads to exposure of the fruits to sunlight. When supports collapse they expose fruit to direct sun. Heat and light cause direct irradiation with sunlight. The fruits fail to turn red and in severe cases turn white and blistered. The exposed side of the fruit turns pale at first and becomes sunken and wrinkled as the fruit ripens. Temperatures above 35 °C inhibit lycopene (red pigment) formation, while carotene continues to be produced at temperature of up to 40 °C, causing the tomato to remain yellow. At temperatures above 40 °C the yellow tissues then turn white.

4.1.3. *Catface*

This is a typical malformation with deep cavities of the fruit which occurs at the blossom end of tomato fruit. These can range from mild to extreme deformations and scarring of the fruit. The fruit will have many bulges and be larger than the unaffected fruit produced by the same cultivar. Although hereditary, occurrence in larger fruited cultivars is mainly worsened by exposure of the plant to cool temperatures at the time of flower initiation. Cultivars may vary in their susceptibility to this disorder. The large fruited cultivar like Moneymaker is very susceptible. High nitrogen has also been recorded as a cause for catfacing.

4.1.4. *Cracked fruits/Growth cracks*

Cracks in tomatoes may radiate from the stem end or may appear in concentric rings on the shoulders of the fruit. The cracks usually form on unripe fruit often cutting deep into the flesh. The shallow ripe cracks are known as bursting. This disorder is hereditary although it is facilitated by continuous rain and heavy dew during maturity of the fruit. It may extend even into harvesting period when there is frequent flood irrigation with small amounts of water. Sudden changes in soil moisture and atmospheric humidity cause this disorder due to alterations in growth rate. It is

however, more severe when foliage is sparse as exposed fruits crack more due to exposure to greater temperature fluctuations from exposure to direct sun rays. Tomatoes grown under cold conditions are more prone to cracking. Temperatures below 10 °C encourage the condition as well. High nitrogen and low potassium result in succulent plants that produce tomatoes that are very susceptible to cracking.

4.1.5. *Blossom end rot (Black bottoms)*

Blossom end rot arises from a localized deficiency in calcium at or near the blossom end, which could be facilitated by several environmental conditions. It is also associated with fluctuations in the soil moisture due to incorrect or poorly scheduled irrigation and poor root development. A brown-black, dry, leathery depression at or near the blossom end occurs during development of the fruit commonly noticeable when the fruits are one-third to one-half full size. Occasionally it appears on the side of the fruits and sometimes produces internal black lesions not visible from the exterior of the fruit. Affected fruits ripen more rapidly than normal fruits. At first a small water soaked light tan spot appears, enlarges and darkens as the fruit size increases. The spot may enlarge to cover as much as a third to half of the entire fruit surface. Large lesions soon dry out and become flattened, black and leathery in appearance and texture. This is due to environmental conditions that cause water stress.

In hydroponic culture the supply of Ca^{2+} is usually more than adequate for plant development and is unlikely to be the primary cause of blossom end rot, however, the uptake of Ca^{2+} can be reduced by high EC, poor aeration or adverse temperature of the root zone. Root cooling to 17 °C in plants grown in rockwool mats reduced blossom end rot incidence and increased the Ca^{2+} content of the fruits (Benoit et al., 2001).

Transport of Ca^{2+} to the fruit is intrinsically low, as most of the Ca^{2+} is transported to leaves by canopy transpiration. A combination of low uptake and low transport of Ca^{2+} to the fruit can cause a low Ca^{2+} status in the fruit (Ho et al., 1999).

Environmental conditions required for high yield, such as light, carbon dioxide concentration and temperature stimulate fruit expansion, but may not increase and may even reduce the transport of Ca^{2+} to the distal tissue of the fruit. Incidence of blossom end rot is determined by the fruit growth response to the environmental conditions during the rapid phase of fruit enlargement.

Symptoms of blossom end rot may not be entirely caused by low Ca^{2+} in the fruit tissue itself, but its interactions with N and P in maintaining the cell membrane permeability and cell wall structure. In an experiment carried out to determine the influence of N source, N application rate and soil moisture on the incidence of blossom end rot, a close relationship was observed between the incidence of blossom end rot and the ammonia concentration in the soil solution (Morikuni and Shimada, 2001). In soil where nitrification had been delayed, the unnitrified ammonia was likely to suppress Ca^{2+} absorption and induce blossom end rot. When only nitrate N was applied the blossom end rot was decreased. A relationship was clearly observed between the incidence of the blossom end rot and Ca^{2+} concentration in petiole sap from the leaf near the trusses, just after blooming. When Ca^{2+} concen-

tration went below 200 mg L^{-1} blossom end rot was more likely to occur. Longer NH_4-N supply increased the amount of fruit with blossom end rot in the winter but had no effect in spring, however, higher NH_4-N concentration in solution in spring greatly increased the number of fruits with blossom end rot (Sandoval-Villa et al., 2001).

Sandy soils are also vulnerable to lack of water and nutrients due to high leaching. Some literature has suggested close spacing in the field and dry winds as encouraging blossom end rot. Factors that influence the uptake of water and Ca^{2+} by the plant definitely promote development of the disorder. This disorder is most prevalent when rapidly growing plants bearing fruits are suddenly exposed to a drought period and when roots fail to obtain nutrients and water due to any form of damage or otherwise. Factors that encourage rapid growth are high nitrogenous fertilizer application or high temperatures (Tabatabaei et al., 2001) and light intensity. Cultivation or hoeing too close to the plant destroys roots interfering with transportation of water and Ca^{2+} predisposes the developing fruit to blossom end rot. These factors favour development of the disorder. When tomato fruits were grown with or without infrared light reflective filters to control temperature, fruits in the unfiltered compartment were 16% larger than those in the filtered compartment, but Ca^{2+} concentration was 12% less. Thirty five percent of fruits in the unfiltered compartment had blossom end rot compared to none in the filtered compartment. High temperature reduced Ca^{2+} accumulation, but increased K^+ concentration in fruits. To control blossom end rot, rapid growth caused by high temperature should be avoided (Tabatabaei et al., 2001).

4.1.6. *Hollowness (Puffiness)*

The fruits are light and soft. Fruits appear slab-sided or angular from the exterior. Various climatic conditions, varietal and nutritional factors cause the malformation. The cavities of a normal fruit are filled with a jelly-like substance which carries the seed between the walls and the core. When hollowness occurs the cavities are not completely filled with the jelly and consequently the fruit becomes puffy or hollow, light in weight, soft and of reduced quality. Cultivars with two or three seed cavities are usually more subject to hollowness. High nitrogen applications in the early stages of growth and incorrect irrigation scheduling facilitate development of hollowness. It is encouraged in plants that set fruits in cool weather although bad or inadequate pollination, fertilization and seed development may contribute to the disorder. These can be due to improper nutrition. Low potassium nutrition, high nitrogen, high phosphorous coupled with low light and low dry matter content of the fruit all encourage development of puffiness. The use of auxin growth regulators for fruit set can also lead to puffiness.

4.1.7. *Uneven colouring (blotchy ripening)*

This disorder is caused by different factors which include insufficient light, virus infection and potash deficiency. Too high or too low temperatures during fruit ripening will cause green or yellow shoulders on ripe tomatoes. Generally high

temperatures and luxuriant growth as a result of nitrogenous fertilizer coupled with cool overcast weather and potassium deficiency are all associated with uneven ripening which may resemble the mottling caused by Tomato mosaic virus (TMV). Tomato Mosaic Virus causes a green-yellow red mottling of the fruits while Tomato Spotted Wilt Virus causes circular spots with concentric red and yellow bands.

4.1.8. *Grey wall (vascular browning; internal browning)*

A greyish-brown discolouration of the fruit wall shows through the healthy tissue of the skin. When the tomato is cut in cross-section, internal browning is evident on the tissue around the vascular bundles. Apart from tomato spotted wilt virus the same conditions that cause uneven ripening will cause this particular disorder. These include TMV, luxuriant growth, heavy applications of nitrogenous fertilizers, low light intensity, moist weather, high soil moisture and low temperature, but also high temperatures and water stress.

4.1.9. *Blackheart*

From the outside the tomato fruit looks healthy, but when cut, the core and other tissues inside are found to be black. The same environmental conditions that cause blossom end rot are thought to cause this disorder.

4.1.10. *Creased stem*

This occurs when too much nitrogen is applied when tomato plants are still very young. Once this occurs fruits become malnourished and become small and unhealthy. They are light in weight and fail to attain the red colour expected by the consumer.

4.1.11. *Internal white tissue*

The white tissues are usually in the outer wall of the fruit which shows when the tomato fruit is cut in half. The problem occurs in the placental area, proximal to the locules. The tissues may extend from the core into the fruit with an increase in the amount of white tissues. There are cultivar differences to susceptibility to this disorder. Poor potassium nutrition and high temperatures or any other factors that impose some form of stress causes internal white tissues. Sweet potato white fly has been involved and tends to increase the incidence of this disorder.

4.1.12. *Rain check*

Numerous tiny concentric cracks (which may coalesce) appear on the shoulders of the fruit. The cracks are rough to the touch. Affected areas may become leathery and remain green when the fruit ripens or may become blackish. If the fruit becomes red the cracks are still visible. The cause is not well known but could be temperature alteration by rain or water uptake which disrupts the shoulder epidermis. The problem tends to be severe when a dry period is followed by heavy rains.

4.1.13. *Blossom drop*

Yellowing of pedicels and calyx leads to the abortion of the flower. This may take place before or after anthesis. The flower withers and turns brown but does not abscise. Stress conditions increase the incidence of the disorder. Any factor that inhibits, pollination and fertilization for example low or high temperature, high relative humidity, excess wind lead to blossom drop. Improper nutrition from deficiencies in fertilizer or excess nitrogen tends to also increase the problem. Foliar diseases and insect damage are some documented causes.

4.1.14. *Zippering*

Very thin longitudinal brown necrotic scars start at the stem scar and extend part or all the way to the blossom end. These long scars have small transverse scars along them resembling a zipper. Mostly one scar occurs per fruit but there can be several, at times a hole open at the locule forms in addition. Under cool weather the anthers may be attached to the ovary wall of newly forming fruits causing zippering. Other weather conditions may also be involved however, susceptibility differs with cultivars.

4.2. Squash

4.2.1. *Chilling injury*

Chilling injury is caused if squashes and pumpkins remain in the field at 10–12.5 °C or are stored below that temperature. Symptoms of chilling injury are sunken pits on the surface and high levels of decay (Cantwell and Suslow, 2001). Severe pitting and slight decay were observed after 12 days of exposure to low temperature (Wang, 1993). The plants may be severely injured and maturity delayed by temperatures below 5 °C for several days.

In squash (*Cucurbita moschata*) the severity of chilling injury in fruits stored at 4 °C was reduced by dipping in 1% $CaCl_2$ or 10 mM sodium benzoate at 20 °C for 30 minutes. Fruits treated with sodium benzoate showed a low incidence (< 10%) of chilling injury following 30 d of storage at 4 °C (Lee and Yang, 1999). The severity of chilling injury in *Cucurbita pepo* (cv Elite) fruits stored at 5 °C and then transferred to 20 °C was reduced by a pre storage treatment with hot water at 42 °C for 30 minutes (Wang, 1993). Chilling injury was further reduced when fruits were preconditioned at 15 °C for 20 days after hot water treatment but before 5 °C storage.

In Zucchini squash (*Cucurbita pepo*) post harvest treatments that reduce chilling injury (temperature conditioning and low O_2 storage) were found to increase endogenous levels of polyamines (Wang, 1993). Exogenous treatment with polyamines by pressure infiltration was shown to increase the tolerance of squash to chilling injury. Both low O_2 storage (1% O_2) and temperature conditioning (2 days at 10 °C followed by storage at 2.5 °C) treatments were effective in delaying the development of chilling injury symptoms. Temperature conditioning reduced the

development of chilling injury symptoms and suppressed chilling induced ethylene evolution in both cultivars of *Cucurbita pepo* (B^+/B^t) and *Precocious Caserta* (B/B) squash (McCollum et al., 1991). Fruits stored under 1% O_2 maintained higher levels of spermidene and spermine than those stored in air indicating that high polyamine levels are positively correlated with the ability of squash fruits to withstand chilling stress (McCollum et al., 1991).

4.2.2. *Freezing injury*

Can occur in vegetables at temperatures below –0.8 °C both in the field and in storage (Cantwell and Suslow, 2001).

4.2.3. *Oedema*

Oedema is a physiological disorder of cucumbers and is most frequently found with pumpkins and winter squash that are subjected to moisture stress. This is most often associated with uneven availability of moisture when immature fruit are enlarging. On winter squash rinds the severity may be enough to make the fruit unmarketable. The fruit surface is often raised with circular shaped lesions that are corky or 'crusty' in appearance and may appear irregularly over the entire surface, or be limited to for example, the shoulder side exposed to direct sunlight. The crusty appearance is similar to the appearance of scab on hard shelled fruit, except that the oedema lesions never appear crater-like or shrunken (Bodnar and Fitts, 2001). On buttercup squash the corking lesions may be circular, spindle or apostrophe shaped. While on butternut squash oedema appears as linear growth cracks usually on the neck portion of the fruit.

4.2.4. *Stigma death*

The development of female flowers may be affected by temperature. Recent experience in the Middle Atlantic States and in New England indicates that high night temperatures (above 20 °C) are associated with failure of female flowers to open and develop properly (Oregon State University, 2002). The ovaries turn yellow and then shrivel and the stigma of the unopened flower exhibits black streaks into the ovary. This reduces the yield of squash.

4.3. Cabbages

Cabbage is a cool season crop belonging to the Cruciferae family. Cabbage is a very important crop in the temperate regions of the world but it is also gaining importance in the high altitude areas of the tropics especially for small scale farmers as it has a fairly long shelf life. Sauerkraut is the main processed product from cabbages (Yamaguchi, 1983). In most of the tropics, cabbage can also be dried blanching is necessary before drying. Optimum range of temperatures for growth of cabbages is 15–20 °C, above 25 °C growth is arrested. Minimum temperature is 0 °C but cold hardened plants can withstand temperatures as low as –10 °C for

short periods of time. Most cultivars are short day plants but others appear to be day length neutral. For a high acceptable quality of cabbage removal of outer wrapper leaves should reveal the typical shape and colour of the cultivar (green, or purple or pale yellow-green), firm, heavy for the size head, and the head must be free of insect, decay, seed stalk and other defects. Leaves should be crisp and turgid (Cantwell and Suslow, 2001). Some disorders caused by environmental factors that impact on quality of cabbages are discussed below.

4.3.1. *Bolting*

This refers to production of a seed stalk instead of a head. In the tropics this rarely happens but it can occur at very low temperatures (especially night temperature) accompanied by extreme variations with day temperatures. This is undesirable as cabbage is grown mainly for the heads. High-veld areas are generally colder therefore cabbages can be grown in summer, autumn and spring to avoid bolting as winter temperature cause bolting even in the tropics. Deterioration in storage can also be associated with bolting (Cantwell and Suslow, 2001).

4.3.2. *Frost injury*

Heavy losses of cabbage occur annually as a result of freezing, both in the field and during storage. Cabbage tissue has one of the highest freezing points (–0.6 °C) found among vegetables (Pierson et al., 1971) even though it is a cool season vegetable. There is no way of determining by examination of a frozen head whether it will exhibit injury when thawed. Immediately after thawing, frozen areas appear water soaked due to suffusions due to water liberated by the thawing of ice in intracellular spaces. If cells have not been killed, some of this water will be reabsorbed and the tissue will only appear slightly wilted or shriveled due to excessive water loss. The margins of lower leaves become flaccid and turn brown and may even die. There is often rupturing of the epidermis on the underside of leaves and cracking of main veins. The veins then become spongy, pithy, and tough, losing their characteristic flavour. Some heads can withstand freezing several times before the effects become pronounced; others are injured the first time. If cells have been killed, water does not re-enter them and is either lost due to evaporation, with attendant drying out of the tissues, or it remains and the tissues become a leaking, disorganized mass which soon succumbs to saprophytic fungi and bacteria attacks. The outer leaves of cabbage appear to be more resistant to frost damage than the inner leaves and stem. They generally thaw without injury. Following prolonged exposure to freezing temperatures, the inner tissues, especially the stem pith, are killed and subsequently become affected with bacterial soft rot. A good quality cabbage should be crunchy and fresh.

4.3.3. *Head cracking*

Cabbage heads crack when approaching maturity during hot months. Uneven provision of soil moisture coupled with high temperature cause cracking. Very dry

conditions followed by sudden high moisture or heavy irrigation after the dry period also leads to cracking. Too rapid growth at high nitrogen levels will lead to coarse, loose heads, poor processing and storage quality and cracking. Cracking may also occur as a result of boron deficiency in the soil.

4.3.4. *Blindness*

Blindness may result from planting blind seedlings from the beginning. The apical meristem is damaged or is missing from the beginning. Physical damage to the growing tip will result in cabbages that will not head due to the absence of the shoot tip. Freezing injury during the initial stage of head formation may lead to blindness (Yamaguchi, 1983). According to Rice and Tindall (1983) temperatures higher that 24 °C hinder head formation and may also lead to blindness.

4.3.5. *Foul smell*

Very dry conditions cause the cabbage leaves to be tough and rough and the cabbage produces a typical strong cabbage smell when cooked. The leaves are supposed to be tender and slightly succulent as cabbage is generally consumed fresh and raw in salads. As a heavy feeder cabbage requires higher nitrogen content than other vegetables. If this is lower than its requirements yields are drastically reduced, storage life shortened, maturity delayed and there is an increase in the 'cabbagey' flavour (foul smell) which is undesirable (Cantwell and Suslow, 2001).

4.3.6. *Tip burn*

Leaf margins become brown and papery later turning dark brown to black and finally necrotic. Injured tissue becomes predisposed to attack by soft rot bacteria. Tipburn mainly due to localized Ca^{2+} deficiency in the head that can be enhanced by several environmental conditions. The Ca^{2+} can be unavailable due to drought or water logging in the soil or when soil fertility is too high due to high levels of N, K and Mg (http://www.cals.ncsu.edu/sustainable/peet/profiles/ppleafy.html). This disorder is believed to be very similar to blossom end rot in tomatoes in not only its causes but also the environmental factors affecting. Conditions favouring include fluctuating growth rates especially rapid growth caused by high temperatures, light intensities and excess nitrogen (http://www.cals.ncsu.edu/sustainable/peet/profiles/ppleafy.html). Late harvesting and wide spacing of crop in the field can also increase the incidence of this disorder.

4.3.7. *Oedema*

These are wart-like swellings that appear mainly on the underside of leaves of cabbage. Over-watering or prolonged rainy weather cause such swellings.

4.3.8. *Button*
This refers to unusually small heads due to premature generative stage. This happens under poor environmental conditions which arrest vegetative growth. Cabbage heads also become small due to boron deficiency and will also be yellow (Yamaguchi, 1983).

4.3.9. *Leaf abscission*

Cabbages are very sensitive to ethylene (Cantwell and Suslow, 2001). Very low levels of the ethylene will lead to leaf fall and yellowing. In the field, if cabbages are exposed to other plants or fruits nearby that produce ethylene the disorder will appear. In storage poor ventilation and mixing with ethylene producing plants and fruits will result in leaf abscission.

4.3.10. *Black speck, pepper spot, petiole spot, gomasho*

Very small to moderate size discolourations in the form of lesions appear on the midrib and leaf veins. In the field black speck is as a result of low temperatures coupled with harvesting over-mature cabbage heads whereas in storage and in transit this is due to exposure to low temperatures suddenly followed by warmer temperatures (Cantwell and Suslow, 2001).

4.4. Sweet potatoes

The sweet potato (*Ipomoea batatas*) is a member of the Convolvulaceae family and is a warm season tender perennial root crop grown as an annual for its storage roots. Other common names include Louisiana yam and Spanish potato. The crop is grown throughout most of the tropics. Sweet potato is the major staple crop in Papua New Guinea (Hartemink et al., 2000). It is also an important secondary food crop for many Eastern and Southern African countries whose staple diets are based on cereals, particularly maize (Gakonyo, 1993). Sweet potato is an important food security crop when maize is in short supply or in years of drought (Mutuura et al., 1992). Sweet potato is grown in tropical, subtropical and warmer temperate regions during the frost-free periods (Yamaguchi, 1993). They are widely grown from 40° N to 40° S and above 2500 m at the equator (Hahn and Hozyo, 1984). It is the most widely adapted of the agriculturally important root crops native to the humid tropics. Sweet potatoes require a minimum frost-free period of 120–150 days and a minimum average daily temperature of 24 °C (Yamaguchi, 1983). It also requires approximately 2 cm of soil moisture per week, uniformly distributed during the growing season. Sweet potatoes are adapted to a wide range of soil textural classes but sandy loams at a pH of 5.3–7.0 are considered ideal (Norman et al., 1995). Heavy clay soils often give low yields of poor quality (Kay, 1973) and irregular tuber shape (Martin, Leonard and Stamp, 1976). However, in spite of their high clay content ($\cong$ 60%) oxisols and histosols are particularly suited to sweet potatoes (Feliciano and Lugo-Lopez, 1977; Chew, 1970). The quality of the sweet potato root is affected by several environmental conditions during the growing

season. The main environmental factors affecting root quality are soil moisture, nutrition, temperature and pests. These will be discussed separately below.

4.4.1. *Freezing injury*

Sweet potatoes are generally considered to be very susceptible to low temperature injury, however, their average freezing temperature of –1.7 °C is lower than that of many hardier vegetables. Sweet potatoes can be exposed to freezing temperatures in the field at harvest if night temperatures fall below 0 °C and dug roots remain in the field overnight. While this is uncommon in the tropics, such temperatures can be encountered during harvest. Sweet potatoes that have been only slightly frozen are characterized by yellowish-brown discoloration of the vascular ring and internal vascular elements and by a yellowish-green water-soaked appearance of surrounding tissues. When exposure has been long enough for extensive ice formation to occur within the tissues, they collapse immediately upon thawing and the root becomes soft and flaccid as water is liberated. Such roots may dry and become brown, discolored mummies, although they usually decay due to infection by blue mold fungus (Clark and Moyer, 1988). Sweet potatoes must be harvested before freezing weather occurs.

4.4.2. *Chilling injury*

Potatoes exposed to moderate chilling may be dug, cured and sold but they should not be placed in long-term storage (Pierce, 1987). Lower temperatures significantly reduce root quality and storage life of sweet potatoes. Low temperatures may cause internal breakdown in storage roots, internal discolouration, an increase in the frequency of roots with decay and sometimes, hard core. Hardcore is a disorder in which roots remain hard after cooking. The cold is thought to modify pectic substances in the middle lamella so that the tissue remains hard during cooking. Hardcore develops in roots exposed to 1.5 °C for as little as one day or 10 °C for at least three days. Chilled roots do not exude latex when cut (Clark and Moyer, 1988).

4.4.3. *High temperature and sunscald*

The sweet potato plants are relatively resistant to the combined effects of heat and sunlight. In contrast to the growing plants, storage roots left exposed to the sun after they are dug are commonly damaged by sunscald. Many harvesting systems involve removing storage roots from the soil and leaving them on the soil surface. This facilitates drying and removal of soil from the roots, which are picked in a second step of the harvest operation. When roots are left in bright sun for as little as 30 minutes, serious sunscald can result. High temperature (greater than 32 °C) can also damage harvested sweet potatoes causing sunscald and eventually breakdown and decay (Pierce, 1987). They become soft near the surface, and within a few days their exposed surfaces may turn purplish brown. The incidence of storage rots, especially *Rhizopus* soft rot, surface rot, *Fusarium* root rot and charcoal rot,

is greatly increased when sun scalded roots are stored (Clark, L. Moyer, 1988). Sweet potatoes left in containers to dry for brief periods in the field can be covered with vines to prevent sunscald.

4.4.4. *Soil structure effects*

Sweet potatoes require well prepared soil worked to a depth of 15 cm, supported by a heavy clay loam subsoil with good drainage. In loose or too deeply ploughed soil the roots have a tendency to become long and slender (Nonnecke, 1989). A firm subsoil beginning at 15 cm provides the right conditions for tuber development. Sweet potatoes produce better quality tuberous roots when following soybeans or other legumes in the rotation. Heavy soils or those with organic matter exceeding 2% are not recommended since the shape may be poor and scurf and black rot fungi persist in such soils. Scurf is a disease characterized by roughened skin. Long rotations should be used to decrease the incidence of scurf and infection from *Fusarium* wilt (Kemble et al., 1996). Heavy clay soils result in rough, irregular roots (Peet, 2002).

4.4.5. *Soil moisture effects and related disorders*

Sweet potato is considered drought tolerant, and an even moisture supply throughout the growing season will enhance the yield and market appearance. Maximum yields have been reported at 20% available moisture on fine soils to 50% in coarse sands. Water logging and fluctuating soil moisture in sweet potato production significantly affect the quality of the roots. Poor aeration caused by poor drainage decrease yields of sensitive cultivars and can cause either souring (tissue breakdown in the storage roots) with severely impeded drainage or water blisters (enlargement of lenticels on the periderm) if the drainage problems is less severe (Peet, 2002). Wet soil conditions at harvest lead to an increase in tuber rots and adversely affect yield, storage life, nutritional and baking quality (Ton and Hernandez, 1978; Akparanta, Skaggsa and Saunders, 1980).

4.4.5.1. *Souring*

Souring is caused by roots being in water – saturated soils for prolonged periods prior to harvest. Souring can result in complete crop loss. Sweet potato roots sustain a high rate of metabolic activity. When soils are saturated with water, the exchange of oxygen and carbon dioxide is inhibited and the roots become asphyxiated. Ethanol and carbon dioxide accumulate in such roots. The result is a high percentage of roots that decay during curing, and surviving roots undergo a greater amount of shrinkage. Excessive soil moisture may also reduce quality factors such as carotenoid pigments, dry matter content and baking quality. Tolerance to flood damage may be due to either less ethanol production (Ahn et al., 1980) or the ability of the storage roots to metabolilize ethanol during and immediately following flooding (Corey et al., 1982).

4.4.5.2. *Water blisters*
Storage roots may develop small raised bumps at the lenticels called water blisters following extended periods of flooded soil. The bumps are white at first and turn brown to black after the roots are harvested. They often develop prior to souring. Cultivars differ in their tendency to develop water blisters.

4.4.6. *Storage root cracking*

Storage roots crack or split in the field, at harvest or even during storage in response to many diverse factors. Growth cracks which develop during root enlargement in the field are probably the most frequently encounted disorder. These are mostly longitudinal and are more common on large than on small tubers. A strong association has been observed between fluctuations in soil moisture and cracking. The incidence of cracking is high when prolonged periods of dry soil conditions are followed by continued wet soil conditions (Clark and Moyer, 1988). Low temperatures (12–16 °C) during the period when storage roots are being set can also lead to increased cracking. It has also been suggested that Boron deficiency or excessive N or lime can lead to cracking. Cracking is more common when sweet potatoes are planted in the same field successively for several years (Clark and Moyer, 1988).

4.4.7. *Soil nutrition*

Soils used to grow sweet potatoes are often of relatively low native fertility or subject to rapid losses of some nutrients by leaching. Nutrient deficiencies are therefore encountered more frequently. Toxicities are also possible in acidic soils, due to an excessive availability of certain elements such as aluminum and manganese. Lime is often used in acid soils to improve overall nutrient availability and plant growth. However, sweet potatoes are subject to serious damage by *Streptomycin impomoea* when the soil pH is above 5.2. The damage caused by this pathogen generally exceeds the potential benefits of liming. Fertilizer recommendations can only be relied on when based on knowledge of the local soil and sweet potato cultivar being grown.

4.4.7.1. *Nitrogen*
Sweet potatoes usually require moderate amounts of N in the presence of other macronutrients and micronutrients for good vine growth and optimum yield of roots of marketable size and shape. Storage roots grown in a soil low or deficient in N may have abnormal skin colour for example white skinned cultivars may develop tan skins (Clark and Moyer, 1988). An adequate supply of N has been found to improve the grades, total yield and quality (protein, carotenoids, low fiber) of sweet potato. Excessive N may increase the number of jumbo roots (Pierce, 1987).

4.4.7.2. *Phosphorus*
Sweet potato uses P efficiently and can extract sufficient amounts of it from soils well supplied with the element. Storage roots are often small and irregularly shaped under P deficiency. Any purple pigmentation present in the storage roots becomes

intensified by the deficiency. Sufficient quantities of P increases the length of storage roots.

4.4.7.3. *Potassium*
Sweet potatoes have a high demand for potassium (K^+), which is required for the enlargement of storage roots. The shape and total soluble solids content of storage roots are also affected by this mineral. Low K^+ reduces the number of roots formed and those that are formed are long, spindly and malformed (Clark and Moyer, 1988; Pierce, 1987). The deficiency has also been associated with greater weight loss and surface rot of roots in storage. Increasing the availability of K^+ tends to cause an increase in the carotene content of storage roots. High concentrations of K^+ can reduce the solids content of storage roots, which can lead to reduced dry matter content and reduced firmness in canned products (Clark and Moyer, 1988).

4.4.7.4. *Calcium*
The effect of Ca deficiency on the ability to produce storage roots varies, depending on the cultivar, from negligible effect to total failure to produce roots (Clark and Moyer, 1988). Calcium deficiency causes soft, very small and misshapen tubers to be formed.

4.4.7.5. *Boron*
Symptoms of B deficiency in storage roots are diagnostic – they are an internal brown spot and blister. The blister caused by B deficiency is characterized by small raised bumps on the root surfaces and plant stunting (Miller and Nielsen, 1970; Peet, 2002; Pierce, 1987). Except for very susceptible cultivars, the blister does not show up until sweet potatoes have been stored for several months. Internal brown spots are similar in appearance to symptoms associated with internal X. They may occur anywhere within the storage root but are most common near the vascular ring. The spots are brown, vary in size, and have indefinite margins.

5. CONCLUSION

The quality of produce on the market is affected by several factors including pre-harvest factors, stage of harvest, transportation, and environmental conditions in storage. This chapter focused on preharvest factors only. The role of these factors have been highlighted showing how quality can be affected. The focus on physiological disorders is also important as it highlights some of the cultural control methods that need to be taken care of in order to produce high quality produce that can be stored for the maximum possible self life. This is not completely exhaustive as t here are also other factors that can cause quality loss that cold not be considered in this chapter.

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EFFECTS OF AGRONOMIC PRACTICES AND PROCESSING CONDITIONS ON TOMATO INGREDIENTS

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1. INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum*) originated in the New World from wild species that are native to the Andean region of South America. In the 16th century, the tomato was taken to Europe. Due to its relationship with poisonous members of the night-shade family, the fruit of the tomato plant developed and was accepted as a food remarkably slowly at first, but then became very popular (Beecher, 1998). Processed tomatoes rank second to potatoes in dollar value among all vegetables produced (Gould, 1992). From 1990 to 1999 the quantity of processed tomatoes significantly increased from 22.8 million tons to 29.6 million tons worldwide (Bilton et al., 2001).

Tomatoes contain modest to high amounts of several nutrients. Regarding the vitamins, tomatoes have remarkable concentrations of folate, vitamin C and vitamin E. In addition, they are the most important source of another constituent, the carotene lycopene, not having any pro-vitamin A activity. They are also known for their content of the pro-vitamin A active β-carotene as well as that of flavonoids and potassium (Beecher, 1998).

Beneficial effects of the Mediterranean diet have been stated manyfold (Nestle, 1995; Kushi et al., 1995). Compared to Northern Europe, in Italy cancer rates have been lower. There, tomatoes are regarded as the second most important source of vitamin C after oranges. In a study of colorectal cancers, based on 1953 cases and 4154 controls, tomato intake was significantly protective on colorectal cancer risk (La Vecchia, 1998).

Among 72 epidemiological studies reviewed by Giovannucci (1999), 57 of them reported inverse associations between tomato intake or blood lycopene level and the risk of cancer at a defined anatomic site. The evidence for a benefit was strongest for cancers of prostate, lung, and stomach (Giovannucci, 1999).

This chapter will focus on major ingredients of tomatoes as affected by agronomic practices and processing conditions.

2. NUTRIENTS

Tomatoes have modest to high concentrations of several traditional nutrients. Besides considerable contents of folate (13 μg/100 g), they are rich sources of potassium (279 mg/100 g) and vitamin C (23 mg/100 g). Regarding the carotenoids, tomatoes and tomato products are the main source of the non pro-vitamin A active carotene lycopene, which is responsible for the red colour. In addition, some other carotenoids

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like the pro-vitamin A active β-carotene and lutein are minor components of tomatoes (Beecher, 1998). Lycopene is mainly associated with the skin of the tomato, where about five times more of this carotenoid (53.9 ± 6.5 mg/100 g, wet basis) was found compared to the whole tomato pulp (11 ± 0.05 mg/100 g, wet basis) (Sharma and Le Maguer, 1996). Own investigations showed a similar relation for lycopene between pericarp (11.1 mg/100 g, wet basis) and pulp (2.7 mg/100 g, wet basis) (Böhm and Bitsch, 1995).

Investigations of different tomato varieties on their contents of antioxidant vitamins resulted in 22–48 mg/100 g vitamin C, which makes fresh tomatoes to an important source of this vitamin. The contents of vitamin E, mainly α-tocopherol, located in the seeds, ranged between 0.1 mg/100 g and 3.2 mg/100 g. β-carotene as pro-vitamin A active carotenoid was analysed in amounts of 1.1–3.7 μg/100 g (Abushita et al., 1997). 98% of the flavonols detected in tomatoes were found in the skin with conjugated quercetin as main components (13.8 ± 0.6 mg/100 g fresh matter). The second flavonol detected in tomatoes, kaempferol, occurred only in minor concentrations (skin: 0.48 ± 0.03 mg/100 g fresh matter) (Steward et al., 2000).

3. EFFECT OF ORIGIN AND VARIETY

The variety highly influenced the contents of secondary plant products and antioxidant activity of tomatoes. Investigating eight varieties, tomatoes with the Crimson gene had highest lycopene content (2.6–4.3 mg/100 g) (Thompson et al., 2000). Scalfi et al. (2000) analysed sixteen ecotypes of Corbarini small tomatoes on their antioxidant activity in the water-soluble fraction and in the water-insoluble fraction. Round shaped tomatoes were significantly more effective than the long ones (Scalfi et al., 2000). In another study, the amount of total carotenoids differed from less than 1 mg/100 g for green salad tomatoes to up to 15 mg/100 g for some cherry tomatoes (Fogliano et al., 2001). 12 tomatoes for fresh consumption (salad tomatoes) were compared to 15 varieties for processing. The latter had significantly higher lycopene concentrations (8.0 ± 1.0 mg/100 g) compared to the salad tomatoes (6.0 ± 0.9 mg/100 g) as well as significantly higher a-tocopherol contents (731 ± 84 μg/100 g vs. 275(44 μg/100 g). In contrast, the concentrations of ascorbic acid did not differ between both groups ($p > 0.05$) (Abushita et al., 2000).

Analysing one variety (Favorita) from different countries, fruits from Spain and South Africa contained 4–5 fold more flavonols than British fruits (Figure 1) (Steward et al., 2000).

4. INFLUENCE OF MATURITY STAGE

Tomatoes harvested at mature green stage had only low contents of lycopene. Lycopene concentrations were significantly higher in fruits harvested at breaker stage and further enhanced in the red ripe stage. Storage at room temperature of tomatoes harvested at green stage or at breaker stage for up to 12 days led to significantly enhanced lycopene concentrations. However, their contents of lycopene were lower

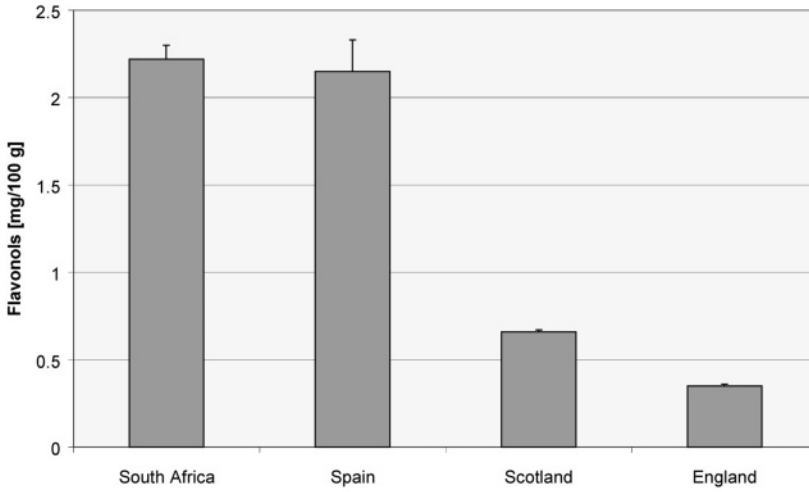

Figure 1. Total flavonol content in Favorita tomatoes from different countries (modified from Steward et al., 2000).

than that of tomatoes harvested at red ripe stage (Thompson et al., 2000). In another study, tomatoes were harvested at five different maturity stages (green, white, yellow, light red, red) (Cabibel and Ferry, 1980). While β-carotene and lutein were present already in the mature green tomatoes, lycopene was analysed first in the light red stage. The contents of β-carotene and lutein did not change in the first two maturity stages. The β-carotene concentration increased in the yellow tomatoes compared to the white tomatoes. In contrast, lutein content first increased in the light red stage. In the light red tomatoes lycopene was analysed first. Its content significantly increased in the red stage, becoming the main carotenoid of red tomatoes (Cabibel and Ferry, 1980).

Observing four ripening stages (green, yellow, pink, red), Abushita et al. (1997) analysed the contents of ascorbic acid, β-carotene and tocopherols. Ascorbic acid content of Floriset tomatoes significantly increased from green stage to yellow stage and decreased while ripening more. In contrast, β-carotene and α-tocopherol as well as β-tocopherol contents significantly increased with ripening stage. γ-tocopherol approached its maximum in yellow fruits, then declined similar to ascorbic acid (Abushita et al., 1997).

Investigations on phenolics were done in five different maturity stages (immature green, mature green, breaker, pink, red) of tomatoes (Buta and Spaulding, 1997). The three predominant phenolics chlorogenic acid, *p*-coumaric acid glucoside and rutin were analysed in all samples. The content of chlorogenic acid (5′-caffeoylquinic acid) declined from immature to mature green, increasing to the breaker stage. This stage showed the maximum of chlorogenic acid with decreasing concentration during further ripening. No large fluctuation in *p*-coumaric acid glucoside occurred during ripening, except in the pulp with increases until breaker stage and then decreasing contents. Rutin concentration significantly declined from immature to mature green stage, being stable while ripening more (Buta and Spaulding, 1997).

5. EFFECTS OF TEMPERATURE

It has long been known that tomatoes ripened at high temperatures failed to develop the red pigment lycopene. For example Rutger tomatoes ripened at 23.5 °C contained 4.4 ± 0.9 mg/100 g lycopene. In contrast, using a maturation temperature of 32.0 °C resulted in a strong reduction of lycopene contents to 0.7 ± 0.4 mg/100 g (Tomes, 1963).

Sub-optimal temperatures were investigated more recently. The authors used the temperatures (each min./max.) 17.8/25.6; 7.2/18.3; 4.4/15.6; 2.8/13.9 °C and harvested the tomatoes after 7/14/21 days. Figure 2 shows the concentrations of lycopene for the different temperatures and harvesting regimens using Early Red Chief tomatoes (Koskitalo and Ormrod, 1972).

Lycopene concentration increased with higher temperature and later harvest. In contrast, β-carotene was not affected by the decline in ripening temperature (data not shown).

In another study (Hamauzu et al., 1998), the effect of post-harvest storage temperature on the conversion of ^{14}C-mevalonic acid to carotenes was investigated. Pericarp sections of tomato fruits were stored at 20/30/35 °C for 5 or 10 days. They were also incubated with ^{14}C-mevalonic acid for 10 hours at these temperatures. Lycopene content was highest at 20 °C and lowest at 35 °C, while β-carotene showed the highest concentration at 30 °C. Regarding the radioactivity, in samples after 10 days of storage that of lycopene was the highest in sections exposed to 20 °C and lowest at 35 °C. In contrast, the incorporation of ^{14}C-mevalonic acid into β-carotene was lowest in sections stored at 20 °C and highest at 35 °C. The conversion rate of ^{14}C-mevalonic acid to β-carotene tended to increase with rising storage temperature while the conversion rate to lycopene tended to decrease with rising storage temperature. At high temperature the conversion of lycopene to β-carotene seemed to be stimulated (Hamauzu et al., 1998).

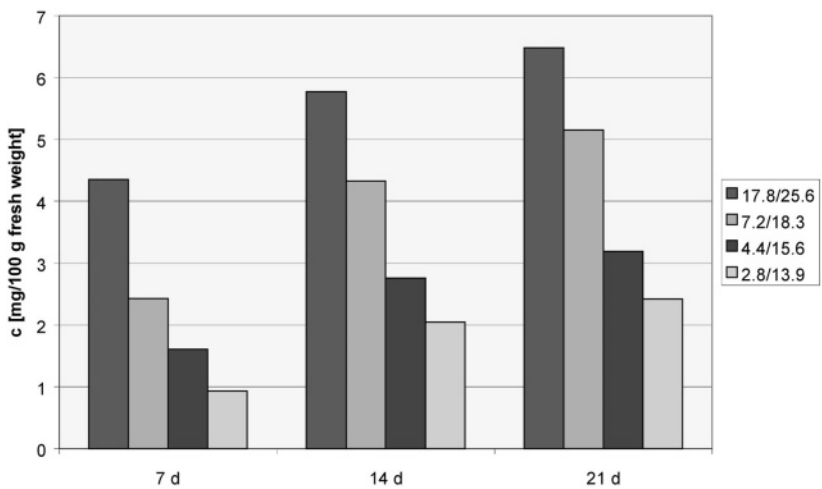

Figure 2. Concentrations of lycopene in tomatoes ripened at different temperatures for different times (modified from Koskitalo and Ormrod, 1972).

6. INFLUENCE OF AGRONOMICAL PRACTICES

The cultivation method is another factor affecting the contents of tomato ingredients. Regarding the carotenoids, tomatoes grown on an open field were compared to those grown under glass or under plastic foil (Cabibel and Ferry, 1980). Figure 3 shows the two carotenoids β-carotene and lycopene in tomatoes of five maturity stages for these three cultivation methods.

For all maturity stages, both carotenoids showed significantly higher contents in field grown tomatoes compared to the in-house grown tomatoes. Lycopene contents in red tomatoes grown under foil were slightly higher than those ripened under glass. These differences have to be further investigated (Cabibel and Ferry, 1980).

In another study, irrigation with water at electrical conductivity of 4.4 dS/m led to a 40% increase of the lycopene content without decrease of the yield. In contrast, fertilisation with different nitrogen sources did not affect the carotenoid content (Fogliano et al., 2001).

Heavy metals in the soil led to an increase of those metals in the tomato leaves but did not affect the contents of carotenoids and total phenolics in tomato fruits (Schicketanz et al., 1999). Thus, heavy metal stress did not induce changes in secondary plant products of the tomato fruit. In contrast to these results, a mechanical wounding led to a 10-fold increase of the contents of the two phenolic compounds E-feruloyl-tyramine and E-p-coumaroyl-tyramine conjugates. These results support a role for hydroxycinnamate-tyramine conjugates as part of the defence system of the plant (Pearce et al., 1998).

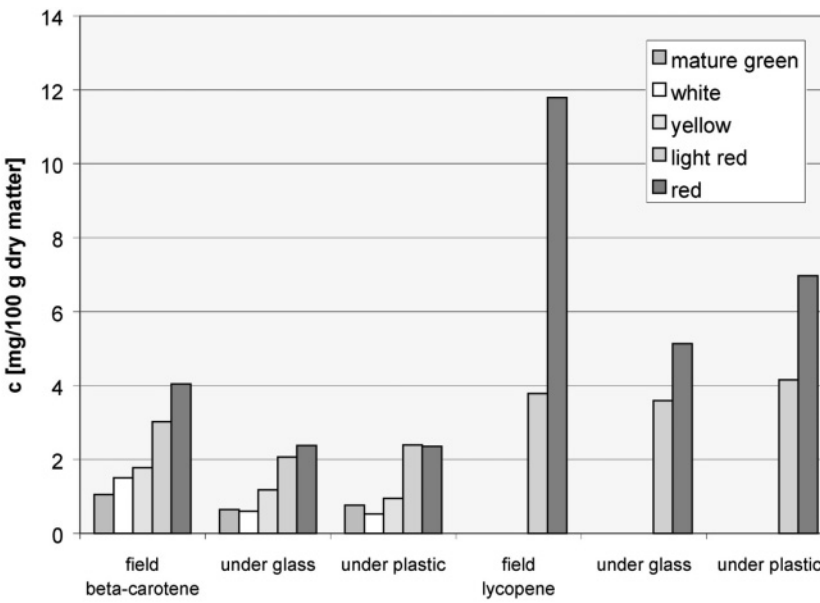

Figure 3. Contents of β-carotene and lycopene in five maturity stages of tomatoes grown in field, under glass or under plastic foil (modified from (Cabibel and Ferry, 1980))

The substrate system is another factor investigated on its influence on tomato ingredients. Four hydroponic systems, based on rock wool and expanded clay as substrates with and without recirculating nutrient solution, were compared to a soil culture system. All tomatoes were cultivated in greenhouses. Tomatoes grown on the soil system contained significantly lower amounts of ascorbic acid and carbohydrates compared to the hydroponic systems (Lippert, 1993). Another study used the sugar/acid ratio to compare tomatoes grown on soil to those grown on rock wool or on expanded clay. The results did not significantly differ between the three substrates (sugar/acid ratios: 6.18–8.46) (Schnitzler et al., 1994). Regarding the contents of 18 major and trace elements of tomatoes grown on soil (target electrical conductivity (EC): 3–4 mS/cm), rock wool (EC: 3–4 mS/cm) and rock wool (EC: 5–6 mS/cm) the concentrations of 9 elements were significantly different depending on the substrate. The concentration of cadmium was 15–30 times higher and that of calcium 50–115% higher in soil-grown fruits than in rock wool-grown fruits (Gundersen et al., 2001).

Carbon dioxide enrichment (700–900 ppm CO_2) during the maturation in a greenhouse affected some quality parameters of tomatoes. Compared to tomatoes grown under control conditions (250–400 ppm CO_2), those grown under CO_2-enriched conditions had lower amounts of vitamin C as well as those of glucose and fructose (Islam et al., 1996).

7. EFFECTS OF STORAGE PERIOD AND TEMPERATURE

Micra RS tomatoes, frozen in the form of cubes, were stored during 12 months at –20 °C and –30 °C. The storage did not affect the level of dry matter, soluble solids, sugars, dietary fibre, total nitrogen, nitrates, nitrites, pH, ash or its alkalinity. In contrast, contents of vitamin C and carotenoids changed significantly. During the 12 months' storage vitamin C decreased to 29% (–20 °C) or 55% (–30 °C) of its basal value. β-carotene significantly decreased to 49% or 68% of its initial value while lycopene losses were 48% and 26%. Figure 4 shows the contents of these three ingredients before and after 12 month storage (Lisiewska and Kmiecik, 2000).

Own storage experiments for 4 months at –30 °C showed a 35% decrease for β-carotene while lycopene was reduced by 50% (Böhm and Bitsch, 1995).

Another study (Sharma and Le Maguer, 1996) investigated the three storage temperatures –20/5/25 °C, varying the storage conditions from vacuum + dark over dark + air to air + light. The largest loss of lycopene (77.6%) resulted after storage for 60 days at 25 °C with air + light. Storage of freeze-dried samples for 4 months at room temperature led to 97% loss of lycopene compared to 73–79% for oven-dried samples (Sharma and Le Maguer, 1996).

Mature green tomatoes were stored at 5/7/12 or 19 °C for 0/3/9/12 or 21 days, then ripened at 19 °C for 3 or 6 days before analysis. The contents of citric acid increased after storage at 5 or 7 °C while they decreased at 19 °C. Malic acid decreased at all temperatures with the greatest decrease occurring at 19 °C (Thorne and Efiuvwevwere, 1988).

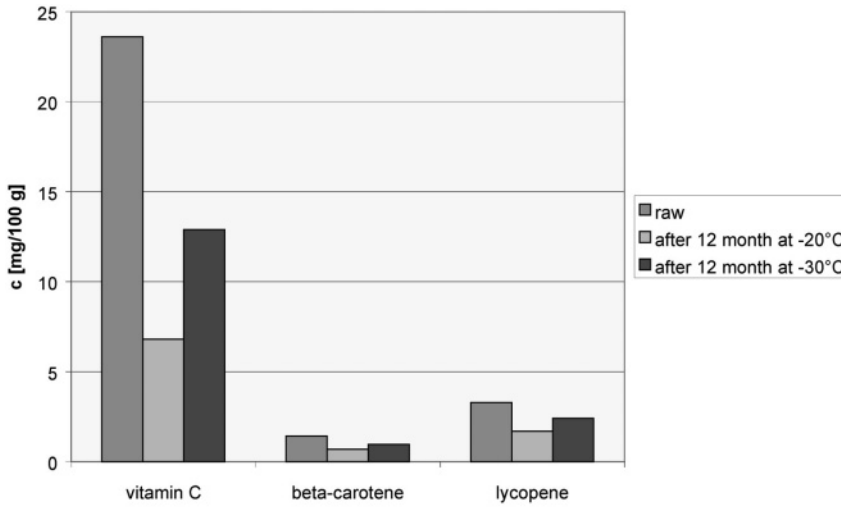

Figure 4. Contents of vitamin C, β-carotene and lycopene in raw tomato cubes and in tomato cubes after 12 months' storage at –20 °C and at –30 °C (modified from Lisiewska and Kmiecik, 2000).

8. EFFECTS OF HEATING

Heating tomato pulp at 100 °C for 120 min at atmospheric pressure led to a decrease in lycopene concentration from 185.5 to 141.5 mg/100 g total solids (24% reduction) (Sharma and Le Maguer, 1996). In another study, thermal treatment at 100 °C for 30 min in water increased the (Z)-isomer percentages by an average of 21 and 27% for β-carotene and lutein. In contrast, lycopene remained stable to isomerisation. Heating trials in 80:20 water/olive oil mixtures led to comparable results (Nguyen et al., 2001).

Cooking tomatoes for 4/8/16 min at 100 °C did not significantly affect the lycopene contents of different varieties (Thompson et al., 2000). Microwave heating (850 W, 3 min) reduced β-carotene by 35% and lycopene by 15% (Böhm and Bitsch, 1995). Regarding the flavonoid quercetin, boiling in simmering water for 15 min resulted in greater reduction (82%) compared to heating in a microwave oven for 1.3 min with 800 W (65%). These reductions in ingredients are mainly due to their extraction from tomato by hot water. In contrast, frying in sunflower oil for 3 min led to a reduction of 35% quercetin (Crozier et al., 1997).

Heat-based processing of tomatoes to tomato paste showed a different behaviour for ascorbic acid, tocopherols, and carotenoids. The ascorbic acid content was reduced by 54.6% while the losses of α-tocopherol (–20.3%) and γ-tocopherol (–32.7%) were lower. In contrast, the concentration of (E)-lycopene significantly increased by 36.9% (all results are based on dry matter). This remarkable increase was mainly explained by the removal of seeds and peels (both without lycopene) and the loss of soluble volatile compounds during water evaporation steps (Abushita et al., 2000). In another study, losses of (E)-lycopene during tomato paste produc-

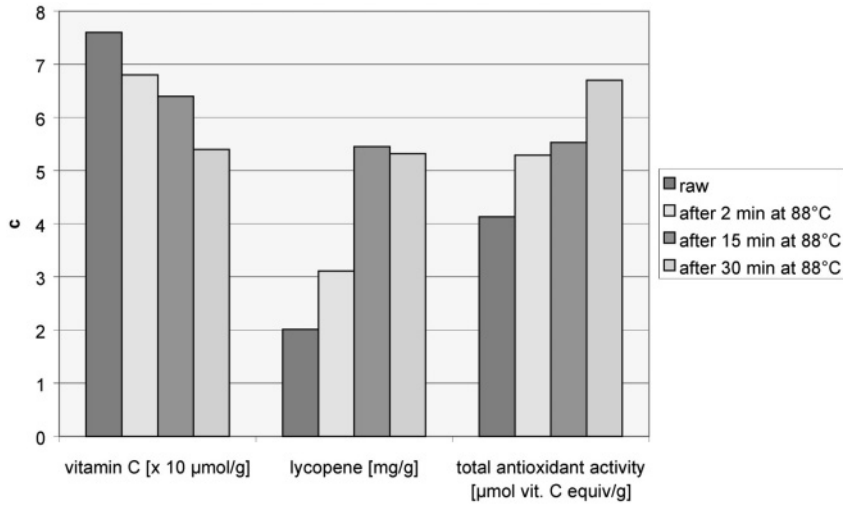

Figure 5. Contents of vitamin C and lycopene as well as total antioxidant activity in tomatoes heated for 2/15/30 min at 88 °C (modified from Dewanto et al., 2002).

tion ranged between 12 and 28% (Takeoka et al., 2001). Recently, Dewanto et al. (2002) investigated how thermal processing affects the nutritional value of tomatoes. Sliced tomatoes were blended and then cooked at 88 °C for 2/15/30 min. Total phenolics and total flavonoids content did not change significantly. In contrast, vitamin C, lycopene and total antioxidant activity were significantly affected by thermal processing. Figure 5 shows the time-course of these parameters.

While vitamin C significantly decreased with increasing heating time, the content of bioaccessible lycopene as well as the total antioxidant activity significantly increased during thermal processing.

9. CONCLUSIONS

Tomatoes are a source of several nutrients. Besides considerable contents of folate, they are rich sources of potassium and vitamin C. In addition, they are the most important source of the non pro-vitamin A active carotenoid lycopene. Further constituents are several flavonoids, pro-vitamin A active β-carotene and potassium.

Origin and variety highly influenced the contents of secondary plant products of tomatoes. Lycopene concentrations, for example, were higher in cherry tomatoes and in tomatoes for processing compared to salad tomatoes. Maturity stage and ripening temperature are two further factors affecting concentrations of nutrients and secondary plant products in tomatoes. Fruits of later maturity stage, ripened at higher temperatures had higher lycopene contents than those not completely ripened or ripened at sub-optimal temperatures.

Regarding the cultivation method, open field grown tomatoes showed higher carotenoid concentrations than those grown under glass or under plastic. Tomatoes grown on soil contained significantly lower amounts of ascorbic acid and carbohydrates compared to four hydroponic systems, based on rock wool and expanded clay as substrates.

Contents of vitamin C and carotenoids were significantly affected by storage for 12 month at –20 °C and –30 °C in contrast to sugars, remaining unchanged. Heat-based processing of tomatoes showed a different behaviour for ascorbic acid and carotenoids. While ascorbic acid decreased with increasing heating time, the content of bioaccessible lycopene increased during thermal processing.

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MODELLING FRUIT QUALITY: ECOPHYSIOLOGICAL, AGRONOMICAL AND ECOLOGICAL PERSPECTIVES

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1. INTRODUCTION

Orchard management is facing new challenges, instigating new research and fruit production strategies. The new market organisation requires to improve the quality of processes, produces and the environment, especially in Europe under current EU regulations. To be more competitive in this new market organisation, growers have to take into account more constraints than earlier. Indeed, they have to adapt their technical choices to the present concerns about environment and fruit quality. On what basis can this adaptation be made? The knowledge reported in the scientific literature is poorly used by orchard managers. In fact the extension services offering advices to growers are often conducting their own experiments to advance empirical knowledge useful to growers (e.g. Giauque et al., 1997). This empirical knowledge is very efficient but takes time to be established. In this new context, growers will have to rapidly adapt their technical management to ensure sustainable horticulture. Scientists must bear responsibility concerning the development of decision support systems, the role of which is thought to increase in the coming years (Knight, 1997). They may improve 'the decision processes that generate cropping systems to incorporate more information, to take into account a wider range of criteria, and to allow for faster adaptation' (Boiffin et al., 2001).

Decision support systems are usually constituted of biological, technical and economic models. They all have to be well adapted to the new objectives and constraints of fruit production. For instance, the economic model has to take into account environmental costs (Doyle, 1997), the technical model has to consider how to create and manage orchards, and the biological model has to consider the effects of orchard management on crop quality. In terms of biological models applied to fruit production, there is a lot to be done as almost none of the current models deal with important aspects such as the response of the plant to orchard management practices, pest attacks and diseases, and consequences on fruit production and quality. Such biological models are now needed to innovate in an integrated view through evaluation, exploration and learning (Boiffin et al., 2001).

To be able to help growers to face unusual situations, the biological model must be, in our sense, process-based. Indeed, process-based models now lead to better predictions in unusual situations than empirical models which can only be used under the range of environmental and agronomical conditions used to develop them. Moreover, only process-based models will be able in the future to easily integrate

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the scientific knowledge issued from functional genomics or to properly consider biotechnological advances.

Our objective is to present the conceptual basis needed to develop such models in horticulture, focusing on fruit quality. Biological models useful to orchard management need to integrate knowledge in several fields of research, mainly ecophysiology which analyses the physiological process of fruit quality elaboration, agronomy which considers cropping operations and their effect on quality, and ecology which analyses the dynamics of pests and diseases. In the first parts of this chapter, the complexity of fruit quality will be underlined and the current knowledge in biological modelling of fruit quality will be presented across several fields of science. Then, we will use the models developed in our team to show how knowledge in these fields can be used at different scales of analysis, from the fruit to the orchard.

2. FRUIT QUALITY: A MULTI-CRITERIA CONCEPT AND A COMPLEX PROBLEM

According to Arthey (1975), 'the quality of a horticultural product is assessed from the relative values of several characteristics which considered together will determine the acceptability of the product to the buyer and ultimately the consumer'. Quality is a multi-criteria concept which is not so easy to consider in modelling. For instance, the quality of fleshy fruits such as pears, cherries or peaches, depends on their sugar content (Leonard et al., 1953; Robertson et al., 1992) and more specifically on the distribution of this content between the different sugars that directly influence fruit flavour components such as sweetness (Robertson et al., 1988; Byrne et al., 1991). Sucrose, glucose and fructose which are the main sugars in the fruits of most plants have different sweetness (Pangborn, 1963; Yamaguchi et al., 1970; Doty, 1976). Moreover, each quality criterion is per se the result of a complex chain of biological processes. Let us consider sweetness. It results from hundreds of processes involved in sugar production in the leaves, loading and translocation in the phloem, unloading in the fruit cells, metabolism in the fruit cells and dilution by the water accumulated in the fruit. The technical operations applied to the orchard influence these processes in a complex way. That is why current agricultural practices result in a wide variability of the sugar contents in fruits from different plots, from trees of the same plot or from one part of the plant to another (Marini and Trout, 1984; Dann and Jerie, 1988). This variability is caused by various factors such as water availability (Besset et al., 2001), microclimatic gradients (Corelli-Grappadelli and Coston, 1991; Marini et al., 1991), leaf area around the fruit (Kliewer and Weaver, 1971; Génard, 1992) and vigour of fruit-bearing shoots (Génard and Bruchou, 1992). Water availability determines the water supply to the fruit, microclimatic factors such as temperature act on carbohydrate breakdown for fruit respiration, whereas leaf area (or vigour of fruit-bearing shoots) and possibly pests and diseases which directly or indirectly reduce carbon pools, act on the level of carbohydrate supply to the fruit. Agricultural practices such as

the density of plantation, fruit thinning, irrigation, or the management of pests and diseases influence the above-cited factors.

It is clear that all the processes involved in the quality of fruits cannot be integrated in biological models. But some degree of complexity is needed to consider quality and the effect of agricultural practices. As presented hereafter, we are just beginning to investigate this complexity and a lot remains to be done in the future.

3. MODELS OF FRUIT QUALITY ACROSS FIELDS OF SCIENCE

In ecophysiology, the priority has been given to modelling growth in dry mass with the development of what has been called 'photosynthesis driven models' (Gary et al., 1998; Marcelis et al., 1998). Such models have been developed for apples (Baumgaertner et al., 1984), grapes (Gutierrez et al., 1985), kiwifruits (Buwalda, 1991), olives (Abdel-Razik, 1989), peaches (Grossman and Dejong, 1994) and consider the plant as made of a few big compartments (leaf, wood, fruit) in which carbon is accumulated. More recently, heterogeneity within the plant has been investigated in tomatoes and peaches (Heuvelink and Bertin, 1994; Heuvelink, 1996; Bruchou and Génard, 1999; Génard et al., 1999a). Modelling fruit growth has also been improved by considering meristematic and non-meristematic tissues as in apple fruits (Austin et al., 1999).

But fruits cannot be restricted to their carbon content or dry mass just because the water is their main component. Only a few models have been proposed to simulate water accumulation in the fruit. Fruit growth has been calculated by integrating numerically the equation for water balance, using water uptake and transpiration per unit of fruit area as a constant (Lee, 1990) or a variable (Génard and Huguet, 1996). In a more mechanistic work, the difference in water potentials in the stem and the fruit has been considered as the driving force in a model of water import rate in tomatoes which also takes into consideration the role of the tomato anatomy (Bussières, 1994). More recently, a model of fruit growth integrating both the dry matter and the water accumulation within the fruit has been developed (Fishman and Génard, 1998) and opened the way to considering the edible quality.

Though taste is now the motto of any advertisement for fruit products, it is still absent from most models (Gary et al., 1998; Van Meeteren, 1998). But there are some exceptions. Génard and Souty (1996) designed a model to predict the sweetness of fruits based on carbon partitioning into various forms of sugars (sucrose, glucose, fructose and sorbitol). Lobit (1999) has designed two models predicting fruit acidity. The first one modelled citric acid production and degradation during fruit development by representing the fluxes through the citrate cycle. In the second one, malic acid content was assumed to depend mainly on the capacity of the cell to store this acid in its vacuole. Both models have been combined with a model of pH calculation (Lobit, 1999) in a global model able to predict the titratable acidity of the fruit (Habib, 2000). This work on acidity is to our knowledge, one of the most complex and interesting in the field of fruit quality modelling.

In agronomy, priority has been given to developing biotechnical models useful

for crop management. These models integrate the effects of cultural practices such as fertilization and irrigation. They are based on ecophysiological or empirical knowledge, but in all the cases, cultural practices and their effects on the system modelled must be included. Many crop modelling groups have used their models to optimize planting date, row spacing, choice of cultivar, and fertilizer application rates for different types of soils (Boote et al., 1996; Bouman et al., 1996). But in most cases, crop simulation models concern annual crops and focus on yield. In fact, biotechnical models focusing on fruits are almost absent (except the models of Doyle et al. (1989) in kiwifruits, Dumas et al. (1995) in processing tomatoes and Gary and Tchamitchian (2001) in fresh tomatoes), and none of them concern essential features of fruit quality such as taste or firmness. Even the different fruit sizes produced by an orchard are almost never considered in biotechnical models, though a grower's benefit is directly related to such classifications. This raises the issue of the variation of fruit quality which is as large within cultivars as between cultivars (Génard and Bruchou, 1992). For a given cultivar, this variation is structured at different levels: within trees, between trees in an orchard, and between orchards. Lescourret et al. (1998a, b, 1999) have recently proposed a biotechnical model on kiwifruit which attempted to account for fruit size variability by considering factors occurring at different levels of organisation (fruit, branch, plant, plot) in response to cultural practices and environmental factors.

In ecology, much research has been done on population growth or disease development and predator-prey relationships and has contributed to strong theoretical bases to study the dynamics of diseases and pests and their effect on crops (de Wit and Goudriaan, 1978; Rijsdijk, 1986; Rabbinge et al., 1989). However, the interactions between disease or pests and crops have not been much studied. Exceptions include the pioneer studies of Gutierrez on various crop-pest systems, especially on cassava (i.e. Gutierrez et al., 1988a, b, c, 1999) and cotton systems (i.e. Gutierrez and Curry, 1989; Gutierrez et al., 1991), as well as those of Rossing (1991) on aphid damage in winter wheat, Blaise et al. (1996) on the effect of disease on grapevine yield, and of Chevalier-Gérard et al. (1994) or Colbach et al. (1999) on the effect of cropping systems on disease development. However, all these studies focus on crop yield and, to our knowledge, there is no model to simulate the effect of diseases or pests on fruit quality.

According to Blaise et al. (1996), optimizing curative strategies is only possible if the consequences of a crop protection decision can be quantified in terms of risks of quality losses through the occurrence of disease or pest. Therefore, combining disease or pest and crop quality models seems to be the actual challenge for the near future (Habib and Lescourret, 1999).

4. THE QUALITY FROM THE ECOPHYSIOLOGIST'S POINT OF VIEW

For an ecophysiologist, quality results from a set of interrelated physiological processes which depend on environmental conditions. At the fruit level, biophysical processes involved in water, carbon, calcium, etc. fluxes and metabolism are the main factors determining fruit quality such as fruit size, sweetness or acidity.

Is it the only level to be considered? No, because fruit quality varies according to different levels of organisation, as mentioned before. In fruit trees, the tree is the key level, since most variations emerge at this level (Marini and Trout, 1984; Habib et al., 1991; Audergon et al., 1993; De Silva et al., 2000), and also because it is the target of most technical interventions. Thus, modelling fruit development with an emphasis on variability within the tree is a crucial step towards improving fruit quality through horticultural practices. The high variability of fruit quality observed at the level of the tree mainly results from sink-source relationships within the tree. We will now analyse these two levels.

4.1. Models of fruit physiology

Size is one of the main parameters of fruit quality. Indeed, the price paid to the grower closely depends on fruit size. Many models have dealt with fruit growth. Some authors have tried to handle the variability observed in fruit growth, either by using a stochastic approach of growth rates (Hall and Gandar, 1996) or by considering the factors influencing the sink strength of the fruit, e.g. the individual number of seeds in the case of kiwifruit (Doyle et al., 1989; Lescourret et al., 1998b). However, these studies usually neglect the effect of the tree on fruit development. Therefore, we developed a model of fruit growth able to simulate dry matter and water accumulation in the fruit according to the water and carbon status of the plant (Fishman and Génard, 1998). Sweetness is also a significant feature of fruit quality. It is more and more considered by producers and consumers and begins to take part in fruit price determination. A model of sugar accumulation in peach simulating the sweetness as a function of fruit development has been proposed (Génard and Souty, 1996). These two models will be presented hereafter: the first model is an example of what can be called a biophysical model, and the second a metabolic model.

4.1.1. *SWAF: a biophysical model of Sugar and Water Accumulation in the Fruit*

The present model is based on the biophysical representation of water and dry matter transport combined with the growth process stimulated by turgor pressure. It simulates the period of fruit growth which does not involve cell division. Fruit flesh is described as one compartment separated from the atmosphere and parent plant by membranes. The parent plant supplies the fruit with water and sugars which are brought through xylem vessels and phloem sieve tubes. The fruit consumes carbon and water through the respiration and transpiration processes. The main state variables of the system are the amount of water (W_{water}) and of dry matter (W_{dry}) in the fruit. The hourly inputs of the model are temperature and relative humidity of the ambient atmosphere, water potential in xylem vessels, and sugars concentration in the phloem sap. Only the main equations of the model are presented hereafter, the readers interested in a complete description of this model will find a more accurate description in Fishman and Génard (1998).

4.1.1.1. *SWAF principles*

The change in the amount of water within the fruit with time (dW_{water}/dt) is the algebraic sum of the water income from xylem (U_x) and phloem (U_p) minus the water outcome due to fruit transpiration (T_f).

$$\frac{dW_{water}}{dt} = U_x + U_p - T_f \tag{1}$$

Fruit transpiration leading to mass loss is assumed to be proportional to the fruit area and to surface conductance to water vapour (Lescourret et al., 2001), and to be driven by the difference of relative humidity in the air-filled space of the fruit and in the ambient atmosphere.

Using the subscripts x, p and f for xylem, phloem and fruit variables respectively, the total phloem and xylem flows into the fruit tissues are:

$$U_x = A_x L_x [P_x - P_f - \sigma_x (p_x - p_f)]$$

and (2)

$$U_p = A_p L_p [P_p - P_f - \sigma_p (p_p - p_f)]$$

L is the hydraulic conductivity coefficient of vascular network membranes, P and p are the hydrostatic and osmotic pressures and σ is a measure of impermeability of the membrane to the solute, ranging from 1 (fully impermeable membrane) to 0. It is known that $p_x = 0$ and $\sigma_x = 1$ (Nobel, 1974). A is the vascular network area assumed to be proportional to the fruit area. The water potentials in the xylem and phloem are assumed equal to the water potential measured in the stem, □. This gives $P_x = \square$ and $P_p = \square + p_p$.

The change in the amount of dry matter (dW_{dry}/dt) is the difference of assimilate uptake from phloem (U_{dry}) and dry matter loss due to the fruit respiration (R_f).

$$\frac{dW_{dry}}{dt} = U_{dry} - R_f \tag{3}$$

Dry matter loss due to fruit respiration is divided into growth respiration, which is proportional to the rate of dry matter income, and maintenance respiration, which is proportional to dry mass (Thornley and Johnson, 1990).

If $s_p < 1$, part of the sugar can be transported from the phloem to the fruit by mass flow. Total uptake of carbohydrates is

$$U_{dry} = U_a + (1 - \sigma_p) \left(\frac{C_p + C_f}{2} \right) U_p \tag{4}$$

where U_a is the rate of uptake due to active or facilitated transport obeying the Michaelis-Menten equation and C_p and C_f are the mean concentrations of the solute in the phloem and the fruit respectively.

Osmotic pressure is calculated from the sugar content according to Nobel (1974).

To calculate the hydrostatic pressure, the following procedure is used: the relative rate of volume (V) growth of the fruit compartment is presented by Lockhart's equation (Lockhart, 1965)

$$\begin{aligned} \frac{dV}{dt} &= V_f(P_f - Y) \qquad \text{if} \qquad P_f > Y \\ \frac{dV}{dt} &= 0 \qquad\qquad\quad \text{if} \qquad P_f \leq Y \end{aligned} \tag{5}$$

where f is the coefficient describing the extensibility of the cell walls and Y is the threshold value that the hydrostatic pressure of the fruit has to exceed before irreversible expansion occurs. The change in fruit volume can also be calculated from equation 1 (with D_w the water density) as

$$\frac{dV}{dt} = \frac{U_x + U_p - T_f}{D_w} \tag{6}$$

Under the condition of steady irreversible growth, equations 5 and 6 must be equal. Setting equations 5 and 6 equal, and inserting the flux from equation 2, the resulting equations for P_f can be solved.

The time-step in the model is one hour. Total fruit mass, volume, and water content may be calculated using the state variables $W_{water}(t)$ and $W_{dry}(t)$.

4.1.1.2. *Using SWAF to explain seasonal and diurnal patterns of peach fruit growth*
A set of model computations was performed to simulate the combined effect of water stress and crop load in peach trees (Figure 1).

Sugar content in the phloem directly influences the rate of its uptake by the fruit and results in lower dry fruit mass and respiration under conditions of high crop load. In this case, the osmotic pressure is lower than with a low crop load, which leads to high fruit water potential and low water uptake. Turgor pressure is lower with a high crop load, mainly in the first period of fruit development, which results in a low growth. Although transpiration decreases with the increase in fruit load, the decrease in water uptake is such with a high crop load that fresh fruit mass is always lower than with a low crop load. Water stress causes a significant decrease in fresh fruit mass but the change in dry fruit mass is negligible because carbon uptake through mass flow is assumed to be low. The occurrence of water stress increases the osmotic pressure which leads to a decrease in fruit water potential and water influx into the fruit. The patterns of variation of carbon and water uptake are quite different, with a maximal carbon uptake occurring one month before the maximal water uptake. This important water uptake during the last month of the fruit development is related to the high transpiration of the fruit during this period.

The diurnal patterns of fruit growth show additional features of the growth process (Figure 2). The model predicts that diurnal patterns of dry and fresh masses of the fruit are not directly correlated. It can be noted that during daytime, when transpiration reaches its maximum value, fresh mass does not increase and even

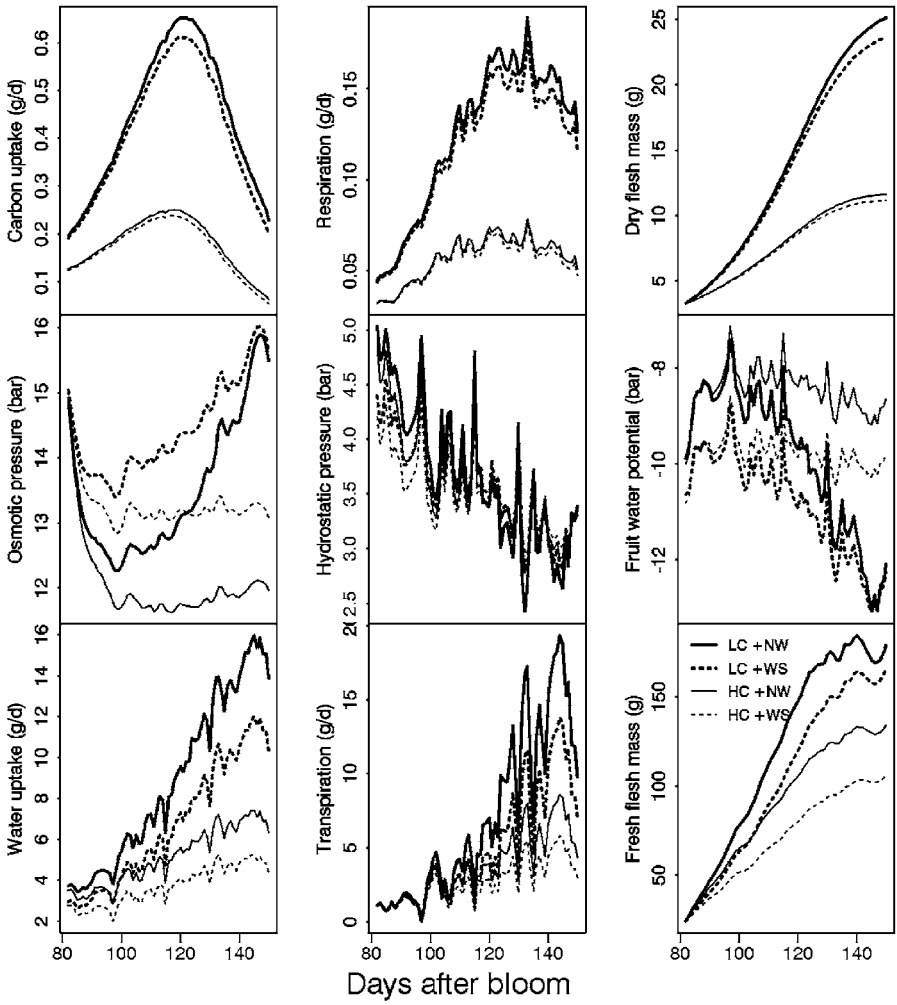

Figure 1. Evolution during the growing season of peach carbon and water components simulated by SWAF as a function of crop load and water stress. Water potential was chosen to vary within the ranges –10 to –2 bar and –12 to –4 for normally watered (NW) and water stressed (WS) trees, respectively. Sugar content in the phloem was chosen to vary within the ranges 0.14 to 0.22 and 0.04 to 0.12 for light (LC) and heavy (HC) crop load, respectively.

decreases, whereas dry matter increases during this period. When transpiration increases, the hydrostatic pressure in the fruit decreases, which causes growth to slowdown and, eventually, to stop. The low plant water potential during daytime reduces the water uptake so that it cannot compensate for the high rate of fruit transpiration; therefore, water balance is equal to zero or is negative during daytime. The most intensive increase in fresh fruit mass takes place during the night.

In conclusion, the SWAF model of fruit growth takes into consideration the fundamental processes and their response to external signals. As SWAF is based

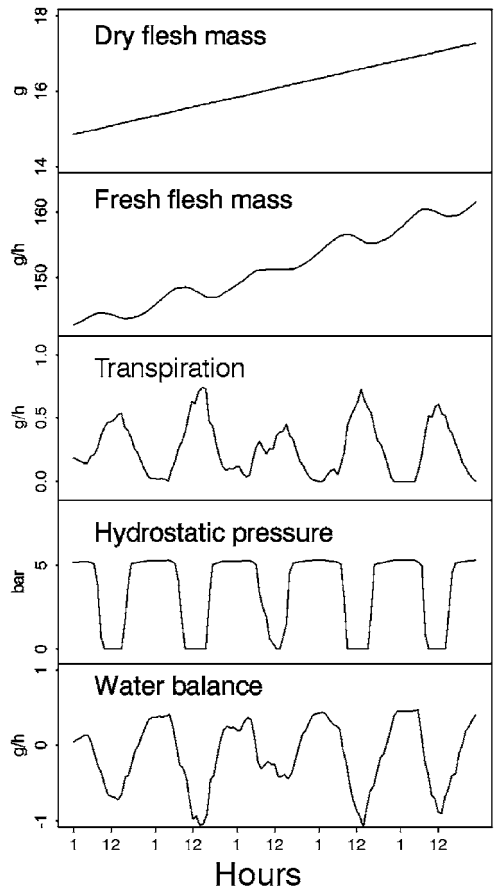

Figure 2. Patterns of 5 days (105–109th days after bloom) simulations obtained with SWAF under normal irrigation and light crop load conditions. The upper graph represents dry flesh mass increase in peach. The second graph shows the changes calculated in fresh mass compared to the calculated peach transpiration, hydrostatic pressure and water balance.

on an analysis of the biophysical processes, it can easily be used in a larger framework than fruit growth. It can be used to simulate concentration in total soluble sugars which is an essential feature of fruit quality. It can also be used to study other aspects of fruit quality such as the occurrence of blossom-end rot which is one of the main physiological disorders of greenhouse pepper. This disorder is considered as the result of low calcium concentrations. The SWAF model has been used to simulate calcium accumulation in the fruit considering that calcium is transported in the xylem by mass flow (Bar-Tal et al., 1999).

4.1.2. *SUGAR: a metabolic model of fruit sweetness*

Many studies on the sugar content of fleshy fruits have focused on compositional changes during fruit growth and maturation (Ishida et al., 1971; Chapman et al.,

1991; Ackerman et al., 1992). The conversion of phloem sugars within the fruit has been studied by Hansen (1970). The main enzymes involved in the sugar metabolism of fleshy fruits have been identified (Yamaki and Ishikawa, 1986; Moriguchi et al., 1992), but little is known about their regulation. Use of carbohydrates by the fruit and changes in sugar contents are probably strongly correlated, which may explain the strong correlations usually noted between sugar content at harvest and the size of fruit from a same tree (Génard et al., 1991 and 1999b). These results were used in the SUGAR model which will be presented in the following section (see Génard and Souty, 1996 for more details). This model is a dynamic deterministic model of carbon use that includes sugar accumulation and synthesis in fruit flesh. The model was designed for peach, but the main principles can be used for other fruit.

4.1.2.1. *Main knowledge in the case of peach and model principles*

Sucrose and sorbitol are the only sugars founds in the phloem sap of trees from the *Prunus* species such as peach trees (Escobar-Gutierrez and Gaudillère, 1996). In the peach flesh, sucrose is the main sugar, glucose and fructose are present in similar quantities and can reach about 25% each of the total sugar content, and sorbitol content is always low (Souty and André, 1975; Brooks et al., 1993). This suggests that sorbitol is almost totally metabolised into reducing sugars when sucrose is only partly hydrolysed into fructose and glucose. But as sucrose accumulates in the fruit, a seasonal decrease in conversion of the sucrose into reducing sugars was assumed. Glucose and fructose are used as substrates for synthesize compounds other than sugars. As more and more soluble sugars accumulate with fruit development (i.e. when the relative growth rate of the fruit decreases), we assumed that this synthesis decreases with the decrease in relative growth rate. Indeed, the periods of low relative growth, such as the ripening period are characterized by low cell wall synthesis (Bouranis and Niavis, 1992; Fishman et al., 1993).

Glucose and fructose are used as substrates for respiration and both sugars are assumed to be used in proportion to their quantity in the fruit. Respiration is calculated as in the SWAF model. Apart from respiration, the carbon flow between two compounds is proportional to the quantity of carbon in the source compound.

The system is represented by the following set of differential equations

$$\begin{aligned}
\frac{dM_{su}}{dt} &= \lambda_{ph}\frac{dM_{ph}}{dt} - k_1(t)M_{su} \\
\frac{dM_{so}}{dt} &= (1-\lambda_{ph})\frac{dM_{ph}}{dt} - [k_2(t)+k_3(t)]M_{so} \\
\frac{dM_{gl}}{dt} &= \frac{k_1(t)}{2}M_{su} + k_2(t)M_{so} - k_4(t)M_{gl} - \frac{M_{gl}}{M_{gl}+M_{fr}}\frac{dM_{re}}{dt} \\
\frac{dM_{fr}}{dt} &= \frac{k_1(t)}{2}M_{su} + k_3(t)M_{so} - k_4(t)M_{gl} - \frac{M_{fr}}{M_{gl}+M_{fr}}\frac{dM_{re}}{dt}
\end{aligned} \tag{1}$$

where M_{su}, M_{so}, M_{gl} and M_{fr} are the amounts of carbon as sucrose, sorbitol, glucose,

fructose, respectively; ($_{ph}$ is the proportion of sucrose in the phloem sugar pool, which results from plant metabolism; $k_1(t)$, $k_2(t)$, $k_3(t)$ and $k_4(t)$ represent the relative rates of sugar transformation for net sucrose transformation into glucose and fructose, net sorbitol transformation into glucose and fructose, and the synthesis of compounds other than sugars from glucose and fructose.

These relative rates are calculated according to the following equations

$$\begin{aligned} k_2(t) &= k_2 \\ k_3(t) &= k_3 \\ k_4(t) &= k_4 \frac{1}{W_{dry}} \frac{dW_{dry}}{dt} \end{aligned} \tag{2}$$

where $k_{1,3}$ is a constant equal to 1 day^{-1}, $k_{1,1}$, $k_{1,2}$, k_2, k_3 and k_4 are parameters, and W_{dry} is the dry mass of the fruit flesh.

(dM_{ph}/dt) and (dM_{re}/dt) are the phloem and respiration flows of carbon into and out of the fruit, respectively

$$\frac{dM_{ph}}{dt} = \sigma_{fl} \frac{dM_{dry}}{dt} + \frac{dM_{re}}{dt} \tag{3}$$

where σ_{fl} is the carbon content of flesh.

Sugar concentrations are computed as

$$C_{su} = \frac{100M_{su}}{\sigma_{su}W_{fresh}}, \quad C_{so} = \frac{100M_{so}}{\sigma_{so}W_{fresh}}, \quad C_{gl} = \frac{100M_{gl}}{\sigma_{gl}W_{fresh}}, \quad C_{fr} = \frac{100M_{fr}}{\sigma_{fr}W_{fresh}}, \tag{4}$$

where σ_{su}, σ_{so}, σ_{gl} and σ_{fr} are the carbon content of 1 g of sucrose, sorbitol, glucose and fructose, respectively. W_{fresh} is the fresh mass of the flesh.

The sweetness of each sugar is computed using its sweetness rating according to Kulp et al. (1991).

Time-step in the model is one day. Daily mean temperature and daily dry and fresh flesh masses are the inputs of SUGAR. Previous studies (Génard and Souty, 1996; Génard and Huguet, 1999) have shown that the SUGAR model predicts the sugar content of peaches with a fairly good accuracy over a wide range of fruit growth rates.

4.1.2.2. *Seasonal variation of sugar concentrations and sweetness shown by SUGAR*
The simulations were performed during the main period of flesh development (two months before maturity for the peach cultivar 'Suncrest'). Figure 3 presents a typical output of the model. Sucrose concentration presents a seasonal increase whereas sorbitol is always low, and glucose and fructose concentrations present almost no change during the season. Total sweetness increases during the season. The effect of an increase in assimilate supply, due to fruit thinning for example which increases leaf to fruit ratio, is mainly an increase in sucrose and sweetness.

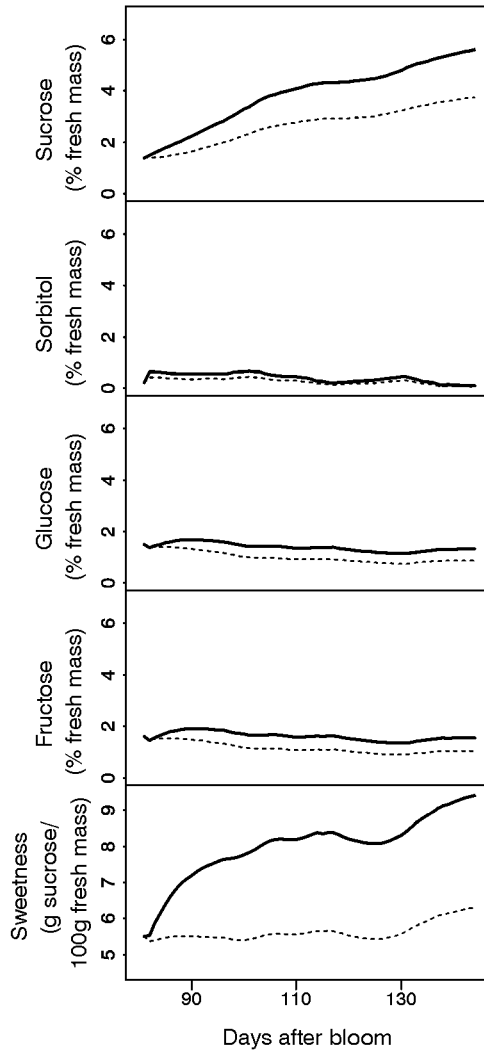

Figure 3. Seasonal variation in sugar contents and sweetness of peach flesh obtained with SUGAR. The bold curves correspond to a 50% increase in carbon supply compared to the dotted curves.

These simulations explain in a comprehensive way the relationship previously noted between flesh sugar contents at harvest and fruit growth (Génard et al., 1991 and 1999b). In accordance with the results presented in these papers, we found a positive relationship between fruit growth and sucrose concentration in flesh at harvest and no clear relationship for reducing sugars.

We computed the seasonal variation of sweetness due to each sugar (Figure 4). The contribution of sorbitol was never significant and that of glucose was weak, except early in the season. Before the hundredth day after bloom, fructose mainly contributed to total sweetness. The importance of fructose was essentially due to

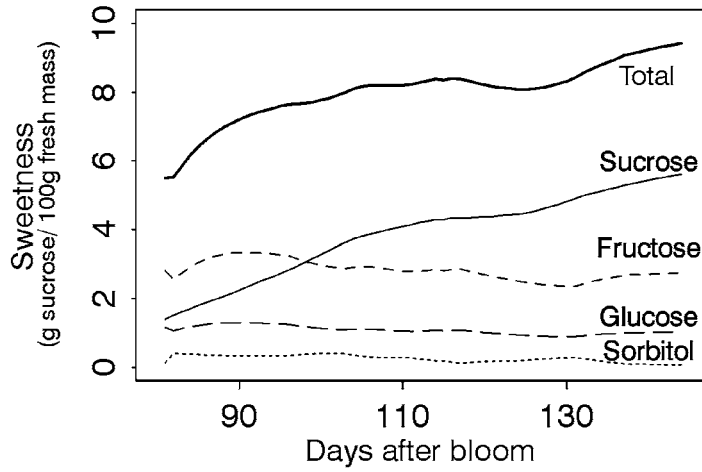

Figure 4. Simulation with SUGAR of seasonal variation in peach sweetness due to each sugar and total sweetness.

its high sweetness rating. Sucrose was the main sweetener after the hundredth day after bloom when fruit flesh contained large amounts of sucrose.

In conclusion, the carbon flow approach used in SUGAR is useful to model sugar accumulation and synthesis during the growth of organs accumulating sugars. It may be easily applicable to other fruit species which do not accumulate starch, such as plums or apricots, but the same approach could be applied to starch accumulating organs. Only mean temperature, flesh water content and dry flesh mass growth curve data are required.

4.2. Modelling fruit quality with regard to source-sink relationships within the plant

Models of carbon assimilation and allocation which include light interception have been proposed for deciduous fruit crops (Seem et al., 1986; Abdel-Razik, 1989; Buwalda, 1991; Wermelinger et al., 1991; Grossman and DeJong, 1994). Such models are useful but they consider the tree as composed of compartments corresponding to the various organs (fruits, leaves, stems, roots, etc.) without describing the within-compartment variability. This serious limitation can be addressed by considering a lower organisation level than that of the tree. Shoots bearing fruit are convenient sub-units because they are large enough to subsume the most important physiological processes on the one hand, and they are the basic units for the most important horticultural interventions on the other. Moreover, they can be considered as relatively autonomous sub-units in terms of carbon flow (Sprugel et al., 1991). Thus, we propose that the shoot bearing fruit is a useful unit for understanding the within-tree variability, and we suggest that modelling isolated shoots is needed as a first step for the sake of clarification.

We present hereafter a simulation model of carbon assimilation by sources and

allocation between sinks in the shoot bearing fruit. The model named 'CaShoo' is described in details in Lescourret et al. (1998a). It was designed to especially analyse the variation of mean peach fruit growth in terms of dry mass between shoots in different conditions (leaf-to-fruit ratio resulting from thinning patterns, light environment; Génard et al., 1998).

4.2.1. *CaShoo: Carbon balance in the Shoot bearing fruit*

The system is divided into three compartments: fruit, one-year-old stem, and leafy shoots, and evolves on a daily basis. The pool of C assimilates available daily is the assimilation of leaves and eventually that mobilized from reserves.

Several authors have reported a feedback inhibition of leaf photosynthesis through the leaf storage carbohydrates (Guinn and Mauney, 1980; Foyer, 1988; Flore and Lakso, 1989). A simple negative linear relationship between the light-saturated leaf photosynthesis $P_l^{\max}$, and the level of reserves in the leaves is used in the model. On this basis, the model uses the formulation of Higgins et al. (1992) to calculate the photosynthesis (P)

$$P = \left\{(P_l^{\max} + p_1) \times \left((1 - e^{\frac{-p_2 \times PPFD}{P_l^{\max} + p_1}}\right)\right\} - p_1 \tag{1}$$

where p_1 and p_2 are parameters, $PPFD$ is the photosynthetically active photon flux density (input data). Carbon assimilation by the fruit is considered on a similar basis.

The carbon is allocated according to organ requirements and priority rules. Maintenance respiration costs are calculated on the basis of the $Q10$ concept and are given first priority. Vegetative and fruit growth are given second and third priority. The daily carbohydrate demand for growth by any organ is the daily potential sink strength devoted to growth (Ho, 1988), as the potential net gain of C plus the C loss due to growth respiration. A very general formulation of daily carbon demand D_i for the growth of a compartment composed of n individual organs i (i.e. n fruits or n leafy shoots) can be written as

$$D_i = n \times \frac{\Delta W_i^{pot}}{\Delta dd} \times \frac{\Delta dd}{\Delta t} \times (CC_i + GRC_i) \tag{2}$$

where $(\Delta W_i^{pot}/\Delta dd)$ is the potential growth of the structural part of the organ in terms of degree-days after full bloom dd, CC_i the carbon concentration and GRC_i the growth respiration coefficient of the organ i.

The following equation is proposed for potential fruit growth rate. It emphasizes the role of fruit history by means of the accumulated growth W_f. It also emphasizes the role of time by means of accumulated degree-days.

$$\frac{\Delta W_f^{pot}}{\Delta dd} = RGR_f^{ini} \times W_f \times \left(1 - \frac{W_f}{W_f^{\max}}\right) \times f(dd) \tag{3}$$

with $f(dd) = 1$ if $dd < dd_{min}$

$$f(dd) = \frac{dd_{\max} - dd}{dd_{\max} - dd_{\min}} \quad \text{if } dd \text{ is between } dd_{\min} \text{ and } dd_{\max}$$

$$f(dd) = 0 \quad \text{if} \quad dd > dd_{max}$$

where RGR_f^{ini} is the initial relative growth rate, $W_f^{\max}$ refers to the limiting final mass and $dd_{\min}$ and $dd_{\max}$ are parameters.

A similar equation has been proposed for potential vegetative growth rate. Finally, if the carbohydrate pool is not empty after allocation to the fruit, it is stored, first in the leafy shoot and then in the one-year-old stem.

4.2.2. *CaShoo, a tool to analyse the behaviour of shoots bearing fruits*

The ability of the model to reproduce the effects of the shoot-to-fruit ratio was studied, by comparing simulation outputs with experimental data. The model outputs were consistent with the general pattern of vegetative growth (Figure 5) which shows no clear differences between shoot-to-fruit ratio treatments.

Corresponding reproductive growth was quite well simulated, with smaller fruit growth for lower shoot-to-fruit ratio (Figure 5). For the lowest shoot-to-fruit ratio, the reserves decreased quickly to reach a nil value at 600 degree-days after bloom and refilled itself after 1200 degree-days after bloom. This results in a high photosynthesis activity during all the season. On the opposite, the reserves are never nil and increase during the season with high shoot-to-fruit ratio which tends to inhibit photosynthesis (Figure 6). This result is interesting for reasoning horticulture practices. The presence of more assimilates than required during summer in case of high shoot-to-fruit ratio, could improve bud initiation and development for the next season.

The model was used to study the effect of source and sink factors on fruit mass at harvest. The factors studied were selected by considering that the source (or sink) strength is the product of a source (sink) size by a source (sink) activity. The "initial" size of leafy shoots (i.e., that established at the beginning of active fruit growth) was chosen as an indicator of source size because it was hypothesised to determine the potential growth of leafy shoots (Lescourret et al., 1998a). The light environment of the shoot bearing fruit was selected as it determines source activity. Its heterogeneity throughout the tree canopy, which has been emphasised by Lakso et al. (1989), Génard and Baret (1994) and Kikuchi et al. (1994), causes a large variation in photosynthesis within the tree (Marini and Marini, 1983). Based on the observations of Davis and Davis (1948) and Batjer and Westwood (1958), which have indicated that the size of the fruit at the early stages of growth seemed to influence fruit growth, the initial size of the fruit was hypothesised to contribute to sink size. Finally, sink activity was considered by comparing early and late maturing cultivars, which have different potential relative growth rates of fruit.

For each cultivar, the previous factors were considered at three levels (low, intermediate and high). One run was performed per combination of factors. The results of the simulations were analysed by analysis of variance (fixed effects). The contribution of the different sources of variation, measured as the corresponding

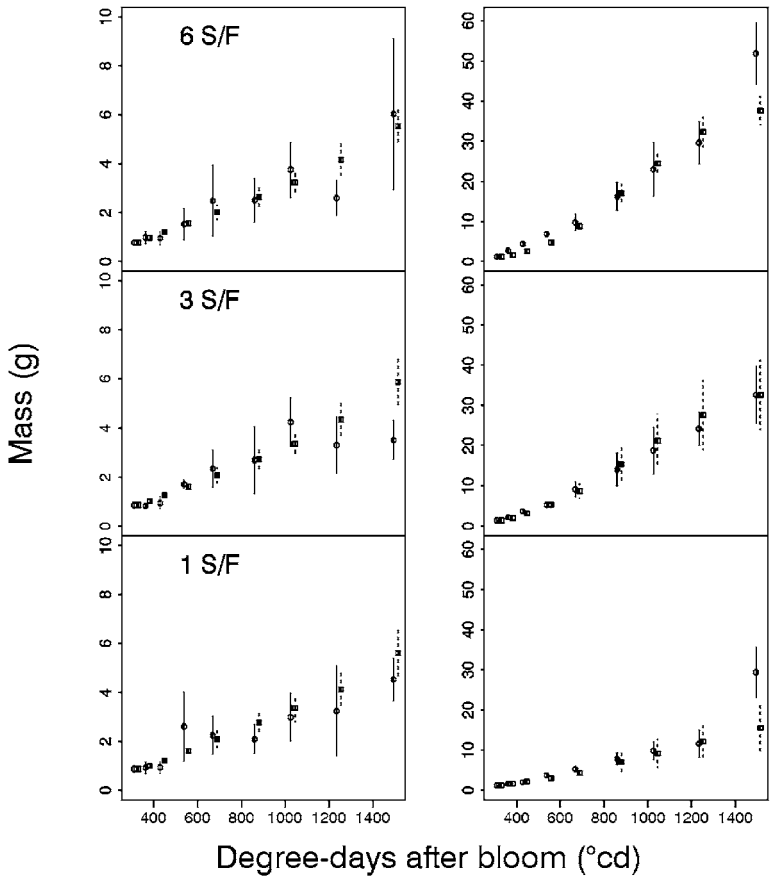

Figure 5. CaShoo data versus experimental data in the 'Suncrest' peach cultivar and several shoot-to-fruit ratios (S/F): variation of shoot and fruit growth (mean ± sd) according to degree-days either observed (circles and full lines) or simulated (squares and dotted lines).

sum of squares divided by the total sum of squares, was examined. The three main effects were significant and explained most of the variation of the simulated fruit size at harvest (Table 1).

The contribution of the initial size of leafy shoots, which strongly influences the development of leaf area, was of the same order of magnitude in late and early cultivars. The contribution of the light environment was greater in the late cultivar, whereas the contribution of the initial fruit size was greater in the early cultivar. The higher sensitivity of early maturing cultivars to initial fruit size is probably due to a stronger sink activity of the fruit resulting from a shorter growing season.

To conclude, the structure of 'CaShoo' allows complex behaviours to emerge from interactions between sources and sinks, such as photosynthetic regulation with fruit load changes. The flux of carbohydrates into a sink, which is a significant determinant of fruit quality, was partly controlled by the sink (for instance actual mass) and partly by the source (for instance leaf area).

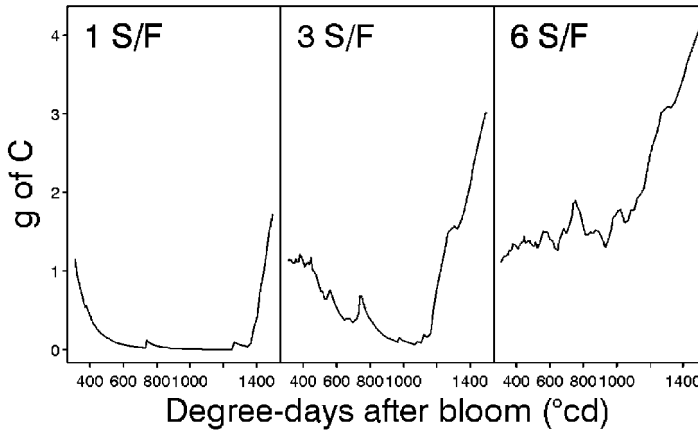

Figure 6. Simulation with CaShoo of daily reserve kinetics in peach shoot bearing fruits for several shoot-to-fruit ratios (S/F).

Table 1. Contribution (%) of different sources of variation of peach size at harvest in two simulated experiments. One experiment concerned an early maturing peach cultivar ('Alexandra') and the other a late maturing cultivar ('Suncrest'). The factors under study were the initial size of leafy shoot, light environment and initial size of fruit.

	Suncrest	Alexandra
Initial size of leafy shoot (LS)	32.2***	30.6***
Light environment (LE)	21.8***	14.6***
Initial fruit size (FS)	27.5***	38.8***
Interaction LS:LE	12.0***	0.9ns
Interaction LS:FS	4.1**	10.4***
Interaction LE:FS	1.7*	3.8**
Residuals	0.7	1.0

*** significant at $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant.

5. THE QUALITY FROM THE AGRONOMIST'S POINT OF VIEW

For the agronomist, fruit quality can be defined by a set of fruit characteristics which can be managed by technical operations. Choosing the technical operations, their intensity and succession is a complex work to which agronomical models can contribute. Two main types of decisions have to be taken to manage fruit production in a plot. The aim of tactical decisions is to adapt the intensity of technical operations to the state of the plant during the season of production. Indicators of the plant vigour, water stress or sanitary state are often used as input in a model designed to simulate the effects of technical operations on the plant. More strategic choices have to be made before crop implantation or the season of production. In this case, the decision concerns an adequate combination of operations in terms of type, intensity and time of application. In the following section, a simple model useful in

tactical decisions about peach tree irrigation and based on an indicator of water stress is first presented. A more complex model useful to study kiwifruit orchard management in terms of strategic decisions is then presented and used to analyse the effects of several combinations of technical operations on fruit size.

5.1. WASIF: a model using WAter Stress Indicator in Fruit management

A micromorphometric technique has been developed to measure the water status of the plant and schedule irrigation (Li et al., 1989). This technique measures trunk shrinkage (Plate 1) which is an indicator of the plant water status as it varies similarly to the water content of the tree (Simonneau et al., 1993). This method helps to determine the water needed to maintain growth of the tree, but do not help *per se* to produce good size fruit. To overcome this difficulty, it can be chosen to use trunk shrinkage and to connect this bio-indicator with a model of fruit growth. Such an approach will be presented hereafter for peach. It can be extended to other aspects of fruit quality such as sugar content (Génard and Huguet, 1999).

5.1.1. *WASIF main principles*

The aim of this model is to simulate the growth of individual peaches during the expansion of flesh cells as a function of water availability. Water availability,

Plate 1. Trunk shrinkage is measured using a linear variable differential transformer (LVDT) mounted on an INVAR frame.

which depends on climate and irrigation, was assumed to be inversely proportional to the maximum daily shrinkage (*MDS*) of trunks measured using the micromorphometric method (Huguet, 1985; Garnier and Berger, 1986).

The model assumes that trees are optimally fertilized and that carbon acquisition by photosynthesis is sufficient for well-irrigated trees to reach full potential fruit growth. The fruit receives a daily solution flow from the plant (F) and loses water by transpiration (T) and carbon by respiration (R). Thus growth is

$$\frac{dW_{fresh}}{dt} = F - R - T$$

Respiration is calculated as in the SWAF model. Transpiration is a function of fruit mass (W_{fresh}), hourly global solar radiation (GR) and skin area of the fruit (Génard and Huguet, 1996).

The model assumes that a maximal flow ($F_{\max}$) is determined by the restricted vascular cross-sectional area of the fruit peduncle. Moreover, the solution flow is considered to increase and decrease with plant and fruit water potential, respectively, which has been stated for xylem flow and is thought to be effective for phloem flow towards the fruit (Lang et al., 1986), though the process involved is more complex. The water potential of a fruit depends on the osmotic potential of its cells, which is usually related to sugar content in the fruit, and on the pressure potential due to the resistance of tissues to deformation. Sugar concentration increases with fruit transpiration per unit mass and with peach mass as indicated by Chapman et al. (1991) and Génard et al. (1991). Pressure potential seems to increase with fruit size (Bussières, 1994). The plant water potential is assumed to be inversely proportional to *MDS*.

Consequently, the model assumes that the flow:

- has a maximal value $F_{\max}$;
- increases with fruit mass (W_{fresh}) and with transpiration per unit mass (T_w), since W_{fresh} and T_w will cause a decrease in the osmotic potential of the fruit;
- levels off at high fruit size, when the fruit pressure potential compensates for the decrease in osmotic potential;
- decreases with *MDS*.

According to the previous assumptions, the flow is computed using the following empirical equation

$$F = A_1\left(1 - e^{-A_2(W_{fresh}T_w)^{A_3}}\right) \quad \text{with} \quad A_i = a_i\left(\frac{MDS}{MDSo}\right)^{b_i}, \quad \text{if} \quad F < F_{\max}$$

and otherwise $F = F_{\max}$

where A_i are empirical functions of the effect of water stress on the daily solution flow F, and a_i, b_i and $F_{\max}$ parameters. *MDSo* is a calibration parameter related to the trunk characteristics of the tree. The product $W_{fresh}\ T_w$ is equal to fruit transpiration (T), which is an important stimulator of fruit growth in the model.

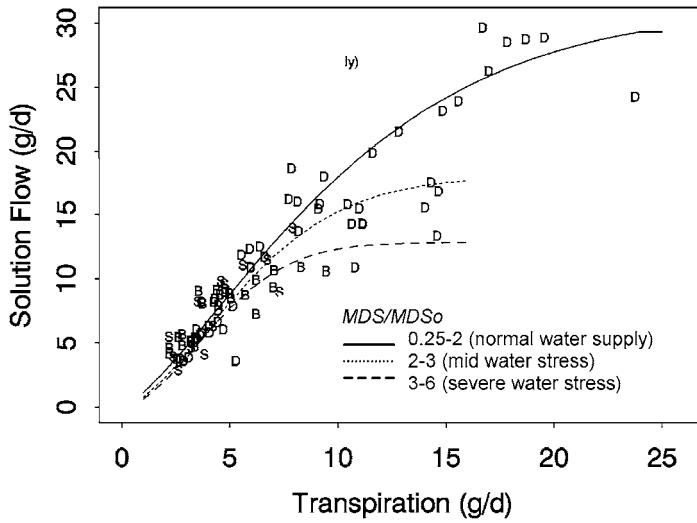

Figure 7. Relationship between solution flow and transpiration for three levels of *MDS/MDSo*. Observed (dots) and simulated (lines) data for 'Dixired' (D), 'BigTop' (B) and 'Suncrest' (S) peach cultivars.

The solution flow computed by the model is very close to the solution flow calculated from the data and obtained for three cultivars (Figure 7). At low transpiration rate the solution flow is not very sensitive to the water supply.

Climate, initial fruit size and *MDS* are the inputs to the model. Time-step in the model is one day.

5.1.2. *Effect of irrigation on fruit growth analysed with WASIF*

An experiment presented by Huguet and Génard (1995) and conducted on 'Dixired' peach trees in Southern France was used to test the model. In this experiment, four-year-old peach trees, on which fruits were highly thinned, were cultivated in containers. Half of them were well watered from 28 May to 30 June and the rest was subjected to water stress. In the stressed treatment, water supply was stopped from 8 to 11 June, and limited to a quarter of the quantity received by well irrigated trees from 16 to 30 June.

The model simulated mean fruit growth per tree (Figure 8). It separated the fruit growth curves of well-irrigated trees from those of stressed trees. The differences between trees were in agreement with field measurements.

The effect of varying MDS was investigated. The simulations showed that fruit mass at harvest sharply decreased when the *MDS/MDSo* ratio increased from 1 to 3. This drop can be due to the great sensitivity of peaches to water stress during the active period of fruit growth (Li et al., 1989). An increase in the *MDS/MDSo* ratio above 3 had a weaker effect on fruit growth (Figure 9). The effect of the period of stress was also investigated. Early stress had a low impact on fruit growth, whereas late stress had a great impact because water stress sharply decreased solution flow in large fruits exhibiting high transpiration (Figure 9).

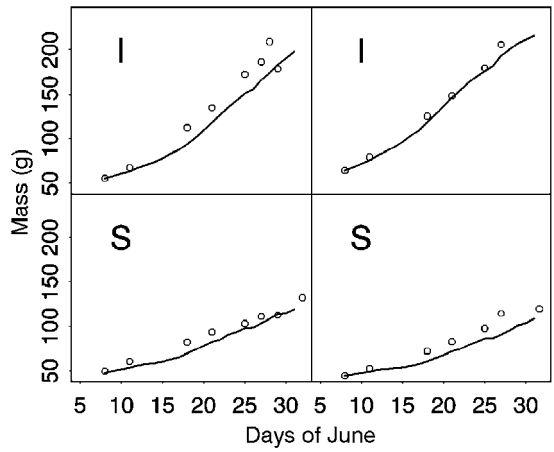

Figure 8. WASIF model: observed (dots) and simulated (lines) data for mean fruit growth in two well irrigated (I) and two stressed (S) trees of 'Dixired' peach cultivar.

In conclusion, this type of model make it possible to manage irrigation day after day according to the information obtained by a bioindicator. It can be applied to species with fleshy fruits having a high transpiration, whereas the fruit growth of species with a low transpiration such as the tomato would probably be better simulated by 'resistance models' where water import into the fruit is mainly controlled by the resistances along the transfer pathway which depend on the fruit radius (Bussières, 1994).

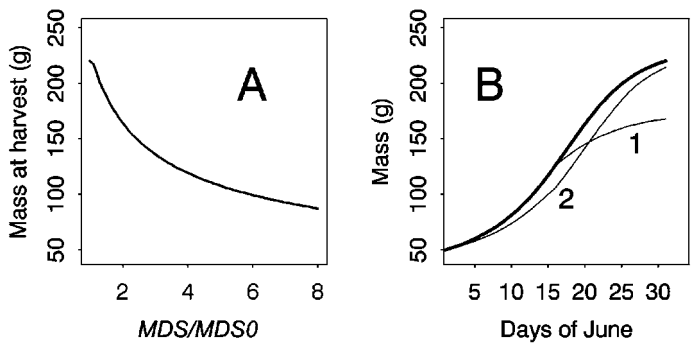

Figure 9. Simulated fruit mass at harvest with WASIF for MDS/MDSo ranging from 1 to 8 (A), and simulated growth curves according to the period of stress (B). In (1) *MDS/MDSo* = 1 for the first fifteen days and 2 for the following days, in (2) the periods are in reverse order. The bold line represents the fruit under reference conditions.

5.2. SIMTECK: a SImulation Model for TEChnical operations in Kiwifruit orchard management

Kiwifruit size is highly variable, especially within vines (Habib et al., 1991; Smith et al., 1994). Accordingly, SIMTECK attempts to account for fruit size variability by considering variation factors occurring at different levels of organisation (flower, cohort of flowers, cane, vine, and plot). The model is made up of sub-models that describe the flowering process in female (Agostini et al., 1999) and male vines, pollination and fertilisation of flowers, and the growth of individual fruits (Lescourret et al., 1998b, c). We will present these submodels, explain their relationships and focus on the way technical operations are incorporated into the global model (Lescourret et al., 1999). By incorporation, we mean the effect of technical decisions on the biological processes. Apart from technical operations, inputs include climatic series (temperature, rainfall, and potential evaporation rate). The output is the size, for a chosen harvest date, of each fruit of an orchard. Time-step in the model is one day. The general principles are presented below. More details can be found in the papers quoted above.

5.2.1. *Flowering model*

Kiwifruit is a fruiting vine that only crops on new shoots originating from one-year-old stems called canes. The flowering model of female kiwifruit works at the cane level, canes being independent units within the plant. It simulates the number and time-distribution of flowers which bloom. The components of the number of flowers are:

- the number of overwintered buds on the cane;
- the number of buds that have burst and will produce shoots. The maximal rates of bud break depend on the location of the buds on the cane (basal, medium and apical), and on their orientation (upwards, sideways or downwards pointing);
- the number of flowers remaining per shoot after abortion.

The time-distribution of flowers which bloom is modelled by a stochastic approach.

As the architecture is more complex in male vines than in female vines, the model operates at the plant level. Another difference is that bud break triggering is based on an estimated bud break rate, without any positional effect.

5.2.2. *Pollination and fertilisation model*

The fertilisation of individual kiwifruit flowers was described as the combination of four random processes:

- a Poisson distributed deposition of pollen on the stigmas. The intensity of the process is computed for each cohort (populations of flowers with the same day of anthesis) in each vine. It is the sum of the pollen produced by all the pollen sources of the orchard (male vines) and received by the targets (the stigmas of the flower) during the effective pollination period. Pollen production, which is

distributed over the period during which the flower of a staminate vine releases pollen, depends on the number and time-distribution of flowers that bloom, which are outputs of the previous model, and on the number of pollen grains per flower, which only depend on the cultivar. Pollen reception is a function of the distance between the source and the target. Rainfall is assumed to temporarily stop the deposition process;
- a binomially distributed selection of fertile pollen grains;
- a random fertilisation of ovules by the pollen tubes conditional upon the presence of N ovules in the ovary;
- a binomially distributed selection of fertile ovules.

The result of flower fertilisation is a number of seeds per flower. Then, the fruit set is assumed to depend on the seed number per flower.

5.2.3. *Fruit growth model*

At any time t of growth, fruit growth (dW_{fresh}/dt) is viewed as a sink strength with two multiplicative components, sink size assessed by means of fruit mass $W_{fresh}(t)$, and sink activity represented by the relative growth rate $RGR(t) = (dW_{fresh}/W_{fresh}dt)$. At any time of growth, $RGR(t)$ is viewed as the product of a reference relative growth rate $RGR_{ref}(t)$ by the effects of the seed number and crop load, f_1 and f_2, assuming that other growing conditions are optimal. These effects concern carbon allocation, and the choice of relative growth rate as a target originates from the assumption based on field observations (Grossman and DeJong, 1995) that fruits are not able to reach their potential growth rate once carbon stress is relieved because the sink size has been reduced.

Thus, growth rate is

$$\frac{dW_{fresh}}{dt} = W_{fresh}(t)\ RGR_{ref}(t)\ f_1\ f_2 \qquad (1)$$

Such a model makes it possible to recover the reference relative growth rate if limitation stops.

The f_1 value is assumed to be constant during the entire growth, whereas f_2 is assumed to be constant during time intervals for which the vine crop load is constant.

To account for the effect of irrigation on fruit growth, a water stress effect is calculated from a simple water balance model at the plot level. This effect is computed as the water supply to water demand ratio, where water supply originates from rainfall and irrigation and water demand is calculated as daily Penman estimates of potential evapotranspiration adjusted by means of a series of kiwifruit crop coefficients measuring the maximal to potential evapotranspiration ratio. The observations of Judd et al. (1989) and Vannière and Huguet (1991) on kiwifruit, as well as those of other authors, have shown that once water stress is relieved, fruit expansion resumes at the same rate as in well-watered vines. Thus, water stress should act on a potential growth rate. This potential growth rate has been defined for each individual fruit conditional on vine load and seed number (equation 1).

5.2.4. *Technical operations*

SIMTECK includes four technical operations: planting scheme and choice of pollenisers, winter pruning, fruit thinning, and irrigation. The block design comprises the choice of cultivars, male-to-female arrangement and planting ratio, and distances between rows and between plants within a row. Winter pruning consists in selecting the number of replacement canes to be left on the vine, and the number of buds to be left on the cane. Thinning takes place at the flower bud stage. The current practice consists in, first, eliminating the aberrant shaped (flat and fan shaped) flower buds. If the remaining flower load is judged too important, lateral flowers, which are always small and have little commercial value (Antognozzi et al., 1991), can be removed till a satisfying flower load is reached. Another technical choice addressed for example by Lahav et al. (1989) concerns the time of thinning, which can take place at the flower bud stage, or just after fruit set when the visual control is easier. Various irrigation tactics can be tested, for example supplying a fixed amount of water and triggering irrigation when the accumulated plant water demand just exceeds a given threshold; or supplying at a fixed frequency an amount of water corresponding to the plant water demand accumulated since the last irrigation.

5.2.5. *Effect of technical operations according to SIMTECK*

The plot simulated comprised six rows and 36 plants per row on a regular scheme with planting distances equal to 6 m between rows and to 4 m within the row (Figure 10). Males of the Tomuri cultivar were found at every third position in every third row, yielding a planting ratio of 1:8.

5.2.5.1. *Planting options, pruning, thinning and irrigation*
Simulated planting schemes included that of the reference plot and a quincunx scheme with identical male to female planting ratio (Figure 10). Fruit size distribution according to the different planting schemes was similar (Figure 11a). Accordingly, yields were close to each other (17.2 vs. 16.8 tonnes/ha). Different planting spacings (5 or 7 m planting distance between rows) resulted in contrasted fruit size distribution (Figure 11b) and yields (24.4 for the shorter vs. 13.1 tonnes/ha for the larger distance).

According to the simulations, Tomuri plants seemed to be less efficient than Matua plants, because of fertility differences, as shown by fruit size distribution (Figure 11c) and resulting yields (17.2 vs. 19.5 tonnes/ha). The more severe the pruning, the lower the number of fruit per vine. Due to the limiting effect of vine crop load on fruit growth, fruit size distribution was right-shifted in the case of severe pruning (Figure 11d), but the difference was slight and the corresponding yield was dramatically lower owing to the small number of fruits (11.5 vs. 25.2 tonnes/ha in the case of light pruning). Thinning simulations were performed at bud stage either with the aberrant shaped flowers removed, or the aberrant shaped and the whole set of lateral flowers. The number of fruits per vine was slightly lower when the lateral flowers had been removed (Figure 11e). In this case, fruit size was slightly favoured, due to the fact that vine load had decreased, but also that fruits with

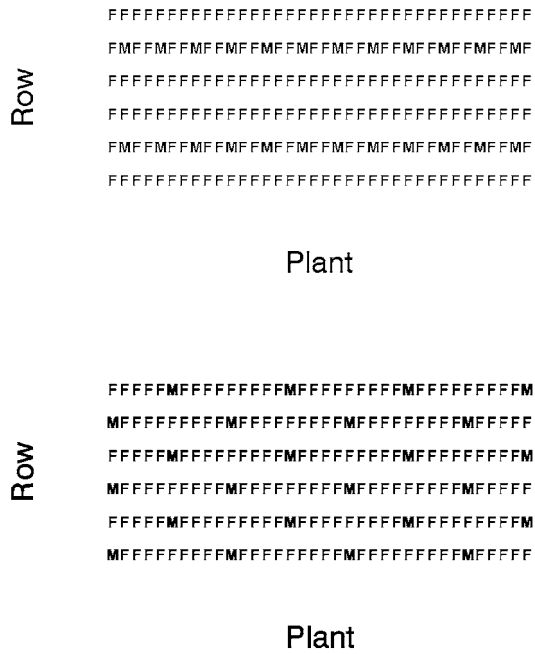

Figure 10. Regular (reference) and quincunx planting schemes (M represents a male plant, F a female plant) as inputs of the SIMTECK model.

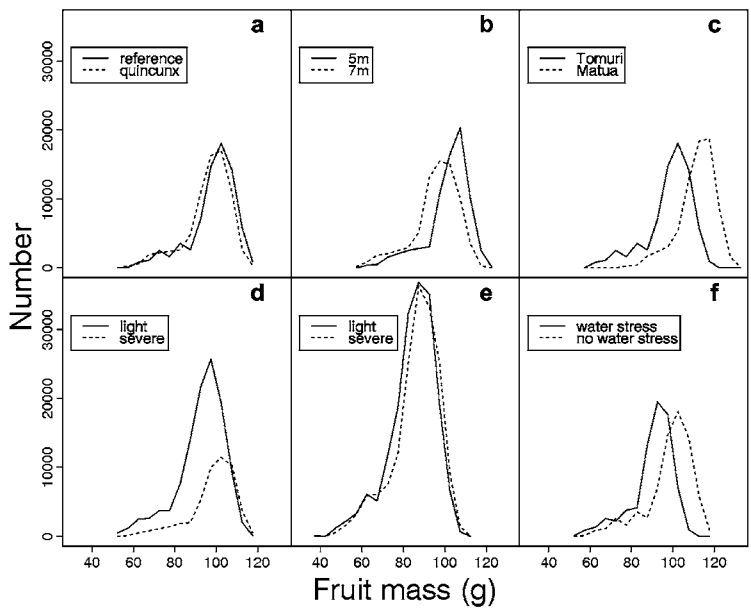

Figure 11. Fruit size distributions according to the planting scheme (a), between-row distances (long = 7 m and short = 5 m, b), cultivar (Tomuri and Matua, c), pruning intensity (d), thinning intensity (e) and water stress (f).

few seeds were removed. However, the difference was negligible, and the overall yield was slightly better when lateral flowers were not thinned (36 vs. 34.4 tonnes/ha).

Two simulated tactics of irrigation were analysed. In the first one, a fixed amount of water (30 mm) was supplied each time the accumulated plant water demand exceeded 30 mm (a) resulting in no water-stressed vine. In the second one, irrigation was triggered each time the accumulated plant water demand exceeded 30 mm and only 65% of the water demand accumulated since the last irrigation was supplied (b) resulting in water stressed vines. In (b), total irrigation water amounted to 427 mm versus 570 in (a), and water demand was frequently unsatisfied. Comparing the effects of irrigation tactics (a) and (b) yielded significant differences, with the distribution of fruit sizes left-shifted for (b) (Figure 11f). In this case, the mean fruit size was 90 g vs. 98 g with no water-stress, and the corresponding yield was 15.7 tonnes/ha versus 17.2 tonnes/ha.

5.2.5.2. *Use of SIMTECK to evaluate management decisions*

This section intends to illustrate how the model can be used to address issues about technical operations and crop performance. For example, it could be useful to evaluate how operations such as pruning and thinning can be managed to make the crop performance of two plots characterised by different planting schemes as close as possible. Using SIMTECK, several technical situations were simulated. Yields and yield values were calculated, the latter was based on a series of prices for year 1997.

In case A, the planting ratio was 1:5. This situation was highly favourable to pollination. It was decided to keep as many fruits as possible on vines, anticipating that the reducing effect of vine crop load on fruit growth would be largely compensated by the large number of seeds that is a major factor of fruit size. Thus, pruning was rather light (60% of replacement canes were kept) as was thinning (only aberrant shaped flower buds were removed). Total yield was 24.6 tonnes/ha and both the percentage and the number of fruits were high in the best grades (Table 2). The yield value was 4900 €/ha.

In case B, the planting ratio was 1:10, which is unfavourable to pollination. Situation B1 consisted in trying to compensate the effect of the anticipated poor pollination on fruit growth by reducing as much as possible the vine crop load.

Table 2. Distribution of fruit numbers (percentages by rows in brackets) among size grades for three simulated technical situations.

	< 70 g	70–90	> 90
A	1086 (1)	9382 (10)	88288 (89)
B1	4018 (6)	14315 (21)	49192 (73)
B2	10977 (9)	48200 (38)	67000 (53)

70 and 90 g are the EEC thresholds for 'First' and 'Extra' grades. The planting ratio was 1:5 for A and 1:10 for B; the ratio of replacement canes kept at pruning was 60%, 40%, and 70% for A, B1, and B2, respectively; thinning concerned the aberrant shaped flower buds for A and B2, as well as the lateral buds for B1.

Pruning and thinning were quite severe (40% of replacement canes were kept, aberrant shaped and lateral flower buds removed). The number of seeds per fruit was dramatically decreased in B1 compared to A and the reduction of the number of flowers, which was stressed by thinning, was not sufficient to compensate the deficit of fruit growth. On the contrary, the resulting small number of fruits was a critical point. Yield was poor (15 tonnes/ha) as was the number of fruits in the best grade: about two times less than for A though these fruits represented a high percentage of the yield (Table 2). Accordingly, the yield value was weak (2400 €/ha).

Situation B2 consisted in testing a less severe pruning option than in B1, i.e. keeping 70% of replacement canes. Because of a higher number of fruits on vines, fruit growth was repressed compared to B1. Accordingly, the proportion of fruits of the best grade was lower (Table 2), but it remained high (76% of the number corresponding to case A). Total yield (26.7 tonnes/ha) was better than in A, but its value (4100 €/ha) lower.

These results show that according to the model, changes in pruning and thinning cannot modify the effect of unfavourable planting options in the simulated example.

In conclusion, SIMTECK focuses on fruit size variability by considering different levels both from a biological and technical point of view. Technical operations are incorporated in the processes to reflect the level at which they are reasoned. The resulting model allows choosing a technical operation in order to reach a given production in terms of fruit size distribution and total yield. In a next step, the sequence of technical operations will have to be compared using economic criteria.

6. THE QUALITY FROM THE ECOLOGIST'S POINT OF VIEW

From the ecological angle, fruit quality can be considered in two ways. First, the quality of wild fruits plays a major role in seed dissemination by animals and is a key factor in the plant population dynamics. Indeed, many traits of fleshy fruits have been interpreted as co-adapted traits of plants that govern the choice of a fruit species by animals (Janzen, 1983). They include mass, palatability and nutrient content of edible tissues (Gautier-Hion et al., 1985). Second, the fruit quality depends on the effect of ecological factors on the state of the plant which bears them. This plant can be subjected to diseases and be attacked by animals, mainly insects with negative consequences on fruit quality. We are now in the framework of disease epidemiology and predator-prey relationships which have been largely studied and modelled (Gillman and Hails, 2000). This relationship between ecology and fruit quality is of particular interest in the framework of Integrated Fruit Production (IFP), which was the innovation of European horticulture in the 1980s (Sansavini, 1997). IFP includes all the field management techniques intended to produce crops that meet both commercial and consumer demands, especially with regard to edible quality, while preserving the environment. IFP calls for adaptating agricultural practices, thus challenging researchers with their capacity to producing data and tools to accompany it. As regards biotechnical models applied to IFP, a lot remains to do as very few of them take into account all the following essential aspects:

– response of the plant to pest attacks and diseases, and consequences on fruit quality;
– regulation of pests in response to the plant, considered as a source of food, and to pest predators and diseases;
– effect of the plant environment as a source of pests, diseases and pest predators;
– response of the whole system to cultural practices, especially pest and disease control.

Our purpose was to investigate the possibility of discussing the above-cited points in an integrated approach. Therefore, we chose the case of pests and a simple and speculative modelling framework basically a predator-prey model (called 'Catiote' and presented in Lescourret et al., 2002). We investigated the effect of a few different crop protection strategies on the behaviour of a simplified orchard system, focusing on basic indicators of fruit quality (fruit mass and sugar content) and environmental quality of the management (number of chemical treatments) that are concerned in the IFP context.

6.1. **Catiote: a predator-prey model under the control of insecticide spraying**

Our simplified system is composed of trees in an orchard (foliage and fruit parts), pests acting on the tree by reducing the leaf area, and their predators. At this stage, pests (and, correspondingly, predators) can belong to a given species or a guild (i.e. guild of foliage eaters). To describe the evolution of fruit growth over time within the foliage/pests/predators subsystem, we used a N-species Lokta-Volterra model (Lebreton and Millier, 1982). To account for a possible effect of plant environment in a broad sense, we added a flow of predators to the fruit trees. It is a rough way to assume that plant reservoirs may have a positive role on the ecological balance (Rieux et al., 1999). We considered two cases:

– the predator migration flow is constant and the predators do not reproduce because of the pests they consume. In this case the response of predators to pests is functional, not numerical. This case will be called 'pest-independent';
– the predator migration flow depends on the local density of pests in a simple way, e.g. linearly, and the reproduction of predators depends on the pests consumed. This case will be called 'pest-dependent'.

The equations of the model were

$$\frac{dFo}{dt} = Fo(\alpha_1 - \beta_{11}Fo - \beta_{12}Pe)$$

$$\frac{dPe}{dt} = Pe(\beta_{21}Fo - \beta_{22}Pe - \beta_{23}Pr)$$

$$\frac{dPr}{dt} = Pr(-\alpha_3) + \gamma_3 \qquad \text{(pest independent)}$$

$$\frac{dPr}{dt} = Pr(-\alpha_3 + \beta_{31}Pe) + \gamma_3 Pe \qquad \text{(pest dependent)}$$

with *Fo*, *Pe* and *Pr* being local 'densities' of foliage, pests and predators expressed per tree for the sake of simplicity, and γ_3 and $\gamma_3 Pe$ being the migration terms. As far as parameters are concerned, α_i is an intrinsic term of population increase or decrease, depending on the sign of α_i, β_{ii} a term of self-limitation, and β_{ij} a term of positive or negative interaction between populations.

Fruit mass and sucrose concentration were the fruit quality traits considered in the simulations. The growth rate of the fruit crop per tree was very roughly described as depending linearly on the tree leaf area *Fo*, thus the mean individual growth rate was

$$\frac{dF}{dt} = \delta \frac{Fo}{n}$$

with *F* being the fruit mass, *n* the number of fruit per tree and □ a parameter.

Sucrose concentration in the fruit was calculated using the SUGAR model presented previously in this chapter.

We modified the basic model to account for a possible chemical control, in a way close to the IFP guidelines. The resulting Catiote model made it possible to trigger insecticide spraying when pest density exceeded a given threshold *y* (reasoned pest control). Input values described the efficiency of the product for the possible targets, pests and predators, and its persistence through the pattern of decreasing efficiency over time. This decrease is assumed to be linear with time.

Simulations were performed for a period of 110 days ended by fruit harvest, using a daily time step. Three cases were investigated where the source of pest control was (i) the plant environment only, (ii) the integrated chemical control only, (iii) a mixture of both.

6.2. Effect of pests, predators and insecticide spraying on fruit quality and environment

When the only source of pest control was the plant environment and when predators were pest-independent, predators and leaf area slowly increased, while pests slowly decreased after a few peaks. Final mean fruit mass was about 70 g and sucrose concentration about 5%. When pest-dependent, predators caused a stronger regulation of the system. The final mean fruit mass and sucrose concentration, about 100 g and 6% respectively, were higher than in the previous case (Figure 12).

Under reasoned chemical pest control, without any predator influence, the developmental trends of pests and leaf area levelled off rapidly but permanent oscillations were encountered. As soon as the effect of insecticide application was over, pests increased, resulting in a new application and so on. The resulting outputs were a mean individual fruit mass of 140 g, a sucrose concentration of 7%, and a number of seven chemical applications.

Combined environmental and reasoned chemical pest control also rapidly yielded flat and oscillating evolution trends of pests and leaf area (Figure 12). The patterns were very similar to the previous case for pest-dependent predators. With pest-independent predators, the oscillations were less frequent. In this situation of double

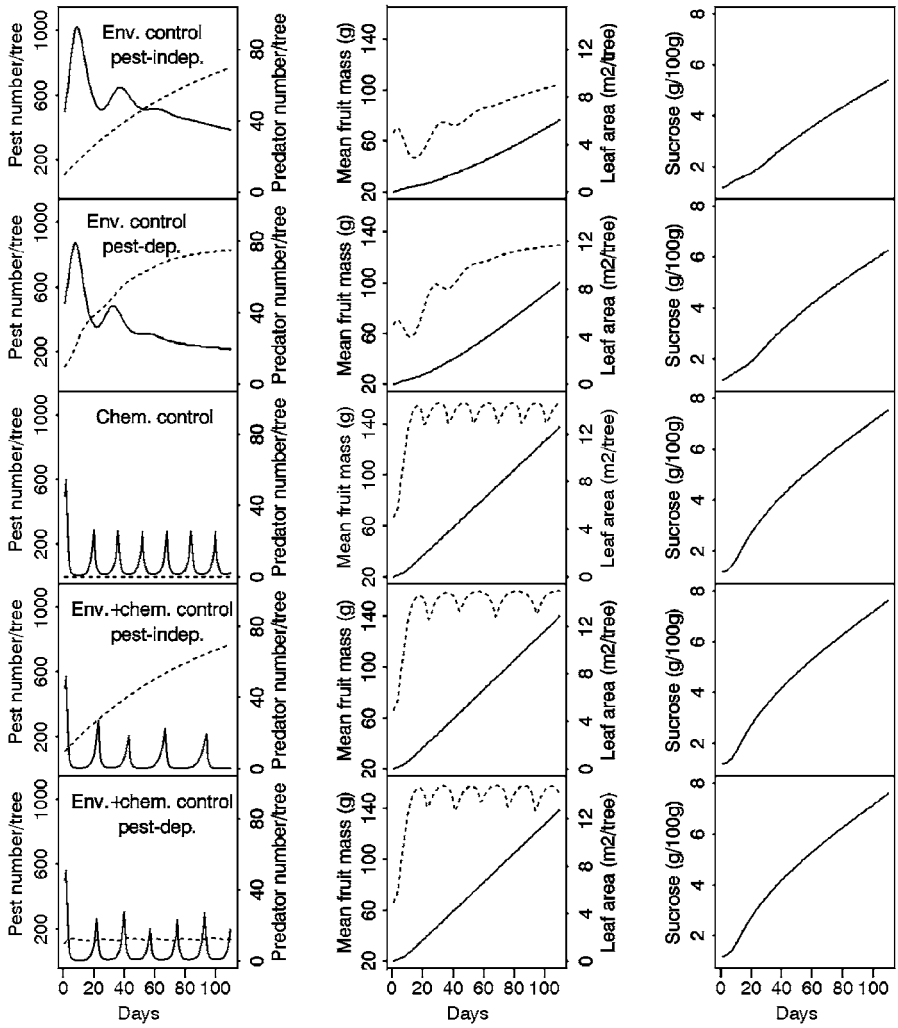

Figure 12. From left to right: temporal evolution of pest (full line) and predator number per tree (dotted line), temporal evolution of mean individual fruit mass (full line) and leaf area per tree (dotted line), temporal evolution of fruit sucrose concentration. From top to bottom: environmental control only with pest-independent predators, environmental control only with pest-dependent predators, reasoned chemical control only, combined environmental (pest-independent) and reasoned chemical control, combined environmental (pest-dependent) and reasoned chemical control.

protection, pest-dependent predators were considerably reduced by the chemical treatments because of their dependence on pests, while pest-independent predators were not affected and increased as in the previous situation of environmental control (Figure 12). As a result, though the final fruit mass and sugar concentrations were high in both cases as in the situation of reasoned chemical control, the number of chemical applications was six for the pest-dependent case, which is a little bit less

than in the situation of reasoned chemical control, and only five for the pest-independent case.

In conclusion, the Catiote model showed it is worth considering an integrated modelling framework combining ecological and agronomical concerns to compare the effect of various protection strategies on fruit quality. Here we focused on reasoned crop protection with the view of its possible environmental impact, roughly indicated by the number of chemical applications. Studying Catiote raised questions about the necessary conditions for pest control. Catiote relies on assumptions that should be carefully examined before concrete use. The Lokta-Volterra model belongs to the class of ecological theories of population regulation that focuses on density-dependent factors, knowing that other classes focus on density-independent factors such as climate, or consider a mixture of both (Dajoz, 1974). By using situations and models that are both speculative, we only aimed at getting an insight into the ability of future models to meet IFP requirements.

7. CONCLUSIONS AND PROSPECTS

We presented several biological models dealing with fruit quality. It is clear that most of the models are mainly restricted to fruit growth, focusing on dry mass, and that research in modelling must now focus on other essential aspects of fruit quality such as acidity, firmness, vitamin content, etc. Indeed, a lot of work has been done by physiologists and technologists on these aspects, but the resulting knowledge has not been integrated in ecophysiological and agronomic models.

The structure of the Catiote model presented in this chapter, shows how it could be possible to consider an ecological approach to deal with environmental problems additionally to fruit quality. But here also, a lot remains to be done, mainly integrating ecophysiological, agronomic and ecological knowledge into a model able to simulate several aspects of quality in response to cultural practices, environment, and pest and diseases. Such an integration has been done previously by Gutierrez and colleagues, and mainly exemplified in cotton and cassava (see Gutierrez, 1996, for a synthesis focusing on the ecological point of view, with numerous referenced papers). However, the agronomic aspects were not completely considered and the quality was not the aim of their models.

We are now developing an integrated model applied to the peach (‘Alexis’ project) which combines some of the models presented here (SWAF, SUGAR, CaShoo) to build a model able to simulate the quality of each fruit in a peach orchard in response to cultural practices and to the occurrence of diseases. Such a model will be object-oriented in order to facilitate its design and its evolution. The advances in computer power make it possible to consider simulation models much more complex than ten years ago.

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SPRAY TECHNOLOGY IN PERENNIAL TREE CROPS

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1. INTRODUCTION

In order to optimise pesticide use, an understanding of the principles and practices of crop spraying and pesticide application is required. Lodeman (1896, cited in Heijne, 1980) defined spraying as 'the throwing upon plants of any fluid or semi-fluid in the form of fine rain or mist'. Over a century later, with an increased awareness of the impact of pesticides, Manktelow (2000) has described successful spraying as the application of appropriate chemicals, at appropriate times and rates, to produce adequate coverage of the target canopy with an effective chemical dose.

Until relatively recently, spray application technology on perennial tree crops was poorly researched. Changes in manufacturing technology have improved sprayer design, but the general concepts of spraying that were introduced at the end of the nineteenth century are still being applied today. The requirement for effective spraying in perennial tree crops is for the chemical to be applied evenly throughout the tree, so that the active ingredient reaches the target. Failure to achieve this results in poor pest/disease control or ineffective uptake of plant bioregulators (PBRs), and usually the chemical is claimed to be ineffective or the argument of pest resistance arises. To avoid this situation, operators need to be aware of the issues involved in relation to both tree and sprayer.

In recent years, many countries have begun to adopt practices to reduce spray pollution from drift and runoff, particularly with the increasing urban encroachment on orchards. Addressing this issue through improved spraying performance and more efficient targeting has not only resulted in reduced chemical usage, but also in a significant reduction in pollution and wastage, benefiting both orchardists and the public at large.

This chapter discusses the development of spray technology in perennial tree crops. Mechanisms of droplet production and factors influencing the efficiency of spraying are discussed in addition to the technological developments that have led to improvements in spray application. While mentioned in passing, the impact of tree and climatic factors is not discussed in depth.

2. THE DEVELOPMENT OF SPRAY TECHNOLOGY

Prior to the 1880's, liquids were applied to plants with watering cans and syringes. The first reports of spraying in perennial crops date back to 1850 when a syringe was used to apply an aqueous solution of flowers of sulphur to grape vines to control powdery mildew (Morgan, 1992). In 1882, Millardet recommended the use of a

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switch of heather to flick copper-lime mixtures over foliage. According to Heijne (1980) the first pump operated sprayer was built in France in 1885, and in 1888 a geared spraying machine was commercially available. Morgan (1992) suggests that the construction of machines specifically for spraying began in 1886 with 'hand operated rotating brushes, bellows air atomisers and hydraulic back pack sprayers whose design has not altered to this day'.

The design of early hydraulic nozzles was simple, with the liquid being restricted as it approached the outlet orifice. Rapid development led to three basic nozzle designs. The fan nozzle, in which the liquid stream was modified by the shape of the outlet orifice, was followed by the development of the impact nozzle where the liquid stream was modified by an obstruction after the outlet orifice. Towards the end of the 19th century, Riley designed the swirl or cone nozzle in which a rotary motion was applied to the liquid stream causing it to break up immediately after leaving the outlet orifice (Morgan, 1992). This nozzle is still one of the main atomising devices used in tree crop spraying.

Initially there was little information available on the range of liquid volumes or the type of spray distribution required to produce the desired biological effect. In the early 1900's sprays were applied by hand-directed nozzles and a 'fine spray' was recommended. In order to spray tall trees, hand-directed nozzles had to be carried on long tubes supplied through flexible hoses. To avoid the need for long hoses, horse drawn wheeled machines were utilised, some with tall platforms to enable operators to reach the tops of trees. The 1930's saw the introduction of adjustable spray guns which enabled a skilled operator to use large spray drops with sufficient momentum to reach the tops of trees or smaller drops in wider sprays for the lower branches. Up to the second world war, mainly contact pesticides were used and many deficiencies in pest and disease control could be ascribed to failure to reach all or most of the target organisms with the spray liquid (Morgan, 1992). Although slow and laborious, in skilled hands the manual lance or gun applications were very effective because of their ability to cope with variations in tree geometry and multi-directional spraying.

In the immediate post war period, 'automatic' spraying methods were introduced with the fixing of hydraulic swirl nozzle assemblies on tractor-drawn machines. These were mounted at the rear of the machine on either low frames or on tall masts the same height as the trees, and were driven by a separate motor or via a power take-off shaft from the tractor. The most long lasting post-war development in tree spraying was air-blast application, first introduced on a large scale in the USA (Morgan, 1992). This meant that the projection of the spray to the trees was no longer dependent on the momentum of the droplets themselves but on their passive transport in a powerful airstream from a motor-driven fan. The first patent for the air-blast principle was granted in 1944, for the 'Speed Sprayer' (Feutrill, 1998). The traditional air-blast sprayer consists of a conventional axial fan fitted with hydraulic nozzles, mounted behind a large wheeled spray vat. As a result of this development, droplets could be much smaller as they no longer needed much momentum, and the same or greater number of droplets could be produced from a smaller volume of liquid. By halving the droplet diameter the same number of drops can be produced from one eighth of the liquid volume.

Traditionally, very large spray volumes have been common when treating orchards or vineyards. Volumes of 2,000–5,000 L/ha for deciduous fruit and 10,000–30,000 L/ha for citrus have been considered standard. There were two reasons for these large volumes. Firstly, it was generally accepted that large spray volumes would help to achieve the required thorough coverage of leaves and stems. Secondly, when using spray guns, only the large drops have enough inertia to reach their target site. However, these high volumes resulted in considerable wastage of the spray through runoff and drift.

Over the last 50 years there has been a trend towards reducing the volume of liquid applied, necessitating the application of discrete droplets. A range of low volume (LV) and ultralow volume (ULV) sprayers have been developed for pesticide application in orchards. Gunn (1980) reports that the early LV and ULV sprayers were able to demonstrate a degree of pest control, but were unable to give adequate control when subjected to severe pest/disease pressures, particularly with the control of red spider mite (*Oligonychus ulmi*) or apple mildew (*Podophaera leucotricha*). However with the move towards Controlled Droplet Application (CDA) technology, Bals (1976) demonstrated that targets could be sprayed more efficiently by selecting optimum droplet size and density for maximum retention and coverage. CDA spray heads can be fitted to air-blast sprayers to give a diverging spray pattern, or on a vertical boom to give a converging spray pattern as described by Oakford et al. (1994a).

Another development over the last 10–15 years includes the tower sprayer which uses an air curtain and rotary atomiser. This system allows horizontal penetration into the canopy which is preferential to the vertical penetration from an air-blast sprayer (Landers, 1999). Tunnel sprayers, developed in both Europe and the USA during the 1990's, offer advantages in trellised and pedestrian orchards (orchards with dwarf trees). The use of a spray collection device at the base of the tunnel results in the ability to recirculate spray with subsequent savings in pesticide and a reduction in drift. The use of tunnel sprayers however is limited due to the restricted tree size and shape on which it can be used (Cross and Berrie, 1995).

With the increasing urban encroachment on orchards and the need to reduce spray pollution from drift and runoff, there has been a worldwide push to increase the efficiency of spray usage. The need to reduce chemical usage on food has given added impetus. In Australia these moves have culminated in the 'Consumers Charter', signed by fruit growers with consumer bodies, to reduce total chemical usage on fruit trees. Oakford et al. (1995) have pointed out the considerable influence that spray technology could have in achieving this goal.

3. MECHANISMS OF DROPLET PRODUCTION

Liquids can be atomised or broken into droplets by a number of methods: hydraulic pressure, centrifugal energy, airshear, kinetic, thermal, and electrodynamic methods. In agricultural spray systems, hydraulic energy (as in conventional nozzles) and centrifugal energy are the most common atomising methods. In addition to breaking up the spray liquid into droplets, the functions of spray nozzles include control-

ling the volume of liquid output, distributing the droplets in specified patterns and providing direction for the droplets (Banks et al., 1990).

3.1. Hydraulic pressure

The use of liquid pressure to produce a spray is one of the oldest and simplest means of atomisation. The conventional hydraulic nozzle is a device with a small hole. When liquid is forced through the orifice under pressure, hydraulic energy spreads it into a thin sheet which becomes unstable, disintegrating into spray droplets. This disintegration process is largely uncontrolled, resulting in a wide range of droplet sizes. There are a range of nozzle types which can be broadly categorised as plain jet, fan, impact, and conical spray (see Alcorn (1993) for a further description of each type). For a given nozzle, a minimum liquid pressure is required to break up the liquid sheet and fully develop the pattern of spray for which the nozzle was designed.

Advantages of hydraulic pressure are that the components are simple, cheap to manufacture and easy to maintain and use. However, the different ways in which the sheet breaks up leads to a wide droplet spectrum, causing problems of drift from the smaller droplets and chemical wastage from the larger droplets. Other disadvantages include rapid wear of components, particularly nozzle tips, and the need to select appropriate nozzle types for a particular job. Care needs to be taken to operate nozzles at the correct pressure and replace worn nozzles to maintain spraying efficiency.

3.2. Centrifugal energy

Centrifugal energy atomisers consist of a spinning disc or a rotating cage in which centrifugal force is used to accelerate liquid and produce droplets. This method of droplet formation is known as Controlled Droplet Application (CDA) and produces droplets whose size can be controlled within very close limits.

In the spinning disc, liquid is fed near the centre of a rotating surface so that centrifugal force spreads the liquid to the periphery where droplets are formed. The droplet spectrum produced depends on liquid properties such as density, surface tension and viscosity, as well as the disc design, diameter, speed, feed position, presence of grooves and teeth on the disc, surface properties of the disc and the presence of multiple discs (Banks et al., 1990). At low flow rates, individual droplets of near-uniform size are formed directly at the disc edge. As the flow rate increases liquid leaves the disc in long continuous threads, or ligaments, which break up into droplets. If the flow rate is increased further the disc edge becomes flooded and the liquid leaves the disc as a sheet. In this case droplet formation is similar to that of hydraulic nozzles and a wide range of droplet sizes is produced.

In rotating cage atomisers, a cylindrical cage that may be porous, perforated, vaned or slotted replaces the spinning disc. The size of the droplets produced is largely dependent on the rotational speed of the cage, however the type of mesh, cage diameter and type of formulation being applied also impact on the droplet spectrum.

With centrifugal energy systems droplet sizes with optimum target collection efficiency can be produced (Harden, 1992), meaning that less volume of total liquid is needed as large drops are not produced. Drift can also be minimised, as there are no droplets at the small end of the spectrum. However, CDA equipment requires constant careful maintenance for optimal efficiency, and the system needs to be understood to be used effectively as flow rate, liquid viscosity and rotational speed are critical for droplet formation.

3.3. Airshear

The airshear principle involves the shattering of a liquid stream with a fast moving column of air. The liquid is sheared into small droplets, with droplet size controlled by the liquid/air ratio. Smaller droplets are produced with reduced liquid flow and higher air velocities. In order to obtain maximum liquid shatter and correct droplet formation, it is necessary to generate air speeds of 65–90 m/sec at the nozzle (Alcorn, 1993). The energy of the motor is used primarily to drive the fan producing the air flow and the ducting is designed to provide maximum air speed at the point of droplet formation. Advantages of this system are the ability to use reduced carrier volumes and the air movement created by the equipment moves droplets to the target and provides energy for impaction/capture. The liquid/air flow ratio must be correctly adjusted and maintained if a narrow droplet spectrum is to be produced.

Airshear can also be used for secondary break up of larger droplets produced initially by hydraulic nozzles (e.g. orchard air-blast sprayers). The orientation of the hydraulic nozzles in relation to the air stream affects the degree of secondary airshear and therefore the droplet spectrum (Harden, 1992).

3.4. Kinetic

Droplets can be formed in either low-energy vibrators or ultrasonic atomisers. The former tends to produce relatively large droplets and are not widely used in pesticide application except where large volumes of pesticide per unit area and minimum drift are required. Liquid is gravity fed through a series of orifices which are vibrated to break up the liquid stream into droplets (Banks et al., 1990). Ultrasonic atomisers use electric and magnetic transducers and can produce uniform droplets as small as 50 micron (μm) in diameter.

3.5. Thermal

Droplets can be produced by vaporising the pesticide in a stream of hot gas which is then cooled by rapid expansion. Condensation produces a dense fog of very small droplets. Such droplets are very susceptible to drift. This system is not suited to the orchard situation.

3.6. Electrodynamic methods

No mechanical energy is required for droplet formation in this system. The liquid stream is fed between two charged plates which subject it to an intensely diver-

gent electrical field. This results in the formation of thin ligaments of the spraying fluid which break into charged uniform sized droplets.

Electrodynamics should not be confused with electrostatics which relies on applying an electrostatic charge to the spray droplets after they have been formed by an atomiser of some sort. Both electrodynamic and electrostatic concepts rely on the theory that charged droplets will be attracted to the nearest earthed object, i.e. the crop, and since all droplets are similarly charged they will tend to repel one another leading to more even distribution of the spray on the target.

4. FACTORS AFFECTING SPRAY EFFICIENCY

A wide range of factors impact on spray efficiency. Climatic conditions, before, during and after spraying have a significant impact, while tree factors such as size, shape, density, spacing, growth stage and morphology play an important role in spray penetration and retention. Sprayer factors contributing to effective coverage and chemical efficacy include sprayer selection and set up, speed of travel, the use of propelled air, air pattern and velocity, and spray nozzle pattern. The addition of spreaders and stickers to the spray solution, choice of carrier medium (oil, water or combination), carrier volume, and the range and size of spray droplets also have a major influence.

4.1. Rate

Chemical labels use the term rate to describe the amount of chemical recommended per hectare, or per 100 litres of spray mix. The problem with rates 'per hectare' is that canopy volume changes with training system and age of tree. Hence the minimum label rate per hectare will not be valid if the canopy sprayed, or the application efficiency of the sprayer used, are markedly different from those used to derive the label rate (Manktelow, 2000). Manktelow also points out that the rate of chemical per 100 litres of spray mix refers to dilute spraying, which wets the canopy to the point of runoff.

4.2. Carrier volume

Volume rate describes the quantity of water applied per hectare to carry the chemical to the target. High volume (HV) or dilute spraying means spraying to the point of runoff and beyond. This uses a large volume of water and is normally done by hand-lance or with conventional air-blast sprayers, producing a wide range of spray droplet sizes. High volume is often described as being > 2000 L/ha.

However the volume should be related to tree size, as larger trees require greater volumes of water to achieve complete coverage and runoff. In Australia a rough rule of thumb in pome fruit orchards is to allow 1000 L of water per hectare for every metre of tree height. Terminology and definitions of volumes commonly used in deciduous tree crops are given in Table 1. However volume is a relative term and depends to a large extent on the crop. For instance, medium spray volume in pome

Table 1. Classification of sprays according to volume per unit area for deciduous tree crops.

Spray volume	Volume applied (L/ha)	Classification	Common droplet sizes (microns)
High (HV)	2000+	Dilute	< 30 to > 500
Semi low/medium	1000–2000	Semi-concentrate	–
Low (LV)	100–1000	Concentrate	100–200
Very-low (VLV)	30–100	–	–
Ultralow (ULV)	< 30	High-concentrate	30–60

fruit orchards is described as 1000–2000 L/ha, whereas in the citrus industry Beattie et al. (1989) describes medium volume as 2000–7000 L/ha.

Low volume or concentrate spray application involves applying less water volume per hectare, but uses nozzles which produce a greater number of droplets with a smaller mean size. The application of fine droplets is critical, since this results in an even distribution of sprays with minimal runoff or waste.

4.3. Droplet size

Bals (1969) stated that 'The efficiency of a spraying machine is inversely proportional to the range of droplets it emits whilst the suitability for a specific problem depends on the actual size of droplets emitted.' The relationship between the size of the spray droplet and its volume (and thus the chemical it contains) is cubic, e.g. a 200 μm droplet contains eight times more spray liquid than a 100 μm droplet. There are a number of terms used to describe droplets, for example diameter, surface area and volume (mass). For orchard sprayers the volume median diameter (VMD) is most frequently used. This term indicates the droplet diameter which divides the spray into two equal portions, i.e. half the volume of the spray will be less than the VMD and half will be greater. While this term is useful in describing a spray pattern it does not provide information on the uniformity of the spray, or on the upper and lower limits of the droplet spectrum.

The traditional air-blast sprayer using conventional hydraulic nozzles produces a wide droplet spectrum, with droplets ranging from < 30 μm up to > 500 μm. Although gravity and wind influence the distribution and deposition characteristics of droplets, those over 300 μm usually fall straight to the ground. Even if intercepted by foliage, droplets of this size are unlikely to be retained as they run or bounce off foliage. As well as being biologically ineffective, these droplets also contain most of the spray volume. Droplets in the 100–300 μm range sediment under the force of gravity, with some displacement with wind, but are far more biologically effective and better retained by foliage. Droplets in the 30–120 μm range are susceptible to drift and are deposited by a mixture of sedimentation and impaction processes (wind, turbulence and gravity). Droplets under 30 μm (aerosols) are carried almost entirely by wind and have insufficient kinetic energy to impact on foliage.

Agricultural engineering has now advanced to the stage where almost any droplet specification can be produced. However, the droplet size requirements of many target

pests are not always clear and there are conflicting requirements in relation to safety, coverage or cost (Heijne, 1980). For example, Himel et al. (1971) suggested that if the spray is driven into the plant canopy, the optimum size for insecticide spray droplets is substantially smaller than 20 μm, while Smith et al. (1975) recommended a droplet size between 140 and 200 μm for controlling boll weevil, because smaller droplets were subject to excessive drift.

4.4. Spray retention

Spray retention is a measure of the percentage of the spray volume applied that is deposited on the target canopy. In most spray systems, but particularly in conventional air-blast application with hydraulic nozzles, only a portion of the spray is actually physically deposited where it is required. Large droplets over 300 μm end up on the ground, leading to soil and ultimately ground water contamination. Small droplets under 30 μm drift out of the target area. This drift is increased by droplets falling below the critical size through evaporation in transport, particularly with water based sprays. Bals (1984) reports that in the UK alone, at least 16 million gallons of spray liquid contribute to general environmental contamination every year through long distance drift.

The deposition of spray droplets is also influenced by air velocity and turbulence, size of the target, angle of the target surface, morphology and physiological state of the target. The optimum droplet size for maximum retention for aqueous solutions is reported to be 100 μm or less (Heijne, 1980) and such a reduction in droplet size would also improve coverage due to an increase in the number of droplets at the same volume application rate. Droplets less than 100 μm tend to adhere to most target surfaces after contact, with oil based droplets being more adhesive than water based droplets. Droplets greater than 100 μm with high contact angles are often deflected off the target (Broadley et al., 1986).

4.5. Coverage

Coverage describes the percentage area of target (e.g. foliage) bearing spray deposits and how evenly these are distributed across the target surface. Coverage is influenced by tree size, growth stage, density and spacing, and is the result of the way the sprayer outputs interact with the canopy being sprayed. Furness (2000) has produced an aid for estimating spray coverage and deposit with agricultural spray equipment and describes the droplet rating chart as the basis of a simple, rapid field technique to ensure that a sprayer is achieving adequate coverage and chemical dose throughout the canopy.

Banks et al. (1990) report that studies with lepidopterous larvae have shown that droplet density and size are important after deposition has occurred. Larvae that had not been contacted directly by spray droplets were able to avoid large droplets of pesticides on the leaves. When droplet density was increased, the number of contacts made by larvae also increased. A similar problem exists with control of fungal diseases. As fungal spores are immobile, the greater the droplet density, the more likely that spores will be targeted.

According to Furness (2000), the number of droplets per unit leaf area (contact density), size and concentration required for good efficacy against pests and diseases with most agricultural chemicals is not known. However, based on experience, optimal densities for most contact chemicals in dilute high volume applications have been determined as at least 200 droplets per cm^2 with fine droplets, or 25–50 droplets per cm^2 or better with medium sized droplets on all parts of the plant.

4.6. Dosage

Dosage describes the amount of chemical deposited per unit area of target and is the result of the interaction between application rate, coverage and the canopy being sprayed. If droplet size and density is known, the dose of chemical reaching the target can be calculated. Furness (2000) describes how this can be done.

When the droplet spectrum is narrow, the calculation of dosage is relatively easy. However, where a wide spectrum of droplet sizes is produced, such as in the output from hydraulic nozzles, it becomes difficult to deliver specific chemical doses to the target. For example, the dosage variation between a 5 μm and a 500 μm droplet is 100^3, i.e. one million.

Good coverage of a sub-lethal dose, or poor coverage of a high dose can both fail to provide control. The rate of chemical applied per hectare, or unit volume of water, does not automatically determine the dosage achieved. Poor sprayer set-up will give poor dosage and coverage. Thus poor control is achieved, even with high application volumes or chemical rates.

4.7. Timing and weather

Timing is a critical consideration in spraying. Any spray application can only be fully effective if it is aimed at the most vulnerable stage of the pest, weed or disease or, in the case of PBRs, the optimum physiological stage of the tree.

Weather conditions before, during and after spraying influence spray coverage, retention and efficacy. Bound et al. (1997a) have described the importance of temperature, both during and several days after spraying, when applying the PBR 6-benzyladenine. Wind speed is a key weather factor. Spraying in moderate to strong wind conditions (> 10 km/hr) is inadvisable, but spraying in light or still wind conditions is often more dangerous. Under these conditions a thermal inversion may cause spray to become trapped. This pesticide cloud is not dispersed and diluted as would normally occur, but stays together in a concentrated form that can move considerable distances. Rain too soon after application will either wash off the chemical or cause re-activation, which may lead to phytotoxicity, while spraying after a prolonged wet period, or under slow drying conditions may result in fruit russeting.

5. IMPROVING THE EFFICIENCY OF SPRAYING

Proper spray application is one of the most demanding cultural practices in tree fruit production, and often one of the most expensive. An example of this can be found in the citrus industry where spraying costs amount to more than one-third of the total production cost for fresh market fruit (Muraro et al., cited in Salyani and Whitney, 1990).

Spray application is a complex process involving numerous interdependent components. Bukovac (1985) outlined the more significant components relating to the efficiency of sprays applied to tree fruits:

- active ingredient (chemical, physical and biological characteristics)
- formulation of the active ingredient
- characteristics of the spray solution
- droplet formation
- spray pattern characteristics
- transport of droplets to the target
- target definition and characteristics
- environmental parameters during spray application and drying
- spray droplet : surface interaction
- spray deposit formation
- penetration of the active ingredient
- translocation of the active ingredient to the reaction site.

Even as recently as the mid 1980's, Hislop (1986) suggested that although the prospect of improving the efficiency of spraying is bright, there is at present no one system to match the proven reliability of the simple traditional sprayer. Bukovac et al. (1986) also stated that current application equipment was inadequate for efficient chemical application, and component processes were poorly understood. An understanding of these components and their interactions is the first step in developing truly efficient spray application systems.

5.1. High volume application

High volume hydraulic spraying has developed in a largely ad-hoc manner from accumulated experience of actual tree spraying operations (Morgan, 1992). Application with hand-lances offers several significant advantages, such as complete and uniform coverage of the tree, minimal over-dosing, and the ability for the operator to compensate for deficiencies in droplet size and delivery of droplets to the target. Droplet size and the interaction of individual droplets with the plant surface are not critical in high volume spraying since a thin film of spray is deposited over the entire plant surface.

Although the move away from hand-lances to air-blast application increased the ease and speed of spraying and was less labour intensive, non-uniform coverage of the target with the accompanying problem of inconsistency in chemical efficacy became an important limitation. During the 1970's and 1980's, orchards in countries such as USA and Australia converted almost entirely to hydraulic, air-blast

sprayers which were developed primarily for application of fungicides and insecticides. Spraying of PBRs followed the pattern set for pesticides in general orchard practice. By the early 1980's it was common in Australia to apply all sprays with an air-blast sprayer using water volumes of 1500 to 2000 L/ha, independent of tree size. Although both Kvale (1977) and Unrath (1978) found that hand-lance sprays were more effective than air-blast sprayers, Koen et al. (1986) showed that this was mainly due to the higher volumes used with hand-lances. Working with PBRs, these workers showed that the effectiveness of the bioregulator ethephon (2-chloroethyl phosphonic acid) improved linearly as the spray volume increased from 1000 to 6500 L/ha at constant ethephon dosage per hectare on large trees. This confirmed work by Bukovac et al. (1986), who had shown that dose-response was a function of carrier volume and concentration. Jones et al. (1988, 1991) also showed that if air-blast sprayers were used on large trees then high water volumes and lower chemical concentrations should be used. If the carrier volume is insufficient to properly cover large trees, increasing the concentration of chemical does not increase the response (Jones et al., 1991). Unrath (1994) confirmed this by demonstrating decreased activity at a fixed concentration of active ingredient (a.i.) as volume decreased.

5.1.1. *Spray thinning highlights deficiencies*

In apple orchards, spray thinning has been established as an accurate method of assessing the value and efficiency of spraying systems. Chemical thinning agents such as ethephon are polar and are not translocated within the tree (Giulivo et al., 1981; Nir and Lavee, 1981), hence coverage is critical in achieving optimal results. Work by Oakford et al. (1991, 1994a, b, 1995) and Bound et al. (1997b) over a ten year period has reinforced the use of thinning as an accurate gauge of spray application effectiveness. Wilton (1996) states that '. . . chemical thinning probably has the most demanding specifications in regard to coverage of all the spraying we do. I therefore believe that if it is possible to obtain satisfactory thinning results with low volume spraying, it should not be too difficult to obtain very satisfactory results for pest and disease control with low volume application techniques.'

Bukovac (1982) suggests that plant bioregulators respond differently as carrier volume is altered, and that spray deposition is less uniform as carrier volume is decreased with air-blast sprayers. He reports that deposit on the lower quadrant adjacent to the spray lane is often 3–5 fold greater than in the top centre of the same tree. With the narrow range for some growth substances between inadequate response and phytotoxicity, such variations in spray deposits result in over-dosing the lower portion while an inadequate dose is deposited in the tops of trees.

5.1.2. *Canopy structure*

Alteration of canopy structure and density can assist in improving coverage. Byers et al. (1984) found that spray deposit increased with a decrease in tree size and was inversely related to canopy density as indexed by light penetration. Ferree and Hall (1980) reported the greatest spray deposit recovery from trees trained to

a trellis and the least from those trained to a pyramid hedgerow. Working with grapevines, Pergher and Gubiani (1995) found that deposition was always lower at the middle height from the ground, corresponding to the position of the permanent cordon in hedgerow-trained vines, where foliage was particularly dense. They suggest that uniformity could be improved by using large output nozzles in the middle portion of the spraying beam. Boucher (1999) discusses the importance of nozzle selection, whatever spray volume is used, to obtain consistent coverage throughout the whole canopy. He suggests that uniform coverage can be obtained by emitting two thirds of the spray volume from the nozzles on the top half of the sprayer.

In some crops it is difficult to alter canopy structure, and tree density is still a major impediment to efficient spray coverage. In the citrus industry, where tree shape and density differ markedly from that in pome and stone- fruit orchards, the low-profile air-blast sprayers do not produce uniform coverage throughout the canopy. Poor deposition in the tops of these canopies is related to the distance the air must flow from the fan to the top of the tree and the large amounts of branches and foliage that filter droplets out of the airflow before it reaches the top of the tree. Cunningham and Harden (1998) have improved spray coverage using sprayers fitted with air-tower conveyors designed to produce even airflows for the full height of the citrus trees being sprayed, rather than the low-profile air-blast sprayers.

5.1.3. *Limitations of high volume spraying*

High volume spraying requires complete coverage of the target, leaving a thin film of spray solution remaining on the plant. Although still in use in many areas today, high volume spraying has many limitations. It is not possible in areas where water is scarce or needs to be transported over difficult terrain. The air-blast sprayers used to apply high volumes are heavy and cumbersome. When full, their weight often causes soil compaction, particularly as sprays are often applied in adverse conditions, following periods of rain resulting in waterlogged soil. The wide droplet spectrum produced by the hydraulic nozzles used in air-blast sprayers operating at high volumes results in wastage of as much as 80% of the spray through runoff and drift.

Slow tractor speeds are required in order to achieve good spray penetration and uniform deposition of spray. The time taken to apply high volume sprays, combined with the need to refill the sprayer tank several times per hectare, raises the cost of spraying and increases the difficulty of applying sprays in a timely manner, particularly when there are weather constraints. Productivity in terms of area covered is not very efficient, Gunn (1980) suggests that in an eight hour day it is only possible to cover 10–12 hectares of orchard.

5.1.4. *Environmental impact*

To improve the efficiency of air-blast sprayers, many manufacturers have employed ducting to direct the spray. However even with these systems a significant proportion of the spray is still lost through drift and runoff. Increasing fan output in

air-blast sprayers in an attempt to improve coverage in dense canopies in grapevines has been shown to actually reduce chemical deposition (Pergher and Gubiani, 1995). Despite all the modifications used to improve accuracy, wastage is still a problem with high-volume air-blast spraying (Campbell, 1985). Spray drift pollution from hydraulic air-blast sprayers, caused by small droplets of < 50 μm was described by Moore (1990), Walklate (1991) and Hobson et al. (1993). According to Fox et al. (1990) this drift can be deposited up to 100 m from the air-blast sprayer source and is a major problem in urban and semi-urban areas.

The high proportion of large droplets produced by traditional hydraulic pressure nozzles used in high volume air-blast spraying means that there is considerable wastage of spray, apart from drift, through either runoff or deflection of large droplets from the target. Splash and runoff can pose a larger problem than drift. As well as leading to possible phytotoxicity through an accumulation of spray liquid at the leaf tip, runoff also leads to soil contamination. Douglas (1995) reported on investigations that found that only 14% of a 10,000 L/ha application, a common rate in citrus orchards, remains on the tree; the rest is lost as drift and runoff. Active ingredients have been detected in both ground water and streams in horticultural areas in Australia as a result of this (Jones et al., 2000).

Praat et al. (2000) report that canopy development has a major influence on spray drift, with 25 times less drift from a fully foliated canopy compared with a dormant canopy. They also found that the proximity of the sprayer relative to the edge of the sprayed block was an additional major factor influencing spray drift.

5.2. Tree row volume

Most chemical applications in tree crops, particularly with air-blast sprayers, have been made on the basis of a specified rate per hectare regardless of tree size or spacing, or foliage density. However it is illogical to apply the same amount of chemical to small trees as to large trees or to disregard the planting density or foliage density. Byers et al. (1971) described the concept of tree-row-volume (TRV) which is based on volume of crop foliage rather than land area. TRV is the volume occupied by the foliage of the crop and is calculated from the height and width of the tree and the total length of row in a hectare. The TRV concept proposed by Byers et al. (1971) has reference to a 'standard' apple canopy, which has been widely accepted in the American literature to consist of trees 6.1 m tall, 7.0 m wide, planted at 10.7 m row spacings (designated as the United States TRV system, or US-TRV). Successful pest and disease control has been achieved on such trees using dilute spray volumes of 3740 L/ha (400 US gallons/acre) and this was used as a 'base spray volume' for US-TRV coverage estimates (Manktelow and Praat, 1997b). The US-TRV calculation assumes that a row of trees can be described as a rectangular box and the volume occupied by canopy per hectare is calculated on that basis. Manktelow and Praat (1997a) suggest that seasonal growth in mature, slender spindle blocks can give a 30% increase in TRV from dormant to full leaf. Wilton (1996) suggests that TRV needs to be calculated two or three times during the season and spray volumes adjusted accordingly to allow for tree growth. Boucher (1999) discusses the use of the spray volume factor (SVF) to adjust TRV calculations. The

Table 2. Spray volume factors in deciduous fruit trees (Boucher, 1999).

Foliage density	Spray volume factor (SVF)
Dormant trees	75
Low density (early season sprays)	100
Medium density	125
High density (mid to late season sprays)	150

SVF equals the number of litres of spray retained by 1000 m^3 of TRV sprayed to run-off and varies depending on the density of the foliage within the tree canopy. Dormant trees have a lower SVF than trees in the middle of the growing season (Table 2).

The method of calculation of TRV used in the USA has been questioned by Wilton (1996) and Manktelow and Praat (1997a, b). They suggest that the US-TRV system doesn't work in New Zealand because New Zealand trees are more triangular in shape than the rectangular US trees. The US system of calculating TRV multiplies tree height by maximum spread and divides this by row spacing and, according to Manktelow and Praat (1997b) over-estimates spray volumes required in New Zealand canopies by up to 70%. By either halving the TRV estimated from a rectangular profile or measuring canopy spread at half-metre height intervals, adding together the stack of smaller rectangles to give a whole tree TRV, they have improved spraying efficiency.

The use of the TRV method to calibrate commercial air-blast sprayers has been demonstrated to reduce variability in thinning (Herrera-Aguirre and Unrath, 1980). Byers et al. (1984) concluded that adjustment of the chemical application rate for an orchard using TRV estimates may greatly reduce the variability in chemical deposits and subsequent responses observed. However, despite the evidence showing the benefits of the TRV system and active promotion over the last 20 years, the majority of orchardists have resisted its uptake due to its complexity.

Furness et al. (1998) have proposed a simpler method of calibration and chemical labelling which is based on a unit canopy size and length of row. This unit canopy row (UCR) for fruit trees and grapevines is defined as 1 m high × 1 m wide × 100 m of row length (100 m^3 of foliage). They claim that this method is a simple alternative to the TRV method which has been rejected by orchardists. The UCR system has been assessed in both Australia and New Zealand and has achieved excellent control.

5.3. Towards Low Volume application

In low volume spraying, the entire plant surface is no longer completely wetted, as in high volume application. Rather, a number of discrete droplets are deposited per unit area to provide adequate coverage. The key to successful low volume application lies in using nozzles which deliver droplets in a narrow range of sizes between 100 and 170 μm in diameter.

Most agricultural chemicals have been developed and rates determined using high-volume spraying techniques. In low volume spraying, maintenance of the dosage rate is important to avoid loss of efficacy of the chemical. McArtney and Hughes (1992) discuss the importance of maintaining a constant product rate per hectare while reducing water volumes. This means that tank concentrations are increased which could increase the risk of phytotoxicity through excess deposition of spray, or failure of the spray through insufficient spray deposited.

With the advent of CDA machines in the 1970's which were able to produce droplets within the 60–100 μm range, there was a marked reduction in both spray drift and the loss associated with large droplets. Several machines had the capability of conforming closely to this range and utilised spinning discs or rotary cages to produce a consistent range of droplets. Further, droplet size could be adjusted by altering flow rates and pressure. This allowed the alteration of spray application when needed, for instance, under warm spraying conditions in some countries it is advisable to increase droplet mean diameter to allow for evaporation effects.

5.3.1. *Application of CDA technology*

Work with CDA machines in Australian orchards in the 1980's demonstrated that they were at least as biologically effective at 200 L/ha as an air-blast sprayer at 6000 L/ha (Oakford et al., 1991). Further trials with both Micron and Micronair CDA machines showed that ultralow volumes as low as 25 L/ha were capable of producing results similar to high volume applications (Oakford et al., 1994a). However, spinning discs and rotary atomisers were not robust, and were subject to frequent breakdowns and damage. This combined with the almost invisible output at ultralow volumes meant orchardists were reluctant to accept this large technological change in spray application, hence uptake of ULV spraying in orchards has been slow, particularly in Australia and New Zealand where tree size tends to be larger than in the UK.

5.3.2. *Application of airshear technology*

Airshear technology, which works on the principle of high air velocity (282–370 km/hr) and low liquid pressures (68-170 kPa) has proved to be more popular, at least in Australia, than CDA technology. These machines are more robust, utilising high speed turbine fans to produce droplets in the 50–130 μm range. While not producing as concise a range of droplet sizes as the spinning disc or rotary cage atomisers, Oakford et al. (1994b, 1995) demonstrated that airshear sprayers were superior to air-blast sprayers using high pressure hydraulic nozzles. Experiments in Australia by Oakford et al. (1995) using airshear have shown that at volumes of 200 L/ha the chemical dosage rate could be reduced by 25%. Oakford et al. (1994b) reported that reducing the output volume from 200 to 100 L/ha significantly depressed the effectiveness of the airshear machine, however Oakford et al. (1995) found 100 L/ha as effective as 200 L/ha. These authors also reported a marked fall off in performance at 800 L/ha. With higher water volumes, airshear nozzles lose their

efficiency due to flooding of the nozzles, affecting the ability of the nozzle to atomise the spray liquid properly. Oakford et al. (1995) suggested that airshear machines are most effective in the range 100–400 L/ha. Although airshear nozzles are sensitive to small changes in flow rate, air speed and fluid properties and are inherently more difficult to control than hydraulic nozzles, the machines used in these experiments produced highly significant results. These scientific findings have been translated to practical use in Australia and New Zealand where airshear machines are commonly used for sensitive operations such as fruit thinning at around 250 L/ha. Airshear technology has also provided a technological bridge for orchardists between ULV and HV.

Many airshear sprayers are fitted with electrostatics, and Moser et al. (1984) showed that electrostatics was an effective way to increase spray coverage. Oakford et al. (1994b) saw no additional effect using electrostatics to charge the spray particles, but concluded that under some conditions, electrostatics may play a role in aiding the attachment of droplets to the target. Efforts to increase application efficiency with electrostatic sprayers have not been as successful in orchards as in row crops (Hogmire and Elliott, 1991).

While providing an efficient method of spray application at reduced water volumes, the airshear system requires the purchase of new expensive machines, often with the additional expense of a high horsepower tractor. This has meant that uptake of this technology has not been widespread. For example, less that 10% of Australian orchardists have chosen to make use of this expensive technology.

5.3.3. *Low volume hydraulic nozzles*

The performance of hydraulic nozzles has greatly improved in the last 10 years with improved spray pattern characteristics. A change of approach in the design of hydraulic nozzles by companies such as Delevan-Delta Inc., has led to the development of hydraulic nozzles which are able to produce a narrower droplet spectrum of fine droplets at lower water volumes than the traditional hydraulic nozzles. These nozzles can now be used at lower pressures, achieving droplet sizes of 100 to 150 μm. Examination of these nozzles fitted to a standard air-blast sprayer has shown that they are able to operate efficiently at low pressure at volumes as low as 200 L/ha (Bound et al., 1997b). The advantage of this technology is that these nozzles can be fitted to existing air-blast sprayers, offering an opportunity for orchardists to convert to low volume spray application for the cost of the nozzles and a low-pressure gauge.

5.3.4. *Reducing dosage rates*

One of the benefits claimed for low-volume spraying is that chemical rates may be reduced. Maber et al. (1984) indicate reductions of up to 50% may be possible. However there is considerable discussion on this matter. Campbell et al. (1988) found that low volume applications were considerably less effective in controlling both apple scab and codling moth in an apple orchard. Cross and Berrie (1990) described the variations found by other workers, and themselves report conflicting results when

reducing dosage rates to one quarter of the full rate for a range of fungicides, insecticides, plant growth regulators and nutrients. Oakford et al. (1995) demonstrated that reducing chemical dosage rates of PBRs to 25% of the full rate is too low, however they achieved good results with a 25% reduction of the chemical dose rate, which is equivalent to 75% of the full rate. Work by Bound et al. (1997b) with low volume, low pressure nozzles fitted to a standard air-blast sprayer confirmed that chemical dosage rates were effective at 75% of the full rate, but a 50% reduction in dosage reduced chemical efficacy.

5.3.5. *Advantages of low volume spraying*

Low volume spraying represents substantial benefits over traditional HV methods. The reduction in spraying time by up to 60% (Oakford et al., 1995) means significant savings to orchardists and allows timing of sprays to be optimised. Environmental issues are becoming more prominent, and any methods which reduce both atmospheric and ground water pollution require serious consideration. Hence, the ability of low volume sprayers to reduce spray wastage and pollution from drift and runoff are of major importance.

5.4. Spray control systems

Spray control systems are based on sensing the presence or absence of target plants and controlling the sprayer output in an on/off manner. Giles et al. (1987) have reported spray volume savings ranging from 28–35% in peaches and 36–52% in apples. Developments in spray control systems have progressed to the stage where sprayer-mounted laser or sonar sensors spot the tree and translate its profile to an on-board computer (Rigo, 1995). Sonar sensors use ultrasonic sensors to image the tree, while laser sensors utilise a single laser to target trees on both sides of the sprayer. As well as turning on the sprayer, the computer controls each of the jets so that only the correct column of spray is applied. This system is capable of saving up to 60% of orchard spraying costs (Anon, 1995).

5.5. Air speed

The importance of the airstream in conveying the spray to the target has been clearly demonstrated (Randall, 1971). Working with air-blast sprayers he demonstrated that higher volumes of lower velocity air produced better coverage than lower volumes of higher velocity air for a given energy input, and concluded that the nature of the airstream produced has a significant effect on coverage. Furness and Pinczewski (1985) found that converging air-jets resulted in greater uniformity of droplet number per cm^2 on grape and citrus foliage compared with diverging air-jets when low volume sprays were applied. They discuss the energy savings and advantages of multiple head air assisted spray machines, and concluded that turbulent air improves the uniformity of spray coverage and improves spray penetration into dense plant canopies. According to Furness (1997) the nature of the airstream is far more important than droplet spectrum and nozzle type in improving spray

coverage and coverage uniformity. Lovelidge (1993) suggests that the air-blast sprayer has outlived its value, stating that its use of a high volume of fast moving air to carry chemicals to the target is excessive for modern orchards and is becoming environmentally and financially unacceptable because much of the spray misses the target. The problem is finding an effective and acceptable alternative. The fitting of ducting to air outlets has allowed more accurate spraying but because the air-blast principle has been retained these sprayers still lose a significant proportion of spray. ULV sprayers have enabled significant reductions in chemical use, however with the spray still carried by a large volume of high speed air as much as 80% misses the target (Lovelidge, 1993).

One of the first machines to produce slow moving air was trialed in the UK in 1992 (Lovelidge, 1993). Air is directed along six 15 cm diameter flexible plastic ducts (three each side), each fitted with a Micron nozzle at the outlet. To ensure maximum spray coverage, the position and direction of the outlets are adjustable. This placement sprayer wasted less spray than standard ULV machines.

The Hydra sprayer, developed in Australia (Furness, 1997), produces a large volume of highly turbulent air at relatively low velocity by axial rotation generated by the fan combined with airstream convergence from adjacent heads. Hollow cone nozzles are located behind the fan and the spray is ducted through the fan. As the spray cloud is directed through the fan it is subjected to high airshear forces near the surface of the fan blades, producing a secondary atomisation resulting in fine 100 μm droplets. Spray coverage is even with a dense spray deposit on 80–100% of upper leaf surfaces and on 70–95% of lower leaf surfaces on both outer and inner canopy. This system is now widely used in the grape industry in Australia and has reduced spraying costs, improved fruit quality and improved pest and disease control.

7. CONCLUSIONS

There has been phenomenal progress in spray technology over the last 30 years. An increased awareness of the issues relating to spray efficiency has led researchers to re-examine factors such as air velocity and pattern, nozzle placement in relation to the crop, and methods of reducing chemical dosage rates. Advances have been made in understanding many of the factors involved in spray efficiency, such as droplet size, coverage, penetration and retention, and machine design.

The increasing prominence of environmental issues combined with the spread of urban areas has forced orchardists to alter their practices in relation to spray application. With the move away from complete coverage of the trees with high volume spraying towards low or ultra-low volume technology, there has been a reduction in drift and runoff, reducing the amount of pollution and wastage. While this has benefited both orchardist and the public, truly efficient spray application systems cannot be developed until there is a full understanding of the interactions of all the components affecting the efficiency of sprays applied to tree crops,

Educating orchardists to apply the TRV system as outlined by Manktelow and Praat (1997b) or the UCR concept described by Furness et al. (1998) will assist

in reducing variability of spray application results. Canopy size calibration adjusts the chemical dose more accurately, so that variability on the target is reduced. This will add to the savings already achieved in both time and wastage, further reducing the cost of spraying.

8. FUTURE DIRECTIONS

Orchardists are becoming increasingly aware of their responsibilities in relation to chemical usage in ensuring that sprays are not causing unacceptable effects on people's health or the environment. The use of low volume spraying with appropriately timed applications of specific chemicals combined with the adoption of integrated fruit production (IFP), and increasing trends towards organic growing are all contributing towards reduced pesticide use. However, there is still considerable room for improvement.

Inadequate labelling of pesticides, especially for concentrate spraying, makes the accurate manipulation of chemical dose difficult. Within the next 10 years, there will hopefully be a change in pesticide labels, with the label rate/ha being replaced with a label rate/UCR. This simple change will remove many inconsistencies in labelling and reduce the dose/ha for most spray operations.

A broad based approach to spray technology research is needed to further improve performance of sprayer technology and reduce the pesticide burden on the environment. Only by encouraging interaction between engineers, plant physiologists, entomologists, plant pathologists and formulation chemists can all the components involved in spraying efficiency be brought together. In this way it may be possible to achieve efficiencies of 90–100% in spraying.

In the interim, the use of systems such as the UCR system combined with improved targeting of sprays through appropriate sprayer selection and calibration, time of application and an understanding of the impact of weather conditions will assist in optimising spray performance and reducing wastage.

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CHESTNUT, AN ANCIENT CROP WITH FUTURE

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1. INTRODUCTION

Chestnut is a multipurpose tree valued for its nut, timber, tannins, as well as forestry landscape. It is distributed mainly in the North Hemisphere (Figure 1), mostly in China, Korea and Japan in the Asia, in Southern-Europe from Turkey to Atlantic Islands and in the United States. It was only recently introduced into Chile, Argentina, Australia and New Zealand.

Chestnut season is traditionally consumed in the autumn and is considered as a fruit announcing the winter arrival. Nuts are usually roasted directly in the fire and consumed with wine as a celebration of the harvest. During Christmas, it is also traditionally used as filling accompaniment for the roast meat. It is also transformed into a luxury product in 'marron glace', an expensive form of candy. Timber, often coming from grafted orchards is a much-loved hardwood, appreciated for its durability and the nice patina in furnitures. Chestnut was more important in Medieval Ages as the nuts could be conserved dried or as flour as a winter food source. At the same time, the tree also produced timber wood for tools,

Figure 1. World distribution of chestnut area.

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furnitures and fuel. The Romans spread chestnut with the grapevines for production of wooden barrels for the preservation and transportation of wine (Bruneton, 1984). In Medieval Ages big plantations for forestry and orchards with dual purpose; fruit and timber were established (Bourgeois, 1992). Some of these plantations can still be found in Toscany (Italy), Galicia (Spain), Tras-Os-Montes (Portugal), Aveyron and Cévennes (France). Monastries are the owners of vineyards and had been the contributors to the establishment of most of these chestnut orchards.

At the end of the 19th Century, chestnut was threatened by ink disease (*Phytophthora* spp.) that encouraged the introduction of Asiatic species and their hybridisation with European species to incorporate resistance. Hybridisation was considered essential in maintaining the positive characteristics of the European species. At the same time Blight (*Chryphonectria parasitica*) was imported in Europe with the introduction of the Asiatic species and devastate many of the orchards. Bruneton (1984) estimated a reduction of 450,000 ha in 1841 to 32,000 ha in 1975 in France, as a result of ink disease as well as competition from more productive woody species as *Pinus*.

The beginning of the 21st Century a number of important studies on chestnut has haulted this decline and revitalised advances in this crop. With a greater understanding and knowledge of the genetics of chestnut and its diseases and considerable improve in propagation methodology; the future of this crop is emerging.

1.1. Origins of chestnut

Vavilov (1951) established as the Centre of Origin of *Castanea sativa* in the Caucasus, Asia Minor (Fourth Origin Centre; Vavilov, 1992). Pitte (1985, 1986) considers that chestnut could be indigenous in the areas where it is presently found, but like other tree crops it was spread from Near Orient toward the Atlantic, first by the Greeks, then later by the Romans. Based on a genetic studies over natural populations of Italy and Turkey, Villani et al. (1991b) established the following hypothesis: (1) Initial spread from the post-glacial refuge in Eastern Turkey to the Western regions was characterised by a low genes flow (40,000 to 1500 years BC.). (2) Diffusion by men from West Turkey, Anatolia and Greece (1500 to 200 years BC.); (3) A second expansion by men toward Italy and Mediterranean area during first and second century.

Camus (1929) first indicated that chestnut could not be spontaneous in France but could be in Spain. Bonnefoi (1984) explained the higher variability found in the Eastern populations of France as due to the influence of the populations of the East Europe or a result of genetic differentiation produced by the different climatic conditions between Eastern and Western France from a common population. More recently, Frascaria and Lefranc (1992) confirmed that the differentiation between Western and Eastern populations in France was due to the commercial flows of nuts in ancient times: Western populations could be influenced by the Iberian Peninsula and Eastern by Italy.

2. WORLD AREA OF CHESTNUT AND PRODUCTION

2.1. Areas of chestnut in the world

There are three main areas of chestnut growing in the world (Table 1): (1) Asia being the most important, mainly in China, where *C. mollissima* is found in the wild as well as in cultivation. (2) Europe and Turkey is the second main area where *C. sativa* is predominant. (3) In North-America *C. dentata* was widespread naturally but nowadays it is being substituted by hybrids with resistance to blight.

According with the FAO (Tables 1 and 2), 289,000 ha of orchards in the world produce 1,800,000 t of chestnuts. China being the most important country in chestnut production with 110,000 ha, but some author (Liu and Zhou, 1999) estimated 670,000 ha, seven times the FAO figure. In France only 5000 ha (Table 1) out of one million ha is cultivated (Morandini, 1958; Bourgeois, 1992; Breisch, 1995). In Spain, chestnut occupies 130,000 ha, more than 46,000 ha is cultivated, but only part of the production (18,000 t) comes to the market (Pereira-Lorenzo and Fernandez-Lopez, 2001).

Recent chestnut areas are localised in South-America, New Zealand and Australia. In Chile, seedlings were introduced from Europe after 15th century (Grau and France, 1999).

In New Zealand chestnut crop is developing rapidly and is mainly based on *C. crenata* × *C. sativa hybrids* (Klinac et al., 1999). In Australia, orchards are also expanding rapidly but mainly based on the introduction of *C. sativa* (Ridley, 1999). In United States, new orchards are being established in California using seedlings of Colossal (*C. crenata*) and present estimated area is 100 ha.

Chestnut production in Asia is twice that of Europe (this includes Turkey as an European producer) (Table 2). The main species to produce nuts being *C. mollissima* followed by *C. sativa*. Turkey and Italy are the main producers of European chestnut, over 50,000 t each, followed by Portugal, France, Spain and Greece.

2.2. Ecology

Chestnut is widespread in Europe, from Turkey to Portugal and Spain, the Azores archipelago being the most Occidental point for *C. sativa*.

It is found at sea level in some areas of Northern Spain upto 1500 m in Granada in Southern Spain, where the latitude could compensate for altitude (Pereira-Lorenzo and Ramos-Cabrer, 2002). But the most frequent range for *C. sativa* in Spain is between 400 m and 1000 m sea level (Table 3). In Southern France, orchards are located lower than 500–600 m (Breisch, 1995).

In Spain, early leafing of hybrids (middle march) restricts its use due to spring frosts in altitudes over 500 m, whereas *C. sativa* cultivars (middle April) could be grown. Similarly, in the Mediterranean region, southerly-facing slopes should be avoided. As the earlier blooming and the frequent spring frosts as well as dry summer are all limiting factors. In Southern Galicia, south facing slopes were destined for

Table 1. Cultivated area of chestnut in the world (FAO, 2002, www.fao.org).

Cultivated area (ha)	Year											
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
China	44500	62000	65000	68000	70000	70000	72000	78000	88000	105000	110000	110000
Japan	37600	36700	35500	34400	33300	30100	29200	28300	27500	26900	26000	26000
North-Korea	5000	5000	5000	5000	5000	5000	5000	5000	5600	5600	5600	5600
South-Korea	36000	36000	39000	30000	37000	37000	42000	50000	45000	37000	37000	37000
Asia	**123100**	**139700**	**144500**	**137400**	**145300**	**142100**	**148200**	**161300**	**166100**	**174500**	**178600**	**178600**
Slovenia			127	125	125	125	125	125	125	125	125	125
Spain	7000	7000	7000	7000	7000	7000	7000	7000	7000	7000	7000	7000
France	12400	7800	7000	7300	7600	7300	7000	4350	4430	4786	5352	5300
Greece	8800	7500	7400	7700	7800	7800	7800	7700	7800	7800	7800	7800
Hungary	380	380	380	380	380	380	380	380	380	380	400	400
Italy	19000	23500	23500	23500	23500	23500	23500	23500	23500	23500	23500	23500
Portugal	15200	15900	16900	18000	18700	19131	19521	19724	19996	28825	28940	25000
Rumania	0	0	0	100	100	100	100	100	100	100	100	100
Turkey	38140	39160	39080	39200	39380	39740	39240	40300	38000	40000	40000	40000
Ex-Yugoslavia	1300	1300	600	600	600	600	600	600	600	600	600	600
Europe	**102.220**	**102.540**	**101.860**	**103.780**	**105.060**	**105.551**	**105.141**	**103.654**	**101.806**	**112.991**	**113.692**	**109.700**
World	**225320**	**242240**	**246487**	**241305**	**250485**	**247776**	**253466**	**265079**	**268031**	**287616**	**292417**	**288425**

Table 2. Chestnut production in the world (FAO, 2002, www.fao.org).

Chestnuts (t)	Year											Average
	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	
China	257000	285000	286000	315000	300000	285000	375000	450000	534631	598185	615000	390983
North Korea	8200	8000	6500	7000	6.500	7.500	7.500	8.500	8.400	8.500	8.500	7.736
South Korea	89.747	101.742	80.994	100.163	93.655	108.346	129.673	109.956	95.768	92.844	90.000	99.353
Japan	32.400	33.600	27.100	32.900	34.400	30.100	32.900	26.200	30.000	27.000	27.000	30.327
Asia	**387.347**	**428.342**	**400.594**	**455.063**	**434.555**	**430.946**	**545.073**	**594.656**	**668.799**	**726.529**	**740.500**	**528.400**
Bulgaria	15	30	50	100	100	350	250	250	300	300	300	186
Slovenia		464	550	500	500	500	500	500	500	500	500	456
Spain	18942	21698	23847	19441	10075	15000	10000	10000	10000	10000	10000	14455
France	12394	14000	13136	12896	11016	10798	9592	11411	12563	13224	13000	12185
Greece	12968	11416	10600	11500	12053	12536	13548	12820	12000	12000	12000	12131
Hungary	1094	756	1130	900	1100	1040	991	973	1029	1015	1100	1012
Italy	66579	69089	67722	69852	71971	68653	72782	78425	52158	70000	50000	67021
Portugal	15713	15102	12825	18682	19194	20423	20643	22022	30811	33359	27000	21434
Romania	0	0	850	900	1000	1000	900	800	900	1000	1000	759
Switzerland	200	200	200	200	0	0	0	0	200	200	200	127
Turkey	81000	85000	80000	76000	77000	65000	61000	55000	60000	60000	60000	69091
Ex-Yugoslavia	2800	1536	1600	1500	1400	1400	1400	1400	1400	1400	1400	1567
Europe	**211705**	**219291**	**212510**	**212471**	**205409**	**196700**	**191606**	**193601**	**181861**	**202998**	**176500**	**200423**
World	**1198104**	**1295266**	**1226208**	**1335068**	**1279928**	**1255292**	**1473358**	**1576514**	**1701320**	**1859054**	**1834000**	**1457647**

Table 3. Number of sampled trees and chestnut denominations in Spain and some characteristics of chestnut producing area in Spain (Pereira-Lorenzo et al., 2001c).

Region	No. Sampled trees	No. Denominations	No. of differents denomination	Range of altitude (m)	Average altitude (m)	Harvesting period	Average date for harvesting
Andalucía	19	13	10	470–850	643	19 Oct–5 Nov (Huelva) 15 Sep–29 Oct (Málaga)	25 Oct (Huelva) 1 Oct (Málaga)
Asturias	301	66	59	10–900	383	3 Oct–23 Nov	27 Oct
Canary Islands (Tenerife)	35	18	16	800–1100	894	9 Oct–11 Nov	1 Nov
Canary Islands (La Palma)	25	14	11	400–1150	628	20 Oct–10 Nov	1 Nov
Canary Islands (El Hierro)	3	3	3	400–1100	850		
Castilla-León	25	8	2	440–950	770	13 Oct–5 Nov	17 Oct
Extremadura	19	4	2	800–1300	1039	29 Oct	29 Oct
Galicia	378	86	86	140–1300	1039	11 Oct–11 Nov	25 Oct.
Total	805	212	189	10–1300			

vineyards and north facing to chestnut orchards. Early-frost in autumn can also reduce the harvest in late bearing cultivars frozen the nuts in the bur after 1st November.

Minimum rainfall for chestnut is 800 mm, so in Spain this is ideal in rainy areas like Galicia (North-western Spain), which has 2000 mm rainfall (Pereira-Lorenzo and Ramos-Cabrer, 2002).

Main limitation for nut production is low temperatures, which enables the female flowers to be fecundated and to develop a normal kernel as was described by Breisch (1995).

Chestnut trees do not thrive in soil rich in active Calcium, basic pH and poor drainage. Therefore it is grown in sandy, poor soil to loamy soil on slopes. It is also found grown in volcanic islands like Sicily, Canary Islands, Madeira and Azores.

Gallardo-Lancho (2001) reported the ecological conditions of Spanish chestnut coppices to include fine earth fraction with means varies between 41% to 58%, sand between 26% and 60%, silt between 28% and 47%, and clay between 12 and 31%. The pH (H_2O) ranged from 4.6 to 5.2.

3. BIOLOGY

Genus *Castanea* ($2n = 24$) belongs to the Family *Fagaceae*, so as other timber production genera like *Fagus* and *Quercus* (Castroviejo et al., 1990). Genus *Castanea* includes 12 species (Dode, 1908 and Camus, 1929, cited by Fenaroli, 1945): *Castanea sativa* Miller, *C. crenata* Sieb. e Zucc., *C. mollissima* Blume, *C. seguinii* Dode, *C. davidii* Dode, *C. henryi* Rehder e Wilson, *C. dentata* Borkhausen, *C. pumila* Miller, *C. ashei* Sudworth, *C. floridana* Ashe, *C. alnifolia* Nuttal y *C. paucispina* Ashe.

Three of the most important crop species are *C. sativa* in Europe, and *C. mollissima* and *C. crenata* in Asia. *C. sativa* is also used for timber production, so is the American species *C. dentata*, a nearly extinct species threaten by blight during 20th century.

Interspecific hybrids were created from *C. sativa* with *C. crenata* or *C. mollissima* in Europe (Urquijo, 1957; Bruneton, 1984; Fernandez et al., 1995). Selected by their resistance to ink disease (*Phytophthora* spp.) and used in new plantations as rootstocks, for production of fruit, timber or both (Breisch, 1995; Pereira-Lorenzo and Fernandez-Lopez, 2001). In United States *C. dentata* was crossed by *C. crenata* and *C. mollissima* to introduce resistance to blight (*Chryphonectria parasitica*) (Anagnostakis, 1992).

3.1. Morphological description

Comparative description between main species can be found in Camus (1929) and revised by Bourgeois (1992) (Table 4). Height of the trees and morphology of the shoots, leaves and fruits are the most informative characteristics. The most vigorous species are *C. sativa* and *C. dentata*, and they are used for timber production. The angular shoots of European chestnut differentiate it from Asiatic ones, while the

Table 4. Main characteristics to distinguish between principal chestnut species (Camus, 1929; UPOV, 1988; Bourgeois, 1992).

Species	Maximum height	Shoots	Buds	Leaves	Fruit
C. sativa	35 m	Thick, angular, reddish, more or less hairy	Thick, ovoid, hairy, phyllotaxy 2/5	Big (10 to 20 cm × 5 to 8 cm), strongly dentate, dark green	Burs in the end of the shoots
C. crenata	20 m	Thin, round, brownish-red and brightly	Small, ovoid, pointed, no hairy, phyllotaxy 1/5	Smaller and narrower (9 to 15 cm × 3 to 3.5 cm), mucronate	Burs in the medium part of the shoot. Broad hilum, 1/4 of the fruit. Bearing at the third year.
C. mollisima	15 m	Thin, brown or grey, slightly hairy	Small, near ovoid, hairy, phyllotaxy 1/5	Long, oval (15 to 20 cm × 5 to 7 cm), slightly dentate	Small nuts, thin and long style
C. dentata	35 m	Thin, slightly angular, greenish-yellow, yellowish-red, no hairy	Small, near ovoid, punted, no hairy	Big, narrow (15 to 20 cm × 4.5 to 5.5 cm)	

mucronate leaf of the Japanese type can distinguish from the Chinese and European species. Interspecific hybrids show intermediate characteristic, which make identification difficult.

Largest nuts come from *C. sativa*, variable sizes in *C. crenata*, medium in *C. mollissima* and small in *C. dentata* (Vossen, 2000). Best flavour is found in the American, Chinese and European chestnuts.

In a study of *C. sativa* cultivars of Northwestern Spain, Pereira-Lorenzo et al. (1996c) the variability of the main morphological parameters defined by the UPOV (1988) were used to distinguish chestnut cultivars (Table 5).

Male catkins varied in length between 4.5 and 42 cm, the average being 18 cm (Table 5, Figure 2). In Spanish cultivars, the most frequent type is longistaminate (44.9%), followed by mesostaminate (35.3%), brachystaminate (12.2%) and astaminate (7.6%) (Pereira-Lorenzo, unpublished).

Male-sterility has been suggested to be related to heavy fruit bearers (Breviglieri, 1955; Miller et al., 1996), but this is not always the case, since some of the best Spanish cultivars are longistaminate such as Pilonga, Temprana and Planta Alajar in Andalucía (Pereira-Lorenzo and Ramos-Cabrer, 2002).

Leaves length varies between 6.2 to 26.8 cm (15.0 cm on average) and the

width ranged from 2.0 to 11.3 cm (4.5 cm on average) (Table 5). The widest point in the leaf is localised at 7.6 cm from the base. Petiole length is 1.7 cm on average, with 0.5 cm the minimum and 3.8 the maximum. Leaves present 17 secondary veins forming a 47° angle with the main vein, but only 15 finished in incisions of margin.

Burs bear two chestnuts on average and the third in the middle usually does not develop, though occassional burs present up to six normal chestnuts. A normal nut by bur was 66% on average (Table 5). Each bearing shoot presents two burs, but occasionally has up to eight. Spines length is 1.23 cm on average, but is highly variable (Pereira-Lorenzo et al., 1996a and c) (Figure 3): (1) few cultivars (14%) showed long spines of 1.56 cm on average. (2) Most of the cultivars (68%) showed medium size of 1.23 cm on average. (3) 19% of Galician cultivars presented short spines of 0.95 cm on average. Most of the burs appeared at the end of the bearing shoots (77% of the samples).

Lateral and central nuts in the bur are significantly different in most of the parameters (Pereira-Lorenzo et al., 1996c). Lateral nuts are longer, broader and thicker than central ones. Average style length was 0.9 and 0.8 cm, respectively.

Variation in weight has been taken in account in selection of cultivars. Smaller nuts can be considered under 10 g, medium between 10 and 15 g and large one over 15 g (Figure 4).

In Galicia (North-western Spain), the mean weight of the central nuts was 10.1 g, and 11.1 g for lateral ones, with a kernel percentage of 76 and 77%, respectively.

Average number of nuts per kg for Galician cultivars (North-western Spain) was 106 (Table 5) *versus* 73 in Andalucía (South Spain) (Pereira-Lorenzo and Ramos-Cabrer, 2002), though sizes of the nuts do not appear to be related with latitude, since in both areas cultivars producing big nuts are cultivated. However, in the South they have mainly propagated cultivars with larger nuts. In Galicia, less than 30% of the nuts are over 32 mm, though some cultivars produce more than 50% of nuts over 36 mm. In the Canary Islands, some cultivars such as Arafero produce 70 nuts per kg while De Sala, Del Haya, or Donosa produce more than 100 nuts per kg (Pereira et al., 2001b).

Normal nuts can vary from 1.2 cm to 4.3 cm in length, 1.0 cm to 4.7 cm in width and 0.7 to 3.9 cm in thickness. The mean values for both lateral and central nuts being 2.8–2.9, 3.2–3.3 and 1.8–2.0 cm, respectively (Table 5). Hilum size can vary between 0.3 × 0.7 cm to 1.3 × 2.0 cm in central nuts and 0.3 × 0.9 cm to 3.0 × 4.1 cm in lateral ones.

Five types of shapes are defined in chestnut, according to the relationship between the width and the length of the nuts (WL = Width/Length*100) in (1) triangular (WL < 100), (2) round (WL = 100), (3) elliptical-triangular (100 ≥ WL < 110), (4) elliptical short (110 ≥ WL < 120), and (5) elliptical broad (120 ≤ WL) (Casabianca and Vincensini, 1981) (Figure 5). These shapes have been used in classification of chesnut cultivars in France and Spain (Casabianca and Vicensini, 1981; Pereira-Lorenzo et al., 1996a).

Penetrations of the inner coat in cotyledons can be upto the depth of 2.7 cm. Such penetrations make it difficult for the nut peeling. European nuts are said to be easiest

Table 5. Variability encountered in the different studied characters of chestnuts, male catkin, leaf and bur in cultivars of North-western Spain (Pereira-Lorenzo et al., 1996c).

Character	No.	Minimum	Maximum	Average	SD	CV
1) LON	5429	4.5	42.0	18.06	4.8	26.39
2) LOL	5534	6.2	26.8	14.5	3.0	20.80
3) ANL	5534	2.0	11.3	4.5	1.0	23.00
4) LPE	5533	0.5	3.8	1.7	0.5	29.85
5) DIS	5533	1.8	15.8	7.6	1.8	24.10
6) NDN	5532	8.0	29.0	17.3	2.9	17.04
7) NIN	5533	8.0	32.0	17.3	3.0	17.21
8) DDL	5533	1.0	26.0	14.6	3.5	23.90
9) DIL	5533	1.0	28.0	14.6	3.5	24.04
10) ADL	5533	19.0	71.0	46.9	7.8	16.67
11) AIL	5533	20.0	77.0	46.9	7.6	16.25
12) ESP	1629	0.2	2.4	1.2	0.3	26.21
13) ERR	458	1.0	8.0	2.3	1.2	52.22
14) NOR	2848	0.0	6.0	2.0	0.9	42.82
15) SEC	2848	0.0	6.0	1.1	0.9	85.58
16) TOT	2848	2.0	8.0	3.1	0.7	21.29
17) CAL	852	51.2	342.6	105.7	37.8	35.73
18) P24	852	0.0	100.0	17.4	25.5	146.80
19) P28	852	0.0	100.0	56.0	27.0	48.17
20) P32	852	0.0	100.0	22.2	23.3	105.29
21) P36	852	0.0	54.8	2.6	6.3	243.14
22) P40	852	0.0	70.4	1.6	6.3	391.30
23) LCC	6390	1.2	4.3	2.8	0.3	12.17
24) LCL	6420	1.6	4.3	2.9	0.3	11.37
25) ACC	6390	1.1	4.7	3.2	0.4	12.75
26) ACL	6420	1.0	4.6	3.3	0.4	12.18
27) GCL	6420	0.8	3.9	2.0	0.3	15.56
28) GCC	6390	0.7	3.5	1.8	0.4	19.95
29) CCC	4563	0.7	3.9	2.0	0.42	20.97
30) CCL	4614	0.9	4.1	2.5	0.45	18.15
31) PEC	2781	0.1	2.3	0.5	0.22	44.00
32) PTL	4562	1.2	26.2	11.1	3.59	32.40
33) PTC	4517	1.3	26.1	10.1	3.49	34.50
34) PPC	1177	27.5	87.5	75.8	6.58	8.68
35) PPL	1177	23.1	90.4	77.1	6.44	8.35
36) PIC	1188	0.4	3.4	1.3	0.43	32.03
37) PIL	1177	0.2	4.9	1.3	0.49	35.83
38) PFC	1192	8.0	53.0	23.9	7.14	30.02
39) PFL	1182	9.0	56.0	25.0	7.75	31.04
40) PLC	1192	13.0	151.0	66.4	23.26	35.02
41) PLL	1182	14.0	163.0	66.5	25.01	37.59
42) POC	1192	32.0	195.0	90.2	25.67	28.46
43) POL	1182	27.0	197.0	91.5	27.37	29.91
44) APC	1043	0.04	2.0	0.9	0.30	34.99
45) APL	1066	0.05	2.0	0.8	0.29	35.07
46) DEN	3323	0.6	1.7	1.0	5.87	5.64
47) PTB	798	0.0	47.5	2.1	5.56	259.89
48) PHE	798	0.0	100.0	22.1	23.22	105.08
49) PPN	798	0.0	100.0	33.5	32.49	97.04

Table 5. Continued.

Character	No.	Minimum	Maximum	Average	SD	CV
50) PRC	798	0.0	94.5	5.4	10.39	191.40
51) PBL	798	0.0	100.0	14.3	19.73	138.01
52) PLS	798	0.0	100.0	16.9	20.52	121.02
53) PS1	798	0.0	60.0	2.1	4.92	231.78
54) PS2	798	0.0	82.5	5.6	11.74	209.02
55) PPM	798	0.0	81.8	8.5	12.54	147.18
56) PPI	798	0.0	25.0	0.3	1.81	576.91

1) LON, Catkin length (cm); 2) LOL, Leaf blade length (cm); 3) ANL, leaf blade width (cm); 4) LPE, Petiole length (cm); 5) DIS, Distance from base to the widest part of the leaf blade (cm); 6) NDN, Number of veins on the right side of the leaf blade; 7) NIN, Idem in the left side; 8) DDL, Number of incisions on the right side of the leaf blade; 9) DIL, Idem left side; 10) ADL, Angle between the main veins and secondary ones (degrees); 11) AIL; Idem left side; 12) ESP; Length of the spines bur (cm); 13) ERR, Number of burs by shoot; 14) NOR, Number of normal nuts by bur; 15) SEC, Number of empty nuts by bur; 16) TOT, Number of total nuts by bur; 17) CAL, Number of nuts by kg; 18, 19, 20, 21, 22) P24, P28, P32, P36 and P40, Percentage of nuts with diameter <24 mm, 24 to 28 mm, 28 to 32 mm, 32 to 36 mm, and 36 to 40 mm; 23) LCC, Length of central nuts (cm); 24) LCL, Idem lateral nuts; 25) ACC, Width of central nuts (cm); 26) ACL, Idem lateral nuts; 27) GCL, Thickness of lateral nuts (cm); 28) GCC, Idem central nuts; 29) CCC, Width of the hilum in central nuts (cm); 30) CCL, Idem lateral nuts (cm); 31) PEC, Penetration of seed coat into embryo in central nuts (cm); 32) PTL, Total weight in lateral nuts (g); 33) PTC, Idem in central nuts (g); 34) PPC, PVC/PTC*100; 35) PPL, PVL/PTL*100; 36) PIC, Total weight in lateral nuts (g); 37) PIL, Idem in lateral nuts; 38) PFC, Seconds to peel central nuts; 39) PFL; Idem lateral nuts (s); 40) PLC, Seconds to remove the inner coat in central nuts; 41) PLL; Idem in lateral nuts (s); 42) POC, Seconds to peel completely central nuts (s); 43) POL; Idem lateral nuts (s); 44) APC, Length of the style in central nuts (cm); 45) APL, Idem lateral nuts, 46) DEN, Density (g/cm3); 47) PTB, Poly-embryonic nuts (%); 48) PHE, Nuts with a gag between cotyledons (%); 49) PPN, Nuts with penetrations of the inner coat in the cotyledons (%); 50) PRC, Nuts with pericarp split (%); 51) PBL, Nuts with *Balaninus elephans* (%); 52) PLS, Nuts with *Laspeyresia splendana* (%); 53), 54) PS1 and PS2, Nuts with *Rhacodiella castaneae* at first step and with black mould, respectively (%); 55) PPM, nuts with *Phoma endogena* (%); 56) PPI, nuts with *Penicillium* (%).

to peel by comparison with Asiatic species. Between cultivars this differences can be quite distinct. Some nuts only need 27–32 s to complete hand peel and upto 195–197 s for the most difficult ones. Average is 90–92 s per nut. The most difficult part of the nut to remove is the inner coat, 66 s on average versus 24–25 s of the pericarp.

A ‘marron’ type cultivar is defined by the low percentage of poly-embryonic nuts. Non ‘marron’ type cultivars produce a higher percentage (>12% of the nuts) of poly-embryonic nuts, which have an inner coat impossible to remove without breaking the nut. A study in Galicia showed that 6% of the samples from grafted trees produced poly-embryonic nuts, as most of the cultivars are ‘marron’ type (Table 5).

Variability of colours can be between yellow-reddish to dark brown, reddish being the most appreciated in the market. In Galicia, 50% of the samples are classified in group CL4 (Figure 6, Table 5), 22% in group CL2, 14% of group CL3, and less frequent groups CL6, CL7 and CL1. 49% of the samples were classified as

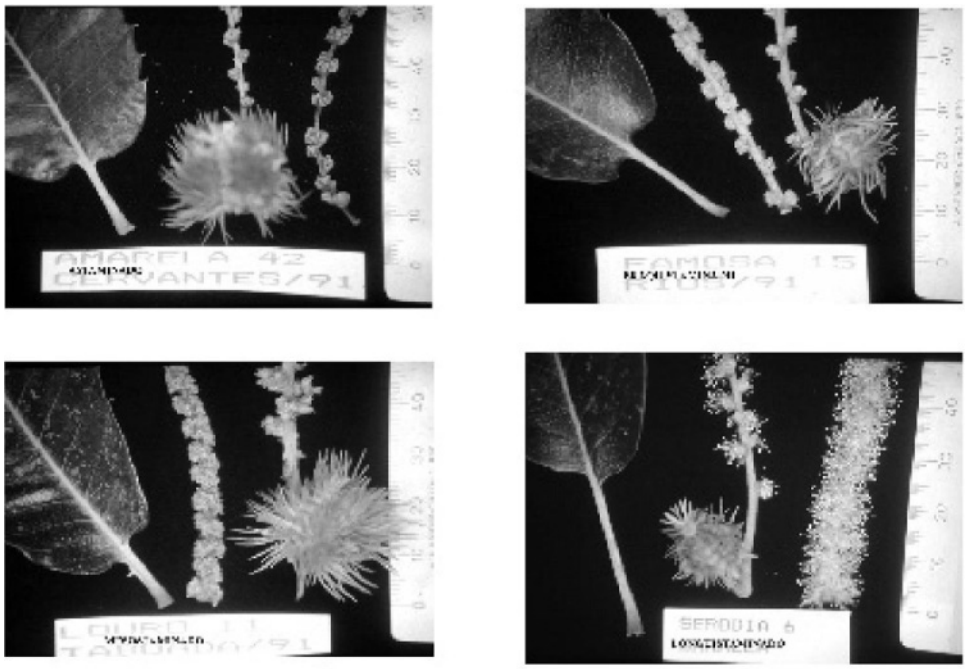

Figure 2. From left to right, up to down astaminate, brachystaminate, mesostaminate and longistaminate catkins.

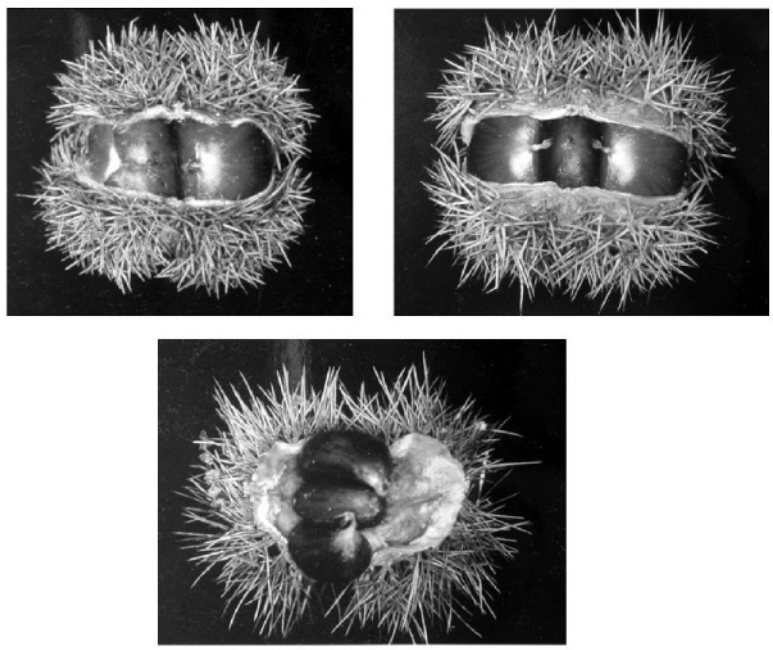

Figure 3. From left to right, up to down, short, medium and long spines of the bur.

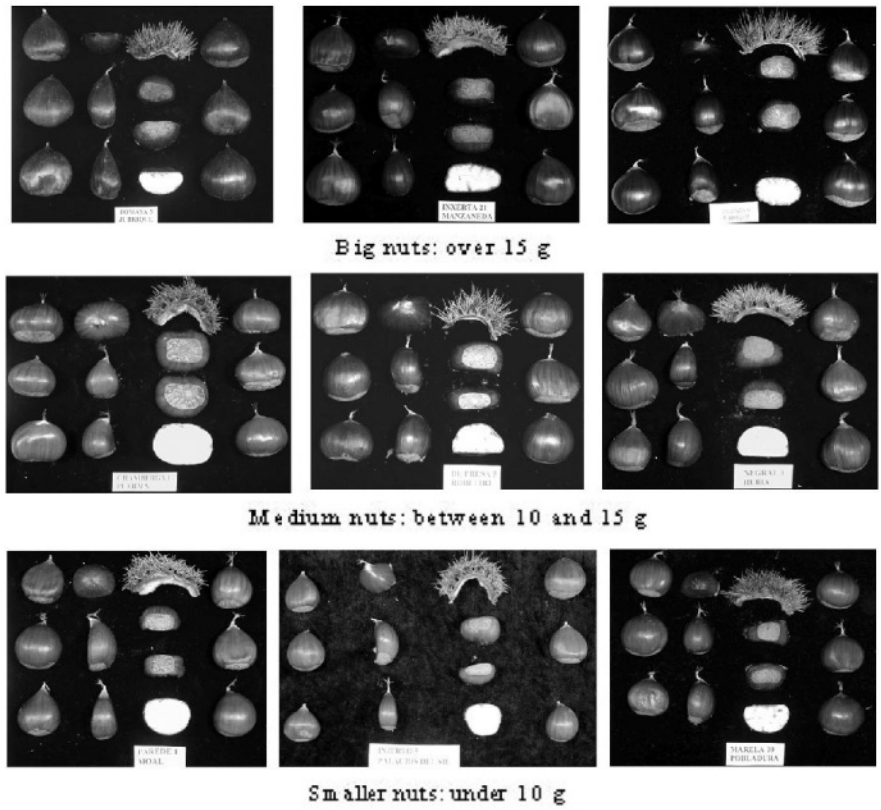

Figure 4. Nut weight variation in Spanish cultivars.

glossy or very glossy. Slight stripes were present in 72% of the samples and conspicuous hilum to pericarp in 88% of the samples. Hairiness near the style is present in only 39% of the samples.

Most samples in North-western Spain showed white cotyledons (71% of the samples), while cream colour was only found in 29%. Sweetness is the most frequent encountered (64% of the samples).

Two chestnut weevils (*Cydia splendana* Hubner and *Balaninus* [*Curculio*] *elephas* Gyll)) are the main insect pests producing losses after harvest. Black mould (*Rhacodiella castaneae* Peyr.) and brown mould (*Phoma endogena* Speg.) are the two diseases causing moulds that damage the nuts during conservation and marketing (Breisch, 1995). In Galicia, *Balaninus* is present in 14% of the nuts and *Laspeyresia* in 17% at harvest (Pereira-Lorenzo et al., 1996a). 9% of the nuts were infected with *Phoma, Racodiella* 8% and *Penicillium* 2%.

Chestnut has been classically described as monoecious with flowers borne on long spikes (catkins) that arise from the leaf axis of current season's growth (Miller et al., 1996). There are generally two types of catkins on the tree, the astaminate or unisexual catkins, and the bisexual catkins. Male flowers only present stamens but female flowers in bisexual catkins have stamen remants in the base of the

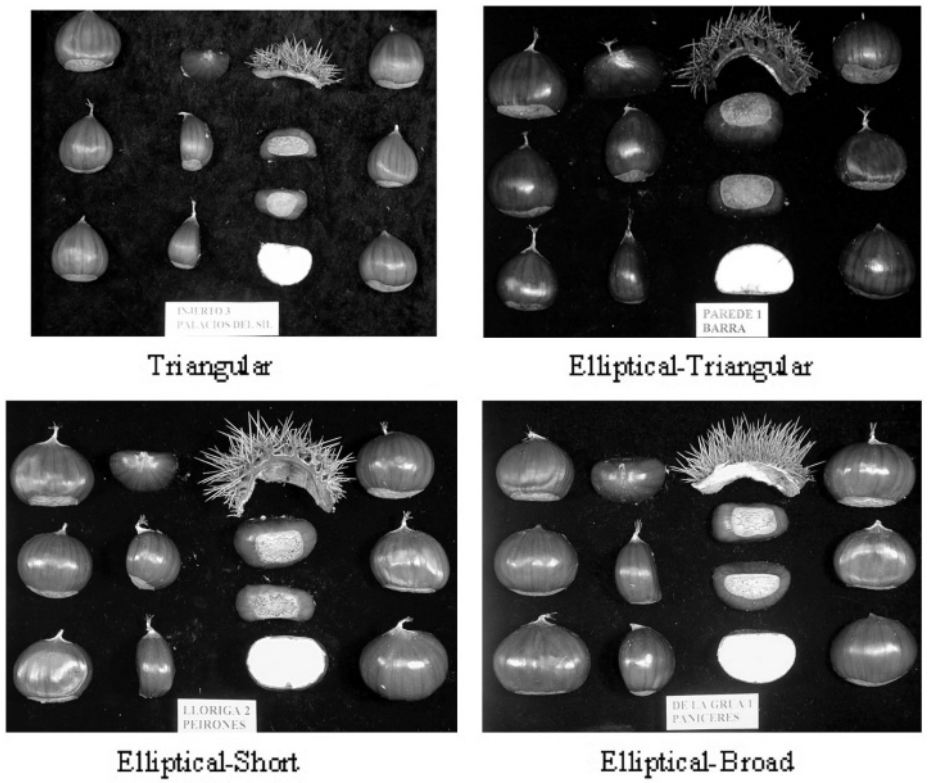

Figure 5. Shape variation in chestnut cultivars.

style (Botta et al., 1995; Valdiviesso, 1999) and some of them can produce fertile pollen. This should be taken into account in controlled crosses.

3.2. Floral biology

Cross-pollination is essential for normal production (Breisch, 1995). In the old orchard, a combination of 2 upto 20 cultivars is practised to ensure pollination. In some orchards, cross-pollinations are achieved from wild trees surrounding the grafted trees. In new orchards, longistaminate cultivars are commonly chosen as pollinators. Some of them produce large nuts.

Chestnuts pollen is transported by the wind in Mediterranean areas, but the contribution from insect pollinators becomes more important as humidity in the environment increases (Breisch, 1995).

Liu and Zhou (1999) have related the abundance of male flowering with the low productivity of Chinese cultivars. This could also be linked to the association made by Breviglieri (1955) between male sterility (astaminate and brachystaminate) and 'marroni' type cultivars. Although it is logical to think that an astaminate or brachystaminate cultivar, which does not produce any pollen, reserved more energy to produce a bigger nut. Astaminate cultivars are less frequent in Spain. A

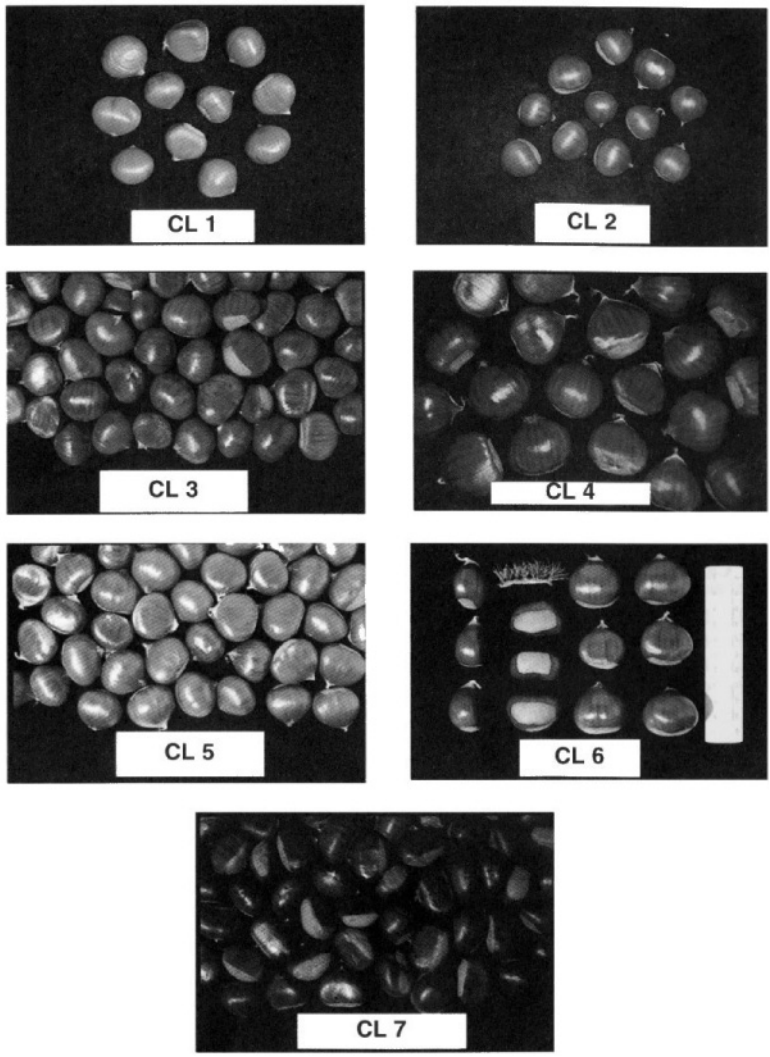

Figure 6. Variation in nut colours found in North-western Spain: CL1, Raigona 3 of Carballeda; CL2, Parede of Fonsagrada; CL3, Raigona 4 of Carballeda; CL4, Amarelante 11 of Manzaneda; CL5, Abarcá 12 of Parada; CL6, Negral 5 of Rubiá; CL7, Verdeá 11 of Folgoso (Pereira-Lorenzo et al., 1996c).

total of 712 accessions corresponding to 159 cultivars studied in Spain showed that only 8% were astaminate, 12% brachystaminate, 35% mesostaminate and 45 longistaminate. Some longistaminate cultivars produce the largest nuts as Pilonga and Temprana from Andalucía. In France, amongst the most important cultivars of *C. sativa* (Breisch, 1995), male sterile cultivars are predominant with 37% of astaminate, 26% of brachystaminate, and 5% of mesostaminate, and 32% of longistaminate cultivars are recommended.

This means there is no close correlation between male sterility and quality of the fruit but perhaps between male sterility and productivity as suggested by Liu and Zhou (1999).

Meiosis in pollen mother cells occurs in the first week of June in Italian cultivars, 10 to 15 days before anthesis (Botta et al., 1995). Pollen viability varied from 81.3 ± 6.1% based on fluorochromatic reaction to 58.2 ± 7.0% on hanging drops and 50.1 ± 4.5% germination on agar media. Pistillate flowers have six to eight styles whose tips present a hollow at full bloom. The ovary presents seven (rarely six or eight) carpels. Each flower has 10 to 16 anatropous ovules. Bearing mono-embryonic seeds (marron type) has been related to the high occurrence of anomalies, such as delayed of embryo sac differentiation and the presence of supernumerary nuclei in the embryo sac (Botta et al., 1995).

3.3. Phenology

Chestnut is a deciduous tree as corresponding to the temperate area where it is cultivated. *Castanea* species are generally cold hardy. *Castanea dentata* and *C. mollissima* have been reported to survive down to –30 °C (Andersen, 1994).

After chilling requirement, bud break begins at the end of March for Asiatic cultivars, and interspecific hybrids (Table 6), being the most precocious, followed by the European cultivars. Interspecific hybrids maintain their precocity contribution origin from the Asiatic. However, some hybrid cultivars of *C. sativa* are as precocious as *C. crenata* (Table 6) such as Garone Negro. New shoots are very sensitive to spring frost, and this limits the use of Asiatic cultivars to orchards located at altitudes lower than 500 m. However, European cultivars can avoid spring frost because of their late bud break.

Flowering time is highly variable with the local climatic condition (Breisch, 1995). Asiatic cultivars and hybrids produce the male flowering between the end of May and the end June, while European cultivars are between mid-June to mid-July. Table 7 show that the time of flowering for Euro-Asiatic hybrids is more precocious than European cultivars.

Precocity is also maintained at harvest time, on the 1st September for *C. crenata* and *C. mollissima*. Most of the cultivars of *C. sativa* produces in October and 1st November, but some cultivars are as precocious as Asiatic species such as Temprana and Pilonga which when cultivated in South Spain (Pereira-Lorenzo and Ramos-Cabrer, 2002).

4. CULTIVARS

4.1. Origin of the cultivars

As Vavilov (1951) described; growers realised a first domestication selecting the best nut from wild populations. With the knowledge of vegetative propagation as grafting, a second domestication began which resulted in fixing the best genotypes.

In chestnut, grafting it is an old technique used for propagating cultivars. According to the high variability found in the old orchards (Pereira-Lorenzo et al., 1996a and b), growers could have propagated multiple local selections from wild populations established previously in the same area (Figure 7). This process is dynamic and it still continues in some countries such as Slovenia; with the aim to improve the quality of the nut production by selecting the most interesting genotypes and propagating them by grafting (Solar et al., 2001).

Most of the cultivars are polyclonal, but some of them have been broadly spread (Pereira-Lorenzo et al., 1996 a and b) such as 'Parede' in Northern Spain (Pereira-Lorenzo et al., 2001a). First study reporting intra-cultivar variability was in the important cultivar Marrone Fiorentino (Borghetti et al., 1983), finding important differences between localities as it was suggested by Morettini and Saccardi (1951) previously.

Morettini and Saccardi (1951) explained this by the fact that the number of cultivars in orchards is high due to its frequency that growers cultivated such seedlings. Some of these seedlings, which produce good nuts, were then used as mother trees for grafting. The best of them are propagated into new localities. As a consequence, the number of local cultivars is often detectably high.

The main characters to be fixed are those related with nut quality. One of the most important could be mono-embryonic nut *versus* poly-embryonic. Mono-embryonic nuts were named 'marroni' type in Italy in Medieval Ages. It could be related with the transformation of this kind of nuts without inner peel in 'marron glacé'. Cultivars producing poly-embryonic nuts are named chestnut type. In Europe, most of the cultivars are 'marron' type (Breisch, 1995; Paglietta and Bounous, 1979; Pereira-Lorenzo and Fernandez-Lopez, 1997a). Nut size, ease of peeling, sweetness, and colour were other characteristics appreciated by growers and consumers.

In China, selection from seedling orchards by grafting the best accessions is a new program developed recently (Liu and Zhou, 1999), and forty excellent cultivars have been introduced in the germplasm bank of the Institute of Botany of Nanjing.

It is reckon that the botanist Pier Antonio Micheli (*Enumeratio rariorum plantarum* (*Manoscr.*), cited by Breviglieri, 1955) was the first to distinguishing the chestnut cultivars based in a study of the bur, fruit, leaf and flower.

In France, Tricaud, Lamy of Lachapelle and, principally Lavialle (1911, cited by Breviglieri, 1955) described 60 French cultivars, using the samples showed in the international exhibition of chestnut celebrated in Limoges in 1910.

Piccioli (1922, cited by Breviglieri, 1955) described nearly 300 Italian cultivars, classified by regions and harvesting time, and Remondino (1926, cited by Breviglieri, 1955) referenced near 1000 denominations, many of them synonymous.

Breviglieri (1955) defined the 'Squeda Castanografica sul Castagno', and established the base work for further studies. He was the first in classifying chestnut cultivars in two main groups: 'marron' and chestnut types. Cultivars 'marron' types produce less than 70 nuts per kg, one or two nuts by bur, mono-embryonic nuts, pericarp of bright colour with stripes, ease in peeling and sweet. In 1977, Bagnaresi et al. described cultivars from Toscana and Emilia Romagna in Italy,

Table 6. Time of bud break and ripening time of chestnut cultivars.

Cultivar	Species	Origin	Time of bud break	Ripening time
Ginyose[1]	*C. crenata*	Japan		First Sept
Ishizuki[1]	*C. crenata*	Japan		First Sept
Tanzawa[1]	*C. crenata*	Japan		First Sept
Tsukuba[1]	*C. crenata*	Japan		First Sept
Bouche de Betizac	Bouche-Rouge × CA04 (*C. Crenata*)		??	15–30 Sept
Précoce Migoule[2]	*C. crenata* × *C. sativa*	France	26 Mar–15 Apr	15–25 Sep
Bournette[2]	*C. crenata* × *C. sativa*	France	26 Mar–15 Apr	20 Sept–5 Oct
Marigoule[2]	*C. crenata* × *C. sativa*	France	26 Mar–15 Apr	5–15 Oct
Maravall[3]	*C. crenata* × *C. sativa*	France	<26 Mar	1–10 Oct
Jiu jia zhong[4]	*C. mollissima*	China	9 Apr	17 Sept
Qin zha[4]	*C. mollissima*	China	9 Apr	20 Sept
Duan zha[4]	*C. mollissima*	China	9 Apr	24 Sept
Jiao zha[4]	*C. mollissima*	China	9 Apr	4 Oct
Jian ding you li[4]	*C. mollissima*	China	10 Apr	24 Sept
Chu shu hong[4]	*C. mollissima*	China	10 Apr	10 Sept
Colossal[5]	*C. sativa* × *C. crenata*	USA		15 Sep–1 Oct
Silverleaf[5]	*C. sativa*	USA		15 Sep–1 Oct
Belle Épine[2]	*C. sativa*	France	1–10 May	5–15 Oct
Bouche Rouge[2]	*C. sativa*	France	>16 May	15–31 Oct
Comballe[2]	*C. sativa*	France	11–15 May	5–15 Oct
Marron Comballe[2]	*C. sativa*	France	11–15 May	5–15 Oct
Sardonne[2]	*C. sativa*	France	11–15 May	25 Sep–15 Oct
Amarelante 1[6]	*C. sativa*	Spain	23 Apr–7 May	26 Oct–11 Nov
Famosaz[6]	*C. sativa*	Spain	21 Apr–7 May	26 Oct–11 Nov
Garrida[6]	*C. sativa*	Spain	15 Apr–7 May	26 Oct–11 Nov
Inxerta1[6]	*C. sativa*	Spain	10 Apr–30 Apr	26 Oct–11 Nov
Longalz[6]	*C. sativa*	Spain	14 Apr–7 May	26 Oct–11 Nov
Louraz[6]	*C. sativa*	Spain	10 Apr–30 Apr	26 Oct–11 Nov
Luguesaz[6]	*C. sativa*	Spain	21 Apr–30 May	26 Oct–11 Nov
Negral1[6]	*C. sativa*	Spain	27 Mar–30 Apr	10 Oct–10 Nov
Paredez[6]	*C. sativa*	Spain	10 Apr–14 May	26 Oct–11 Nov
Raigona2[6]	*C. sativa*	Spain	21 Apr–30 Apr	26 Oct–11 Nov
Rapadaz[6]	*C. sativa*	Spain	3 Apr–7 May	26 Oct–11 Nov
Venturaz[6]	*C. sativa*	Spain	17 Mar–7 May	26 Oct–11 Nov
Tempranaz[7]	*C. sativa*	Spain		15 Sep–4 Oct
Pilongaz[7]	*C. sativa*	Spain		17 Sep–4 Oct
Arafero[8]	*C. sativa*	Spain		31–Oct
De Sala[8]	*C. sativa*	Spain		11–31 Oct
Manso[8]	*C. sativa*	Spain		11–31 Nov
Castagna della Madonna[4]	*C. sativa*	Italy	1–15 May	15–25 Sep
Sarvaschina[4]	*C. sativa*	Italy	<28 Mar	25 Sep–5 Oct
Salenga[4]	*C. sativa*	Italy	25–30 Apr	25 Sep–5 Oct
Temporiva[4]	*C. sativa*	Italy	28–30 Apr	25 Sep–5 Oct
Bracalla[4]	*C. sativa*	Italy	26 Mar–15 Apr	5 Oct–15 Oct
Garrone Nero[4]	*C. sativa*	Italy	27 Mar–15 Apr	5 Oct–15 Oct
Pelosa Grossa[4]	*C. sativa*	Italy	28 Mar–15 Apr	5 Oct–15 Oct
Pelosa Piccola[4]	*C. sativa*	Italy	29 Mar–15 Apr	5 Oct–15 Oct
Garrone Rosso[4]	*C. sativa*	Italy	16–30 Apr	5 Oct–15 Oct
Gentile[4]	*C. sativa*	Italy	17–30 Apr	5 Oct–15 Oct

Table 6. Continued.

Cultivar	Species	Origin	Time of bud break	Ripening time
Gioviasca[4]	*C. sativa*	Italy	18–30 Apr	5 Oct–15 Oct
Marrone di Chiusa Pesio[4]	*C. sativa*	Italy	19–30 Apr	5 Oct–15 Oct
Marrone di Val Susa[4]	*C. sativa*	Italy	21–30 Apr	5 Oct–15 Oct
Marrubia[4]	*C. sativa*	Italy	23–30 Apr	5 Oct–15 Oct
Neirana[4]	*C. sativa*	Italy	24–30 Apr	5 Oct–15 Oct
Marrone di Luserna[4]	*C. sativa*	Italy	20–30 Apr	15 Oct–25 Oct
Marrone di Villar Pellice[4]	*C. sativa*	Italy	22–30 Apr	15 Oct–25 Oct
Siria[4]	*C. sativa*	Italy	26–30 Apr	15 Oct–25 Oct
Spinalunga[4]	*C. sativa*	Italy	27–30 Apr	15 Oct–25 Oct
Frattona[4]	*C. sativa*	Italy	2–15 May	15 Oct–25 Oct
Gabiana[4]	*C. sativa*	Italy	3–15 May	15 Oct–25 Oct
Rossastra[4]	*C. sativa*	Italy	4–15 May	15 Oct–25 Oct
Verdesa[4]	*C. sativa*	Italy	5–15 May	15 Oct–25 Oct
Amarelal[9]	*C. sativa*	Portugal	25 Apr–3 May	Sep–Oct
Longal5[9]	*C. sativa*	Portugal	4–17 May	Oct
Verdeal[9]	*C. sativa*	Portugal	4–10 May	Oct
Martainha2[9]	*C. sativa*	Portugal	25 Aprl–10 May	Oct

[1] Paglietta and Bounous, 1979; [2] Bergougnoux et al., 1978 and Breisch, 1995; [3] Bergougnoux et al., 1978, Chapa, 1987 and Breisch, 1995; [4] FAO (in press); [5] Vossen, 2000; [6] Pereira-Lorenzo and Fernandez-Lopez, 1997c; [7] Pereira-Lorenzo and Ramos-Cabrer, 2002; [8] Pereira-Lorenzo et al., 2001b.

Table 7. Blooming time of Euro-Asiatic hybrids and European cultivars.

Cultivar	Species	Origin	Male blossoming	Female blossoming
Bouche de Betizac[1]	Bouche-Rouge × CA04 (*C. Crenata*)	France	20 May–5 Jun	20 May–10 Jun
Précoce Migoule[1]	*C. crenata* × *C. sativa*	France	15–25 May	15–30 May
Marigoule[1]	*C. crenata* × *C. sativa*	France	10–25 May	15 May–5 Jun
Maravall[1]	*C. crenata* × *C. sativa*	France	15–30 May	15 May–5 Jun
Bournette[1]	*C. crenata* × *C. sativa*	France	25 May–10 Jun	20 May–5 Jun
Famosaz[2]	*C. sativa*	Spain	10–30 Jul	2–30 Jul
Longalz[2]	*C. sativa*	Spain	16–23 Jul	25 Jun–10 Jul
Amarelante 1[2]	*C. sativa*	Spain	25 Jun–9 Jul	25 Jun–16 Jul
Rapadaz[2]	*C. sativa*	Spain	19 Jun–16 Jul	5 Jun–31 Jul
Famosaz[2]	*C. sativa*	Spain	10–30 Jul	2–30 Jul
Inxerta[2]	*C. sativa*	Spain	12 Jun–16 Jul	5 Jun–24 Jul
Loura[2]	*C. sativa*	Spain	25 Jun–16 Jul	5 Jun–16 Jul
Luguesa[2]	*C. sativa*	Spain	25 Jun–10 Jul	10–31 Jul
Negral[2]	*C. sativa*	Spain	25 Jun–9 Jul	5 Jul–16 Jul
Parede[2]	*C. sativa*	Spain	18 Jun–9 Jul	5–26 Jun
Presaz[2]	*C. sativa*	Spain	5 Jun–17 Jul	5 Jun–17 Jul
Raigona[2]	*C. sativa*	Spain	19 Jun–30 Jul	14 Jun–30 Jul
Venturaz[2]	*C. sativa*	Spain	25 Jun–17 Jul	
Verde[2]	*C. sativa*	Spain	25 Jun–16 Jul	14 Jun–30 Jul

[1] Bassi and Pellegrino, 1991; Pereira-Lorenzo and Fernandez-Lopez, 1997c.

Figure 7. Grower re-grafting an old tree to change the cultivar without removing the tree. The same tree has been grafted at least four times.

including data of productivity. Paglietta and Bounous (1979) made a new classification of Italian cultivars in 'marron' or chestnut types, differentiating two types of marroni in Italy: Marrone Fiorentino or Casentinese and Marrone Piemontesi and synonymies.

Solignat et al. (1975) and Solignat and Chapa (1975a and b) described the French cultivars, and classified them into main or local interest. Those descriptions are updated by Bergougnoux et al. (1978) but focused in clonal selection of the main cultivars, and including hybrids that were used in the new plantations mixed with European chestnut as in Cevennes (Figure 8).

Bergougnoux et al. (1978) clarified the term 'marron' and is now internationally accepted that it should be used for those cultivars producing less than 12% of the fruits poly-embryonic, naming those cultivars producing more than 12% as chestnut type.

In 1987 the Official inventory of French cultivars was published and to promote some of them (Chapa, 1987) describing 13 recommended cultivars, 29 secondary and 13 in experimental stages.

In 1988, UPOV (Unión Internacional para la Protección de las Obtenciones Vegetales) published a guideline to test the distinction, homogeneity and stability of chestnut cultivars so as interspecific hybrids by 39 characteristics. Main characteristics in classification were the male and female flowering, percentage of

Figure 8. Orchard in Cevennes mixing interespecific hybrids with local cultivars.

poly-embryonic nuts, shape, colour and size of the nuts. Pereira-Lorenzo et al. (1996a) confirmed the importance of the size and the shape of the nuts, but also the type of male flowering and the spines of the bur.

Several studies of Portuguese cultivars were recently conducted by Pinto et al. (1990), Pimentel (1990), Pimentel et al. (1992), Gaspar et al. (1992), Gomes and Pinto de Abreu (1992), Gomes et al. (1993a y b), Guerner and Valdiviesso (1993), Pimentel-Pereira and Torres-Pereira (1993) and Valdiviesso (1999).

Chinese cultivars described by Liu et al. (1992) and Liu (1993). Liu and Zhou (1999) identified the excellence of six famous Chinese cultivars (*C. mollissima*) out of 28 reported; namely 'Chu shu hong', 'Jiu jia zhong', 'Duan zha', 'Qin zha', 'Jiao zha' and 'Jian ding you li'. All of them produce nuts over 10 g (Jiao zha over 20 g), easy peeling, and excellent kernel quality highly recommended for cooking and roasting.

Though the crop in Switzerland has been mostly abandoned, some cultivars could be localised by Conedera et al. (1993).

In Spain, Elorrieta (1949) was the first in reporting Spanish cultivars. In 1993, Fernandez and Pereira published the first inventory for Galician cultivars in Northwestern Spain. Later, it presented the variability and the description of those cultivars (Pereira-Lorenzo et al., 1996a and b; Pereira-Lorenzo and Fernandez-Lopez, 1997 a and b). These studies had been extended to other important areas for nut production as Asturias, Castilla-León, Extremadura, Andalucía and Canary Islands (Pereira-Lorenzo et al., 2001c).

4.1.1. *Hybrids as direct producers*

Recently French researchers have focused in the breeding of interspecific hybrids resistant to ink diseases as direct producers (Bergougnoux et al., 1978, and Breisch, 1995). In Spain, hybrids are considered an alternative in Atlantic areas where they show very good adaptability (Fernandez et al., 1992) and with sufficient quality as size and mono-embryony, and harvesting before 20th September. In United States, the use of hybrids such as Colossal are highly recommended in new orchards due to the high incidence of blight in United States (Vossen, 2000).

4.2. Main cultivars in nut production

Although variability in chestnut cultivars is very high, some of the cultivars have increased their importance because of their superior quality in the present market as the size of the nuts and 'marron' type, i.e. less than 12% of poly-embryonic nuts. In some areas of Spain, growers do not collect nuts smaller than 10 g.

Some cultivars in Spain such as Parede (Pereira-Lorenzo et al., 2001a) have been propagated profusely during last 200 years but it is difficult to explain their popularity because they produce quite small nuts. Perhaps Parede presents a better adaptability to areas where temperatures are too low to bear fruits or it is more resistant to natural conservation.

Fresh market demands big size of nuts and mono-embryonic. Peeling can be solved with modern machines. Most of the best cultivars combine these characteristics, but they are localised in specific areas where they were selected since little experimentation has been performed in this crop to promote the best clones.

Most of the best cultivars recommended in France for new plantations are *C. sativa* cultivars, producing nuts between 12 and 18 g and most of them 'marron' type (Table 8). Some cultivars as Comballe and Marron Comballe produce a high percentage of poly-embryonic nuts but they are the most important in the French production due to the quality of the kernel. Five out of 26 recommended cultivars are hybrids between *C. sativa* and *C. crenata*, one a clone from *C. crenata* and one from *C. mollissima*. They are planted in combination with European cultivars (Figure 8). In France the cultivars recommended for altitudes lower than 500 m, are all hybrids or Asiatic species resistant to spring frost.

In Northern Spain, the most popular cultivars are Amarelante, Negral, Famosa, Longal, Ventura, Garrida, Loura and Luguesa (Table 9). In Extremadura, Central Spain, Injerta and Verata are cultivated and in Southern Spain, Planta Alajar, Temprana and Pilonga are the best. In Canary Islands, the most widespread cultivars are Mulata in Tenerife and Jabuda in La Palma (Pereira-Lorenzo et al., 2001b). Arafero, Castagrande, Picudo and Polegre produce biggest nuts.

In Portugal, Longal has been promoted as the best cultivar for fresh market and industry. The production of this important cultivar can be found in the international market. Normally it is combined with Judia but other alternatives can be selected such as Amarelal and Verdeal (Table 10). Although denominations of

Table 8. Recommended French cultivars for new orchards (Breisch, 1995).

Cultivar	Origin	Poly-embryonic nuts (%)	Weight (g)	Use
Bouche de Betizac	Bouche-Rouge (*C. sativa*) × CA04 (*C. crenata*)	8–25	15 → 18	Fresh
Maridonne	Sardonne (*C. sativa*) × CA04 (*C. crenata*)	5	15–18	Experimentation
Marigoule	*C. crenata* × *C. sativa*	5	15 → 18	Fresh
Precoce migoule	*C. crenata* × *C. sativa*	20–40	15–18	Fresh
Bournette	*C. crenata* × *C. sativa*	5	12–18	Fresh
Iphara	*C. crenata*	5	15 → 18	Fresh
CA75	*C. mollissima*	5	10–12	Pollinizator
Merle	*C. sativa*	12	12–18	Fresh, intermediate rootstock
Aguyane	*C. sativa*	12	12–15	Fresh
Dorée de Lyon	*C. sativa*	20	12–18	Fresh
Laguépie	*C. sativa*	20	15–18	Fresh
Précoce Ronde des Vans	C. sativa	12	10–15	Fresh
Sardonne	*C. sativa*	12	15–18	Fresh
Arizinca	*C. sativa*	5	12–15	Fresh and industry
Bouche rouge	*C. sativa*	5	15–18	Fresh and industry
Comballe	*C. sativa*	>20	15–18	Fresh and industry
Insidina	*C. sativa*	5	12–15	Fresh and industry
Marron Comballe	*C. sativa*	20	15–18	Fresh and industry
Belle Epine	*C. sativa*	5	12–18	Fresh and transformation, pollinizator
Marron de Goujounac	*C. sativa*	5	15–18	Fresh, pollinizator
Montagne	*C. sativa*	5	15–18	Fresh, pollinizator
Tricciuda	*C. sativa*	5	15–18	Industry
Verdale (Delsol)	*C. sativa*	5	12–15	Industry, pollinizator
Marron de Chevanceaux	C. sativa	5	12–15	Natural 'marron'
Pellegrine	*C. sativa*	5	10–15	Natural 'marron'
Imperiale	*C. sativa*	20	15–18	Pollinizator

cultivars are coincident in North Spain and Portugal as Longal, Amarelal and Verdeal, no studies have been conducted to establish the genetic relationship between them.

In California, the principal cultivar is Colossal (*C. sativa* × *C. crenata*) grown with Silverleaf (*C. sativa*), Nevada (*C. sativa* × *C. crenata*), Eurobella (*C. sativa* × *C. crenata*, or Colossal seddlings as pollinator) (Vossen, 2000). Colossal produces very large and sweet nuts (40 nuts/kg) but some are poly-embryonic. Sylverleaf produces also large and sweet nuts but the shell tends to be cracked. Nevada and Eurobella produce smaller nuts than Colossal.

Italian cultivars have been well described by Breviglieri (1955). Since then, clones have been selected from the most important cultivars. Some of them include

Table 9. Main quality characteristics of the most important Spanish chestnut cultivars of *C. sativa* (Pereira-Lorenzo and Fernandez-Lopez, 1997c; Pereira-Lorenzo and Ramos-Cabrer 2002; and Pereira-Lorenzo et al., 2001b).

Region	Cultivar	Nuts/kg	Poly-embryonic nuts (%)	Central nut weight	Lateral nut weight (g)	Use
Andalucía	Dieguina	87	8	10	14	Marron
Andalucía	Helechal	80	7	12	14	'Marron glace'
Andalucía	Pilonga	63	8	15	18	'Marron glace'
Andalucía	Planta Alajar	71	11	12	16	'Marron glace'
Andalucía	Temprana	61	12	15	19	'Marron glace'
Andalucía	Tomasa	44	14	22	25	Fresh
Andalucía	Vazqueña	73	4	11	16	'Marron glace'
Galicia	Amarelante	83	0	11	13	'Marron'
Galicia	Famosa	88	0	13	13	'Marron'
Galicia	Garrida	74	2	14	15	'Marron glace'
Galicia	Inxerta	82	0	12	13	'Marron'
Galicia	Loura	85	1	14	14	'Marron'
Galicia	Longal	119	0	9	10	Marmalades, purées
Galicia	Luguesa	84	1	11	14	'Marron'
Galicia	Negral	106	1	9	11	Marmalades, purées
Galicia	Parede	133	0	8	9	Marmalades, purées
Galicia	Presa	95	1	10	13	Fresh
Galicia	Raigona	128	13	8	9	Marmalades, purées
Galicia	Rapada	98	3	10	11	Fresh
Galicia	Ventura	85	0	12	13	'Marron'
Canary Islands	Arafero	72	5	15	15	'Marron glace'
Canary Islands	Castagrande	99	6	13	14	Fresh
Canary Islands	Picudo	89	5	13	13	'Marron'
Canary Islands	Polegre	80	3	14	13	'Marron glace'

Table 10. Most important Portuguese cultivars of *C. sativa* recommended for new orchards, Pinto de Abreu et al. (1990) and Valdiviesso (1999).

Cultivar	Nuts/kg	Poly-embryonic nuts (%)	Use
Martainha1	63	2	Industry
Martainha2	88	80	Fresh
Longal5	63	1	Industry
Longal6	58	4	Industry
Amarelal	73	20	Fresh
Verdeal1	68	6	Industry
Judia	55	14	Fresh

the term 'Marrone', indicating as Breviglieri defined (1955) nuts of big size, monoembryonic, bright clear colour, easy peeling and good flavour. However, other cultivars not denominated 'Marrone' also produce good nuts (Table 11).

5. ROOTSTOCKS

Traditionally, growers have used seedlings (in Europe of *C. sativa*) coming from seeds germinated under the grafted trees to establish the new orchards. These rootstocks present a very good compatibility and are very rustic. Recently, Soylu and Serdar (2000) have initiated in Turkey a selection program of mother trees as producers of seedlings for chestnut rootstocks with good emergence rate, growth and drought tolerance. However, they are expected to be susceptible to ink disease.

At the beginning of the 20th century, researchers from France, Spain, Italy and Portugal introduced seeds of *C. crenata* from Japan and *C. mollissima* from China into Europe. These species are resistant to the ink disease, but their nuts were not appreciated by growers because of the poor peeling. Also they were not particularly vigorous to produce timber. Later, they were tried as rootstocks, but incompatibility appeared. Interspecific hybrids were made and some clones were selected as rootstocks, combining resistance to ink disease, easy propagation, good compatibility and rusticity. Nevertheless, most of the hybrid rootstocks did not combine all these characteristics.

Compatibility in chestnut was studied in the past by Urquijo (1957) and later by Jaynes (1979), who advised on the need for further studies on specific rootstock-scion combinations. More recently, Breisch (1995) pointed out different compatibility relationships between French rootstocks and cultivars (Table 12). Pereira-Lorenzo and Fernandez-Lopez (1997a) found a strong incompatibility between Marigoule and Spanish cultivars. That incompatibility was shown at the beginning of the third growth of the cultivars grafted by budding. The scion died while the rootstock was alive (Figure 9). Local incompatibility by weak union at the graft point can be observed due to the lack of fibrous connections.

Only France and Spain commercialise their resistant hybrid rootstocks propagated by stooling, cuttings or *in vitro* culture. Five French (Breisch, 1995) and four Spanish hybrid clones (Pereira-Lorenzo and Fernandez-Lopez, 1997a and Pereira-Lorenzo et al., 2000) are recommended. Between Spanish rootstocks, CHR-151 (HS) has been broadly used and it propagates very well by *in vitro* culture (Miranda-Fontaíña and Fernandez-Lopez, 1992).

Resistance to the ink disease is from low to very high resistance for French rootstocks (Breisch, 1995), while the Spanish hybrids varied between very resistance to medium level (Fernandez-Lopez et al., 2002).

Breisch (1995) indicated the high sensitivity of French rootstocks to early spring frosts (Table 12). Spanish hybrid rootstocks in traditional orchards localised between 400 and 600 m, which were evaluated during the last 14 years (Pereira-Lorenzo et al., 2000), showed resistance to frost. They are very sensitive during

Table 11. Main Italian cultivars.

Variety name	Synonyms	Origin	Nut size (g)	Nuts/kg	Poly-embryonic nuts (%)	Use	Reference
Marrone Fiorentino	Casentinese, Toscano	Toscana	14–22	55–65	–	All	Breviglieri, 1955
Frattona		Piamonte	–	94	39	–	Bounous et al., 1988
Gabbiana1		Piamonte	–	154	4	Dried	Bounous et al., 1988
Gabbiana2		Piamonte	–	138	6	Dried	Bounous et al., 1988
Rossastra1		Piamonte	–	135	3	Dried	Bounous et al., 1988
Rossastra2		Piamonte	–	151	1	Dried	Bounous et al., 1988
Spinalunga		Piamonte	–	133	4	Dried	Bounous et al., 1988
Temporiva		Piamonte	–	158	20	Dried	Bounous et al., 1988
Nzerta1		Calabria	–	85	0	–	Antonaroli et al., 1983
Nzerta2		Calabria	–	80–120	0	–	Antonaroli et al., 1983
Curcia1		Calabria	–	135	0	–	Antonaroli et al., 1983
Ricciola2		Calabria	–	80	0	–	Antonaroli et al., 1983
Ricciola3		Calabria	–	80	0	–	Antonaroli et al., 1983
Inserta		Calabria	–	140	0	–	Antonaroli et al., 1983
Castagna di Montella		Campania	–	54–80	0–2	–	Bassi and Sbaragli, 1984
Marrone Castel del Rio		Emilia-Romagna	–	57–92	0–6	–	Bassi and Sbaragli, 1984
Marrone di Castiglione dei Pepoli		Emilia-Romagna	–	59–100	0–6	–	Bassi and Sbaragli, 1984
Marrone di Montepastore		Emilia-Romagna	–	61–80	0–5	–	Bassi and Sbaragli, 1984
Palummina		Campania	–	64	2	–	Bassi and Sbaragli, 1984

sprouting, but were not when used as rootstocks. Their compatibility was excellent (Figure 10).

Other alternatives evaluated as rootstocks in Spain are the following hybrid clones (Pereira-Lorenzo y Fernandez-Lopez, 1997a): CHR-137 (125), CHR-31 (2), CHR-149 (90025), CHR-147 (431), CHR-167 (19) and 776, and hybrids CHR-137 and CHR-162, which were previously recommended by Urquijo (1957).

Table 12. Hybrid rootstock resistant to the ink disease recommended in Spain (Breisch, 1995) and Pereira-Lorenzo and Fernandez-Lopez (1997a).

Roostock	Resistance to ink disease	Resistance to early frost	Compatibility	Vigour with the cultivar	Compatibility with hybrids	Compatibility with *C. sativa*
Ferosacre CA90	***	0	**	5	Précoce Migoule, Bouche de Bétizac, Bournette, Maridonne	Belle Épine, Bouche Rouge, Comballe, Bastellicacciu, Impériale, Précoce des Vans, Sardonne
Maraval CA74	**	**	*	2	Bournette, Maridonne, Précoce Migoule	Dorée de Lyon, Goujounac, M. De Chevanceaux, Montagne, Verdale CA577 and CA756, Sardonne, Précoce des Vans
Marigoule CA15	***	**	*	4	Précoce Migoule, Maridonne	Sauvage Marron, Précoce Monteil
Marlhac CA118	**	*	**	3	Maridonne, Précoce Migoule, Bouche de Bétizac, Marigoule	Bastellicacciu, Belle Épine, Verdale CA577, Bouche Rouge, Précoce des Vans, Sardonne
Marsol CA07	*	**	**	4	Bouche de Bétizac, Bournette, Maridonne, Marigoule	Marron Comballe, Belle Épine, Sauvage Marron, Précoce des Vans
CHR-162 (7521)	***	**	***	5		Tested with Spanish cultivars
CHR-151 (HS)	**	**	***	4		Tested with Spanish cultivars
CHR-168 (110)	**	**	***	5		Tested with Spanish cultivars
CHR-161 (100)	**	**	***	–		Tested with Spanish cultivars

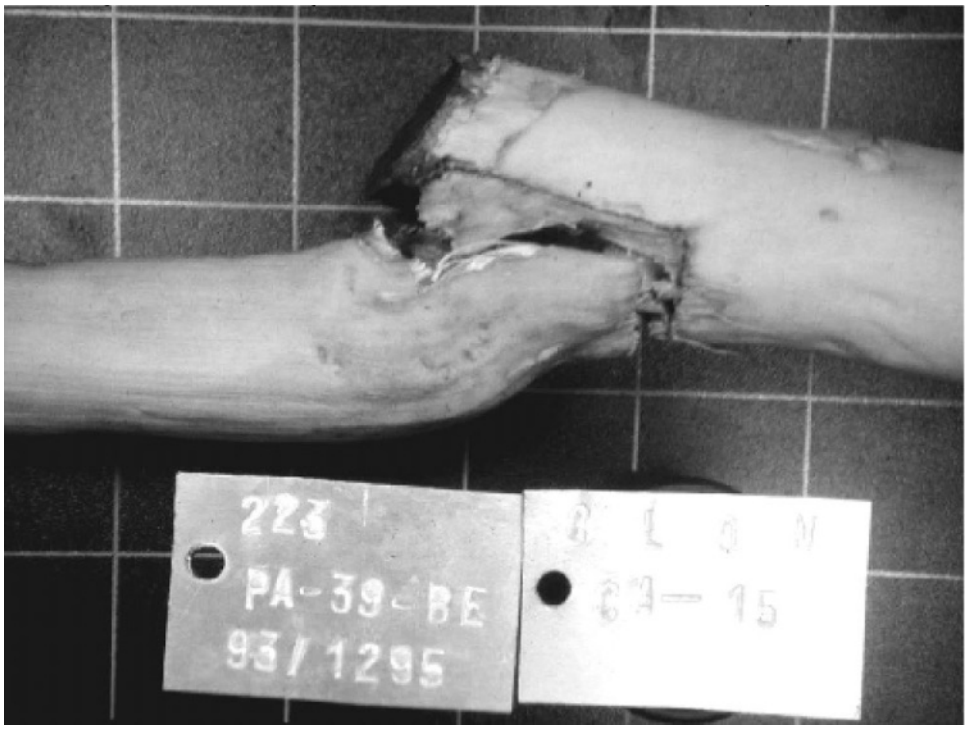

Figure 9. Local incompatibility between *C. sativa* cultivar and interspecific hybrid rootstock (Pereira-Lorenzo and Fernandez-Lopez, 1997a).

6. PLANTATION AND MANAGEMENT

Chestnut is a traditional crop that has declined in importance due to diseases and the low profitability, especially in the mountainous old plantations. However, its stable fresh market and industry encourage the growers to establish new plantations and maintain the old orchards in some areas of Europe. Chestnut orchards are being considered as an alternative in non-traditional areas such as Australia and California.

6.1. New orchards

New orchards should be established on slopes to allow good drainage. For nut production it is essential to have mechanisation to reduce the necessity of hard labour, and thus this could only be practiced on gentle slopes. If we do not have such conditions then it is limited only for timber plantations.

Chestnut is species of long-term investment where returns are limited. Some examples of intensification have been investigated, using irrigation and frost defence or planting chestnut in hedges (Breisch, 1995). The main way to establish new orchards is to use some of the following traditional models: Three different options can be chosen for the cultivation of chestnut; timber, nut or nut and timber

Figure 10. Perfect compatibility between *C. sativa* cultivar and interspecific hybrid rootstock HS (Pereira-Lorenzo et al., 2000).

production (Pereira-Lorenzo and Fernandez-Lopez, 1995). It is clear that steep slopes should be dedicated solely to timber production. To produce nuts, minimum conditions are essential to facilitate the crop. The differences between an orchard to produce nuts only from others to produce nuts and timber are: (1) For nut production, mechanisation is essential, which limits the crop to the best fields. (2) Stands for

timber production should be established in areas where rainfall does not limit the growth, more than 1000 mm in Spain. When it is under 1000 mmm, growth is normally reduced. Between 800 and 1000 mm we recommend nut production exclusively. (3) When the growers have limited labour time due to other activities, perhaps nut and timber production should be the alternative. These kind of orchards can also provide pasture.

Some considerations must be taken in establishing new orchards: (1) Plants must be protected during the first years. Normally, chestnut orchards are established in open fields, where wild animals and livestock can enter. Damages from wild animals and livestock can be a problem. Protection with local shrubs like genista or whin can also help avoid sun damage and early frost. (2) Irrigation must be provided to each plant regularly during the first two years in summer when it is necessary.

6.1.1. *Orchards for nuts*

In medium to fertile soil, chestnut trees develop a big crown and pruning becomes necessary to increase accessibility of sunlight. In this situation, 10 × 10 m to 12 × 12 m is recommended (Figure 11).

In Southern Spain, to increase the production during the first years, plantations at 5 × 5 m are established (Figure 12). But that compels to intensive pruning, which reduces production, but maintains a very high quality of nuts in terms of

Figure 11. Orchard established to 10 × 10 m in Galicia.

Figure 12. Orchard established to 5 × 5 m in Ronda, Andalucía.

size. When the trees develop too much, then as much as half of the trees should be removed.

In orchards established in the main producing region of Spain with local cultivars grafted over hybrid rootstocks, the annual growth indicates that it will take at least 30 years to cover the ground. Here, we suggest a much higher densities of 8 × 8 m or 9 × 9 m (Pereira-Lorenzo et al., 2000).

New plantations can be made with grafted plants or, as it was done traditionally, by planting first the rootstocks and then grafting them after the 3rd or 4th year. Differences between the two methods are clear in the early stages of the orchard. Grafted plants are more expensive but they create a more regular orchard. When grafts are made in the field, the success rate is generally not reliable and some of the rootstocks must be re-grafted the following year, thus producing irregular developmental stages of the trees. But because of the long-life of chestnut plantations growth of both kinds of plants will equilibrate when orchard matured (Pereira-Lorenzo et al., 2000).

6.1.2. *Fruit and timber production*

When rainfall and quality of the soil is adequate, we can establish orchards to produce nuts and timber. Plantation distances should be closer than in orchards for nut production only. This will increase competition between trees and also will improve conformation of the trees. We recommend 7 × 7 m. Of course,

nut production will be delay for some years while the trunk and crown is forming.

There are two possibilities in producing quality timber. The most interesting, according with the higher prices obtained in the timber market is forming a long trunk up to 7 m free of branches. It will take at least 40 years to cut a good trunk, more than 40 cm width. But in Galicia, the best qualities, more than 70 cm width are obtained after 80 to 90 years. The advantage of this system is the addition of annual production of nuts. Such system is used in Galicia, Spain, with Garrida and Loura cultivars and in Cuneo, Italy, with cultivars 'marron type' (Figure 13).

Other possibility is forming a trunk 2 to 3 m long and pruning over that point to obtain 4 to 6 vertical branches (Figure 14). After 20 years we can obtain more than 20-cm width logs. And when the tree is removed part of the stump can also be used. Such poles are often used for vineyard trellis, hops trellis, fences, charcol making or, in general, as outdoor timber without any treatment.

6.2. Management of ground

Four systems are used: (1) In humid areas pasture is the most common since it can be used for animals. (2) In drier areas, pasture could not be maintained without irrigation, which limit its use as intensive orchards (Figure 15). In some situations farmers grow wheat or rye during spring as a complementary crop which will help to limit weeds and incorporating organic matter. A first ploughing is made before sowing the spring crop and one before collecting the nuts. Though spring crops are no being any more, growers continue ploughing the ground, which increase soil erosion. (3) The use of herbicides can be a possibility but the ecological importance of chestnut growing must prevent its excessive use. In intensive orchards herbicides are recommended on the inter-lines. But for this system it is only useful when the orchard is irrigated. (4) The last alternative is to use minimum inputs by natural grass cover the ground and two mows must practice to control the vegetation, avoid fires and facilitate the harvest.

6.3. Fertilisation

The best suggestion for fertilizer input can be found in Breisch, 1995. Average values for macro- and micronutrients in leaves can be found in Table 13.

The lack of copper is related with granite soils, extremely poor in nutritive elements, and produce symptoms of reddish-brown in the secondary veins top of the leave (Breisch, 1995). In South Korea, the lack of Boron is related with the early fall of young burs after flowering and in France, necrotic parts of the bark after hard pruning in orchards located in poor soils areas.

Chestnut can be found in soils of high acidity, as much as pH 4,5, but soils with a pH higher than 6 it is recommended to rectified with calcium magnesium carbonate.

For soil amendments to get a minimum level of fertility with recommendation given by Breisch (1995) are quantities of 200 to 300 kg/ha of P_2O_5, 150 to 300 kg/ha

Figure 13. Grafted orchard to produce nuts and timber forming a trunk up to 7 m free of branches in Galicia.

of K_2O. In poor soils can be recommended quantities of 100 kg/ha of magnesium sulfate and 5 to 20 kg/ha of copper sulfate.

Organic matter should be added when the level is lower than 2%, and the minimum is 50 t/ha.

Figure 14. Grafted orchard to produce nuts and timber pruning intensively over the graft point in Galicia.

6.4. Forming new trees

Two traditional systems (Pereira-Lorenzo and Fernandez-Lopez, 1995) and three new ones (Breisch, 1995) have been proposed for new plantations.

(1) Traditional systems:

Nut production. New plants are pruned to form a trunk with four to six branches well distributed during forming prune, and branches are forced to open. Cutting back of the four or six branches down to halve their sizes, we can obtain eight or twelve smaller branches well distributed around the trunk (Figure 16).

Frequently, graft is made in the field after four years to obtain a strong trunk and a well-developed radical system. Graft is made at 2 or 3 m above ground using bark graft or cutting back the tree over four to six buds to obtain four or six small branches where pipe graft is practice (Figure 17).

Nut and timber production. A trunk of 7 m long must be formed from the grafted plant and pruned to remove branches more than 6 cm in diameter. Grafted plants with cultivars as Garrida, Loura and Parede are good because of their ease in forming good trunk. A much higher density than the nut-only orchards is required, 7×7 m, for increasing competition between the crowns. No pruning of the crown is

Figure 15. Irrigated orchard in North Portugal.

Table 13. Macro- and micronutrients in leaves related to dry matter (Breisch, 1995).

Element	Concentration
Nitrogen	1.8–2.5%
Potassium	6–10‰
Calcium	8–12‰
Magnesium	2–4‰
Phosphorus	3–4‰
Manganese	0.3–1‰
Copper	10–15 ppm
Iron	60–100 ppm
Zinc	25–35 ppm
Boron	40–50 ppm

necessary. Although nut production is delayed and reduced considerably, quality of the nuts is maintained (Pereira-Lorenzo and Fernandez-Lopez, 1995).

(2) New systems proposed for chestnut are the Japanese pruning, central leader and laterals in a plain (Breisch, 1995). Japanese pruning is an easy way to train chestnut tree to a globlet shape where the branches come from the base of the trunk. It allows a fast bearing, an easy pruning tree. The central leader is to promote a main axe while the lateral branches appear along in avoiding a heavy competition by removing the ones too close the others. Using a plain trellis, lateral branches of the central leader can be orientated in a direction to facilitate harvesting.

Figure 16. Young orchard in Galicia pruned in vase.

Figure 17. Pipe graft in new small branch after prune.

6.5. Management of old orchards

Main problems with old orchards are the aging related with the lack of pruning. Then, the trees begin to produce smaller nuts and to emite rejects from the roots loosing the cultivar (Figure 18).

Traditionally, one third of the crown of the old trees are pruned each 8 to 10 years (Figure 11). This allows a third in full production, a third beginning to produce and the other third renewing. In some areas, this operation is accompanied by regrafting old trees with a new cultivar according to the demand of the market.

To recovering an abandoned old orchard, first one must consider cutting back the old trees in order to produce vigorous stumps for the grafting (Figure 19). Excessive strong prunes accompanied additional grafting can often kill the old tree (Figure 20).

In some areas as in Switzerland (Figure 21) or in Galicia old orchards are recuperated as part of the traditional landscape.

7. PEST AND DISEASES

Two main diseases, ink disease (*Phytophthora* spp.) and blight (*Chryphonectria parasitica*) threaten chestnut. The most important insect pests are the weevils *Balaninus* and *Laspeyresia*. In Asia, gall wasp is considered a very important pest in Japan and Korea, and recently also in the United States.

Figure 18. Abandoned orchard in Galicia.

Figure 19. Recovering old trees from stumps after cutting back.

Figure 20. Too strong prune accompanied additional grafting can often kill the old tree.

Weevils reduce the harvest considerably. Chemical treatments can be applied to the trees before harvest but they compromise the woodland ecology because chestnut it is an extensive crop occupying large areas. Two methods for controling weevils are practiced: (1) Some collectors 'jobbers' apply methyl bromide to kill weevils before delivering the nuts to the industry or central markets. (2) 'Curatura' is an

Figure 21. Recovered orchard in Switzerland.

old way to prepare nuts for a better conservation. Nuts are soaked in water long enough to kill weevils and washes the spores in the pericarp. In industry, nuts are soaked in water for 45 minutes at 50 °C (Breisch, 1995).

7.1. Ink disease

This disease produces root and collar rot on seedlings and adult chestnuts plant. Seedlings producing chlorosis in leaves that do not fall and are killed rapidly. In adult trees, chlorosis in leaves and burs remaining in the crown, dark necrosis in the collar under the bark, which gave the name of this disease (Figure 22).

Phytophthora species were reported in Europe since 1726 in Spain and in 1838 in Portugal (Vannini and Vettraino, 2001). Two species have been reported to cause ink disease: *P. cinnamomi* and *P. cambivora* and they are considered the most pathogenic to *C. sativa*, followed by *P. citricola* and *P. cactorum*, both are also present in chestnut stands.

Some authors showed a higher incidence of ink disease in orchards of South facing slopes with poor soils (Martins et al., 1999) and the applications of lime and manure can reduce the activity of this disease (Portela et al., 1999).

Hardy et al. (2001) pointed out that it is unlikely to eradicate *P. cinnamomi* where

Figure 22. Ink disease killing an old tree.

the disease is already established and the importance to avoid the spread of this pathogen into uninfected areas.

7.2. Chestnut blight

Chestnut blight it is caused by *Cryphonectria parasitica* (Murr.) Barr (Syn. *Endothia parasitica* [Murr.] And.). It became the major disease of chestnut due to the sen-

sitivity of *C. dentata* and less *C. sativa* to this fungus. Blight destroys the bark and the cambium and causing the death of the branches or the tree over the wound when the disease girdles around them (Figure 23) (Anagnostakis, 1987; Heiniger and Rigling, 1994).

It was first observed in Europe in Genova, Italy in 1938, spreading quickly through Italy and other European countries (Robin and Heiniger, 2002) less so in Southern UK, Netherlands, Central and Southern Spain and the Canary Islands. Blight almost eliminated the American chestnut (*C. dentata*) but European chestnut recovered rapidly due to the natural occurrence of hypovirulence dsRNA hypovirus CHV1. Recently, Allemann et al. (1999) have isolated five different CHV1 subtypes.

Biological control is practised in Europe applying hypovirulent strains hypovirus to growing cankers using Grente's method (Grente and Berthelay-Sauret, 1978).

8. PROPAGATION

8.1. Seedlings

Chestnut tree is naturally spread by seedlings, and this is the main way to produce plants for forest plantations and traditional rootstocks. In both cases, they should be promoted in areas over 500 m, where ink disease is less frequent. In areas

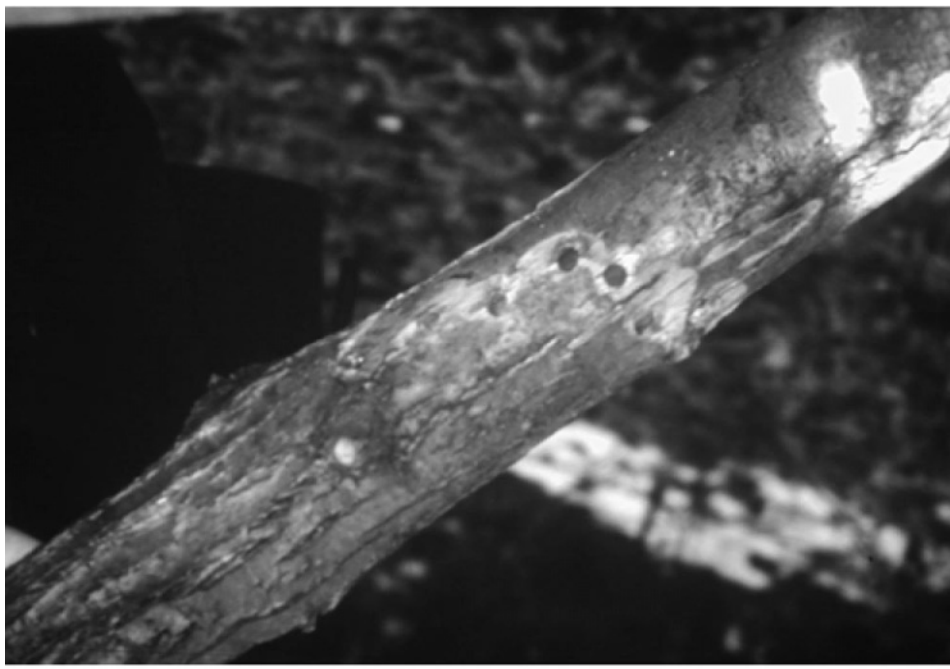

Figure 23. Chestnut blight and hypovirulent traitment.

where ink disease is rampant, disease resistant hybrids should be used in both for plant and rootstocks.

Chestnut, preferably coming from origin regions are collected and disinfected by soaking with fungicide. It is important to avoid problems like development of diseases such as *Sclerotinia, Phoma* and *Penicillium*, as well as weevils of *Laspeyresia* and *Balaninus* during storage. After soaking, seeds are slightly dried. They can be stored in plastic bags and stratified between 0 and 4 °C. Requirements for stratification varies with the genetic origin, between 2 and 3 months for breaking seed dormancy (Soylu and Serdar, 2000).

Chestnuts are germinated in seeds beds using perlite, cork pine or peat as substrate. Alternative, they can be germinated directly in containers such as wax paper pots or plastic containers, preferably with more than 400-cm^3 capacity. If germination is made in December in heated greenhouses, seedlings can be sold in containers in May to June.

They can also be established in the ground. In this case, it is good practice to prepare 30 cm-elevated beds with 1.5 m width covered with black plastic. Cutting the main root can facilitate seedlings. Seeds are planted 20 cm apart. The black plastic prevents weeds but it could also increase incidence of ink disease. Other problems are birds, rabbits and rodents. Seedlings can be sold next winter.

8.2. Grafting

Chestnut cultivars have been traditionally propagated using bark graft in spring (Figure 24) or pipe bud (Figure 17) and continue to be used in new orchards when *in situ* graft is made. At present days, nurseries prepare grafted plants using different types of grafts.

Different methods of grafting in chestnut have been published by Urquijo (1957), Bergougnoux et al. (1978), Lagerstedt (1979), Turchetti and Gemignani (1981), Chapa et al. (1990), Fernandez (1990), and Pereira-Lorenzo and Fernandez-Lopez (1997a). Huang et al. (1994c) studied the grafting relationships between cultivars of *C. dentata, C. mollissima* and *C. crenata* over seedlings from *C. mollissima*.

A study about five grafting methods (Chip, Cleft and Whip in spring, and Patch and T-bud in summer) showed that summer methods are easier and more effective since the higher temperatures causes rapid healing (Pereira-Lorenzo and Fernandez-Lopez, 1997a). However, summer grafting is not ready for the market until the following winter. If necessary, spring graft can produce plants for the same winter, although they need a second year growth to get to comparable growth with those summer-grafted plants.

8.3. Layering

It was the main way to propagate hybrids in France (Bergougnoux et al., 1978; Breisch, 1995) and Spain (Miranda-Fontaíña and Fernandez-Lopez, 1992). Mother plants are established to 3 × 0.5–1.5 m. They develop during four years to accumulate starch in the collar and roots. In winter they are cut back to the ground level. From adventitious buds, new shoots are developed the following spring. In

Figure 24. Bark graft in spring.

May, leaves from the lower part of the shoots are removed and girdled with wire with hormones applied. Afterwards, the part is covered with a mound of soil to produce etiolation. When the leaves fall in the autumn, new rooted shoots are severed. Main problems of this propagation system are the low number of propagules obtained from the mother plant, labour intensity and unfit plants produced because of the vigorous aereal part in relation to the limited root system (only the 5 to 10% of the total dry matter; Miranda-Fontaíña and Fernandez-Lopez, 1992). Clonal variation is an important factor in the number of shoots by mother plant; range from 11.1 to 23.3 and in the number of shoots with a well-developed rooting system, and between 30.5% and 49.6% (Miranda-Fontaíña and Fernandez-Lopez, 1992).

8.4. Micropropagation

Several *in vitro* propagation methods have been developed since 1980s when applied to chestnut (Viéitez and Viéitez, 1980 a and b; Viéitez et al., 1983). It is useful for propagating hybrids for forest and rootstocks and is used in France and Spain as a commercial technique (Breisch, 1995; Miranda-Fontaíña, 2001). Main problems are the high cost of the facilities, skilled technicians and the variability of rootings between clones.

Basically, *in vitro* culture has been used in the propagation of axillary shoot or buds (Chevre et al., 1983; Sánchez and Viéitez, 1991; Sánchez et al., 1997a and b; Ballester et al., 2002). However, potential of somatic embryogenesis has been pointed out for chestnut micropropagation and as a tool in genetic engineering programmes (Xing et al., 1999; Ballester et al., 2002).

In vitro culture is based on the establishment of axillary shoot or buds from juvenile and mature material (Viéitez and Viéitez, 1980a; Sánchez et al., 1997a and b). After several subcultures of *in vitro* multiplication, some micro-cuttings are induced to root. Acclimatisation from the *in vitro* culture system to soil, seedlings are grown in greenhouse under fog system.

8.5. Cuttings

Chestnut has been considered traditionally difficult to obtain hardwood cuttings (Viéitez et al., 1987; Viéitez and Ballester, 1988). Techniques involving rejuvenation by hard pruning over mother plants, the use of softwood cutting in spring under fog system and hormones inductions have increased the percentage of rooting. Some nurseries are trying to incorporate these methods to substitute layering method in propagating hybrids for forest or rootstocks (Ponchia and Howard, 1988; Chapa et al., 1990; Gautan and Howard, 1991; Fernandez et al., 1992; Jinks, 1995). Clonal variation in rooting capability must be taken in account, because it can vary between 16% and 90% in hybrid clones (Fernandez et al., 1992).

9. BREEDING

9.1. Variability

Important efforts are being made in studying chestnut variability using morphology characteristics based on Breviglieri's (1955) 'Scheda Castanografica', after in the UPOV chestnut guideline (1988) and, more recently, applied to the Spanish cultivars (Pereira-Lorenzo et al., 1996a) and different chestnut species (Oraguzie et al., 1998).

The first molecular markers based studies in chestnut were by isoenzymes (Sawano et al., 1984) over 16 clones (10 Japanese, 3 Chinese, and 2 hybrids). Wen and Norton (1992) studied four isoenzyme system and identify 22 Chinese cultivars. Genetic analysis with isoenzymes were performed by Bonnefoi (1984), Malvolti and Fineschi (1987), Fineschi et al. (1990a and b) and Huang et al. (1994a).

In Europe, genetic variability between natural chestnut populations was studied by Pigliucci et al. (1990a and b), Villani et al. (1991a and b, 1993) and Aravanopoulos (2002). These works established a gene flow from Orient (Turkey) to the Occident (Italy). In United States similar studied were performed on wild populations of the American chestnut (Huang et al., 1994b, 1996b and 1998) using both isoenzymes and RAPDs.

In Portugal, analysis of the genetic variability in cultivars was studied by isoenzymes (Pereira et al., 1999), with RAPDs by Valdiviesso (1999) and RAPD and ISSR by Goulao et al. (2001). In Spain, the genetic variability between *C. sativa, C. crenata, C. mollissima* and interspecific hybrid clones was studied by isoenzymes by Fernandez-Lopez (1996). Pereira (1994) and Pereira-Lorenzo et al. (1996b, 1997b) studied the variability of the Galician chestnut cultivars by isoenzymes confirming the high variability found by morphology. However, Fineschi et al. (1994) showed a relatively high degree of homogeneity both among individuals of the same variety and among varieties of the same area but a high genetic distance between geographic areas in Italy.

Studies about the American species *C. pumila* var. ozarkensis showed that the level of diversity is lower than in Chinese and European chestnut populations, this perhaps reflect the mortality caused by blight (Dane et al., 1999). Huang (1998) showed a negative correlation between genetic distance and geographic distance between chestnut populations in America and suggest a limited gene flow and possible geographical isolation. Populations from Alabama showed a high level of genetic diversity and this could be related with glacial refugia.

Linkage relationships of isoenzymes and morphological traits in interspecific crosses were found (Huang et al.,1996a). Recently, molecular maps have been developed (Kubisiak et al., 1997 and Casasoli et al., 2001), opening a new way to the knowledge about the genetics of chestnut.

9.2. Propagation

Incompatibility between some interspecific hybrids and cultivars from *C. sativa* (Breisch, 1995; Pereira-Lorenzo and Fernandez-Lopez, 1997a; Craddock and Bassi, 1999) are restricting the use of these as rootstocks resistant to ink disease.

In micropropagation, though important advances have been made, the selection of culture medium, carbon source, rooting stage and acclimatisation needed further studies (Ballester et al., 2002). Somatic embryogenesis as an alternative to clonal system is being explored for chestnut, as well as a tool in genetic engineering programmes (Carraway and Merkle, 1997; Xing et al., 1999, Ballester et al., 2002).

Improvement in propagation by cuttings has reduced the practice of layering. However, propagation by cuttings needs expensive facilities such as heated greenhouses with fog system, and clonal variation in rooting reduces the importance in transferring this technique from research to nursery production.

9.3. Hybrids

Chestnut breeding in Europe began with the production of hybrids resistant to the ink disease (*Phytophthora* spp.) to substitute the indigenous species. Initially, seedlings from Asiatic species *C. crenata* and *C. mollissima* were introduced between 1917 and 1940 (Elorrieta, 1949; Fernandez et al., 1993) as a way to combat the ink disease, which was threatening the European chestnut orchards. Resistance of the Asiatic species was confirmed later, but these species were in many respect inferior to the European species *C. sativa*; i.e. less vigour, lower quality of the nuts, bad affinity with the local cultivars, sensitive to early spring frost and summer drought, difficulty in adapting to climatic characteristics of some areas in Europe (Elorrieta, 1949; Fernandez-Lopez, 1996). In Spain, a program of hybridisation between *C. crenata* with *C. sativa* were carried out by Gallastegui (1926) with the aim of combine the best characteristics of both species.

Between 1942 and 1958 (Urquijo, 1944, 1957; Fernandez et al., 1993), first generations of hybrids were obtained using *C. sativa* as female progenitor, and *C. crenata* or *C. mollissima* as male. Between 1953 and 1958, 40 clones of second and third generation of open pollination hybrids were selected by resistance to ink disease and, amongst these, some as rootstocks to local cultivars and some as direct producers (Urquijo, 1944). A new selection by resistance to ink disease was developed between 1954 and 1965 with over 10000 seedlings coming from a few families whose exact origin is unknown but were supposed hybrids.

Most of the hybrids were transfered to the CTIFL between 1959 and 1962. In 1989 a new program began to identify the mixture of hybrids clones with the aim of selecting the best material.

Results showed that some clones were interesting for timber, nut production or as rootstocks (Fernandez et al., 1992, 1993; Fernandez-Lopez, 1996; Pereira-Lorenzo and Fernandez-Lopez, 2001).

Asiatic species were introduced in France in 1925 and they showed a high tolerance to ink disease but poor adaptation to soil and weather conditions. Schad et al. (1952), beginning a breeding program to create and select interspecific hybrids obtained from open or controlled crossing. Some of the French clones became very popular as direct nut producers (Bergougnoux et al., 1978; Breisch, 1995) but occasionally incompatible with clones of *C. sativa* (Breisch, 1995; Pereira-Lorenzo and Fernandez-Lopez, 1997; Craddock and Bassi, 1999). A new hybridisation program has been initiated in France to obtain resistant, vigorous clones that produce high quality nuts (Guedes-Lafargue and Salesses, 1999).

9.4. Pest and diseases

Most of the studies about the ink disease on chestnut during recent years were focused in the better knowledge on the species identification, pathogenicity and climatic and human factors predisposing trees to ink disease infection (Vanini and Vettraino, 2001). Recently, molecular studies allow more rapid and objective identification of *Phythophthora* taxa, between them those which affecting chestnut (Cacciola et al., 2001).

Efforts have been made to detect ink disease spread in chestnut using aerial photography (Martins et al., 1999).

Some works studied the level of resistance of *Castanea* spp. to the ink disease (Guedes-Lafargue and Salesses, 1999; Fernandez-Lopez et al., 2002).

Phosphite, the anionic form of phosphonic acid $(HPO_3)^{-2}$ induce strong and rapid defense responses in plants infected by *P. cinnamomi* and it is being trial as a foliar applicant or injected into trees (Hardy et al., 2001).

The main origin of resistance to blight is coming from Asiatic species, mainly *C. mollissima* (Hebard and Stiles, 1996). Two or three genes probably control resistance in the Chinese species, and they are only partial dominant. American Chestnut Foundation has developed a backcross-breeding program to restore the American chestnut *C. dentata*. By the third backcross it is getting progeny on average 15/16 American, which eventually exhibit entirely American characteristics in later generations.

10. MARKET AND PROCESSING

10.1. Harvesting

Harvesting is the hardest and costly work in chestnut since it is made by hand in most orchards. Some alternatives have been proposed but it has been used only in few occasions.

Owners, normally with the help of the family or friends collect chestnut production. Large orchards contract gangs paid on daily basis and only harvesting biggest nuts can be profitable.

Different methods have been suggested to improve chestnut harvesting such as vacuums, brushes and nets.

A good preparation of the ground is necessary to allow accessibility for machines. The main problem with vacuums and brushes are the big quantity of burs, leaves and pebbles collected that must be discarded later. Some traditional machinery has been developed (Figure 25). An additional problem it is that when chestnuts are mixed with soil this can be the increase level of diseases associated with conservation.

Most interesting appear to be the system proposed by Frenchs, installing nets under the trees elevated from the ground with sticks (Breisch, 1995). This is expensive and time consuming, but the sanitary status will be excellent.

When nuts are harvested by machines or in some humid areas where nuts do not fall from bur, it is necessary to isolate them. Some machines have been developed to do that. Traditionally, nuts into burs are accumulated in small constructions (Figure 26) or in heaps (Figure 27) in the orchard area. After some weeks, burs begin to rot and then it is easier to collect the nuts.

Figure 25. Traditional machine to spare chestnuts from burs.

Figure 26. Small construction to store nuts into the burs in Galicia.

Figure 27. Heaps of nuts into the burs in La Palma, Canary Islands.

10.2. Processing

In Northern Spain, trees of the largest orchards belong to a number of different owners as it was reported in other chestnut areas of Italy (Bruneton, 1984). In these areas, each family harvest their production and they sell to a collector 'jobber' who accumulates quite big amounts of chestnuts in a warehouse. Jobbers will disinfect the insects, wash and brush the nuts and, in most of the cases, they classify chestnuts in different categories: over 4-cm width, between 3 and 4 cm and less than 3 cm. After that, nuts are packed in bags of 50 kg, 5 kg, 1 kg, or 250 g, depending on the market. They also send to industries in big pallets.

In Southern Spain as in France and Italy, growers are grouped into associations to prepare chestnut after harvesting. These associations have better installations where 'curature' or washing in hot water to eliminate diseases and insects is the best and healthy way to prepare chestnut. The nuts are graded and packed for marketing.

Chestnut industries buy from jobbers or associations to transform the chestnuts. Main transformation is peeling. Nuts can be peeled burning or steaming them. Second method is considered more appropriate to nuts destined to 'marron glace'.

Most of the peeled nuts are frozen. After, they can be sold in big pallets to other industries or packed in small bags. Frozen nuts can be later transformed or cooked. Biggest nuts are used for the production of 'marron glace'; peeled nuts are boiled in high concentrated solution of sugar until it replaces intracellular content.

'Marron glace' being the most expensive product based on chestnut but not the most important by quantity. Intermediate sized nuts can be transformed in natural 'marron' or 'marron' in different liqueurs.

11. CONCLUSIONS AND RECOMMENDATIONS

1. Knowledge about chestnut has increased in recent years and it will encourage its use as an economical and social alternative in orchards and forest.
2. Complementary studies are necessary in pollination compatibility, agronomic evaluation of the best cultivars, studies about compatibility groups in *Chryphonectria parasitica* and genetic heredibility of main agronomic characters.
3. Selected material should be accessible to growers. Only in France selected material are propagated, and Spain is trying to establish such procedure.
4. The lack of essays in different locations of the best cultivars of Europe is limiting their recommendation in new orchards, though it improved the Origin Denominations.
5. Most of cultivars grown in Europe and North America are based on *C. sativa* and interespecific hybrids with *C. crenata*. Cultivars of *C. mollissima* are mainly cultivated in China and less frequently in France.
6. Propagation by cuttings or *in vitro* micropropagation can substitute layering when costs are reduced. Quality of the plants is better than that obtained by layering.

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IMPROVEMENT OF GRAIN LEGUME PRODUCTION IN SEMI-ARID KENYA THROUGH BIOLOGICAL NITROGEN FIXATION: THE EXPERIENCE WITH TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS* A. GRAY VAR. LATIFOLIUS)

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1. INTRODUCTION

1.1. Food security concerns in sub-Saharan Africa

Although agriculture is the main stay for the majority of households in Africa, food security still remains the greatest challenge. There seems to be no prospects for sustainable agricultural growth for increased productivity and African agriculture has been 'diagnosed' as having a 'chronic and anaemic rather than episodic growth performance'. According to the World Bank (2001), agriculture share of gross domestic product (GDP) in Africa ranges from 3.6–63.6% but with very low annual average percentage growth ranging from –4.9 to 7.7% (Table 1). Generally, small-scale farmers who produce for subsistence dominate the agricultural sector in Africa. Indeed, a large proportion of farm households aim simply to produce enough food to meet household needs and many often fail to meet even this limited goal. According to Bongaarts (1994), there are 186 million hungry people in Africa.

Between 1950 and 1995, Africa's population grew at an average annual rate of 2.6% and more than trebled, reaching 561 million. This rate of increase is historically unprecedented among major regions of the world over comparable periods of time (Bloom and Sachs, 1998). Despite the fact that there is no relationship between prevalence of hunger in a given country and its population the challenge is to produce enough food to feed the populations. For example, for every densely populated and hungry nation like Bangladesh or Haiti, there is a sparsely populated and hungry nation like Brazil and Indonesia.

The causes of hunger in Africa should be viewed from two angles: long-term and short-term. In the long-term, poor populations have limited income and are unable to purchase or produce, on continuous basis, the amount and quality of food needed for good health. Many people are too poor to buy food that is available or lack ability and resources to grow it themselves (Lappe et al., 1998).

On the other hand, short-term food insecurity is frequently the result of crisis (e.g. civil strife and HIV/AIDS pandemic) or seasonal food shortages due to factors such as drought. In many African nations, the drive to social developments is being greatly undermined by human capital degradation caused by the HIV/AIDS pandemic, rising armed conflicts and civil strife, ethnic and cultural upheavals and the burden of staggering national debt, all of which have become a major factor in food emergencies. For example, FAO (1999) reported several countries facing exceptional food emergencies (Table 2).

R. Dris and S. M. Jain (eds.), Production Practices and Quality Assessment of Food Crops, Vol. 1, "Preharvest Practice", pp. 163–188.

Table 1. Agricultural share of GDP in Africa in 1999 and annual average percentage agricultural growth, 1990–99.

Countries	Agricultural share GDP in 1999	Annual average percentage growth, 1990–99 (constant 1995 US $)
Guinea-Bissau	63.6	3.3
Central African Republic	55.1	3.6
Burundi	52.2	–1.3
Ethiopia	48.9	–
Tanzania	47.6	3.3
Mali	46.5	2.8
Rwanda	45.7	–1.9
Sierra Leone	44.4	0.3
Uganda	44.2	3.7
Cameroon	43.5	4.7
Togo	43.0	3.8
Nigeria	41.2	2.8
Niger	40.4	3.7
Comoros	38.7	–0.2
Benin	38.2	5.5
Chad	37.9	6.2
Malawi	37.6	7.7
Ghana	35.8	3.5
Gambia	33.2	2.7
Burkina Faso	32.1	4.7
Mozambique	31.6	3.9
Madagascar	30.0	1.4
Kenya	27.0	0.9
Mauritania	25.2	5.6
Cote d'ivoire	23.8	1.4
Guinea	23.1	4.5
Sao Tome and Principe	20.7	4.1
Senegal	17.9	1.1
Zambia	17.3	–4.1
Eritrea	16.0	–
Equatorial Guinea	16.0	5.3
Swaziland	15.8	–0.3
Namibia	12.8	4.1
Cape Verde	12.0	4.1
Congo Republic	10.1	2.3
Mauritius	8.0	1.6
Angola	6.7	–4.9
Seychelles	4.1	–2.1
South Africa	3.8	0.9
Botswana	3.6	0.9
Congo, Dem. Rep.	–	1.0
Gabon	–	–1.4
Lesotho	–	0.7
Zimbabwe	–	3.5

Source: World Bank: World Development Indicator Database.

Table 2. African countries facing food insecurity and contributing factors.

Country	Contributing factor
Angola	Civil strife, population displacement
Burundi	Civil strife and insecurity
DRC	Civil strife, internal displaced persons (IDPs) and refugee
Eritrea	IDPs and returnees
Ethiopia	Drought, large number of vulnerable people, IDPs
Guinea-Bissau	Civil strife, population displacement
Kenya	Weather adversaries in parts of the country
Liberia	Impact of civil strife, shortage of farm inputs
Mauritania	Localised deficits
Rwanda	Insecurity in parts of the country
Somalia	Drought and civil strife
Sudan	Civil strife in the south
Tanzania	Food shortages in several regions
Uganda	Civil strife in parts of the country, IDPs and refugees

Source: After Joubert et al. (1999).

The real causes of hunger therefore are poverty, inequality and lack of access to food and land. With the rapid population growth, there is increasing cultivation of available land, which has important implications for ecosystem sustainability and biodiversity. In many areas, farmers are moving into lands less productive and more fragile and soil fertility is declining appreciably. For many years agricultural production in Africa has been increasing largely due to the movement of populations into new lands. There are still many countries (e.g., Nigeria, Mozambique, Democratic Republic of Congo and Uganda), where there remains a very large reservoir of high potential agricultural land; however, many other countries (e.g., Kenya, Rwanda, Malawi) have reached the extensive limit and are experiencing a reduction in the size of land holdings, increasing landlessness and increasing soil degradation. In many countries, however, domestic food production regularly falls short of national food requirements. Thus, annual food imports have become necessary to complement domestic production. With increasing competition in the allocation of hard currency for various alternative imports (e.g. energy) and persistent food production shortfalls, an increasing number of people will become more vulnerable to malnutrition and starvation unless domestic production is increased. For example, Eicher (1990, 1999) stated that by the year 2010, the number of chronically undernourished people in Africa would be 300 million, compared to 200 million during the period 1988 to 1990. This scenario is made worse considering that opportunities for extensive land cultivation and off-farm employment are limited against a background of low levels of technology adoption by farmers. Food for the urban poor depends upon jobs, income and low prices, while food for most of the rural poor depends upon their own labour and productivity of their often limited or fragile land resources.

1.2. Food security challenges in sub-Saharan Africa: the role of agricultural research and development

The Green Revolution helped Asia and Latin America to achieve self-sufficiency in food production (Chrispeels, 2000) and is a clear manifestation of the role of agricultural research and development in providing solutions to food insecurity problems. Unfortunately, sub-Saharan Africa missed the Green revolution and crop productivity per unit area are of land is the lowest in the world. For example, the production of sweet potato, a staple crop, is 6 t ha^{-1} compared to the global average of 14 t ha^{-1} while the average of maize is 1.7 t ha^{-1} compared to a world average of > 3.5 t ha^{-1}. Besides, for sub-Saharan Africa the solution is not a matter of fixing the distribution problem to solve food insecurity. Purchasing power too is a problem and most African countries cannot afford food export prices. In these circumstances, there is need for improved agriculture to bridge this yield gap and avoid food insecurity or dependence on foods imported from other countries.

There is urgent need for the development and use of agricultural biotechnology in Africa to help counter famine, environmental degradation and poverty. Africa must enthusiastically join the biotechnology revolution to probably compensate for the bi-passed Green Revolution. African countries need to have a forward-looking biotechnology policy. Biotechnology advances offer tremendous opportunities for increasing yields, reducing pest damage, protecting the environment and improving the nutritional value of many crops. The application of biotechnology to the problems of the poor will not be straightforward and the models from developed countries will probably not be applicable. Agriculture in developing countries does not need to be 'modernised' although it needs to be improved and environmentally friendly. The developing countries can skip the high input unsustainable phase through which agriculture is now passing in the developed countries and proceed from the bottom-up, not from the top down. Crops have to be created that fit not only in the agroecology of the poorest regions often characterised by marginal heterogeneous environments, but must also in the social and economic systems. Agricultural research has to start with studying farming systems (on-farm research), asking farmers-men and women-what they want, allowing the farmers to make choices between often conflicting objectives such as higher yields versus yield stability, and examining the possibility of marketing the excess production. Will the crop be used by women in their kitchen gardens or by men in their cash crop fields? Aid workers have to begin by soliciting the help of the farmers to describe farming practices and analyse these practices to pinpoint problem areas and opportunities. Together, the aid workers and farmers have to generate a range of choices that farmers could implement. The major objective of this approach is transfer of technology to empower the farmer to improve production.

Increased investment in agricultural research and development is a must but this should be backed by political stability, sound economic policies and invigorated institutions tailored to geological realities. There are many aspects of providing food for the poor that are well beyond the control of either laboratory scientists or agricultural advisors in the field. Poor countries must realise that agriculture can be an important engine of economic growth and therefore must invest more

in agricultural research. The governments need to encourage agricultural development and create rural infrastructure that will permit crop surpluses to be marketed. If agricultural productivity is very low in Africa for ecological and climatological reasons, perhaps the real lesson is that growth should be led much more by outward-oriented industry and services, rather than blindly making yet another attempt to transplant integrated rural development strategies from other parts of the world without customising them to Africa's unique conditions. However, a food programme for Africa must be intimately related to the needs of the rest of the world. The aim should not only be self-sufficiency but should be to become a major net supplier to the rest of the world. No matter how successful Africa's efforts are to industrialize, it remains a fact that Africa will be for many generations, primarily a producer of agricultural and other primary products. We must learn to do it well and on a rapidly growing scale. This will require massive frontal attack, not only on the research needs but also on the practical problems of production, storage and marketing (Eicher, 1999).

It should be noted that much of the needed food can be produced by smallholder farmers using improved technologies (Uphoff and Altieri, 1999). In fact, new rural development approaches and low-input technologies spearheaded by farmers and non-governmental organisations are making a significant contribution to food security at household, national and regional levels in some parts of Africa (Pretty, 1995). Yield increases are being achieved by using technological approaches, based on agroecological principles that emphasize diversity, synergy, recycling and integration and social processes that emphasize community participation and empowerment (Rosset, 1999). When such features are optimised, yield enhancement and stability of production are achieved, as well as a series of ecological services such as conservation, improved natural pest regulation mechanisms, etc. (Altieri, 1996). These results can be used to achieve food security and environmental preservation in developing countries, but their potential and further spread depends on investments, policies, institutional support and attitude changes on the part of policy makers and scientific community (Binswanger, 1998). Failure to promote such people-centred agricultural research and development due to diversion of funds and expertise will forego an historical opportunity to raise agricultural productivity in economical viable, environmentally benign and socially uplifting ways. It is clear that food security for a growing African population is more important than food self-sufficiency and it should, therefore, be the major thrust in formulating new agricultural policies.

1.3. The arid and semi-arid areas of Kenya – their extend and climatic conditions

The arid and semi-arid lands (ASALs) cover approximately 80% of the total land surface of Kenya (Figure 1) and support slightly over 25% of the population, owning over 50% of Kenya's livestock population (Langat and Magwata, 1994). About 60% of the country's cattle population, 70% of its sheep and goats, and over 100% of the camels are found in the ASALs (Brown, 1994). ASALs are subject to low and erratic rainfall with great intra-and inter-annual variations. This variability becomes

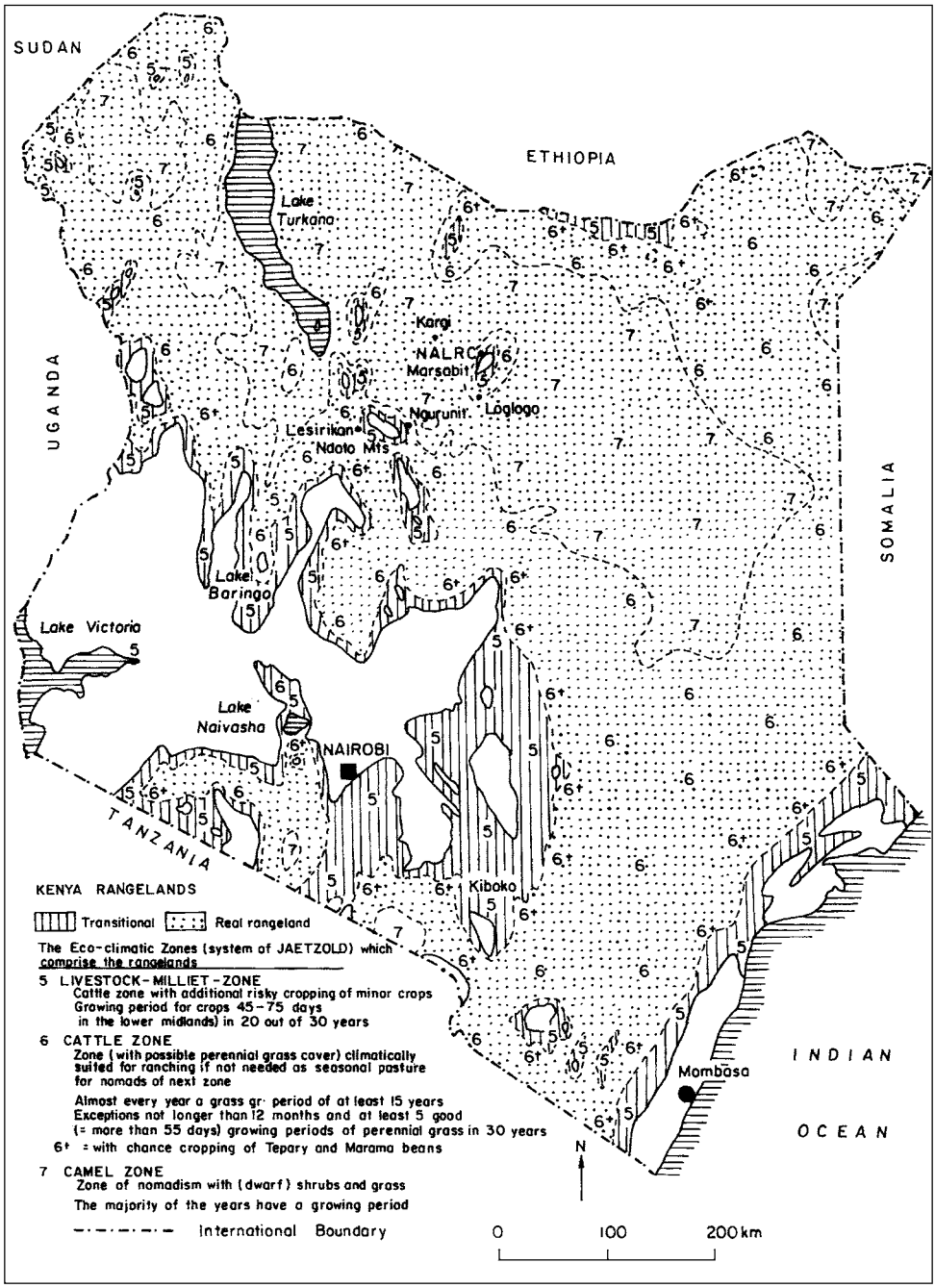

Figure 1. Eco-climatic classification of semi-arid and arid lands of Kenya (Source: After Hornetz, et al., 1992).

even greater as the mean annual rainfall decreases. Characteristic of these areas is the high intensity storms (over 40 mm day^{-1}), which produce considerable runoff in the absence of tree or bush cover. The rainfall regime is generally bimodal with two peaks in November and April. The total annual rainfall ranges between 120–700 mm (Jaetzold, 1994), while potential evapotranspiration ranges between 1800–2500 mm. Evapotranspiration is a direct function of the prevailing temperatures and altitude. Most of the ASALs are found at an altitude below 1500 m. Soils are generally classified as sandy loams to loamy sands, and light to medium texture inherently low in fertility and cation exchange capacity. These soils are prone to compaction and capping. The native vegetation is mainly of the Acacia-thorn savannah (semi-arid areas) and dwarf shrub (arid areas) type.

In the greater part of the arid areas, permanent rivers are lacking and only seasonal rivers prevail. In these drier parts, underground water resources are more important although quantities are generally very low. Water quality and quantity for both human and livestock consumption is often insufficient. Drought, which is a recurrent phenomenon in these arid areas, causes great livestock and crop losses resulting in famine, destitution, poverty and heavy reliance on famine relief handouts. Because of the natural climatic limitations in the ASALs, livestock rearing remains the single most important source of subsistence for the inhabitants of the arid lands. Agropastoralism, ranching and pure pastoralism are the main forms of land use. Crop farming is, however, practised in the eco-climatic zone 5, or the so called 'boundary zone of rainfed agriculture' (Shisanya, 1996; Jaetzold and Schmidt, 1983), although there is normally a high risk of crop failure. Large tracts of land in the arid zone are under National Parks and Game Reserves. The exploitation of these marginal areas certainly requires a certain level of management skills to make them more productive and sustainable – skills that the new settlers in these areas do not possess (Quaye, 1994). The eventual mismanagement of such lands results in degradation and a subsequent decrease in the productivity of the agricultural systems.

1.4. The need for agroecologically suitable 'marginal' food crops for the sub-Saharan African drylands – the Kenyan example

Kenya's ASALs have become the destination of unprecedented immigration, which has led to land use conflict and resource degradation (Hornetz, 1997). In the last three decades or so, there has been considerable human migration from the high potential areas to the medium potential areas because of population pressure in the former areas. The influx of population from the high potential areas to the medium and low potential areas is likely to accelerate (Shisanya, 1999). Today, it can be observed that settlers are moving into the wetter parts of the Ranching zone, i.e. Lower – Lower Midland agroecological zone 6 (Shisanya, 1996). This zone is only suitable for rainfed marginal cropping cultivation. According to the government policy as outlined in the five-year Development Plan one third of the projected increase in agricultural food production in Kenya was expected to come from the new acreage largely in these marginal areas (Republic of Kenya, 1993). Thus, it would seem that these areas will continued to play an important role with regard to both human settlement as well as the production of subsistence food crops.

The current National Development Plan stresses the fact that ASALs will receive increasing attention albeit at a higher cost in recognition of their important contribution to the national development (Republic of Kenya, 2002).

The major environmental factors limiting crop production in the ASALs of Kenya are high potential evaporation and rainfall, with the latter being variable and unpredictable in space and time (Keya, 1998). It should be noted that these areas have only short to very short agrohumid periods (AHPs) (potential growing periods) of about 40–45 and 85–105 days (Hornetz et al., 2000) with low soil moisture supply during the main part of the rainy season and frequent dry spells (Hornetz, 1997). Apart from environmental limitations, the new farming communities in these ASALs lack the background knowledge in selecting crops and farming strategies well suited to the stabilization and maximization of food production in their diminished rainfall circumstances (Shisanya, 1999). As Hornetz (1997) had earlier observed, the new communities in these ASALs are unconscious of the carrying capacity of their new fields, hence are aggravating the problems of desertification and finally causing food crisis and famine.

Though it can be observed in the relatively wetter parts of the drylands that smallholder farmers have started to cultivate drought resistant leguminous crops like pigeon peas (*Cajanus cajan*) and cowpeas (*Vigna unguiculata*), there is still a lack of ecologically adapted crops in the potential cropping areas (Hornetz, 1997). This is particularly the case in the drier parts of the agroecological zones Lower-Lower midland 5 and 6, according to Jaetzold and Schmidt (1983), which have until recently been dominated by extensive grazing systems. The reason for this is that on the one hand the pastoralists have little interest in cultivating food and fodder crops, while on the other hand the new settlers from the high potential areas generally possess seeds of crops with longer vegetation cycles and less ability to adapt to high temperature and water stress typical of their new environment (Hornetz, 1997).

A number of researchers have proposed various strategies that need to be adopted in Kenya for the development of sustainable food production systems in the ASALs and at the same time reducing the vulnerability of small-scale farmers to crop failures (Jaetzold and Schmidt, 1983; Zoebisch, 1986; Hornetz, 1988; 1990; 1991a; 1991b; 1997; Maingi et al., 1999; Hornetz et al., 2000; Gitonga et al., 1999; Shisanya, 1996; 1998; 2002; Maingi et al., 2001). Some of the proposed agroecologically suitable farming strategies include: ‘water harvesting’ systems (e.g. Matuta), run-off irrigation, agroforestry and the introduction of drought resistant ‘minor crops’, and use of biofertilizers. Already, some of these proposed measures have been operationalised in the ASALs of Kenya (Shisanya, 2002; Maingi et al., 2001; Hornetz et al., 2000 and Gitonga et al., 1999).

2. IMPROVEMENT OF TEPARY BEAN YIELDS THROUGH BIOLOGICAL NITROGEN FIXATION IN SEMI-ARID KENYA

2.1. Biological nitrogen fixation: potentials and limitations

There has been tremendous interest in biological nitrogen fixation as an alternative to mineral fertilisers in agricultural systems (Gitonga et al., 1999; Hornetz et al., 2000; Maingi et al., 2001; Shisanya, 2002). The Kenyan government has encouraged complementation of inorganic fertilisers through alternatives such as biofertilizers (using N_2 – fixing agents) as pointed out in the National Development Plan (Republic of Kenya, 2002). Biological nitrogen fixation (BNF) contributes to productivity both directly, where the fixed N_2 is harvested in grains or other food for human and animal consumption or indirectly through enhancement of soil fertility (Giller and Cadish, 1995). Biological nitrogen fixation represents a particularly renewable source of N for many farming systems (Peoples and Jensen, 1999). Global estimates of BNF to soil nitrogen vary from 135–175 million tonnes annually (Paul, 1988). The calculated annual amounts fixed range from 3–160 kg N ha^{-1} yr^{-1} shoot N for annual pasture species, 37–128 kg N ha^{-1} yr^{-1} for lucern, and 14–160 kg N ha^{-1} yr^{-1} for pulses (Peoples et al., 2001). Other sources of nitrogen accumulation in the soil include decomposition of organic matter, action of lightning and use of N fertilisers among others.

Legumes of all categories contribute to N fertility in various cropping practices including fallow, agroforestry and rotational systems. Low input legume-based agriculture exists in a continuum between subsistence farming and intensive arable and pastoral systems. Pastoral systems reliant solely on fixed N are capable of moderately high production with modest N losses (Ledgard, 2001). The principal factors regulating BNF can be summarised in terms of environmental or management constraints to legume growth (basic agronomy, nutrition, water supply, diseases and pests) or result from local practices that impact on percentage N fixation (Peoples and Jensen, 1999). The low moisture contents in soils of Sahelian Africa can reduce nodule functioning in symbiotic legumes through drought-induced collapse of lenticels, decreased nitrogenase activity, and reduced respiratory capacity of bacteroids and decline in leghaemoglobin content of nodules (Guerin et al., 1990). In many parts of the world, reduced water supply limits fixation in the field. In the semi-arid tropics, crops often suffer water stress for various periods during the growth cycle (Hornetz et al., 2001). Drought can also affect longevity of introduced rhizobia and a decline occurs with low moisture and soil desiccation (Hornetz et al., 2000). Consequently, nodulation fails to occur through loss of infection sites due to changes in the morphology of infectible root hairs (Sprent and Sprent, 1990). In the tropics, legumes have the ability to produce nodules on more acid soils and soils deficient in phosphorus (P) and calcium (Ca), and other nutrients than in temperate countries (Shisanya, 2002). In the temperate regions nodulation is poor in seasons characterised by low light intensity (Ledgard, 2001).

A study in Australia found that N fixation was primarily regulated by biomass production and that both pasture and crop legumes fixed between 20 and 25 kg shoot N for every tonne of shoot dry matter (DM) produced (Peoples et al., 2001).

Although pulses often fixed more N than pastures, legume – dominant pastures provided greater net inputs of fixed N, since a much larger fraction of the total plant N was removed when pulses were harvested for grain than was estimated to be removed or lost from grazed pastures. The conclusions about the relative size of the contributions of fixed N to the N – economies of different farming systems depend upon the inclusion or emission of an estimate of fixed N associated with the nodulated roots (Peoples et al., 2001). The net amounts of fixed N remaining after each year of either legume – based pasture or pulse crop were calculated to be sufficient to balance the N removed by at least one subsequent non – legume crop only when below – ground N components were included. This has important implications for the interpretation of the results of previous N fixation studies undertaken all over the world, which have either ignored or underestimated the nodulated root when evaluating the contributions of fixed N to rotations.

Few studies have assessed the direct effects of drought on legume symbiotic performances in Africa. A study by Schulze et al. (1991) in the desert and savannah areas of Namibia has shown that symbiotic Acacias spend more water per unit carbon assimilated than legumes. The water spent on carbon assimilation probably represents the cost of supplying extra carbohydrate for N_2 fixation (Danso et al., 1992). There is a trade-off in photosynthesis, when plant stomata are open, allowing CO_2 to diffuse inward; O_2 and H_2O diffuse outward to the atmosphere (Schlesinger, 1997). The loss of water relative to photosynthesis is often expressed as water use efficiency (WUE), i.e.:

$$\text{WUE} = \frac{\text{mmole of } CO_2 \text{ fixed}}{\text{Moles of } H_2O \text{ lost}} \tag{1}$$

or

$$\text{WUE} = \frac{\text{mol } CO_2 \text{ fixed}}{\text{Mol } H_2O \text{ transpired}} \tag{2}$$

For most plants, WUE (Equations 1 and 2) typically range from 0.86 to 1.50 mmol/mole depending on environmental conditions (Ceulemans and Mousseau, 1994). High water loss per CO_2 fixed is generally not a problem so long as plenty of water is available for transpiration (Nobel, 1991). Symbiotic response to physiological stress is a highly variable parameter in which tolerance or susceptibility seems to result from heritable factors in both the host and the rhizobium (Bitangi, 1985). In soil, a particular bacterial strain has to survive fluctuations in temperature, moisture and ion content, and able to compete successfully for nutrients with a wide variety of microorganisms (Hornetz et al., 2000). Although few legumes are able to tolerate rising temperatures (Hornetz et al., 2001), the thermal effects of high temperature in Sahelian Africa in particular are likely to adversely affect legume symbiotic activity (Hornetz, 1990). Plants that grow in arid regions and their microsymbionts must have mechanisms for temperature tolerance (Hornetz et al., 2001).

2.2. The tepary bean

Tepary bean, which belongs to the genus *Phaseolus* and subfamily Papilionoideae of the family *Leguminasea*, originates from the semi-deserts and deserts of NW-Mexico and SW-USA. It is native to the Sonoran Desert and has been grown here for over 5000 years, predominantly by means of 'flood water farming agriculture' (Nabhan and Felger, 1978). The first scientific papers on tepary beans appeared at the beginning of the 20th century (Freeman, 1913; Hendry, 1919), the emphasis being placed on the botanical and ethno-historical aspects. The period 1915–1930 could be referred to as the 'tepary boom' (Nabhan and Felger, 1978) because it was the first time that the crop was incorporated into the Dryland Farming Project at the Arizona Agricultural Experimental Station.

From 1960, there were increased research activities on tepary beans, particularly the biochemical and biophysical characteristics of the crop (Coyne and Serrano, 1963; Sullivan and Kinbacher, 1967). The drought, heat and disease resistance properties of the crop were exploited and used in cross-breading programmes with other less environmentally-hardened *Phaseolus vulgaris* L. varieties during the 1970s (Mok , 1970). Tepary beans seem to possess capabilities for drought-resistance and gives good yield in arid regions that are too dry for other beans. The results of biochemical analysis (Coyne and Serrano, 1963) showed that the plant produces high amounts of soluble solids such as glucose and sucrose in under sufficient or insufficient water supply.

2.3. State of the art in tepary bean research in Kenya

The government of Kenya has recognized the important role the ASALs play and will continue to play with respect to human settlement as well as production of subsistence crops (Republic of Kenya, 1993). Currently, food production in the ASALs has lagged behind population growth and as such there is an urgent need to step-up food production for the expanding population (Bohlool et al., 1992). One of the major steps towards increasing food production in the ASALs is the use of modern technologies in agriculture and selection of suitable crop cultivars. Dow (1989) emphasized the need for research on drought tolerant crop species of short vegetative cycle, e.g. *Pima-Papago* maize (*Zea mays*) varieties (Tohono *O'odham* Z16) and tepary beans (TB) (*Phaseolus acutifolius*), as one of the special issues in development related to drought, desertification and food deficit in Africa. Unfortunately, the use of the above mentioned technologies in the ASALs have not been adequately adapted because of socio-economic constraints (Shisanya, 1999). Most farmers in the ASALs are resource poor and cannot afford the required inputs, mainly in the form of chemical N fertilizers. Legume-*Rhizobium* has been exploited elsewhere as a substitute for the N fertilizers (Maingi et al., 1999; Gitonga et al., 1999; Hornetz et al., 2000; Maingi et al., 2001). This technology uses the *Rhizobium*-legume symbiosis that has become particularly important because it has shown very high rates of N_2 fixation (Zargar and Kahlon, 1995).

There is very little research work that has been carried out on the effectiveness of N_2 fixation of indigenous (natural) and inoculated (host-specific) rhizobia strains

on drought-adapted legumes, e.g. TB in semi-arid SE-Kenya (Gitonga et al., 1999; Maingi et al., 2001). The study by Pilbeam et al. (1995) found significant N increases in the plant tissues of drought-adapted cowpea (*Vigna unguiculata* L. Walp) in the study area. Additionally, N fertilizer application had no significant effect on the dry matter yield and N content of common bean (*Phaseolus vulgaris* L. cv. B9). A limiting factor in the dry lands of southeast Kenya is that the prevalent soils, i.e. Fluvisols, Luvisols and Ferralsols (Eichinger, 1999) release important available plant nutrients only after a very short period of cultivation and through leaching (Hornetz, 1997). Systematic research on the eco-physiological demands, drought resistance and yield potentials of TB in potential cultivation areas in the ASALs of southeast and northern Kenya began in the late 1980s and intensified in the 1990s (Hornetz, 1988, 1990, 1991a, b, 1997; and Shisanya, 1996, 1998). No research attention has been given to N_2 fixation in TB, which has recently become a prominent legume crop among resource poor small-scale farmers in SE-Kenya (Shisanya, 1999). The main objective of this study was therefore to investigate N_2 fixation in TB through inoculation by different host-specific rhizobia strains under the semi-arid environment of SE-Kenya.

2.4. Biological nitrogen fixation experiments with tepary bean in semi-arid Kenya

2.4.1. *Experimental site*

The experiments were carried out at KARI Kiboko sub-centre (latitude 02°12′ S, longitude 37°43′ E, altitude 975 m a.s.l.), located at about 160 km southeast of Nairobi, the capital town of Kenya (Figure 1). The soils of the study area are well drained Fluvisols, Ferralsols and Luvisols (Eichinger, 1999). The soil pH of the experimental field is 7.9 (measured in 0.01 ML^{-1} $CaCl_2$). Rainfall is bimodally distributed, with median monthly maximum in April (126 mm) and November (138 mm). The medial annual rainfall is about 582 mm yr^{-1}. The SR (October–January) generally has more rainfall and are more reliable than the LR (March–June) (Musembi and Griffiths, 1986). The lengths of the agrohumid periods for drought-adapted crops are 50–55 days (LR) and 65–70 days (SR) (Jaetzold and Schmidt, 1983). Average monthly temperatures are highest in February (24.3 °C) and October (23.4 °C) (KMD, 1984), prior to the onset of the rains in March and November, respectively.

2.4.2. *Soil sampling and analyses*

Soil samples were collected to a depth of 60 cm using a soil auger before planting. Five sub-samples were collected from each plot, bulked, mixed thoroughly in polythene bags and transported to the laboratory. Sub-sample was air-dried while the remainder was refrigerated at 4 °C. The air-dried samples were sieved (2 mm) and a sub-sample was further ground to pass through a 0.25 mm sieve for total C and N analyses. Carbon and nitrogen contents in the soil were determined. The

colorimetric method described by Anderson and Ingram (1993) was used for soil organic C. Total N was measured colorimetrically following Kjeldahl digestion (Bremner and Mulvaney, 1982). Soil P was determined following the method of Foster (1995). Analyses of soil N and P indicate a deficiency of both nutrients (0.7 mg N/g soil and 3.0 mg P/kg soil in 0–60 cm soil depth, respectively). These values are below the threshold values of 2.0 mg N/kg soil and 4.5 mg P/kg soil for optimum crop growth in tropical soils, respectively (Kapkiyai et al., 1999). The C/N ratio and CEC are 11.7 and 7.8 mmol/kg, respectively.

2.5. Greenhouse experiments

Glass growth tubes were used as the growth containers while the growth medium was nutrient free vermiculite. Pre-germinated seeds (Somarsegaran and Hoben, 1985) were transferred into the glass growth tubes, one seedling per tube. Uninoculated control plants were used as material control to detect contamination over the growth period in the greenhouse experiment. The Rhizobia were routinely grown on yeast extract mannitol (YEMA). For inoculation purposes, the rhizobia were grown in yeast extract mannitol broth (YEMB). Isolation, presumptive and authentication tests were conducted according to the methods described by Somasegaran and Hoben (1985). The tests carried out were Gram staining, growth of isolates on YEMA, growth on YEMA plus Bromothymol blue (BTB) and growth of isolates YEMA plus Congo red media. The plates were incubated in darkness at 20 °C for 3–5 days. The commercial Rhizobia strains used, i.e. R446 and R3254 were obtained from Microbiological Resource Centre (MIRCEN), University of Nairobi, while strains R578 and R579 were obtained from the Radizin Institut, Iserlohn, Germany. A native strain RTB was isolated from Kiboko soils where tepary beans had been grown.

For assessment of effectiveness of the various Rhizobia strains in nitrogen fixation, a total of seven treatments were used. The experimental design was a 7×7 Latin square with 7 treatments. This design was chosen because it eliminates row and column effects from the experimental error thus increasing the power of ANOVA (Steel and Torrie, 1981). Each treatment constituted 7 replications. The treatments were as follows:

(1)	Nitrogen (N)	tepary bean grown in N rich medium
(2)	Control	no inoculation of tepary bean and no N
(3)	R578	tepary bean inoculated with rhizobium strain R578
(4)	R446	tepary bean inoculated with rhizobium strain R446
(5)	R579	tepary bean inoculated with rhizobium strain R579
(6)	R3254	tepary bean inoculated with rhizobium strain R3254
(7)	RTB	tepary bean inoculated with rhizobium strain RTB

Nodule assessment was carried out in the greenhouse as described by Gitonga et al. (1999). The number of Rhizobia in the soil was determined using the MPN plant infection technique as described by Beck et al. (1993) (equation 3)

$$MPN = (\mathbf{m} \times \mathbf{d})/\mathbf{v} \quad (3)$$

where: **m** is the most likely number, **d** is the lowest dilution, and **v** is the aliquot used for inoculation

The plants in the glass growth tubes were harvested after 30 days. The plant material was separated into shoots, roots and nodules and dried to constant weight at 70 °C. The dry weights were determined using a high precision digital Sartorius weighing balance (U6100-D2, Sartorius, Göttingen).

2.7. Determination of nitrogen concentration in plant tissues

Due to high cost of nitrogen analysis, final field harvest plant samples were analysed for nitrogen. The plants were dried to constant weight and the dry matter weights recorded. Thereafter, the dry matter was separated into above ground (shoot) and below ground (root) tissues. The shoot, root and seeds were ground into powder using a grinding mill type 1029-A (Yoshida Seishakusho Co. Ltd., Japan). Nitrogen was analysed in the shoot, root and seeds using the highly sensitive automatic nitrogen-carbon analyzer (SUMIGRAPH NC-90 A). 10 mg of each sample were weighed into a boat and inserted into the reaction tube of the analyzer that was then sealed. The analyzer completely combusted the sample in a circulating oxygen current with an oxidation catalyst, converting the component nitrogen and carbon to N_2 and CO_2 gases, respectively for detection by a TCD gas chromatograph and simultaneous quantification by the data processor. Nitrogen concentration of the sample was calculated as follows:

a) Using equation (4) below, nitrogen coefficient F_N was obtained per peak integral value of N_2 for the known standard sample.
b) The calculated coefficient F_N was used in equation (5) to calculate the total nitrogen concentration, which was then recorded. The percentage N concentration was multiplied by the respective plant tissue dry weights to get the actual N concentrations as mg N/g dry matter.

$$F_N = \frac{N_{ST}}{NP_{ST} - NP_{BL} \times 100} \quad (4)$$

$$T_N\ (\%\ N) = \frac{F_N \times (NP_{SA} - NP_{BL})}{SA} \quad (5)$$

where:

N_{ST} = Nitrogen content of the standard sample
NP_{ST} = N_2 peak integral value of the standard sample
NP_{BL} = N_2 peak integral value of the operating blank
NP_{SA} = N_2 peak integral value of the unknown sample
SA = weight of the unknown sample in mg.

2.8. Field experiments

The greenhouse experimental design and treatments were replicated in the field. The experiments were carried out during the LR season of 1999 (April–June) and SR season of 1999/2000 (October–January). Land preparation was done by a disc plough followed by disc harrowing. A basal dose triple superphosphate (TSP) fertilizer was applied at the rate of 40 kg ha^{-1} on all the plots to alleviate phosphorus deficiency. The crop was dry planted on 1st of April and 31st October 1999 during the two seasons, respectively. Each experimental replicate constituted a 3×3 m plot. Tepary beans were sown in rows 50 cm apart with spacing of 20 cm giving a plant density of 100,000 plants ha^{-1}. This is the recommended density for TB in these semi-arid areas of SE-Kenya (Shisanya, 1998). Undamaged seeds of uniform colour and size were selected for the experiments. Before planting, inoculation with the respective rhizobia was carried out by adding gum *Arabica* sticker material to the filter mud carrier (Kibunja, 1984). They were thoroughly mixed with tepary bean seeds and a little water added. For each of the treatments 3 seeds were planted per hole. During the first weeding (7 days after planting (DAE)) plants were thinned to two per hole. At 10 DAE, when the plants were about 20 cm above ground, calcium ammonium nitrate (CAN) powder (26% N) was top-dressed on N treatment plots at the rate of 40 kg ha^{-1}.

Plants were sampled at 21 DAE, 42 DAE and at physiological maturity (70 DAE). Four plants were randomly sampled from each treatment replicated and various parameters assessed. The plants were dug up for nodule counting and determination of below and above ground biomass. Plant samples and nodules were dried to constant weight at 70 °C in an oven. Dry weights were determined using a high precision Sartorius balance. Average plot yield per treatment was used to calculate yield ha^{-1}. All data were subjected to ANOVA using the statistical computer package STATGRAPHICS and treatment means separated using Duncan's multiple range test at $P \leq 0.05$ level (Steel and Torrie, 1981).

2.9. Results

2.9.1. *MPN counts*

The most likely number of Rhizobia specific to TB was calculated from the MPN results (Table 3), according to the method of Vincent (1970) (see also

Table 3. Nodulated units in tepary bean MPN greenhouse experiment.[a]

Soil dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
No. of tubes with nodulation	4	4	3	0	0	0	0	0	0	0

[a] Number of replications (n) = 4; Dilution steps = 10; Lowest dilution = 10^{-1}; number of tubes with nodulation = 11 (Source: after Shisanya, 2002).

equation 3). The Rhizobia capable of nodulating TB in the soils of SE-Kenya was 1.0×10^2 cells per gram of soil.

2.9.2. *Nodulation status and dry weight of TB in greenhouse experiment*

There were significant differences ($P \leq 0.05$) in nodule number and hence nodule dry weight between the treatments (Table 4). Treatment R3254 had the highest number of nodules and hence nodule dry weight. At harvest, no significant differences in root and shoot dry weights between N and R3254 treatments were observed. However, these treatments differed significantly in the two parameters from the other treatments (Table 4).

Table 4. Effectiveness of rhizobia strains in nitrogen fixation in tepary bean during greenhouse experiment.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
Nitrogen (N*)	3 c	2.8 d	0.7 a	5.6 a
Control	0 d	0.0 e	0.2 c	1.5 c
TB + R3254	40 a	17.8 a	0.8 a	5.8 a
TB + R446	10 b	7.1 b	0.5 b	4.2 b
TB + RTB	14 b	10.5 b	0.6 b	4.3 b
TB + R578	4 c	3.0 d	0.5 b	3.8 b
TB + R579	7 c	6.1 c	0.6 b	4.0 b

Means ($n = 7$) followed by the same letter down the column are not significantly different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen medium incorporating 5 mM $Ca(NO_3)_2$ $4H_2O$ in the solution.
(Source: after Shisanya, 2002)

2.9.3. *The LR season field results*

The total amount of rainfall received during the growing season was 223 mm. There were significant differences ($P \leq 0.05$) in nodule number and the corresponding nodule dry weight between treatment R3254 and the rest of the other treatments (Table 5). Treatment R3254 had the highest number of nodules at this stage of growth. The rest of the treatments had the same number of nodules. However, no significant differences in total plant dry weight between the different treatments, 21 DAE, were observed (Table 5).

The pattern observed 21 DAE was similar to that at 42 DAE, except for the pod dry weight and plant dry weight (Table 6). Treatment R3254 had significantly ($P \leq 0.05$) higher pod numbers per plant and hence pods dry weight. There was no significant difference ($P \leq 0.05$) in plant dry weight between the N and R3254 treatments. The other treatments had similar plant dry weights (Table 6). At final harvest, the R3254 treatment had significantly ($P \leq 0.05$) higher pod dry weight, plant dry weight, 100 seed weight, seed weight per plot and grain yield (Table 7).

Table 5. Effects of rhizobia inoculation on growth and nodulation of TB 21 DAE during the LR season of 1999.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Plant dry weight (g plant^{-1})
TB + N*	10 b	4.9 b	2.8 a
Control	10 b	4.8 b	2.2 a
TB + R3254	16 a	7.8 a	2.7 a
TB + R446	10 b	4.7 b	2.6 a
TB + RTB	10 b	4.8 b	2.5 a
TB + R578	10 b	4.6 b	2.7 a
TB + R 579	10 b	5.0 b	2.4 a

Means (n = 28) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder. Source: After Shisanya, 2002.

Table 6. Effects of rhizobia inoculation on growth and nodulation of TB 42 DAE during the LR season of 1999.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Pod number (plant^{-1})	Pod dry weight (g plant^{-1})	Plant dry weight (g plant^{-1})
TB + N*	20 b	5.2 b	24 b	25.3 b	11.6 a
Control	20 b	5.0 b	22 b	19.7 b	8.5 b
TB + R3254	43 a	10.4 a	35 a	43.4 a	11.8 a
TB + R446	20 b	5.6 b	18 b	18.6 b	7.5 b
TB + RTB	20 b	5.3 b	21 b	23.4 b	9.5 b
TB + R578	20 b	5.5 b	23 b	25.3 b	9.6 b
TB + R579	20 b	5.4 b	23 b	18.8 b	8.3 b

Means (n = 28) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder. Source: After Shisanya, 2002.

2.9.4. *The SR season field results*

The total rainfall amount received during the growing season was 507 mm. Treatment R3254 had a significantly higher number of nodules per plant and hence nodule dry weight (Table 8). Like in the previous season, there were no significant differences in plant dry weights between the treatments, 21 DAE (Table 8).

At 42 DAE, tepary bean treated with R3254 had significantly ($P \leq 0.05$) higher nodule number; nodule dry weight, pod number and pod dry weight than the other treatments (Table 9). Like in the LR season. No significant differences were observed in plant dry weight between treatments N and R3254 at 42 DAE. However, at final harvest treatment R3254 had significantly ($P \leq 0.05$) higher pod dry weight, plant dry weight, 100 seed weight, seed weight per plot and grain yield

Table 7. Effects of rhizobia inoculation on growth and grain yield of TB at final harvest 70 DAE during the LR season of 1999.

Treatment	Pod dry weight (g plant^{-1})	Plant dry weight (g plant^{-1})	100 seed weight (g)	Seed weight (g plot^{-1})	Grain yield (kg ha^{-1})
TB+N*	2405.3 b	503.3 b	12.6 b	1776.4 b	935 c
Control	1945.3 b	419.4 b	12.5 b	1446.3 b	874 c
TB + R3254	3413.3 a	663.7 a	14.5 a	2827.4 a	1409 a
TB + R446	1949.3 b	402.1 b	12.5 b	1447.3 b	786 c
TB + RTB	1954.4 b	390.9 b	12.3 b	1454.3 b	824 c
TB + R578	1806.0 b	324.3 b	12.4 b	1333.7 b	782 c
TB + R579	2271.3 b	464.9 b	12.9 b	1700.3 b	1029 b

Means followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder.
Source: After Shisanya, 2002.

Table 8. Effects of rhizobia inoculation on growth and nodulation of TB 21 DAE during the SR season of 1999/2000.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Plant dry weight (g plant^{-1})
TB + N*	12 b	4.9 b	4.3 a
Control	10 b	4.7 b	3.0 a
TB + R3254	30 a	11.2 a	4.0 a
TB + R446	10 b	4.0 b	3.7 a
TB + RTB	14 b	5.1 b	3.5 a
TB + R578	12 b	5.0 b	3.3 a
TB + R579	15 b	5.2 b	3.3 a

Means ($n = 28$) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder.
Source: After Shisanya, 2002.

(Table 10). A similar trend as above was observed during the LR season (Table 7).

2.9.5. *Results of plant tissue N concentration*

The data in Table 11 show the N concentration in various plant tissue parts at final harvest during the LR and SR seasons. Plant tissues of treatment R3254 had consistently significant higher N concentrations than N and other treatments.

2.10. Discussion

The results obtained from Gram staining and growth of the isolate from TB root nodules in YEMA conformed to the standard cultural and morphological charac-

Table 9. Effects of rhizobia inoculation on growth and nodulation of TB 42 DAE during the SR season of 1999/2000.

Treatment	Nodule number (plant^{-1})	Nodule dry weight (mg plant^{-1})	Pod dry number (plant^{-1})	Pod dry weight (g plant^{-1})	Plant dry weight (g plant^{-1})
TB+N*	20 b	13.2 b	21 b	18.7 b	14.2 a
Control	19 b	13.0 b	17 b	14.4 b	9.5 b
TB + R3254	47 a	32.0 a	22 a	39.8 a	14.4 a
TB + R446	20 b	13.3 b	12 b	16.4 b	8.5 b
TB + RTB	20 b	13.0 b	20 b	15.1 b	11.3 b
TB + R578	20 b	13.2 b	32 b	15.7 b	10.0 b
TB + R579	18 b	12.8 b	15 b	17.3 b	9.5 b

Means (n = 28) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder. Source: After Shisanya, 2002.

Table 10. Effects of rhizobia inoculation on growth and grain yield of TB at final harvest 70 DAE during the SR season of 1999/2000.

Treatment	Pod dry weight (g plant^{-1})	Plant dry weight (g plant^{-1})	100 seed weight (g)	Seed weight (plot^{-1})	Grain yield (kg ha^{-1})
TB+N*	1871.4 b	518.9 b	12.2 b	1415.3 b	978 b
Control	1437.3 b	333.3 b	11.5 b	1069.4 b	850 c
TB + R3254	3978.0 a	755.3 a	13.3 a	3126.3 a	1576 a
TB + R446	1641.4 b	372.3 b	11.6 b	1304.3 b	793 c
TB + RTB	1512.6 b	333.7 b	11.7 b	1133.9 b	848 c
TB + R578	1569.0 b	329.1 b	11.5 b	1167.7 b	792 c
TB + R579	1730.3 b	410.0 b	12.0 b	1316.0 b	998 b

Means followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26 % N) powder. Source: after Shisanya, 2002.

teristics of *Rhizobium* sp. described by Vincent (1970) and Somasegaran and Hoben (1985). The isolate from tepary bean (RTB) did not absorb Congo red at all. On BTB medium, a colour change to yellow indicated production of acidic substances, which diffused into the medium. A change to blue colour would have indicated production of alkaline substances, which diffuse into the medium. This is common with fast-growing *Rhizobium* sp. and slow-growing *Bradyrhizobium* sp., respectively (Somarsegaran and Hoben, 1985). These tests helped in the screening of rhizobial isolates for contamination (Gitonga et al., 1999) and enabled the rejection of impure cultures.

From the MPN greenhouse experiment, the population of rhizobia specific to tepary bean was 1.0×10^2 cells g^{-1} of Kiboko soil (Table 3). These numbers are

Table 11. N concentration at harvest in TB plant tissues during the LR season 1999 and SR season of 1999/2000.

Treatment	LR season 1999			SR season 1999/2000		
	Shoot	Root	Seed	Shoot	Root	Seed
	N concentration (mg N/g dry matter)					
TB+N*	1.5 b	0.6 b	2.5 b	1.5 b	0.8 b	2.5 b
Control	1.1 b	0.5 b	2.0 b	1.3 b	0.6 b	2.0 b
TB + R3254	2.3 a	1.8 a	3.8 a	2.1 a	2.0 a	3.8 a
TB + R446	1.7 b	0.8 b	2.9 b	1.5 b	0.9 b	2.8 b
TB + RTB	1.6 b	0.6 b	2.8 b	1.5 b	0.8 b	2.9 b
TB + R578	1.3 b	0.7 b	2.3 b	1.4 b	0.7 b	2.5 b
TB + R579	1.5 b	0.8 b	2.4 b	1.5 b	0.9 b	2.3 b

Means (n = 4) followed by the same letter down the column are not statistically different ($P \leq 0.05$) by Duncan's multiple range test.
* Nitrogen (N) fertilizer was top-dressed 10 DAE at the rate of 40 kg ha^{-1} of CAN (26% N) powder.

above the threshold (50 rhizobia cells per gram of soil) at which inoculation may be excluded (Thies et al., 1991). The soils were collected during the dry season and it is also expected that during the rains rhizobia along with other soil micro-organisms, Rhizobia population number would increase (Gitonga et al., 1999; Maingi et al., 2001). These results support the findings of Nambiar et al. (1983) that most tropical soils have rhizobial population of at least 100 rhizobia cells per gram of soil capable of nodulating the legumes grown in such soil.

The number of nodules on the tepary bean in all the treatments was counted regardless of their size. The greenhouse results (Table 4) obtained on nodulation indicated that tepary bean inoculated with *Rhizobium* strains R3254, RTB and R446 had good nodulation, while those inoculated with strains R578, R579 and N treatment had poor nodulation. There was no nodulation in the control treatment. Overall, treatment R3254 had significantly higher ($P \leq 0.05$) nodule number than the other treatments (Table 4). These nodules were effective in N_2 fixation as evidenced by the pink colouration of the nodules indicating the presence of leghaemoglobin (Sprent and Sprent, 1990; Amara et al., 1995;). Nodule number is always used as a measure of infectiveness (Beck et al., 1993). The high number of nodules per plant in tepary bean inoculated with strain R3254 was evidence for the high infectiveness of this particular *Rhizobium* strain.

The plant test is the only confirmatory test for rhizobia (Vincent, 1970). Glass growth tubes were used for plant tests. This method, besides the Leonard jar assembly, has become a standard method for testing nodulation and nitrogen fixation under greenhouse conditions (Beck et al., 1993). The method is economical in water use and reduces the chances of bacterial contamination (Beck et al., 1993). The tubes occupy very little space in the greenhouse and provide good depth for growth and development of roots. Nitrogen production due to algae growth on media and in water has been shown to be minimal (Hornetz et al., 2000). Plant dry weight was used indirectly to estimate N_2 fixation in the present study. This method is the

best for screening large number of plants for nitrogen fixation in nitrogen free media (Halliday, 1984). The method is inexpensive and easy to use. However, this method is not sensitive enough to be used in soils with high nitrogen content. Sometimes other factors besides nitrogen do not permit the nitrogen fixed to be translated into increased dry matter yield (Danso, 1985). The method also cannot be used to compare nitrogen fixation in different varieties or species because the yields are also genetically determined (Danso, 1985).

In the greenhouse experiment, there was no significant difference ($P \leq 0.05$) between the N treatment and R3254 in shoot dry weight at harvest (Table 4). The rest of the other treatments had similar shoot dry weights at harvest. In field experiments, at 21 DAE, there were no significant differences in plant dry weights of tepary bean under field conditions over the two seasons (Tables 5 and 8). This could imply that the tepary bean plants were still deriving their nutrients from the seed and none had an advantage over the other. Similar observations have been documented in the study area by Gitonga et al. (1999) in green gram and Maingi et al. (2001). At final harvest, tepary bean inoculated with strain R3254 proved to be superior to the other treatments. This is an indication that commercially available *Rhizobium* strain R3254 had a significant effect on N_2 fixation compared to indigenous rhizobia (Wani et al., 1995). These results contradict those of Pilbeam et al. (1995), who reported that inoculation did not improve yield of common beans in the study area. It is appreciated that N_2 fixation by common beans is notoriously variable (Graham, 1981; Piha and Munns, 1987), depending on many biotic and abiotic factors. High soil temperatures and the incompatibility of indigenous rhizobial strains are contributory factors in the failure of beans to nodulate and so fix N_2 in semi-arid areas of Kenya (Pilbeam et al., 1995).

At 42 DAE over the two seasons, there was no significant difference ($P \leq 0.05$) in plant dry weight between the N and R3254 treatments (Tables 6 and 9). The other treatments had similar plant dry weights, which were significantly lower ($P \leq 0.05$) than the N and R3254 treatments. At final harvest, the N fertilized plots had significantly lower grain yield compared to treatment R3254 over the two seasons (Tables 7 and 10). Soil mineral N is often a major limitation to crop growth and productivity. Apparently this was not the case in the present study since increasing supply of mineral N by fertilizer additions had no significant effect on dry matter production or grain yield in both seasons. However, uptake of N may be constrained by low root length density, which is typical of tepary bean (Shisanya, 1998), and/or inadequate soil moisture content by which mineral N can move to the plant root (Pilbeam et al., 1995). Further, the aspect of fast mineralization of N under such semi-arid conditions cannot be ruled out (Hornetz, 1997; Eichinger, 1999). According to Ledgard et al. (1985), Danso et al. (1986, 1988), Hardarson et al. (1988) and Launauce (1996), among others, for temperate and cold climates, it seems that very little N is transferred to the plants in the short term period because of mineralization.

Plant tissue analysis on N concentration showed that plants inoculated with rhizobium strain R3254 were significantly enriched with tissue N concentration during the vegetative cycle compared to the other treatments (Table 11). This can be interpreted that strain R3254 is very infective and effective in N_2 fixation in

TB under the semi-arid environmental conditions of SE-Kenya. This would in the long run contribute to the sustainability of crop production system in the study area. The *Rhizobium* strain R3254 could be very useful to the resource poor farmers of SE-Kenya who farm purely for subsistence purposes without applying any fertilizer into the soil.

2.11. Chapter summary

This chapter highlighted the food security concerns in sub-Saharan Africa, their contributing factors and possible ways of ameliorating the situation. It is recognised that Africa must embrace agricultural biotechnology to help counter famine, environmental degradation and poverty. Biotechnology does offer tremendous opportunities for increasing crop yields, reducing pest damage, protecting the environment and improving nutritional value of crops. An example of how tepary bean legume yield has been increased in semi-arid Kenya through biological nitrogen fixation has been illustrated. It has been demonstrated that higher yields of tepary bean are achieved by inoculation with a commercially available infective and effective *Rhizobium* strain R3254. This strain is able to increase yield over and above nitrogen fertiliser application. It therefore offers a cheaper alternative to the resource poor farmers of semi-arid Kenya who cannot afford the expensive artificial N fertiliser.

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IMPACT OF OZONE ON CROPS

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1. INTRODUCTION

Ozone (O_3) is regarded as one of the most damaging air pollutants to which plants are exposed (Thompson, 1992). Over large rural areas of industrialized countries, its average monthly concentration increased during the last century to between 20 and 80 ppb (Lucas et al., 1993; Heath, 1994a). During episodes of severe air pollution, concentrations as high as 400 to 500 ppb have been monitored (Seinfeld, 1989). Ozone is a secondary pollutant resulting from photochemical reactions (mainly volatile organic compounds and nitrogen oxides). Under favourable meteorological conditions, ozone may accumulate in the troposphere and reach a level that causes significant decrease in growth and yield of ozone-sensitive species in many parts of the world. The problem of phytotoxicity is well established in Europe (Jäger et al., 1992) and North America (Heck et al., 1988). More recently, high concentrations of ozone have also been measured (Wahid et al., 1995).

Ozone enters the leaves through the stomata and diffuses within the apoplast. In this microenvironment it is intensively reactive and produces high levels of toxic compounds such as hydroxyl and superoxide radicals, hydrogen peroxide and other reactive oxygen species (Heath and Taylor, 1997; Pell et al., 1997). These active oxygen species react with proteins, lipids, and plasma membrane. Antioxidative defence activity systems may prevent this damage. Impact on plant crop yield ranges from minimal visible symptoms to substantial inhibition of productivity, including reduced photosynthetic capacity, enhanced rate of maintained respiration, and increased retention of fixed carbon in source leaves (Lefohn, 1992; Alscher and Wellburn, 1994). These plant responses can affect the plants' abilities to respond to further stress attacks. The action of ambient O_3 on the plant defence system enhances attack by pathogens but may lead to induce resistance (Sandermann et al., 1998). In addition, the impact of O_3 is profoundly influenced by other environmental factors.

Agricultural yield loss in the USA is approximately 1 to 2 billion dollars each year (US EPA, 1998). In addition to this considerable yield reduction, damaging of forest ecosystems and a reduction of lung functions in healthy people and people with an impaired respiratory system have been demonstrated. Despite the economic significance of these effects on crops, the mechanisms of the action of O_3 are still poorly understood.

A number of points discussed below have been described in an earlier review that describes the role of O_3 in phytotoxicity (Laurence and Weinstein, 1981; Gunderian, 1985; Heagle, 1989; Treshow and Anderson, 1989; Smith, 1990; Sandermann, 1996).

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2. THE TROPOSPHERICAL OZONE

For many years experts thought that tropospherical O_3 was formed exclusively in the stratosphere. It was assumed that O_3 was transported into the troposphere until it was destroyed through deposition (Junge, 1963). Experimental data have demonstrated that the photochemical production of O_3 from nitrogen oxides and volatile organic compounds is the major source of tropospherical O_3 (Gunderian, 1985). The overall reactions of the photochemical theory are well known (Stockwell et al., 1997). However, the exact relationship between precursor levels and O_3 concentrations is highly complex and not yet completely understood. Knowledge of this relationship is important to develop effective strategies for controlling ambient O_3 reductions. The production of O_3 depends on light intensity, whereas decomposition of O_3 also takes place in darkness. In winter, anthropogenic emission of NO and hydrocarbons are able to destroy O_3, whereas in summer, due to intensive sunshine anthropogenic O_3 is formed. Air polluted with O_3 is not confined to urban localities but also affects many rural locations.

Tropospheric O_3 levels are dependent upon the hour of day, season, geographical location and meteorological conditions (US EPA, 1986). At low elevation sites, a clear 24-hour periodicity is usually observed, with low concentration at night and maximum levels before midday. At high elevation sites, diurnal variation is not observed. Average daytime concentrations ranges from 35 to 55 ppb in summer, but at peak episodes of severe air pollution concentrations from 400 to 500 ppb have been measured.

Many O_3 dose exposures have been applied (Lefhon, 1992; Musselman et al., 1994). Examples of exposure indices are 7 or 8-hour seasonal average, the sum of all hourly mean concentrations above a threshold of 30, 40 ppb over a defined period, SUM06, SUM08 (sum of all mean concentrations above 60, 80 ppb), and AOT40 (sum of all hourly average O_3 concentrations above 40 ppb, for a defined period). A limit AOT40 value of 5.3 ppm has been proposed for crop plants, calculated for a period of 3 months at day-light hours (Fuhrer et al., 1997).

3. OZONE UPTAKE

Ozone is transported to the surface of plants by turbulent atmosphere. In leaves, cuticles represent an impermeable barrier (Kerstein and Ledzian, 1989) and thus, the main route of O_3 entry into the leaf is via the stomata. Stomatal apertures change in response to a multiplicity of environmental and internal factors in order to achieve fine adjustment of the gaseous conductance of leaves. It is difficult to generalize about the stomatal apertures caused by O_3 because the same concentration sometimes, can, in different circumstances, cause stomata to open or to close, and dose-response relationships may be erratic. Reduced stomatal conductance is commonly observed after O_3 exposition (Mansfield and Freer-Smith, 1984; Winner et al., 1988; Mansfield and Pearson, 1996). However, the control of stomatal aperture by O_3 has still to be elucidated. The flux of O_3 from the troposphere into leaves depends on different resistances at various levels. High O_3 concentrations are usually

observed in spring and summer and under these conditions (high irradiance, high temperature, low wind speed) boundary layer and stomatal resistances are high and, thus, limit O_3 uptake (Musselman et al., 1994).

Only the O_3 that are absorbed inside the plant directly affect metabolism. Analysis of O_3 uptake by plants is the key to understanding the phytotoxicity impact of O_3 on physiological and growth processes in plants. The dose-response curves are plots of physiological response against O_3 absorption. When O_3 uptake (Mansfield and Free-Smith, 1984; Reich, 1987; Winner et al., 1991) was used as the basis for comparison, responses in all plant species became comparable despite the differences in antioxidant status. This finding suggests a common biochemical mechanism for O_3 phytotoxicity.

Since O_3 penetrates the leaves via stomata, the first target of O_3 or its reactive species are the plasma membrane (Heath, 1980; Heath, 1987; Heath, 1994b; Heath and Taylor, 1997) of the mesophyll cells, which are severely damaged (changes in permeability, fluidity, and ionic and metabolic disturbances) if the detoxifying system of the apoplast is overcharged.

4. FATE OF OZONE

The fate of O_3 upon entry into the leaf is not well known. In order to study the reactions that may occur within the leaf it is necessary to know the chemical reactions of O_3 *in vitro*. However, a chemical reaction that participates in aqueous solutions does not necessarily take place in living organisms. It has been reported that, in aqueous solutions, O_3 decomposes to form reactive oxygen species (ROS), including $OH\cdot$ and $O^{2\cdot-}$ radicals (Grimes et al., 1983; Byvoet et al., 1995) but the question remains whether production rates are important in the leaves (Runeckles, 1992; Heath and Taylor, 1997). In addition, O_3 reacts with various solutes of the apoplast fluid, at reaction rate orders of a greater magnitude than with water (Lyons et al., 1999).

It has been reported that O_3 concentrations in intracellular spaces are close to zero (Laisk et al., 1989). This means that O_3 is absorbed and rapidly decomposed in the cell walls and plasmalemma (Figure 1). It does not penetrate into deeper layers of cells. O_3 decomposition at the cell wall and plasma membrane indicates detoxification, but the O_3-generated reactive oxygen species can be implicated in phytotoxic responses. Toxic oxyradicals such as superoxide anion ($O^{2\cdot-}$) have been suggested as being responsible for injury caused by O_3 (Alscher and Hess, 1993). The evidence for the role of $O^{2\cdot-}$ in O_3 phytotoxicity is indirect and controversial. Increased SOD activity in bean as being a result of O_3 exposure has been reported (Lee and Bennett, 1982). Electron paramagnetic resonance spectroscopy can be used to observe the spectra of free radicals directly. Free radicals measurements have been attempted by Mhelhorn et al. (1990), although identification of the specific radicals has not been provided. Direct observations of radical signals have revealed the appearance of a signal with the characteristics of the isotropic superoxide anion signal during exposure to low levels of O_3 (Runeckles and Vaarnou, 1997). Thus, initial reaction of O_3 could result in the

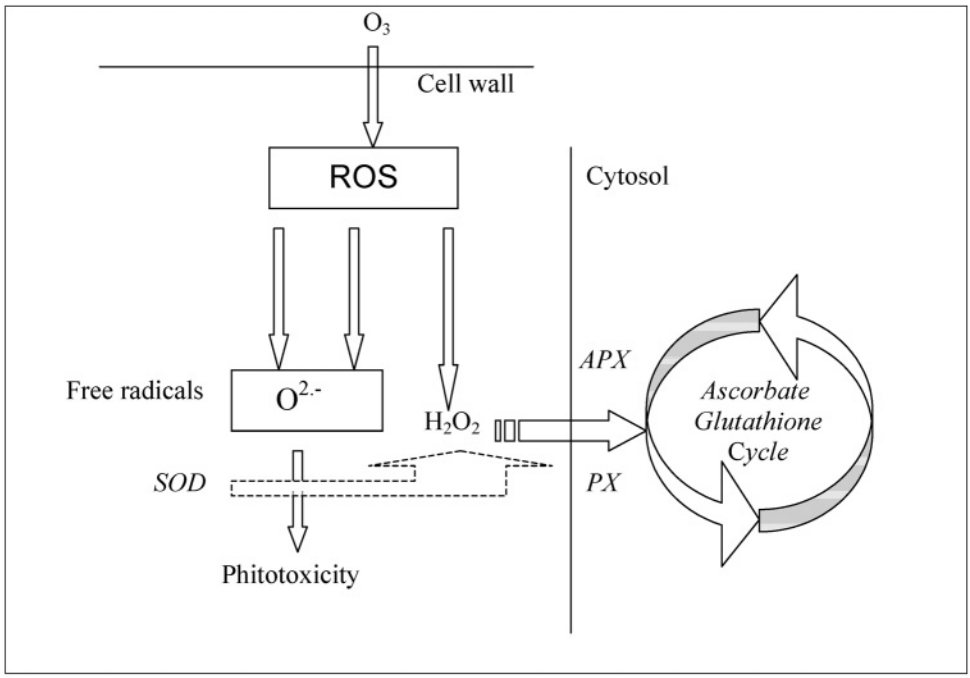

Figure 1. The postulated mechanism of ozone phitotoxicity within cell wall and cytosol and the enzymes that can react with the O_3-generated reactive oxygen species (after Mhelhorn, 1990; Alscher and Hess, 1993).

production of active oxygen species (Grimes et al., 1983; Pryor and Church, 1991; Byvoet et al., 1995).

5. OXIDATIVE STRESS

During normal metabolism of plant cells a variety of activated oxygen species are formed (Rubinstein and Luster, 1993; Asada, 1994; Fridovich, 1995). Oxygen toxicity is due primarily to activated oxygen species rather than to molecular oxygen itself (Scandalios, 1994). Oxidative stress is caused by exposition to reactive oxygen intermediates, such as superoxide anion ($O^{2\cdot-}$), hydrogen hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). Oxidative stress is an unavoidable by-product of aerobic life style, because activated oxygen species are formed whenever molecular oxygen oxidizes electron carriers chemically.

ROS are important metabolites, participating in the metabolism, growth and development of plant cells (Alscher and Hess, 1993). ROS production is stimulated by environmental stresses such as exposure to high temperature, heavy metals, herbicides, extremes of temperature, UV radiation, and air pollutants including O_3, and they are produced in response to invasion by various pathogens (Alscher and Hess, 1993; Foyer and Molineaux, 1994; Dangl et al., 1996; Hammond-Kosack and Jones,

1996). ROS are therefore implicated in most, if not all, stress responses. The importance of oxidative stress is that ROS are implicated in various deleterious effects. In plants, ROS have been implicated in wound responses, decreased photosynthesis, root growth, yield, and senescence (Scandalios, 1994).

Superoxide anion, hydroxyl radicals and singlet oxygen have very few characterized functions in plant cells, except perhaps in senescence and cell death. Hydrogen peroxide has many important metabolic roles, since it has been demonstrated to function on plant defence responses (Mehdy et al., 1996) such as oxidative cross linking of cell pathogens, direct pathogen killing, activation of host defence-related genes, host cell death, and down-regulation of host defence-related genes. In addition, it is now generally accepted that H_2O_2 is implicated in lignifications during plant development (Olson and Warner, 1993; Nose et al., 1995).

Nitrogen oxide is a free radical gas with a well-characterized signal role in mammalian systems. It is now clear that NO is also a major signal molecule in plants (Durner and Klessig, 1999). NO can be synthesized during environmental stress responses at the same time as H_2O_2 and it may be that cellular effects reflect responses of both H_2O_2 and NO. However, the full range of biological functions for these two signalling molecules has still to be catalogued and determining the ways in which they interact will need to be elucidated.

The extremely short lifetime of ROS makes their production analysis on plants a very difficult task. Spin label, advanced molecular genetic techniques, and digital imaging of ROS are power tools for investigating the development of oxidative stress in leaves and for the regulation of ROS metabolism.

The functions of calcium, jasmonic acid, salicylic acid, salicylic acid-signalling and jasmonic acid-signalling pathways on O_3-induced oxidative stress will need to be elucidated.

6. PHYTOTOXICITY OF REACTIVE OXYGEN SPECIES

The O_3-generated reactive species include $OH^{\cdot}$, $O^{2\cdot-}$ and H_2O_2. The hydroxyl radical is one of the most reactive species of oxygen and no protective mechanisms are known, except α-tocopherol (Heath, 1980). The free radical hydroxyl is also formed by the chemical reaction of H_2O_2 and $O^{2\cdot-}$ in the presence of transition metals (Haber-Weis reaction). The hydroxyl radicals are responsible for a great part of O_3 phytotoxicity since they react rapidly with protein, lipids, and DNA causing cell damage (Iqbal et al., 1996). Hydroxyl radicals can modify proteins making them more susceptible to protein attack (Casano et al., 1994). Once damaged, proteins can be broken down further by specific endopectidases (Casano et al., 1994). Hydrogen peroxide can cause DNA breakage and can also inactivate thiol-containing enzymes such as thioredoxin-modulated enzymes in the chloroplast stroma (Charles and Halliwell, 1981; Hagar et al., 1996). Although $O^{2\cdot-}$ anion and H_2O_2 can inactivate various macromolecules directly, it is their conversion to hydroxyl radical that accounts for their main toxicity. Superoxide and H_2O_2 in the presence of transition metals (Haber-Weis reaction) lead to hydroxyl radical formation. Hydroxyl radicals can cause damage to all kinds of biological macromolecules. These hydroxyl

radicals account for the phytotoxicity since they react rapidly with proteins, lipids, and DNA causing rapid cell damage. Damage of leaves due to the air pollutant O_3 is also mediated through the production of ROS (Kangasjärvi et al., 1994; Foyer and Mollineaux, 1994; Foyer et al., 1995). Extracellular space is actually considered the site of action of O_3.

7. DEFENSES AGAINST THE OXIDATIVE STRESS

Since most ROS are highly reactive and lead to perturbation in enzyme activities and membrane damage, they are not compatible with cell function and their generation is frequently considered to be deleterious and harmful. To protect against damage caused by oxidative stress, cells possess a variety of antioxidant systems. Cell antioxidants include non-enzymatics (ascorbic acid, carotenoid pigments, glutathione and α-tocopherol) and enzymes (glutathione reductase, superoxide dismutase, ascorbate peroxidase, and catalases).

To limit plasma membrane damage and subsequent injury responses, O_3 and associated oxygen intermediates formed during O_3 decomposition must be neutralized in apoplast space (Figure 1).

The spontaneous disproportionation of superoxide radicals is dependent on pH values and the maximum rate is observed at the pH of 4.8 (Asada, 1994b). At high pH values the spontaneous reaction becomes low.

Superoxide dismutases (SOD) are enzymes that eliminate superoxide radicals:

$$2\ O^{2\cdot -} + 2\ H^{+} \rightarrow H_2O_2 + O_2$$

Three types of SOD have been found which differ in their prosthetic metals (Inzé and Montagu, 1995).

Apoplast contains peroxidases and SOD activities (Castillo et al., 1986; Rainieri et al., 1996; Lyons et al., 1999) (Figure 2). The importance of these enzymes in scavenging O_3 reaction products is at the present moment unclear.

Ascorbic acid is the most abundant antioxidant in leaf apoplast space (Horemans et al., 2000). Ascorbic acid in the apoplast could minimize ozone injury by two types of reactions. Direct ozonolysis between ascorbic acid and O_3 has been postulated (Chaimenides, 1989), although a recent report suggests that this reaction is not the major pathway (Jakob and Heber, 1998). Ascorbic acid is also involved in the enzymatic deactivation of peroxides generated during O_3 decomposition (Castillo and Greppin, 1988). Ascorbic acid is oxidized by H_2O_2 to monodehydroascorbate (MDHA) radical, which disproportionates into AA. The role of AA in protecting plants against oxidative stress is based on the positive relationship between leaf AA and O_3 resistance (Menser, 1964; Lee et al., 1984) and the hypersensibility of a mutant of *Arabidopsis thaliana* defective in AA biosynthesis (Conklin et al., 1997). Monodehydroascorbate oxidase activity has been described in apoplast (Polle et al., 1990; Luwe et al., 1993), which reduced DHA to AA. However, contradictory data have been observed regarding the physiological significance of this enzyme.

In the ascorbate-glutathione (Halliwell and Asada) cycle (Halliwell and

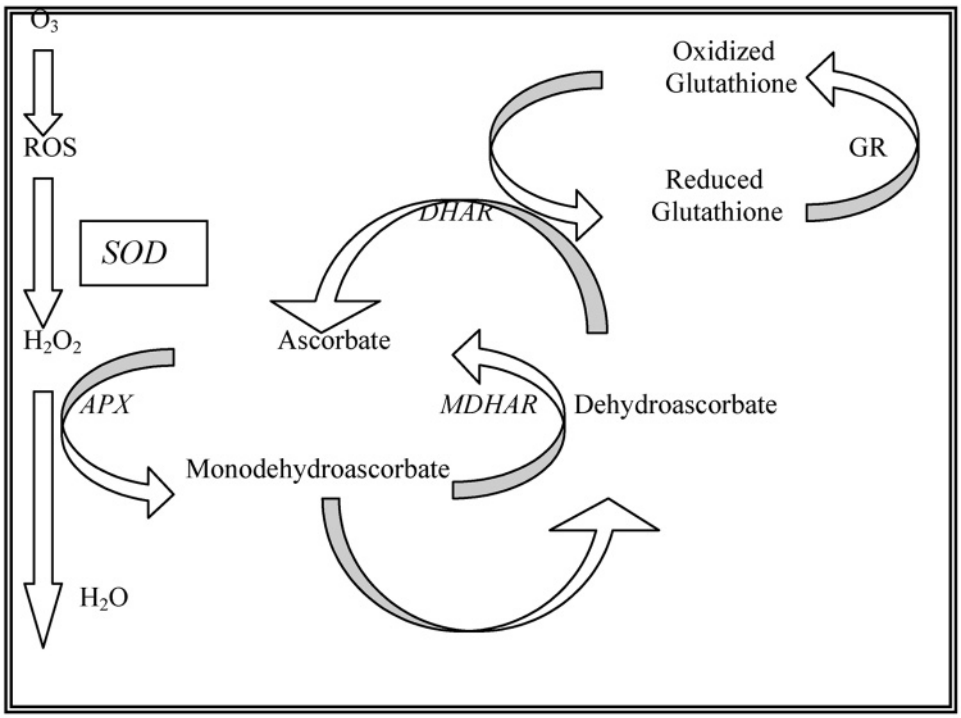

Figure 2. The ascorbate-glutathione (Halliwell-Asada) cycle (after Halliwell and Gutteridge, 1989; Asada, 1994b).

Gutteindge, 1989; Asada, 1994), two enzymes are involved in the detoxification of reactive oxygen species. The primary scavenger is the enzyme superoxide dismutase (SOD), which converts superoxide radical. to H_2O_2. Hydrogen peroxide is eliminated by catalases and peroxidases. Ascorbate peroxidase (APX) is considered to be the most important scavenger. The product of SOD is eliminated by ascorbate peroxidase, at the expense of oxidizing ascorbate to monodehydroascorbate. The enzyme uses ascorbic acid as a substrate and forms part of the ascorbate-glutathione (Halliwell-Asada) cycle. Other enzymes of the ascorbate-glutathione cycle are monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR).

8. ETHYLENE AND PLANT RESPONSES TO O_3

Ozone phytotoxicity has been related to ethylene emission (Tingey et al., 1976). When plants are subjected to a variety of stresses they often exhibit symptoms of exposure to ethylene. Exposure of plants to O_3 leads to stress ethylene production. It is generally accepted that there is a broad correlation between stress ethylene formations and sensitivity to O_3 (Wang et al., 1990). Exposure of plants to exogenous ethylene enhances their sensitivity to O_3, whereas pre-treatment with

aminoethoxyvinyl glycine to inhibit stress ethylene biosynthesis makes plants less sensitive (Mehlhorn et al., 1991). Ozone may react with volatile compounds emitted by plants into the protoplast. Interactions of O_3 with ethylene or other hydrocarbons are thought to be part of the mechanism leading to injury. Ozone-sensitive species were found to produce more ethylene during O_3 treatment than ozone-tolerant plants (Wellburn and Wellburn, 1996). In summary, stress ethylene is considered to be the major cause of accelerated senescence and leaf abscission (Mehlhorn et al., 1991).

9. LEAF VISUAL INJURY

Visual injury to crop plants caused by O_3 can range from severe necrosis and death of much or all the exposed tissue, to mild chlorosis (Heagle et al., 1973; Deveaou et al., 1987; Heagle et al., 1987; Mulchi et al.; 1988, Heggestad, 1997). Acute injury usually involves necrosis, varying from general large die-back areas, to small areas, stipples or flecks; such injuries occurring as a result of fumigation with high doses of O_3. Chronic injury results from exposures to sub-acute dosage. Chlorosis and death of isolated cells are generally observed in these conditions. However, visible injury is not dependent upon O_3 concentration or exposure time (Heck et al., 1966). In the last few decades, the effects of O_3 on crops have been made by visual procedures. The principal drawbacks of this procedure are subjectivity, inconsistency, and low specificity.

10. PHYSIOLOGICAL EFFECTS

Elevated atmospheric O_3 frequently has harmful effects on the photosyntheticperformance of agricultural crops (Heck et al., 1983, Amundson et al., 1987; Lehnherr et al., 1988; Violin, 1998; Donnelly et al., 1998, 2000). Reduction in chlorophyll content (Köllner and Krauss, 2000) and carboxylation efficiency (Reid and Fiscus, 1988; Farage et al., 1991) contribute to the observed decrease in net photosynthesis under elevated O_3. The loss of Rubisco activity induced by elevated O_3 apparently results from a decrease in the quantity of Rubisco present (Pell et al., 1992; Baker et al., 1994). Moreover, exposure to O_3 typically reduces effective leaf area, thus decreasing light interception and the quantity of assimilate available to support the growth of economic yield component. O_3-induced disruption of cellular metabolism may also promote a loss of chlorophyll and increase senescence (Grandjean and Furher, 1989; Fangmeier et al., 1994; Djanperä et al., 1998).

11. MECHANISM OF OZONE ACTION

The precise mechanism of O_3-induced injury in plants is poorly understood (Pell et al., 1992; Heath, 1994; Schaudner et al., 1997; Heath and Taylor, 1997; Pell et

al., 1997; Dizengremel, 2001). The fate of O_3 upon entry into the leaf is not well known. It has been demonstrated that toxic ROS are formed in the cell wall. The phytotoxicity of O_3 is due to its high oxidant capacity (redox potential = +2.07 V) and its capacity to generate other toxic species such as $OH^{\cdot}$, $O^{2\cdot -}$, and H_2O_2. These toxic substances attack the composition, structure and function of the plasma membrane giving place to the primary effects of O_3. H_2O_2 can be transported through membranes and can induce the generation of other toxic species and signal chain with messenger molecules. H_2O_2, salicylic acid, jasmonic acid, and calcium have been proposed in signal pathways for plant defence reaction and may act as second messengers. The mechanism interactions of these molecules are not yet clear.

12. METHODOLOGY FOR STUDYING IMPACT ON AGRICULTURAL CROPS

In order to estimate the impact of ambient O_3 on agricultural crops, several methods have been used. These methods include controlled environment exposure, field exposure, and field plot systems. Sufficient details may be obtained from Manning (1999) and EPA USA (1997) reviews and references therein. The most used method is the open-top chamber (Heagle et al., 1973). These cylindrical open-top chambers consist of a metallic frame, plastic film panel, and a set-up to sample the air inside the chamber uniformly. The incoming air is circulated with a fan to reduce the temperature increase at midday and the entrance of ambient air above the OTC. Sometimes, a baffle (fustrum) is added to the top portion of the OTC to reduce downdraft. The growth environment within the OTC will differ in terms of temperature, relative humidity, irradiance, and wind speed and these parameters are usually measured inside and outside. The OTC technique is used with a range of concentrations above and below the ambient air.

Several dose levels of O_3 are used to formulate the dose-response relationships for yield. Linear polynomial and non-linear Weibull models are normally used to quantify the dose-response relationships (Kickert and Kruppa, 1991).

The difficulty of transferring results gain under partially controlled conditions to a more complex situation in the field is well known. Concern about the relevance of plant responses to plant exposures from plants grown in chambers has existed for many years (Lewis and Brennan, 1977). However, OTC are considered the best compromise in simulating natural systems because the experimental unit is replicable, a range of treatments are available, control of exogenous factors is possible, and the simulation of field losses is possible. Also, the simulating of field losses due to O_3 is relatively accurate (US EPA, 1996).

One major concern about OTC has been their modification of microclimatic conditions. In OTC, daytime temperatures can increase, while photosynthetic active radiation and wind speed can decrease (Heagle et al., 1988). However, modification of microclimatic conditions does not appear to affect relative plant responses to O_3 (US EPA, 1996).

In addition to the aforementioned concerns, constant air flow and turbulence within the chamber have been criticized (Kimball et al., 1997) and thus, it is

suggested, exposure-response curves derived under chamber conditions can not be used directly to estimate quantitative effect under chamberless (ambient) conditions. More experimental work must be done in order to give an answer to this question.

13. CROP YIELD LOSS

There is a considerable volume of published data on the effect of polluted air on a wide range of agricultural yield reduction (Adams et al., 1988; Heagle, 1989; US EPA, 1996). The International Cooperative Program on Effects of Air Pollutants on Natural Vegetation and Crops (Kärenlampi and Skärby, 1996) and the USA National Crops Loss Network (US EPA, 1986; Adams et al., 1988; Heagle, 1989; US EPA, 1996) are the principal programs dedicated to studying the impacts of O_3 on crops. These and other reports have demonstrated that air polluted O_3 showed a significant decrease in yield losses; these losses being influenced by crop species and cultivars, moisture stress and other growth conditions. To sum up, yield losses ranged from near 0 up to 39%. Complete reduction of yield has been observed during O_3 episodes (Velissariou, 1999).

14. INDICATORS OF OZONE STRESS

Initial studies on the effect of O_3 on crop species were centred on the reduction of agronomical yield. Later on, in order to understand the action mechanism of O_3 this interest area of agronomical yield has been displaced by other injury effects such us physiological responses. The physiological changes observed in the presence of O_3 polluted air are reduced net photosynthesis, increased respiration rate, membrane lipid peroxidation, enhanced rate of senescence, reduced transpiration, and inhibition of translocation to roots (Cooley and Manning, 1987; Darrall, 1989; Heath, 1994b; Dizengemel and Pertins, 1994; Mudd, 1996; Taylor and Ferris, 1996; Sandermannn, 1996; Langebartels et al., 1997; Heath and Taylor, 1997; Pell et al., 1997; Schraudner et al., 1997). The most important of these techniques are spectral reflectance, fluorescence and videography.

Imaging techniques (reflectance and near infrared imaging) are used for earlier detection of plant stress induced by tropospheric O_3 (Chaerle and van der Straeten, 2000). Stress induced physiological damage in plants is manifested in altered reflectance spectrum. Several spectral indices have been proposed as stress indicators (Hunt et al., 1987). Leaf spectral reflectance as a rapid method for detecting O_3 stress has been used for crop species (Runeckles and Resh, 1975; Schutt et al., 1984; Carter et al., 1992; Williams and Ashenden, 1992). A decreased infrared reflectance is detectable for clover leaves after O_3 treatment, although no visible foliar damage symptoms could be observed (Kraft et al., 1996). Ozone-treated leaves showed lower water content, photosynthetic pigments, and PSII activity (Rudorff et al., 1996).

Measurement of chlorophyll *a* fluorescence is a technique for measuring incipient O_3 effect in the field (Schreiber et al., 1998 and references therein). This

technique is rapid, non-invasive, and non-destructive and requires relatively inexpensive instrumentation. The utility of fluorescence measurements in the field as an indicator of plant responses to environmental conditions is well established (Bolhar-Nordenkampf et al., 1989). Fluorescence measurements are mainly used to determine activities of photosynthetic apparatus, such as the fluxes of absorbed photons, trapped energy or transported electron. Therefore, fluorescence measurements can be used before any changes in the visual appearance of leaves have occurred or even when no changes in their chemical composition can be detected. The ratio of variable fluorescence to maximal fluorescence is used to estimate maximum quantum yield of PSII. This parameter, also called photochemical efficiency estimates any disturbance in electron transfer between PSII and PSI. Ambient O_3 exposure causes a decrease in the photochemical efficiency (Guidi et al., 1997). Photochemical and non-photochemical quenching coefficients are also utilized to detect the primary acceptor of PSII oxidation and heat emission (Reiling and Davison, 1994). Both parameters are modified by the presence of O_3.

Fluorescence imaging is a newly developed tool (Lichtenthaler and Miehé, 1997; Oxborough and Baker, 1997). It is used to identify the primary site of damage and to characterize some features of O_3 damage when leaves are exposed to O_3. High resolution image of bean leaves exposed to O_3 fumigation showed localized decreases in PSII. Photochemical efficiencies were accompanied by an increase in minimal fluorescence level, which is indicative of PSII inactivation (Leipner et al., 2001).

It is expected that the application of chlorophyll fluorescence and chlorophyll fluorescence imaging will be very successful in detecting the incipient effects of O_3 stress.

Various other techniques have been used for the assessment of O_3 injury to plants. The following techniques have been utilized: chlorophyll losses, ethylene emission, products of lipid peroxidation, ion leakage, antioxidative enzymes and free radical metabolites (Tingey et al., 1976; Lee et al., 1982; Bors et al., 1989; Floyd et al., 1989; Langebartels et al., 1991; Pitcher et al., 1991; Yalpani et al., 1994; Glick et al., 1995; Sharma et al., 1996). These techniques are not specific for O_3 injury, since other factors (heavy metals, herbicides, high temperature, and UV radiation) causing oxidative stress induce similar results.

15. OZONE CONTROL

In addition to crop yield loss, damage of forest ecosystems and a reduction of lung function in healthy people and people with an impaired respiratory system have been demonstrated (Schmieden and Wild, 1995; Sandermann et al., 1997, 1998; US EPA, 1998). Because of these serious environmental problems as a result of the presence of O_3 in the lowest layer of the atmosphere many efforts have been made to reduce the level of O_3. National and international limits for ambient O_3 exist, but are usually exceeded in North America and Western Europe. Despite massive and costly efforts, countries in Europe and North America still experience a severe problem (Photochemical Oxidants Review Group, 1990; National Research Council, 1991). Ground-level O_3 is the most difficult to control of the

air pollutants (US EPA, 1998). This difficulty is mainly due to two facts: (a) O_3 is not emitted directly into the atmosphere by specific sources. Ozone-forming volatile organic compounds include gasoline vapours, chemical solvents, combustion fuels and consumer products. (b) The O_3-precursos can be transported hundreds of km by atmosphere winds. Unfortunately, up to now the control of O_3-precursors has not been successful.

16. AMELIORATION OF O_3-INDUCED INJURY

16.1. Chemical protective agents

Diverse groups of chemical compounds have been utilized to protect plants against O_3 injury. These groups include antioxidants, antitranspirants, growth regulators, fungicides, herbicides, and antisenescence agents (for a recent review see Manning, 1999). Many reports suggest that the chemical agents protect against O_3, but more experimental data are necessary to demonstrate the proposed reduction of injury symptoms clearly. The most frequently used are antioxidant ethylene diurea, the systemic fungicide benomyl, and the antioxidant ascorbic acid.

Ethylene diurea is a protective chemical that prevents the onset of foliar injury and estimated production loss by O_3. This chemical compound is applied as a foliar spray, a soil drench or by direct injection (Weidensaul, 1980; Kostka-Rick and Manning, 1992). The delay of senescence is the most usual response. When properly used, ethylene diurea is a good tool for detecting the phytotoxicity O_3 concentrations and loss of yield. Ascorbic acid is an antioxidant in plants (Horemans et al., 2000). Application of ascorbic acid to leaves increases the concentration of ascorbic acid in cell walls and results in considerable protection from O_3 injury (Freebairn and Taylor, 1960). The systemic fungicide, benomyl, shows antiozonant and antisenescence in plants (Manning et al., 1979). However, separation of fungicides from O_3 protection action is necessary to avoid confusion.

The use of protective chemicals in commercial agriculture requires a toxicological study prior to determining the dose-response relationship required to determine appropriate treatments in the field. The advantages of the utilization of chemical protectives are low cost, good reproducibility of experiments, and no chambers are required. The disadvantages of this method are that ambient O_3 concentrations and environmental conditions must be measured, and finally, it is not possible to calculate the dose-response relationship.

16.2. Elevated CO_2 ameliorates the impact of O_3

In C_3 species, the photosynthetic process is not CO_2-saturated under present-day conditions because the atmospheric concentrations of *ca* 370 ppb is well below the saturation point of *ca* 670 ppb. In short-term experiments, increased atmospheric CO_2 may increase photosynthetic rates in C_3 species, provided that sufficient water and nutrients are available (Long et al., 1993). In long-term experiments, some

species show a progressive decline in photosynthetic rate under elevated CO_2 (Sage et al., 1989; Miglietti et al., 1996). Elevated CO_2 has been shown to reduce the extent of O_3-induced visible damage in some species (Rudorff et al., 1996; Mulholand et al., 1997), but does not necessarily prevent reduction in photosynthetic rate (Balaguer et al., 1995). In order to explain the protective influence of elevated CO_2 several mechanisms have been proposed: (a) partial stomatal closure may reduce the flux of O_3 into leaves (Allen, 1990); (b) increases in Rubisco content and activity under elevated CO_2 may compensate for O_3-induced damage (Reid et al., 1998); and (c) improved assimilate may increase the production of antioxidants (Rao et al., 1995).

16.3. Transgenic plants

Manipulation of antioxidative capacity could be a valuable way of obtaining ozone-tolerant plants. Screening analysis following plant transformation has shown that with overexpression of superoxide dismutase or glutathione reductase in important agronomical species such as tobacco, tomato, and potato, the transformant is more resistant to photo-oxidative stress (Rennember and Polle, 1994; Foyer et al., 1995). The responses of transformants to O_3 are dependent upon the type of isoenzyme and the exposure regimen. Tobacco plants overexpressing superoxide dismutase and glutathione reductase do not show enhanced tolerance to ambient O_3 levels (Pitcher et al., 1991), whereas tobacco overexpressing superoxide dismutase shows less visible foliar injury upon exposure to O_3 (Van Camp et al., 1994). Unfortunately, the ecological significance of ambient O_3 on these transformed enzymes has still to be elucidated. The study of these transformed plants will increase our present understanding of O_3 phytotoxicity and will help to obtain O_3-tolerant plants.

17. CONCLUSIONS

Tropospheric O_3 has a negative impact on growth, development, and productivity of crops. Effects of O_3 have been observed in a wide range of physiological characteristics, such as accelerated senescence, decreased photosynthetic assimilation, decreased productivity, and reduced carbon allocation to roots. Different responses to O_3 have been observed in related species and, hence, it is thought that the initial mechanism of O_3-induced stress on crops is uniform. A better understanding of the effect of O_3 and O_3-generated reactive oxygen species is necessary for an insight into the impact of O_3 in crops. A great effort must be made in order to decrease the concentrations of O_3 air pollution. In the absence of an effective emission control of O_3 precursors, it is expected that ambient O_3 concentration will increase in the future. The responses of crops to increased tropospheric O_3 level could play an important role in determining crop yielding. Given that O_3-induced stress imposes considerable constraints on crop yield production, there is a need for continuous research in this area. More work is necessary to better our understanding of signal pathway and gene expression implicated in O_3 responses.

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SAFFRON QUALITY: EFFECT OF AGRICULTURAL PRACTICES, PROCESSING AND STORAGE

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1. INTRODUCTION

1.1. History

Saffron, the most expensive spice worldwide, is comprised of the dried stigmas of the plant *Crocus sativus* Linnaeus of the *Iridaceae* family, a sterile triploid not found in the wild.

According to the definition given by FAO (Food and Agricultural Organisation) it forms 'a loosely matted mass of dark, reddish-brown flattened threads, amongst which a few narrower yellow ones can be distinguished. The upper, enlarged part of the flattened threads is the stigma of the flower, the lower narrower portion is the style' (FAO, 1986).

Saffron is mainly used as a spice that imparts colour to food but its medicinal and dyeing properties are also well known and appreciated.

C. cartwrightianus, a possible progenitor of *C. sativus*, as well as more than 80 other species belonging to genus *Crocus* originate from the eastern Mediterranean basin from where the cultivation of the plant was spread to other parts of the 'Old World'. Many of the *Crocus* species occur in the Aegean islands and Crete (Greece) that may be considered as 'the birthplace' of the cultivated plant, highly appreciated in the early civilizations of those areas for its exceptional properties. Famous fresco fragments exhibited today in the archaeological museums in Heraklion (Crete), Santorini and that in Athens offer evidence for the ritual significance and the use of *Crocus* plant in the every day life of the prehistorical natives. In addition, written information on pottery tablets give unequivocal evidence for the participation of the plant material in the economy of the Cretan kings of Knossos (1500–1450 B.C). The small amounts of the final product, the dried stigmas, reported on those records, equivalent to a few grams up to half a kilo, indicate that it commanded continually through the centuries an exceptional high commercial price.

The ancient Greek name 'krokos' survived in the current language of this small country to characterise both the plant and the spice whereas the word saffron of Arabic (or old French?) roots (that means 'yellow') that may dates even back to the Assyrian empire (2300 B.C) and comes from the name of a town called Azupirano (Saffron town) (Basker and Negbi, 1983) is used in many languages ('safran' in French and German, 'saufuran' in Japanese, 'azafran' in Spanish, 'zafora' in Greek). The Hebrew word found in Bible is 'karkom' and the Chinese names are 'fan-hong-hua' (foreign red flower) or 'zang-hong-hua' (Tibet red flower).

R. Dris and S. M. Jain (eds.), Production Practices and Quality Assessment of Food Crops, Vol. 1, "Preharvest Practice", pp. 209–260.

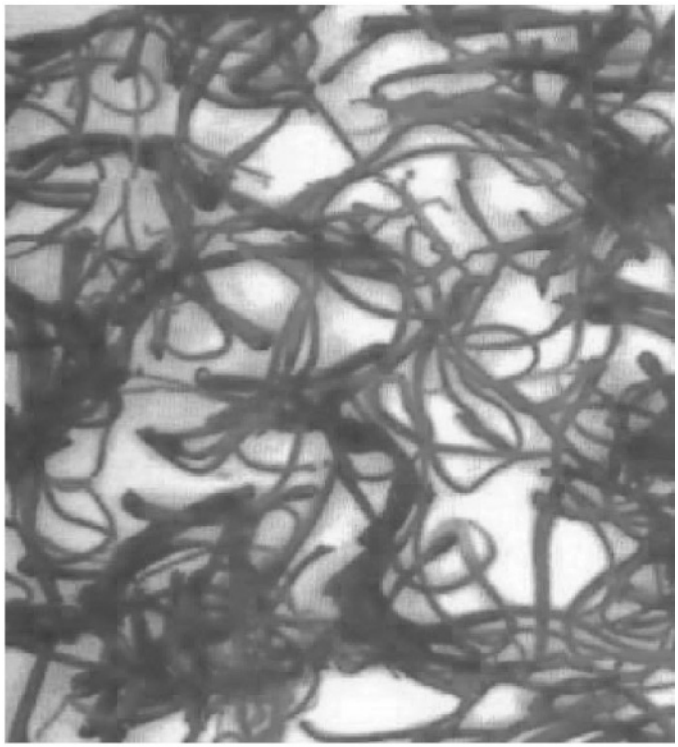


Figure 1. Dried stigmas of saffron.

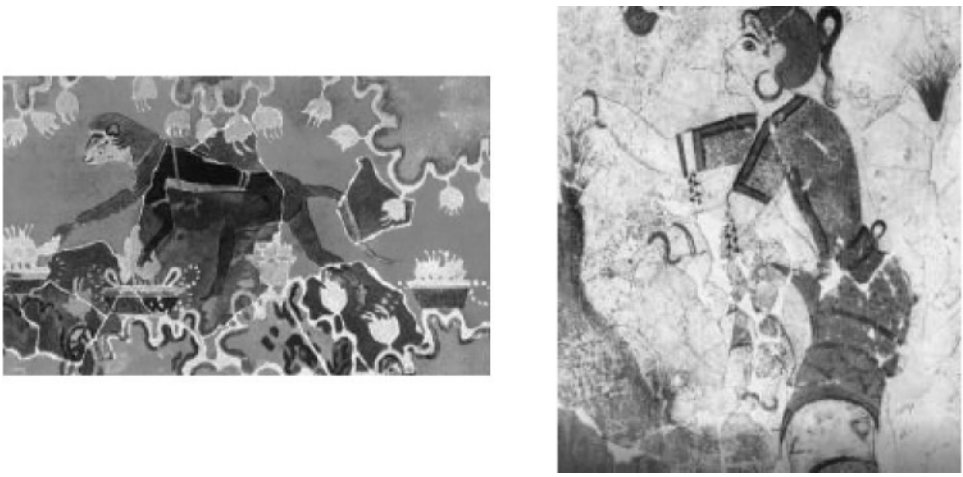


Figure 2. On the left, a monkey among *Crocus* flowers (archaeological museum of Heraklion, Crete). On the right, a woman-collecting *Crocus* flowers (archaeological museum of Thira, Santorini).

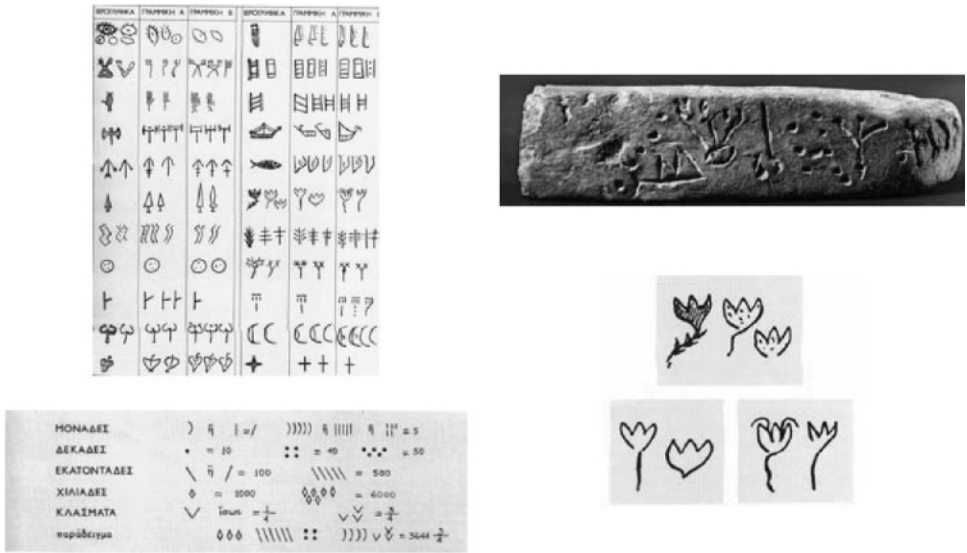


Figure 3. *Crocus* symbols in scruples: hieroglyphic, linear A and B.

1.2. Commercial interest

Today, the cultivated species, *C. sativus*, is found in many countries such as Azerbaijan, Greece, India, Iran, Italy and Spain. Moreover, China, Israel, Japan and Mexico show their interest in its cultivation whereas in countries such as France, Austria or Germany, with a history in growing, it is found only sparingly owe to the enthusiasm of certain individuals. A vivid interest is observed in many countries to increase the production of the valuable saffron ingredients. Moreover, investigations on alternative sources of saffron are carried out (Kamikura et al., 1985; Pham et al., 2000; Radjabian et al., 2001). Among the latter, cape jasmine (*Gardenia jasminoides* Ellis) is a serious competitor, since its extracts contain the same colourings but not the aromatic compounds. In international legislation saffron is regarded as a 'natural colour' (Green, 1995).

Private sector mainly conducts saffron trade worldwide so that statistical data available are incomplete. An annual rate of 50 tons at a cost of 50 million dollars was reported at early 90s (Oberdieck, 1991). A recent review on production and export (Negbi, 1999) gives data that mainly concern the 70s and 80s. Prices for saffron small size sachets (0.25–1 g) are even ten times higher the raw saffron price. Swings and fluctuations of the traded quantities are related to changes in the consumer trends, industrial choices and production in various countries with regards to socio-economical or climatic factors. In any case saffron cultivation relies heavily on labour since no step of the whole production line is mechanised so far (Galigani and Pegna, 1999). Its exceptionally high price is, thus, self-evident. According to data available till the beginning of 80s (Sampathu et al., 1984), Spain

almost monopolised the world trade (>90%) though its production accounted for the 52% of overall saffron production. According to the same source India and Greece produced the 21.2%, and 13.2%, respectively, and other countries much less (Italy, 7.5%; France 6.1%). Spain is also a great importer of Iranian and Indian saffron (Green, 1995). A more recent ranking (Saltron et al., 1999; Alonso et al., 2001) shows significant changes in production trend: Iran (80 tons), Kashmir (10 tons), Greece (6 tons), Spain (3 tons), Morocco (1 ton). The major markets are Western Europe, North America, Saudi Arabia and the Gulf Emirates. The USA imported 1.5 tons (~$198000) in 1969 (Papanikolaou, 1997), 3 tons in 1992 and 8.3 tons in 1994! The price of the spice was around $2000/kg ten years ago (Sujata et al., 1992). Variation in exports is characteristic in saffron trade in India, e.g. 1485 kg in 1969 and none five years later. An increase in exports in line with the international trend is reported in early 90s (1991–1992, 3724 kg) (Dhar and Mir, 1997). The authors relate saffron production and prosperity of the community in charge, in comparison to other common plant products. Domestic prices ranged from 700–1000 dollars per kilogram. Stamen trade seems to add value to the cultivation since a significant quantity seems to be exported every year (11904 kg in 1991). Raina et al. (1996) gives rather high values for the Indian production (~30 tons valued at $20 millions). Fluctuations in production are also observed in Spain. Basker and Negbi (1983) report 56 metric tones in 1972 and a decline to 20 metric tones in 1980 due to product failure. The Saffron Growers' Co-operative of Kozani (Greece) (personal communication) that hosts approximately 1300 members gives valuable statistical data that indicate a continuous increase in the commercial price of the product ($272 in 1985 and $600 in 2000). The 93% of the commercial price of the product returns to the growers, who are very satisfied from their profits. The price of the product in small packets is steadily two folds higher than that of the bulk product. The amount of Greek saffron is approximately 5 tons. An increased production (ca. 7 tons) was observed in the years 1996 and 1997. The worst harvesting period the last decade was the year 2001 when a sudden climatic change in the middle of the harvesting period destroyed half of the expected production. It is worth mentioning that best clients for Greek saffron are Spain (almost half of the production in large packing of dried stigmas), France (>1 ton) and Italy (~0.5 ton). A steady market of 100–300 kg in Germany and Sweden and an increasing trend in Greece are also observed in the last ten years. The above mentioned prices are surprisingly much lower than those reported by Tammaro (1999) who gives a figure of $4000/kg of dried stigmas and a much higher price for retail packed Italian saffron. High prices in Milan market ($304–312/kg raw saffron) are found since 1965–1968. The situation in Morocco was recently presented by Ait-Oubahou and El-Otmani (1999), who expressed very interesting views on the economics of this cultivation. Though the ancient Cilicia (Asia Minor) was famous for the high quality saffron, no place in Turkey has a systematic cultivation of saffron crocus. Only Safranbolu, a city near the Black Sea coast, produces a few kilograms (10–12) of saffron annually (Sampathu et al., 1984). This place, as indicated by name, used to be an exporter of saffron to central Europe in the past.

1.3. Uses

There is not a systematic study on the history of saffron use in the various countries, yet. Most scientists in review articles and research papers refer to the same quotations found in Homer, the Greek classic poets, Plinius and Dioskorides, or abstract evidence from the medieval and more recent records on folk medicine recipes. The exceptional presence of saffron as a condiment in the Roman (*see* the famous cookbook, De la Coquinaria, Apicius, 1st century B.C), Byzantine and Arabian cuisine coincides with its esteemed culinary position in the medieval courts of central Europe and Britain. Inspectors for keeping saffron free from fraud were in charge in Venice and Germany. Saffron preparations in the Ayurvedic, Siidha and Unani (Greek) systems of traditional medicine of India and in the Chinese therapeutic are credited with several exceptional properties. As a dye, it is reported to color the official clothes of kings, priests and other eminent persons at various places in different historical periods.

Food uses. Saffron culinary uses are related to three important properties, i.e. colouring power, bitter taste and flavour that is characterised as floral with a fatty herbaceous undertone. The colour of aqueous or alcoholic extracts is bright yellow and is due to the presence of glucosylated C20 carotenoids, the crocins and their aglycon, crocetin. Bitter taste is attributed to picrocrocin whereas safranal is the major volatile component of saffron aroma. More about the chemistry of saffron constituents is given in part 2.

The spice is used today in culinary, bakery and confectionery and also in alcoholic and non-alcoholic beverages. Certain dishes are traditionally seasoned with saffron such as paella, French bouillabaisse (fish and shellfish stew), arroz con polo, bacalao a la viz caina, risotto Milan style, pastries in Greek islands, baked products in Sweden, Central Europe, Britain etc. Modern recipes can be found in contemporary local culinary books (Tammaro, 1994; Voutsina, 1999) or in relevant electronic sources (e.g., www.astaspice.org; www.medusa.maich.gr; www.safinter.com; www.neda.net; www.saffron.gr). The amounts needed in all cases are extremely low (1–10 mg/kg) except for alcoholic drinks and meat (20–200 mg/kg). In the European Community saffron extract is not considered as additive and is included in the 'natural extract' category (Ghorpade et al., 1995). This is also the case in the Code of Federal Regulations of the U.S.A where it is listed as a colour additive exempt from certification (Title 21, Part 73, sec 73.500). Extracts or dried material are used in formulations, sometimes patented, to flavour liquors, cordials (e.g. Boonenkamp, Benedictine, 'sand pear' vermouth and other bitter drinks) and sauces (Basker and Negbi, 1983; Sampathu et al., 1984; Knewstubb and Henry, 1988; Timberlake and Henry, 1986; Rees, 1988; Oberdieck, 1991; Iborra et al., 1992a; Dufresne et al., 1999; Negbi, 1999; Selim et al., 2000). Tsimidou and co-workers examined the stability of saffron pigments under various conditions (Tsimidou and Tsatsaroni, 1993; Orfanou and Tsimidou, 1995; Tsimidou and Biliaderis, 1997; Selim et al., 2000). Recently, Pham et al. (2000) reported on the antioxidant activity of crocin, a property desired in food industry. Interest in food

medicines and in functional foods is expected to attract the attention of consumers to saffron the next years.

Medical and pharmacological uses. Many therapeutic properties have been credited to saffron that was considered to be a kind of panacea. In general, saffron, its extracts and tinctures are used in folk medicine as an antispasmodic, eupeptic, gingival sedative, carminative, diaphoteric, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue, but its extensive use as an abortifacient decreased following reports of fatalities (Consorti, 1980; Basker and Negbi, 1983; Sampathu et al., 1984; Ríos et al., 1996). In Ayurvedic preparations is used as a tonic and a promoter of non-specific immunological defence ('Rasayan') (Dhar and Mir, 1997; Ghosal et al., 1989). In the Chinese therapeutic it is used to treat some disorders of the central nervous system (Abe and Saito, 2000; Soeda, et al., 2001). Toxicity of saffron is well known above 0.5 g and death has been reported after ingestion of 1.5 g. The medicinal use of saffron and other herbs and spices has declined in the 20th century but since the 90s saffronologists enhanced their efforts towards this direction. Abdullaev and co-workers (1993, 1999, 2002) reviewed the cytotoxic, anticancer and antitumor properties of saffron and its active ingredients in, mainly, *in vitro* studies but also *in vivo* studies. Effects to brain functions have been recently investigated (Ni, 1992; Suigiura, 1995; Abe et al., 1999; Soeda et al., 2001). Researchers working on the medical properties of saffron itself (or of other saffron plant material, e.g. corms) are mainly coming from producing countries: Greece (Liakopoulou-Kyriakides et al., 1985; Liakopoulou-Kyriakides and Skubas, 1990; Morjani et al., 1990; Tarantilis et al., 1992 and 1994a); India (Nair et al., 1991a and 1994; Salomi et al., 1991; Premkumar et al., 2001); Spain (Garcia-Olmo et al., 1999; Escribano et al., 1999a, c and 2000a) or from countries with long tradition in herbal medicine such as Japan (Abe and Saito, 2000; Soeda et al., 2001). The active constituents of saffron are considered to be crocins. Other compounds not so intensively examined in the case of saffron but known to present significant anti-stress/anti-anxiety properties such as mangicrocin may also significantly contribute to the therapeutic properties of the spice (Ghosal et al., 1989). Saffron pigments have been also used as light stabilisers for photosensitive drugs (Nagy et al., 1990). The authorised European Commission suppressed the monograph on saffron from the European Pharmacopoeia in 1987 because the product was considered as of 'no common European interest'. Since 2002 a monograph (code 1624) on saffron for homeopathic preparations appeared in the above source.

Other applications. The use of saffron for textile dyeing declined with the advent of synthetic dyes. Recent papers indicate an interest for dyeing natural expensive textiles (silk, wool, and cotton) with it. Takaoka et al. (1992) studied comparatively the colour of extracts from saffron and *Gardenia jasminoides* Ellis and the effect of pH and Na-L-ascorbate on colour yield on silk. The same group (1991) found that the colour properties of silk and wood dyed with the saffron extract were increased by mordanting with compounds containing Al and Sn. Tsatsaroni and Eleftheriadis (1994) studied the dyeing and fastness properties of saffron on cotton and wool. Very small amounts of tincture of saffron are used in the preparation of

oriental-type perfumes (Sampathu et al., 1984). Crocetin, a saffron pigment, is used as a label in hybridisation probes in the detection of DNA molecules (Narayanan et al., 1996).

2. SAFFRON CHEMISTRY IN RELATION TO QUALITY

The chemical composition of dried saffron stigmas has been extensively studied since the end of 19th century.

2.1. Proximate composition

Proximate composition of dried stigmas of saffron is given in Table 1. Sampathu et al. (1984) referred to the vitamin content of different samples of saffron focusing on riboflavin (3.4–5.6 μg/g) and thiamine (0.3–0.4 μg/g). The % composition of saffron ash according to various reports (Sampathu et al., 1984; Tarantilis et al., 1990; Alonso et al., 1998c) is: 34.46% K_2O – 8.56% Na_2O – 10.01% P_2O_5 – 7.12% SO_3 – 2.89% Cl. Magnesium (~565 mg/100 g), calcium (~150 mg/100 g), iron (25.4–76.5 mg/100 g), manganese (0.24–5.7 mg/100 g), copper (1.5–27.7 mg/100 g) and zinc (0.5–15.2 mg/100 g) are also reported.

However, the quality and consequently the commercial value of saffron is based on the estimation of colouring power, the bitter taste and the aroma, therefore, the responsible compounds are detailed below.

2.2. Pigments

A large number of C_{20} carotenoid pigments, have been isolated from the dried stigmas of saffron. Pfander and Schurtenberger (1982) have examined the possible biosynthetic pathways of saffron carotenoids and suggested their dependence on age and origin of the product. The main pigments are the glycosidic forms of crocetin

Table 1. Proximate composition of saffron (after Sampathu et al., 1984; Ríos et al., 1996).

Constituent	Percentage
Water	10–12
Mineral matter	5–7
Fat	5–8
Protein	12–13
Reducing sugars	20
Free sugars	Trace
Starch	6–7
Pentosans	6–7
Gums and Dextrins	9–10
Crude fibre	4–5
Crocin pigment	8–9
Essential oil	0.3

(8,8′-diapocarotene-8,8′-dioic acid) (Figure 4), *D*-glucose and *D* gentiobiose being the sugar moieties. These derivatives are known as crocins.

α-Carotene, β-carotene, lycopene and zeaxanthin are also present in trace amounts (Sampathu et al., 1984) and mangicrocin, a xanthone-carotenoid glycosidic conjugate (Ghosal et al., 1989) has also been identified.

Crocetin is insoluble in water and in most organic solvents, with the exception of pyridine, from which it separates in red coloured leaves (m.p. 275 to 276 °C). When rubbed with a drop of concentrated H_2SO_4, crocetin produces a strong indigo colour, turning violet, then brown after a few seconds (Sampathu et al., 1984). Crocetin monomethyl ester (β-crocetin) melts at 218 °C and separates as reddish yellow plates from a mixture of chloroform and methyl alcohol. Besides the *trans* crocetin isomer, 13-*cis*-crocetin isomer (Speranza et al., 1984) has been reported. The structure of dimethylcrocetin $(C_{11}H_{14}O_2)_2$, a purple compound, prepared by alkaline hydrolysis in methanol of an extract of saffron, has been elucidated by Fourier transform-IR and Raman spectroscopy and also crystal structure analysis (Tarantilis et al., 1994c). This compound, which crystallises in the orthorhombic space, has the all-*trans* configuration and forms a long planar conjugated system. Crocetin is reported to occur to a limited extent freely in the spice (Speranza et al., 1984). Côté et al. (2000) have reported on the properties of a glucosyltransferase which is involved in the glucosylation of crocetin. This enzyme (uridine-5′-diphosphoglucose-crocetin 8,8′-glucosyltransferase), which glucosylates the carboxylic ends of crocetin, has been isolated from cell cultures of saffron. The glucosylation of crocetin into crocin by UDP-glucose takes place according to the following sequence: monoglucosyl ester; monogentiobiosyl and diglucosyl esters; gentiobiosylglucosyl ester and crocin (Dufresne et al., 1999).

2.2.1. *Crocins*

Saffron colouring properties are mainly attributed to the water-soluble crocins. The chemistry of the digentiobiosyl derivative, known as α-crocin or crocin 1, was dated back in the beginning of 19th century. Karrer, Kuhn and their co-workers achieved isolation in the crystalline state in early 30s. Since then many other derivatives have been identified in the aqueous or alcohol extracts of saffron. Structures of major crocins as reported by various investigators (1, 2: Pfander and Wittwer, 1975a, b; 3: Pfander and Rychener, 1982; 4: Speranza et al., 1984; 5: Morjani et al., 1990; 6, 7: Tarantilis et al., 1994b, 1995; 8: Pfister et al., 1996;) are shown in Figure 5.

Tarantilis et al. (1995) used HPLC-UV-visible photodiode-array detection on-line with mass spectrometry for the analysis of crocetin glycosides (crocins), carrying

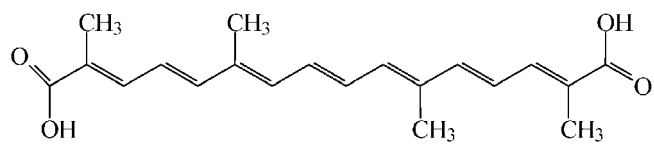
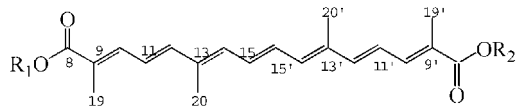
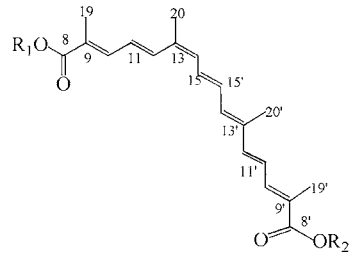


Figure 4. Chemical structure of crocetin.



I



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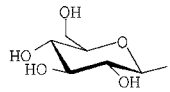
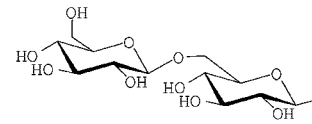
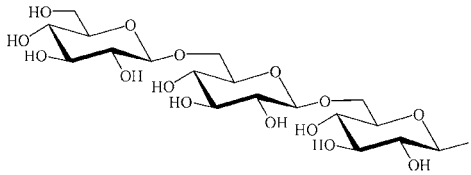


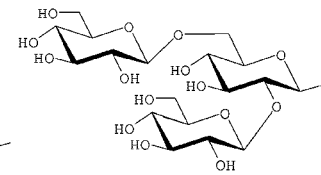
β-D-glucosyl-
A



β-D-gentiobiosyl-
B



tri-(β-D-glucosyl)-
C



β-D-neapolitanosyl-
D

Moiety	Compound	Reference
$R_1 = R_2 = \beta$-D-gentiobiosyl (B)	Crocetin-di-(β-D-gentiobiosyl) ester (Crocin 1)	1, 2, 3, 4,
R_1 = three β-D-glucosyl (C or D), $R_2 = \beta$-D-gentiobiosyl (B)	Crocetin-tri-(β-D-glucosyl)-(β-D-gentiobiosyl) ester Or Crocetin-(β-D-gentiobiosyl)-(β-D-neapolitanosyl) ester	6, 7, 8
$R_1 = \beta$-D-gentiobiosyl (B), $R_2 = \beta$-D-glucosyl (A)	Crocetin-(β-D-gentiobiosyl)-(β-D-glucosyl) ester	1, 2, 3, 4
$R_1 = R_2 = \beta$-D-glucosyl (A)	Crocetin-di-(β-D-glucosyl) ester	1,2,3,4
$R_1 = \beta$-D-gentiobiosyl (B), R_2=H	Crocetin-mono-(β-D-gentiobiosyl) ester	1, 2, 3, 4
$R_1 = \beta$-D-glucosyl (A), R_2=H	Crocetin-mono-(β-D-glucosyl) ester	1, 2, 3, 4
$R_1 = R_2$= Methyl	Dimetylcrocetin	4, 5

Figure 5. Chemical structures of major crocins present in *C. sativus*.

one to five glucose molecules, and differentiated their *trans* and *cis* isomers. Van Calsteren et al. (1997) reported also on the structure of some already known crocetin derivatives from saffron stigmas and compared their findings with carotenoids present in *Gardenia jasminoides* seeds.

Crocins dissolve readily in water to give an orange-red solution. This is the reason for its application as a food colorant. Crystals of crocin (m.p. 186 °C) contain water, which is only given up on prolonged drying in vacuum at 100 °C. On acid hydrolysis in absence of air, crocins yield crocetin and glucose, while hydrolysis with alcoholic ammonia results in crocetin and gentiobiose (Sampathu et al., 1984; Solinas and Cichelli, 1988). Crocins are extremely sensitive to dilute aqueous potassium hydroxide giving a quantitative yield of crocetin (potassium salt). Crocins, as a-carotene, dissolve in concentrated sulfuric acid, forming a deep blue solution, which on standing changes to violet, red and finally brown. Crocins are coloured green by nitric acid (Sampathu et al., 1984). The absorbance maxima of crocins are at about 440 nm in distilled water (ISO, 1993). The antioxidant properties of crocin have also been investigated (Pham et al., 2000). Weber and Grosch (1976) have reported a carbonyl compound as a product of crocin bleaching during co-oxidation with linoleic acid by a soybean lipoxygenase. On the other hand, quenching properties of water extracts of saffron may not be related to crocins but to other ingredients (Kumar and Nultsch, 1985).

The levels of each pigment in saffron vary due to the different origin of the plant and the overall processing conditions and storage length. Pfander and Rychener (1982) found that crocin 1 represents the 40–45% of the saffron aqueous extract, followed by the crocetin-(β-D-gentiobiosyl)-(β-D-glucosyl) ester (35%), the crocetin-di-(β-D-glucosyl) ester (ca. 10%), as well as the crocetin-mono-(β-D-gentiobiosyl) and mono-(β-D-glucosyl) esters (each ca. 2%). On the other hand, Alonso et al. (2001) examined the content of Spanish, Indian and Iranian saffron in crocin derivatives and gave results for *trans*- and *cis*-crocins (*trans*-crocin: 0,46–12,12%; *cis*-crocin: 0,04–8,53%; *trans*-(β-D-gentiobiosyl)-(β-D-glucosyl) ester: 0,01–9,44%; *cis*-(β-D-gentiobiosyl)-(β-D-glucosyl) ester: 0,01–2,26%).

2.3. Bitter compounds

The colourless glycoside picrocrocin ($C_{16}H_{26}O_7$, 4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is the major bitter compound of saffron. The compound was firstly crystallised (m.p. 156 °C) and separated by Winterstein and Teleczky in 1922. Kuhn and Winterstein (1934) examined its chemical properties. UV absorption maxima for picrocrocin are at 254 nm/ε = 7124 (Alonso et al., 1999a) or 250.5 nm/ε = 10100 (Buchecker and Eugster, 1973). The latter in their work on the absolute configuration of picrocrocin, reported the following structure, with the R-configuration at the aglycon O link.

The formation of picrocrocin is related to degradation of zeaxanthin (Pfander and Schurtenberger, 1982). Its decomposition gives rise to compounds responsible for the aroma of saffron. Removal of the sugar moiety takes place during processing (drying, storage) of saffron (Zarghami and Heinz, 1971; Sampathu et al., 1984; Iborra et al., 1992a, b; Iborra et al., 1993; Raina, 1996; Ríos et al., 1996;

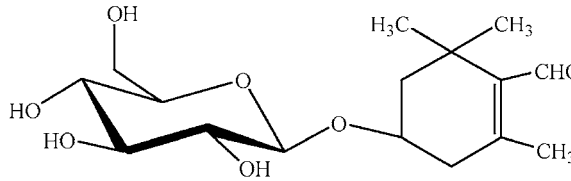


Figure 6. Chemical structure of picrocrocin.

Straubinger et al., 1997; Straubinger et al., 1998) or even during flower development (Himeno and Sano, 1987). The hydrolysis of picrocrocin may also occur due to the activation of β-glucosidase. Iborra et al. (1992a) proposed a method for the immobilisation of this enzyme in order to increase the rate of hydrolysis yielding thus higher levels of safranal. Himeno and Sano (1987) studied the picrocrocin content in intact stigmas of saffron during the flower development and observed a stepwise increase in the content before anthesis. Then, an appreciable decrease in picrocrocin level coincided with the appearance of volatiles. Alonso et al. (2001), who examined several samples of Spanish, Indian and Iranian saffron found their picrocrocin content: 0.79–12.94% in Spanish saffron, 1.07–2.16% in Indian and 2.18–6.15% in Iranian saffron.

2.4. Volatiles

The characteristic delicate aroma of saffron comes from an essential oil, which mainly contains terpene aldehydes (Zarghami and Heinz, 1971). Safranal is the main responsible compound for the aroma of saffron, even though there are other more volatile constituents which provide saffron with its final odour (Zarghami and Heinz, 1971; Sampathu et al., 1984; Curro et al., 1986; Tarantilis and Polissiou, 1997; Sraubinger et al., 1998). Tarantilis and Polissiou reported the presence of 23 volatile components 13 of which were ketones and 6 aldehydes. The molecular weights of these compounds ranged from 122–208 and their presence was directly related to the method of volatile isolation. Five compounds, namely, 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde (safranal) (*ca*. 70%); 3,5,5-trimethyl-2-cyclohexen-1-one (isophorone) (*ca*. 14%); 3,5,5,-trimethyl-3-cyclohexene-1-one (isomer of isophorone) (5%); 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4%); and 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (isomer of safranal) (3%) were the major compounds the chemical structures of which, elucidated by MS, are given in Figure 7.

These volatiles with a common nucleus possibly originate from picrocrocin and are formed either by enzymic activity or by oxidative degradation process. A scheme for the formation of safranal has been repeatedly presented in literature (Figure 8). According to this scheme, an intermediate volatile precursor of safranal namely 2,6,6-trimethyl-4-hydroxy-1-carboxaldehyde-1-cyclohexene (HTCC) may be formed.

The levels of safranal and of the other aroma compounds of saffron vary depending mainly on the conditions of processing, storage and the methods of analysis of saffron. In any case, safranal is the most abundant volatile component in the stigmas of saffron (>60% of essential oil) (Zarghami and Heinz, 1971; Corradi

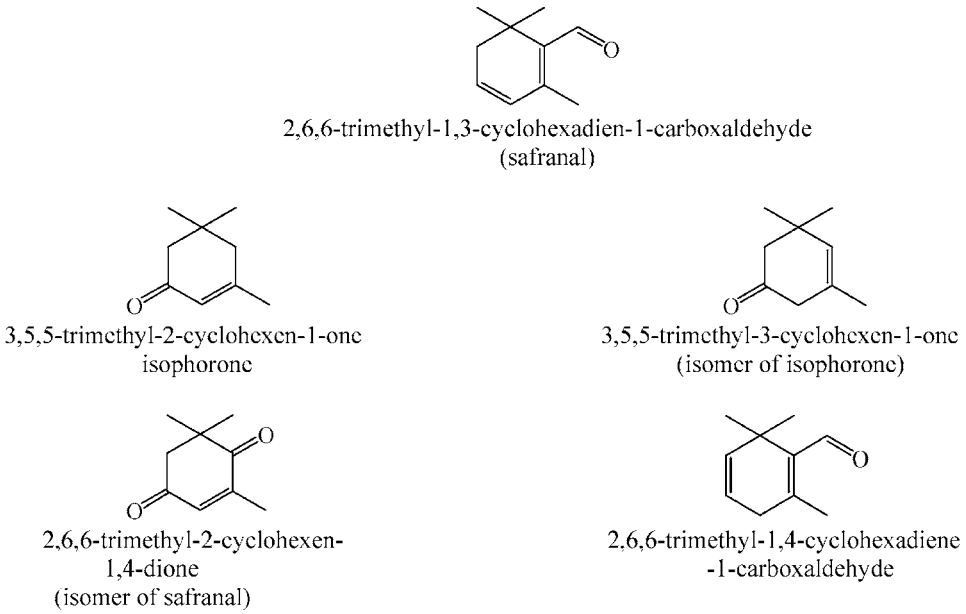


Figure 7. Chemical structures of major volatile compounds present in *C. sativus*.

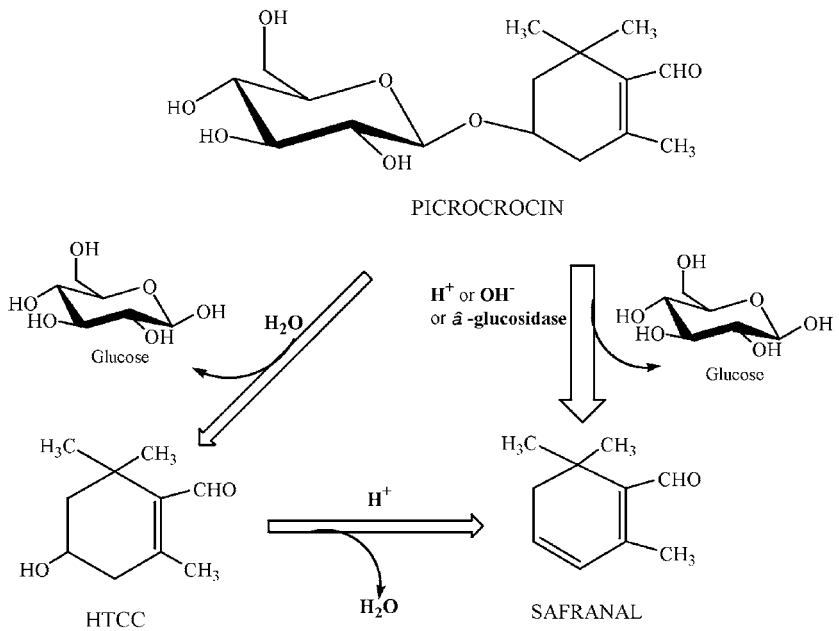


Figure 8. Schematic representation of picrocrocin's degradation.

and Micheli, 1979; Curro et al., 1986; Roedel and Petrzika, 1991; Tarantilis et al., 1997; Straubinger et al., 1998) though even lower levels have been reported (*ca.* 30%) (Zareena et al., 2001). Some minor volatile compounds are believed to derive from non-volatile precursors such as carotenoids, when the latter are exposed to heat, light, oxygen or activity of β-glucosidase (Tarantilis et al., 1995; Raina et al., 1996; Straubinger et al., 1997; Straubinger et al., 1998). The presence of such compounds may affect negatively the aroma of saffron.

2.5. Isolation, synthesis and analysis of crocins, picrocrocin and volatiles

Several solvents have been used in order to extract the saffron constituents. Methanol-water (Koyama et al., 1988; Tarantilis et al., 1995; Orfanou and Tsimidou, 1996; Straubinger et al., 1997; Lozano et al., 1999; Soeda et al., 2001), ethanol-water (Buchecker and Eugster, 1973; Pfander and Wittwer, 1975; Speranza et al., 1984; Gómez et al., 1987b; Himeno and Sano, 1987; Visvanath et al., 1990; Oberdieck et al., 1991; Sujata et al., 1992; Loskutov et al., 2000) diethyl ether (Zarghami and Heinz, 1971; Pfander and Schurtenberger, 1982; Tarantilis and Polissiou, 1997; Zareena et al., 2001) petroleum ether (Tarantilis et al., 1994b) or aqueous extracts of saffron (Basker and Negbi, 1985; Alonso et al., 1990; Iborra et al., 1992a; Iborra et al., 1992b; Orfanou and Tsimidou, 1996; Tsimidou and Billiaderis, 1997; Selim et al., 2000) have been used. Among these, water was found to be the solvent of choice in the case of crocins, and picrocrocin extraction whereas safranal and HTCC were better extracted with less polar solvents.

2.5.1. *Crocins*

Isolation procedures for crocin 1 or for crocins have been developed by many investigators (Weber and Grosch, 1976; Speranza et al., 1984; Iborra et al., 1992; Pfister et al., 1996; Soeda et al., 2001; Zareena et al., 2001; Zhang et al., 2001). Isolation procedures for crocins from gardenia seeds have also been reported by Pfister et al. (1996), Van Calsteren et al. (1997) and Pham et al. (2000). Some useful protocols are given in Table 2.

Detailed chromatographic and spectroscopic analysis in the work of Straubinger et al. (1997) indicated the presence of the following glucosides: paracetylated digentiobiosyl ester of crocetin (fraction I), paracetylated β-D-gentiobiosyl-β-D-glucopyranosyl ester of crocetin, (2*E*,4*E*)-2-methyl-6-oxohepta-2,4-dienoic acid β-D-gentiobiosyl ester, 3,5,5-trimethyl-cyclohexenone derivatives (fraction II) and (4*S*)-4-(hydroxymethyl)-3,5,5-trimethylcyclohex-2-enone-β-D-glucopyranoside (fraction III).

A novel xanthone-carotenoid glycoside, mangicrocin ($C_{45}H_{50}O_{19}$) has been isolated by Ghosal et al. (1989) as an optically active, yellowish-brown amorphous powder. The compound was extracted from homogenised stigmas of saffron using a mixture of solvents (Et_2O, EtOAc, BuOH), analysed with column chromatography and TLC and finally, identified by its MS and NMR spectra. Its chemical structure is given in Figure 9.

Synthesis of crocins depends heavily on biotechnology (e.g., Himeno and Sano,

Table 2. Recommended protocols for crocin isolation.

Step	Action
	Weber and Grosch (1976)
1	Removal of fat in a Soxhlet apparatus saffron (2.5 g) + Et_2O
2	Extraction with MeOH (2 × 100 ml)
3	Crude crocin + celite (2 g) purification on column chromatography (silic acid, Mallinckrodt 100 mesh, in Et_2O (1.8 cm × 30 cm) a. removal of impurities (100 ml Et_2O + 100 ml Et_2O:MeOH, 70:30,v/v) b. crocin elution with 20% methanolic Et_2O
4	Evaporation *in vacuo* Adjustment of pH with 5 ml 0.05 M Na_3PO_4 (pH 6.5)
5	Further purification with column chromatography (Sephadex G-10 column, 22 cm × 1.8 cm) Equilibration of column with the same buffer as above.
6	Collection of fractions containing pure crocin
	Speranza et al., 1984
1	saffron (10 g) Successive extraction with *n*-hexane (100 ml), Et_2O (100 ml), Et_2O satur. with H_2O (200 ml), AcOEt (150 ml), aqueous EtOH 70% (300 ml)
2	The ethanolic extract a) evaporation of ethanol, b) lyophilisation (residue: 5.1 g)
3	Purification on column chromatography: silica gel 60 (230–400 mesh, 1300 g) Eluent: AcOEt-EtOH-H_2O, 6:3:1, v/v/v
4	Collection of fractions containing crocins – Evaporation to dryness (residue: 0.7 g)
5	Further separation on preparative HPLC (Lichrosorb RP-18, 250 × 10 mm, 7 μ Eluent: ACN-H_2O, 20–45% ACN in 15 min, flow rate 5 ml/min, 440 nm *[crocin 1: UV(H_2O) λ_{max} = 462, 443, 410 nm]* *[13-cis crocin: UV (H_2O) λ_{max} = 462, 437, 410, 328 nm]*
	Straubinger et al. (1997)
1	saffron (8.8 g) successive extraction in a Soxhlet apparatus with petroleum ether (250 ml), Et_2O (250 ml), MeOH (3 × 250 ml) evaporation under reduced pressure to dryness
2	Fractionation with multilayer coil countercurrent chromatography (75 m × 2.6 mm) Eluent: $CHCl_3$: MeOH:H_2O, 7:13:8, v/v/v
3	Combination of 70 fractions (× 10 ml each) I: 1–12; II: 13–29; III: 30–34; IV: 35–64; V: 65–70 (study of the first three)

1987; Koyama et al., 1988; Isa et al., 1990; Sarma et al., 1990, 1991; Isa and Ogasawara, 1991; Dufresne et al., 1997; Bhagyalakshmi, 1999; Zhao et al., 2001). The *in vitro* synthesis of crocin (and also picrocrocin and safranal) in stigma-like structures developed by young stigmas and ovaries of *C. sativus* was first reported by Himeno and Sano (1987). The stigma-like structures were formed and grown for 20 weeks on a Linsmaier and Skoog medium supplemented with napthaleneacetic

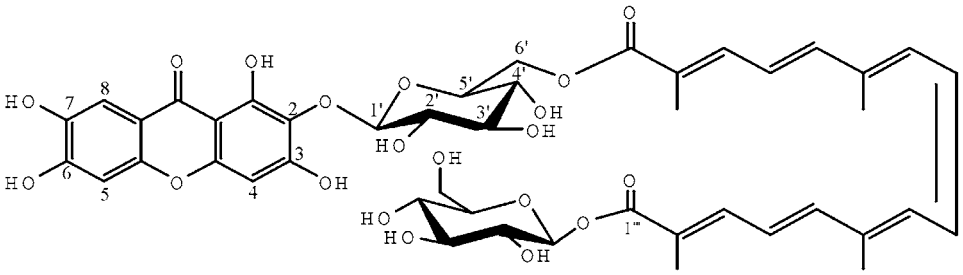


Figure 9. Chemical structure of mangicrocin.

acid (NAA) and kinetin (Kn). The content of the secondary metabolites in dried stigma-like structures were considerably less than that in the intact natural stigmas of saffron. Koyama, et al. (1988) reported on the synthesis of crocin and picrocrocin in stigma-like and style-like tissues and its control by auxin and cytokinin. The stigma and style portions of the plant were induced in various media. In all cases, the HPLC analysis of the constituents of these tissues showed that they contained crocin and other pigments equal to that of the intact stigmas. Fakhrai and Evans (1990) supported the production of secondary products from specialised cultured organs rather than from disorganised callus or suspended cultures to eliminate problems of downstream processing. Still, many parameters such as frequency of stigmas development and continuous proliferation have to be optimised. The authors used various organs of the plant, which were incubated in basal Whites media supplemented with many combinations of various growth regulators. All floral parts had the potential to produce stigma-like structures depending on a critical range of levels and a combination of growth regulators. However, it was suggested that each explant has an inherent potential for the production of stigma-like structures. A similar study with the above was taken on by Ebrahimzadeh, et al. (2000), who investigated the *in vitro* production of stigma-like structures by various floral organs of *C. sativus*. The stigma-like structures regenerated directly of indirectly through merismatic tissues. The direct regeneration of stigma-like structures from stigma explants was also studied by Sarma et al. (1990, 1991). Apart from producing new tissues, the above workers proceeded with the analysis of the pigment content of stigma-like structures. They observed that the crocin and picrocrocin contents were lower by 8 and 6 times, respectively, than in the natural stigmas, while safranal was not detected either in the stigma-like or in the natural stigmas. Visvanath et al. (1990) studied first the potential of callus cultures of saffron for the production of crocin, crocetin, picrocrocin and safranal. Callus cultures were obtained from floral buds on Murashige and Skoog's medium supplemented with sucrose, 2,4-dichlorophenoxy acetic acid and kinetin. The cultures led to the formation either of red globular callous (RGC) or red filamentous structures (RFS). TLC and spectrophotometric analysis showed that both of them contained picrocrocin at higher concentrations than the natural stigmas, whereas their crocin content was less. The safranal content of RGC was similar to that of the natural grown stigmas of saffron. 13-*cis* Crocin may be formed by photoisomerisation of crocin 1 (Speranza et al., 1984).

Several studies have dealt with spectrophotometric methods of analysis of saffron constituents (Buchecker and Eugster, 1973; Dhar and Suri, 1974; Pfander and Wittwer, 1975; Ruppolt, 1975; Amelotti and Mannino, 1977; Corradi and Micheli, 1979a,b; Corradi, 1981; Corradi et al., 1981; Speranza et al., 1984; Basker and Negbi, 1985; Gómez et al., 1987b; Himeno and Sano, 1987; Alonso et al., 1990; Sujata et al., 1992; Tarantilis et al., 1995; Orfanou and Tsimidou, 1996; Straubinger et al., 1997; van Calsteren, 1997; Alonso et al., 1999b; Lozano et al., 1999).

Spectrophotometric estimation of crocin content (or colouring power of saffron) is based on the absorbance at 440 nm of a 1% aqueous extract of the spice. For more accurate figures a value for molar extinction coefficient of 89000 (Speranza et al., 1984) may be employed. The method of extraction may have a significant impact on the value of colouring strength obtained spectrophotometrically (Basker and Negbi, 1985). Orfanou and Tsimidou (1996) reported on the possible misleading which may occur in the evaluation of the colouring power of saffron when using different preparation procedures for the extraction of crocins. The authors proposed the application of derivative spectroscopy as an alternative approach for the evaluation of the colouring strength of commercial saffron.

The profile of crocins has been mainly examined by HPLC (Pfander and Rychener, 1982; Kamikura et al., 1985; Himeno and Sano, 1987; Solinas and Cichelli, 1988; Sujata et al., 1992; Morimoto et al., 1994; Tarantilis et al., 1994c, 1995; Pfister et al., 1996; Alonso et al., 1999b; Li et al., 1999; Lozano et al., 1999; Alonso et al., 2001; Radjabian et al., 2001; Zareena et al., 2001).

Pfander and Rychener (1982) first reported the separation of crocetin glucosides. The authors used two different columns for the analysis: (1) a LiChrosorb SI 60 (Merck, Darmstadt, Germany) (7 μ) and (2) a LiChrosorb RP-18 (Merck) (5 μ). The eluent in the case of normal phase analysis was either a mixture of ethyl acetate-isopropanol-water (56:34:10) (v/v/v) or ethyl acetate-*n*-hexane (70:30) (v/v). The reversed phase system included methanol-water (60:40) (v/v) as a mobile phase. The latter system offered a perfect separation of diglucosyl and digentiobiosyl esters within a short time, but it was limited to the separation of non-acetylated esters. In any case the normal phase system though being time-consuming proved to be more suitable for the separation. Himeno and Sano (1987) and Sujata et al. (1992) used similar composition of solvents (20–90% aqueous ACN and 20–80% ACN) to elute pigments from reversed phase columns. In both cases, crocin 1 was the first compound eluted, followed by crocetin esters and crocetin (detection at 443 nm). The retention trend of various crocins was in accordance with that reported by Morimoto et al. (1994). A more distinct resolution of crocetin esters was achieved by Tarantilis et al. (1994b). The authors using a reversed phase column (LiChroCART 125 × 4 mm, Superspher 100 RP-18, Merck) in combination with gradient elution (20–100% ACN, 0.5 ml/min) managed to distinguish between *cis*- and *trans*-crocins. The retention times reported for *cis* and *trans* crocins ranged from 8.5 to 15 min whereas *trans* and *cis* crocetin were eluted much later (t_R: 20, 20.5 min, respectively). The detection wavelength was set at 440 nm. In another work, Tarantilis et al. (1995) combined the HPLC system with MS spectrometry in the electrospray and thermospray modes and identified the major crocins. The ^{1}H-NMR spectrum of *trans*-crocin 1 in DMSO-d_6, as reported by Speranza et al.

(1984) gives the following data: δ 7.35 (2H, d, J = 11.4), δ 6.66 (2H, dd, J = 11.4, 15.0), δ 6.82 (2H, d, J = 15.0), δ 6.53 (2H, dd, J = 8.0, 2.5), δ 6.86 (2H, dd, J = 8.0, 2.5), δ 1.97 (2H, s), δ 2.00 (2H, s), δ 5.42 (1H, d, J = 7.5), δ (1H, dd, J = 7.5, 7.5), δ 3.0–3.3 (7H, m), δ 3.4–4.0 (2H, m).

2.5.2. *Picrocrocin*

According to Tarantilis et al. (1994b), the isolation of picrocrocin is achieved initially by successive extraction of saffron components in a Soxhlet apparatus with light petroleum, diethyl ether and methanol. Picrocrocin, which was present in the diethyl ether extract, was purified in the Soxhlet extractor, diluted with methanol and passed through a 0.45 μ filter. Finally, it was analysed by HPLC using three different conditions (*see* Table 3) and detected by UV-Vis. Except for its absorption maxima at 250 nm (broad band due to the α, β-unsaturated cycloaldehyde moiety), picrocrocin also exhibited a shoulder at 350 nm. Himeno and Sano (1987) reported the *in vitro* synthesis of picrocrocin in stigma like structures of saffron. The main analytical methods that have been applied to the analysis of picrocrocin include thin layer chromatography (Iborra et al., 1992b; Sujata et al., 1992) and HPLC (Sujata et al., 1992; Tarantilis et al. 1995; Alonso et al., 2001). Different investigators give spectroscopic characteristics of picrocrocin. Iborra et al. (1992b) referred to the IR spectrum of an aqueous solution of picrocrocin in caesium chloride plates: 3400, 2950, 1620, 1410, 1360, 1080 and 1040 cm^{-1} in deutered water and 1410, 1350, 1075 and 1035 cm^{-1} in water. The latter also reported ^{1}H-NMR and ^{13}C-NMR spectra of this compound.

2.5.3. *Volatiles*

The common extraction processes for the volatile components of saffron are steam distillation (SD), solvent (hydrophilic to hydrophobic), vacuum headspace (VHS) or dynamic headspace (DHS) extraction. Tarantilis and Polissiou (1997) have examined the effects of the extraction method on the composition of the obtained essential oil. After identification of the volatile compounds with GC-MS analysis, they came to the conclusion that the higher the temperature of the extraction, the more drastic the changes in the structure and the nature of the ingredients of each volatile fraction. Apart from this, Loskutov et al. (2000) showed that safranal as being water-insoluble, is better extracted with hydrophobic solvents while its concentration in the extracts remains stable with time. However, such methods for extracting volatile components are highly destructive for the sample. A non-destructive technique for the isolation of safranal and oxysafranal (HTCC) from the solid matrix has been proposed by Semiond et al. (1996) and Lozano et al. (2000). It refers to the application of a supercritical fluid (CO_2) to the extraction of the above volatile oils directly from saffron powder within a short time.

Several methods have been proposed for the qualitative and quantitative analysis of the aroma compounds of saffron. Estimation of safranal content can be achieved by measuring the absorbance at 330 nm (ISO 3632, 1993) of an aqueous extract of saffron or by chromatographic methods. The spectrophotometric estimation is not

selective and accurate as the chromatographic one because other compounds absorb at 330 nm (Tarantilis et al., 1994b, 1995; Orfanou and Tsimidou, 1996). Zarghami and Heinz (1971) were the first to investigate the chemical composition of the essential oil using a gas chromatograph coupled to a mass spectrometer. The HPLC and GC conditions of analysis are tabulated in Tables 3 and 4.

Different modes for sample injection have been used. The chromatographic peaks are identified using spectroscopic data from MS, IR or NMR spectra libraries (Zarghami and Heinz, 1971; Petrzika and Roedel, 1991; Tarantilis and Polissiou, 1997; Straubinger et al., 1998; Lozano et al., 1999, 2000; Alonso et al., 2001b). The major fragments of safranal ($C_{10}H_{14}O$) as identified by MS spectra were the following: *m/z* (%) 107 (100), 91 (69), 121 (66), 150 (59), 79 (21), 65 (13), 135 (12) (Tarantilis and Polissiou, 1997). Lozano et al., 2000) after using a different extraction procedure, did not detect the ion with *m/z*: 135, while they matched the molecular ion of safranal with the fragment having *m/z*: 91. Concerning the IR spectrum of safranal there have been reported absorptions at 2830, 2720 and 1720 cm^{-1} which indicate the presence of an unconjucated aldehyde moiety in the molecule (Zarghami and Heinz, 1971).

2.6. Constituents of other *C. sativus* plant organs

Except for the chemistry of dried saffron, several investigators have studied the chemical composition of different organs of *C. sativus*. Various flavonoids have been identified in the flowers, tepals and leaves of many *Crocus* species (Poldini et al., 1979; Harborne and Williams, 1984; Kubo and Kinst-Hori, 1999). Kaempferol, astragalin, helichrysoside, kaempferol-3-O-β-D-glucopyranosyl-(1 → 2)-β-D-6-acetylglucopyranoside and kaempferol-3-O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside myricetin, petunidin, and delphinidin-3,5-diglycoside have been reported in pollen, petals and tepals of *C. sativus* (Garrido et al., 1987; Song, 1990; Ríos et al., 1996).

Gao et al. (1999) reported on the isolation and characterisation of some phenolic glycosides and a γ-lactone glucoside from the sprouts of the plant. The compounds found were elucidated as 2,4-dihydroxy-6-methoxyacetophenone-2-β-D-glucopyranoside, 2,3,4-trihydroxy-6-methoxy-acetophenone-3-β-D-glucopyranoside and 3-(S)-3-β-D-glucopyranosylbutanolide. Escribano et al. (1999a) isolated a proteoglucan of 30 kDa from corms of *C. sativus*. The polysaccharide part of the molecule was found to contain 36.4% rhamnose, while the protein backbone is composed by aspartic acid/asparagine, alanine, glutamic acid/glutamine, glycine and serine, which make up the 60% of the total amino acids.

3. SAFFRON QUALITY. TRADE STANDARDS AND OTHER CRITERIA

Herbs, spices and their derivatives are used in the food, pharmaceutical and cosmetic industry. Their trade obeys certain rules set either by the relevant legislators or by trade organisations and other interesting parties. The last decade harmonisation of international legislation facilitated the quality control of these precious products

Table 3. HPLC analysis of safranal and other volatile components of saffron.

Extraction procedure	Column	Elution conditions/ detection system	Reference
50% aqueous EtOH	Lichrosorb RP-18. 5 μ	Linear gradient elution (20–90% ACN in 20 min), 1 ml/min, 308 nm	Himeno and Sano, 1987
Saffron powder (100 mg) + 1 ml of int. standard (75 ppm undecanal/ACN), 60 °C/30 min, derivatisation with 2.4-dinitrophenyl-hydrazine, (125 mg in 50 ml ACN)	Bondapak C18	Gradient elution (55–90% ACN in 18 min, 90–99% ACN in 15 min), 0.7 ml/min, 365 nm	Solinas and Cichelli, 1988
80% cold, aqueous EtOH (5 ml) centrifuge: 5000 g/10 min, wash: twice with 5 ml of 80% EtOH each	Sep-PAK-C18, 15 cm × 4.9 mm	Linear gradient elution (20–80% ACN) or Isocratic elution 76% ACN 0.5 ml/min, 308 nm	Sujata et al., 1992
Saffron (20 g) successive extraction in Soxhlet apparatus with light petroleum, diethyl ether (0.03% BHT), MeOH:	LiChroCart 125 mm × 4 mm Superspher 100 RP-18, 4 μ	Linear gradient (20–100% ACN in 20 min), 0.5 ml/min, 308 nm	Tarantilis et al., 1994b
Ground saffron (400 mg) + 50% aqueous MeOH (200 ml), stirr. for 1 h: 25 °C/dark, centrifuge: 30000 g/20 min 100 ml of extract + 100 ml of int. standard (0.2 mg/ml of 4-nitroaniline)	Nova-PACK RP-18, 15 cm × 3.9 mm, 4 μ, 6 nm	Linear gradient elution 20–70% MeOH, 1 ml/min, 310 nm	Lozano et al., 1999
a) Saffron powder (200 mg) in the extractor cell (0.5 ml) + 0.70 ml CO_2/min (17 MPa, 70 °C, 30 min) 0.8 ml of extract + 0.2 ml int. standard (0.2 mg/ml of 4-nitroaniline in 50% MeOH) b) Saffron residue (200 mg) + 50% aqueous MeOH (50 ml), stirr: 1 h/room temp./dark, centrifuge: 30000 g/20 min 8 ml of extract + 20 ml int. stand. (same as above)	Lichro-CART RP-18, 125 mm × 4 mm, 5 μ, 10 nm	Isocratic elution: 20% MeOH in 10 min Gradient elution: 20–70% MeOH, 1%/min, 1 ml/min, 310 nm	Lozano et al., 2000
a) Ground stigmas (100 mg) + 80% aqueous EtOH (3 ml), (3reps), centrifuge: 8000 rpm/ 5 min, wash: 80% aqueous EtOH b) 100 mg of ground stigmas + 100% aqueous ACN (3 ml), (3 reps) centrifuge: 8000 rpm/5 min c) the same as in (b) except for time of injection (1 h after extraction)	RP-C18 25 cm × 4.9 mm, 5 μ	Isocratic elution: 100% ACN, 1 ml/min 308 nm	Loskutov et al., 2000

Table 4. Gas chromatographic analysis of the volatile compounds of saffron.

Column	Elution conditions/detection system	Reference
SE-30 5%, 3 m stainless steel	150 °C, 30 ml N_2/min, FID	Sujata et al., 1992
SE-30 3% on Chromosorb-W Glass (1.8 m × 6.35 mm)	80–200 °C, 6 °C/min, 30 ml N_2/min, sensory detection	Raina et al., 1996
FFAP (50 m × 0.32 mm), 0.52 μ	60 °C for 8 min-240 °C for 30 min, 2 °C/min, 3 ml He/min, FID (290 °C)	Tarantilis and Polissiou, 1997
Ultra-1 methyl silicone (25 m × 0.32 mm), 0.50 μ	70 °C for 3 min-150 °C for 1 min-200 °C for 10 min, 2 °C/min, 3 ml He/min, FID (230 °C) and 60 °C for 1 min-280 °C for 10 min, 5 °C/min, FID (260 °C)	Tarantilis and Polissiou, 1997
SGE-BP-21	100–230 °C, 3 °C/min, 230 °C for 10 min, 40.1 lb He/in^2, TD-MS	Alonso et al., 1996, 1998a
DB-5 coated fused silica capillary	60–300 °C, 5 °C/min, 1.5 ml He/min, MS	Straubinger et al., 1998
DB-1 capillary (30 m × 0.25 mm × 0.25 μm)	60–200 °C, 4 °C/min, 200 °C for 5 min – 280 °C for 25 min, 10 °C/min, He, MS	Zareena et al., 2001

that afford adulteration, misbranding and other illegal treatments since ancient times.

In general, the principal criteria of a trade quality standard (e.g., ISO, EN, AFNOR, etc) are organoleptic, botanical and physicochemical ones, and also the content of microorganisms, especially those that are pathogens. Methods of analysis for foreign matter, moisture content, total ash, non-volatile extract, etc. are found in a series of ISO generic standards. Itemised standards for certain products are also published, as it is the case of saffron. The Committee of *Codex Alimentarius* has published jointly with ISO a Code for the Hygiene of Spices. European directives on food contaminants and on methods of decontamination for plant origin products (e.g., irradiation) also apply to herbs and spices. Packaging and preservation techniques are very important to maintain the valuable characteristics of spices. Unfortunately, the majority of the world-wide available quantities of spices are sold without the required precaution, saffron not being an exception.

Dried saffron is traded in the form of whole or cut threads (stigmas+part of the style) or as powder. Different commercial grades are found in the various countries, some of them distinguished for their exquisite organoleptic characteristics. In India, four commercial types are known. *Shahi* consists of stigmas only, *Mongra* consists of stigmas with style and few filaments, *Laccha* of broken stigmas, styles, filaments and other portions of floral tissue whereas *Gucchi*, mainly from Kishtwar region, of whole stigmas with style tied in bundles (Dhar and Mir, 1997). Spanish saffron has been classified in various ways by different interesting bodies. As Alonso et al. (2000) recently reviews it the three most known grades are *Mancha, Río* and *Sierra*. The designation of 'protected origin and protected geographical indications' was awarded to *the Azafrán de la Mancha* recently (Commission Reg.

464/2001, OJ L66, 8.3.2001, p. 29). Two years earlier the *Greek 'red saffron'* has also gained the same designation under the name '*Krokos Kozanis*' (Commission Reg. 378/99 OJ L. 46, 20.2.1999, p. 13). Grades A and B are the two commercial names for Iranian saffron. Quality evaluation of powdered saffron is more difficult (Marini et al., 1992).

The International Organisation for Standardisation (ISO) issued a specific standard for saffron ISO 3632 in 1975, revised in 1980 that was technically improved in 1993. In ISO 3632 1&2 (1993) trade standard definitions as well as requirements for saffron quality and methods of analysis are given. Other relevant standards for the analysis of spices and condiments useful for the control of saffron quality characteristics are ISO 928:1980 (determination of total ash); ISO 930:1980 (determination of acid-insoluble ash); ISO 941:1980 (determination of cold water-soluble extract); ISO 948:1980 (sampling); ISO 1871:1975 (General directions for the determination of nitrogen by the Kjeldahl method); ISO 5498: 1981 (Agricultural food products-Determination of crude fibre content-General method).

For the purpose of Part 1 of the standard entitled 'Specification' the following definitions apply:

a. Saffron in filaments are the stigmas of *C. sativus* Linneaus, dried, dark red in colour and trumpet shaped, serrated or indented at the distal end. The length is between 20 mm and 40 mm. The stigmas may be isolated or joined in pairs or threes at the end of the portion of the style, which is white/yellow in colour.
b. Saffron in cut filaments are the stigmas of *C. sativus* Linneaus with styles removed and completely detached from each other.
c. Yellow filaments are dried yellow stamens of the flowers of *C. sativus* Linneaus.
d. Floral wastes are yellow filaments that are unattached and separated, pollen, stamens, parts of ovaries and other vegetable matter. The only mineral matter permitted is sand, earth and dust.
e. Saffron in powder is saffron obtained by crushing the filaments.
f. Moisture and volatile matter content are loss of mass determined under the conditions described. It is expressed as a percentage by mass of the sample.
g. Colouring strength is mainly due to its crocins content, as measured by its optical density at about 440 nm.
h. Bitterness is mainly due to its picrocrocin content, as measured by its optical density at about 257 nm.
i. Flavour is mainly due to its safranal content, as measured by its optical density at about 330 nm.

Saffron in filaments is classified into four categories, on the basis of its floral waste and extraneous matter content.

Saffron should have a specific flavour that is slightly bitter and a little pungent and should be free from foreign flavour. It should be free from living insects, insect fragments and rodent contamination visible to the naked eye or using the required magnifying instrument in certain cases. If the magnification exceeds X10, this fact shall be mentioned in the test report.

Trade requirements for saffron in filaments or in powder form as given by ISO 3632-1:1993 are presented in Tables 5 and 6.

Table 5. Classification of saffron in filaments.

Characteristic	Categories			
	Extra I	II	III	IV
Floral waste, % (m/m), max	0.5	4	7	10
Extraneous matter, % (m/m), max	0.1	0.5	1.0	1.0

Table 6. Chemical requirements for saffron, in filaments or in powder form (after ISO 3632-1-1993).

Characteristic	Requirement	
	Saffron in filament	Saffron in powder form
Moisture and volatile matter,% (m/m), max	12	10
Total ash, % (m/m), on dry basis, max	8	8
Acid-insoluble ash, % (m/m),on dry basis, max		
Categories I and II	1.0	1.0
Categories III and IV	1.5	1.5
Solubility in cold water, % (m/m), on dry basis, max	65	65
Bitterness expressed as direct reading of the absorbance of picrocrocin at about 257 nm, on dry basis, min.		
Category I	70	70
Category II	55	55
Category III	40	40
Category IV	30	30
Safranal, expressed as direct reading of the absorbance at about 330 nm, on dry basis		
All categories		
Min.	20	20
Max.	50	50
Colouring strength, expressed as direct reading of the absorbance of crocins at about 440 nm, on dry basis, min		
Category I	190	190
Category II	150	150
Category III	110	110
Category IV	80	80
Total nitrogen, % (m/m), on dry basis, max	3.0	3.0
Crude fiber, % (m/m), on dry basis, max	6	6

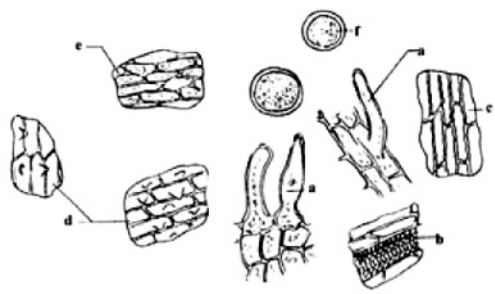
Saffron in filaments (or threads) or in powder form should be packed in containers that do not affect the content. Commercial and botanical names, name and address of the producer or packer, trade mark, batch or code number, commercial category and other information required by the client (harvest year, packing date etc) are required on labelling.

According to the Part 2 of the above standard the test methods and the analytical procedures are described in details. To spare saffron sample (2×5 g) provision for the exact order of test procedures is necessary for repeating certain analyses in the case of disputes. In the 1975 edition of ISO standard a dichromate number that is, the concentration of a $K_2Cr_2O_7$ solution that gives the same colour intensity as a 0.01% saffron solution, was included (Corradi and Michelli, 1979b). Identification tests include reaction with sulfuric acid and diphenylamine and thin layer chromatography. There are underway collaborative trials of an ISO task force for the development and standardisation of TLC and HPLC procedures for the detection of artificial water-soluble colourings (Saltron et al., 1999). The use of instrumental analytical procedures for grading saffron is gaining interest (Curro et al., 1986; Solinas and Cichelli, 1988; Sujata et al., 1992).

Microscopic observation of typical anatomical elements is valuable to test the authenticity of powdered saffron in a) distilled water, b) in sodium or potassium hydroxide solution and c) in aqueous iodine/iodide solution. The alkali treatment destroys the greatest part of the starch and also the cellular elements (sclerous elements, vessels, fibres and epidermis) become easier to observe. On the other hand the starch grains are stained blackish blue or violet with iodine/iodide solution.

Among the diagnostic characters of at most importance are the upper epidermis of the stigmas with small papillose protuberances and the large pollen grains.

The determination of the major organoleptic characteristics of saffron (picrocrocin, safranal and crocins) is carried out in an oversimplified way by spectrometric evaluation of an aqueous extract at three characteristic maxima 257, 330 and 440 nm, respectively. The drawbacks of the procedure have been often criticised by many investigators (e.g., Amelotti and Mannino, 1977; Basker and Negbi, 1985;



a: papilla
b: vascular bundles
c: epidermis of a style
d: upper epidermis
e: parenchyma
f: pollen grain

Figure 10. Anatomical structure of powdered saffron (After ISO standard 3632-2/1993).

Tarantilis et al., 1994b, 1995; Orfanou and Tsimidou, 1996; Alonso et al., 1998b). The flavour threshold, flavour profile and psychophysical scaling of saffron flavour had been reported by Narasimhan et al. (1992) who attempted the quality evaluation of the commercial product. A procedure for the application of FT-Raman spectrum of solid saffron as a means of product standardisation has been recently proposed (Aminzadeh, 2000). Tristimulus colorimetry has been also examined as a means for evaluating colouring power of dried saffron *in situ* (Cuko, 1997; Mitsopoulou, 2000; Carmona et al., 2002).

Concerning the morphological characteristics of the spice, typical length of the spice ranges from 20–40 mm whereas the upper part of the stigma is usually 2–3 mm thick (Sampathu et al., 1984; Rees, 1988; Raina et al., 1996; Mitsopoulou, 2000).

In accordance to the international concern for food safety the European Committee issued a directive on the Food Hygiene (93/43/EOK) so that since 2000 all the member states have adopted the Hazard Analysis at Critical Control Points Systems (HACCP) in the food industry. At the same time Good Manufacturing Practice Manuals are becoming common practice in the food sector, even to the small-scale rural food industries (Fellows et al., 1995). Such manuals rather assist than regulate the industrial practice. In the sphere of Total Quality Management (TQM) any HACCP system should be in accordance with the relevant ISO 9000 quality standards as well as with standards related to the environmental management.

Further requirements for saffron quality are found in the international food legislation as well as in certain pharmacopoeias.

The chemical and sensory analysis of the tissue culture saffron indicated that the pigments produced were the same as those in natural stigmas, although their levels were much less (Sarma et al., 1991). Flavour profile analysis showed that the important characteristics (sweet, floral, spicy and fatty notes) were very low as compared to the commercial sample. Herbaceous notes-harsh/acrid and barky dominated. According to Visvanath et al. (1990) the quantity of picrocrocin in red globular callous and red filamentous structures was higher than that in natural stigmas. The safranal content was comparable to that of stigmas whereas the crocin content was much less in cultured product. No dependence of crocin and picrocrocin levels on the medium was observed so far whereas safranal and HTCC appear after drying (Himeno and Sano, 1987).

3.1. Criteria for detection of fraud

Saffron, the most expensive spice worldwide, has been subjected to different types of admixture with organic or inorganic materials since the antiquity.

In modern times adulteration of saffron with an array of ways was experienced. In the early sixties Spanish saffron was found to be impregnated with salts, mainly with potassium nitrate (18–20% w/w KNO_3) as a means to increase weight of the spice without changing its appearance (Lowell, 1964). Such types of adulterants include the use of glycerin, borax, glucose and fructose (Yan et al., 1983) and other salts. A modified diphenylamine reagent, heating (sparkling explosion in the presence of nitrates), the use of a polarising microscope, a flame test for potas-

sium were the qualitative tests used to detect fraud. Quantification was achieved through the application of Devarda method and colorimetric methods used for nitrate. The water and alcohol insoluble matter weight was also useful in this case. Numerous saffron adulteration cases were reported by Stahl and Wagner (1968), who experimented on the combination of microanalytical methods to indicate the characteristic saffron components. Oberdieck (1991) and Alonso et al. (1998a) summarised the most frequent adulteration practices:

(1) Misbranding or falsification of origin
(2) Admixture with old saffron or with style material
(3) Admixture with stamens previously cut and dyed
(4) Impregnation with substances to increase weight (syrups, honey, glycerin, oils, potassium hydroxide, saltpetre, Glauber's salt, Seignette's salt, borax, lactose, starch or glucose)
(5) Parts of other plants with or without colouring power
(6) Animal substances (fibers of salted and dried meat)
(7) Threads of coloured gelatin; organic colourings and colouring materials derived from tar.

Among plant materials adulteration with dried petals of *Carthamus tinctorius*, L seems to be common even in recent times (Seidemann, 2001). Other plant materials reported are *Calendula officinalis* L., stigmas from other crocuses (*C. vernus* L., *C. esperiosus* L) that are generally shorter and without colouring properties, strips of petals from *Papaver rhoeas* L., *Punicam granatum* L., *Arnica montana* L., and *Scolymus hispanicus* L., stamens from carnations, ground red pepper, sandlewood dust and longwood particles and also curcuma. Molecular genetic methodology indicated that the nucleotide sequence may differentiate *C. sativus* from three typical adulterants used in the South East Asia, namely, *C. tinctorius*, *Hemerocallis fulva*, L. and *Hemerocallis citrina* Baroni (Ma et al., 2001). Two phenylpropanoid glucosides, verbascoside and poliumoside, determined by HPLC, have been used to identify *Buddleja officinallis* Maxim. yellow natural colorants in foods containing crocetin derivatives in the Chinese market (Aoki et al., 2001). Many other crocuses may be used as potential adulterants (e.g., *C. haussknechtii* Boiss) (Radjabian et al., 2001). Frequent seems to be the adulteration of saffron in the Italian (Corradi, and Micheli, 1979a, b; Corradi, 1981) and Spanish market. The main reason is that the product is imported in small amounts of 1 kg or even less so that analysis of all batches becomes difficult. Adulteration with mixtures of synthetic dyes such as tartrazine, Ponceau 4R, azorubine, sunset yellow and erythrosine were detectable even with TLC procedures (e.g. Corradi et al., 1981; Alonso et al., 1999a; Carmona et al., 2002). Many HPLC procedures have been also reported for the detection of synthetic dyes (Lozano et al., 1999; Saltron et al., 1999). Authentication of saffron aroma through the establishment of a fingerprint of the volatiles has been attempted by Alonso et al. (1998a) or by safranal analysis by isotopic ^{13}C (Semiond et al., 1996). The latter gives a clear discrimination between the synthetic and the natural counterpart but no definite conclusion about the geographical origin. Even the presence of mangicrocin, a xanthone-carotenoid glycosidic conjugate from saffron has been discussed as a biomarker for the control of authenticity (Ghosal et al.,

1989). As biomarkers may be also used crocetin derivatives present in the stigmas of other crocuses but not identified in *C. sativus* (Rychener et al., 1984). Extracts from saffron and Gardenia fruit seemed to contain the same yellow pigments (Kamikura and Nakazato, 1985) so that the latter is a potential substitute or adulterant.

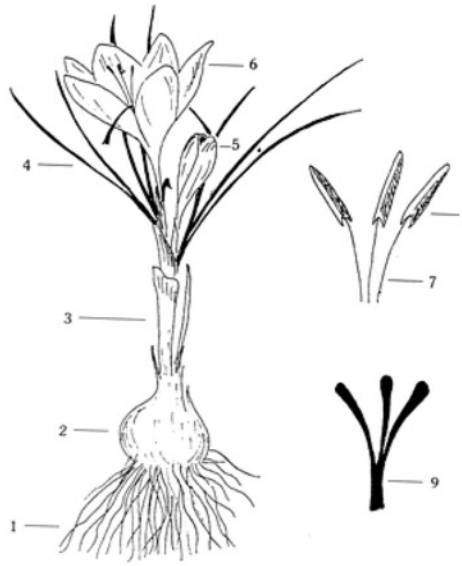
4. EFFECT OF AGRICULTURAL PRACTICES ON QUALITY

The concept on the quality of agricultural products has been substantially changed since the 80s. The quality of raw materials is definitely considered as a prerequisite for the quality of the final product and, consequently, agricultural practices are equally important to technologies applied to ensure food wholesomeness and safety. Regardless the size of the plant, the 'state of art' of agricultural practices should be scientifically supported. In the case of saffron, control of its authenticity is not the panacea. Agricultural practices do vary from producer to producer and from country to country though the argument 'all are doing the same from the father to the son' is common. Therefore, the implementation of parameters, such as propagation and cultivation practices, control of pests and diseases and also the harvesting and handling of flowers till the fresh stigmas are separated, on the spice quality are discussed on the basis of literature, experimentation and personal experience.

4.1. Propagation practices

A full description of the botany, taxonomy and cytology of *C. sativus* L. and its allies has been repeatedly detailed by Mathew (1999). Information can be also found in papers on the biochemical evolution of *Iridaceae*. The saffron plant is propagated by the cormlets (or daughter corms) formed by the mother corm. The corm that is up to 5 cm in diameter, is depressed-globose, flattened at the base with fibrous tunics. The fibres are very slender and finely reticulated, extended at the apex of the corm into a neck up to 5 cm long. The corms remain dormant during the summer months and grow at about the end of the summer season (Sampathu et al., 1984). The plant flowers (1–4 per plant) appear in autumn from October to late November. They are fragrant, deep lilac-purple with darker veins and a darker violet stain in the white or lilac throat. The pistil is in the center of the flower from which the style arises. The style in the upper part is divided into three deep red branches, or three-lobe stigmas. Three yellow anthers also arise from the throat of the flower. Growth of leaves (5–11, green, 1.5–2.5 mm wide) roots and, in parallel, sprouting of mother corms follow up till February. Death of mother corms and intensive growth of daughter corms is observed in this period. From March to May leaves, mother corm and roots are completely dried up and the bulb remains dormant till the end of summer.

The low fertility of *C. sativus* is partially due to an irregular meiosis and also because the ovarian transmitting tissue prevented penetration of the ovules (Chichiricco and Grilli Caiola, 1986). The vegetative type of reproduction leads



1. Root
2. Bulb
3. Shoot
4. Leaf
5. Bud
6. Open flower
7. Stamens
8. Anthers
9. Three-lobe stigmas

Figure 11. The saffron plant (after ISO standard 3632-1/1993).

to a quite uniform genetic material in spite of diversity in origin and morphological differences of the plant parts. Thus, improvement of the genetic material is restricted unless future breeding programmes involving fertile allies (e.g., *C. cartwrightianus, C. oreocreticus* and *C. thomasii*) are carried out (Grilli Caiola, 1999) or other fertilisation routes are explored (Chichiricco, 2000). Plant improvement has been also attempted through irradiation of corms. Modernisation of saffron crocus cultivation except for reducing labour and introducing mechanisation (Galigani and Pegna, 1999) requires improved starting material. The use of gibberillins plus other growth regulators when corms were dormant (June–July) accelerated formation of more flowers (Azizbekova et al., 1978 and 1982; Kabdal and Joshi, 1978; Farooq and Kaul, 1983; Chrungoo and Farooq, 1984; Chrungoo and Farooq, 1989) and consequently of saffron itself. The effect of gibberellic acid is reported to stimulate starch breakdown in favour of reducing sugars and pentoses (Chrungoo and Farooq, 1989, 1991). Increase in leaf length, and the number of daughter corms is reported after an overnight dipping of mother corms in an aqueous solution (10–200 ppm) of 2,4-dichlorophenoxy acetic acid (Kabdal and Joshi, 1978). *In vitro* corm production is another means for increasing the reproductive capacity of the plant (Ilahi et al., 1987). Plessner et al. (1990) found that growth regulators such as cytokinins, in particular zeatin, and auxin 2,4D are essential for develop-

ment of bud explants. Ethylene and ethaphon (an ethylene releasing agent) pre-treatment inhibited leaf development but on the other hand induced corm production as well as dormancy. Sprouting and corm production was also enhanced after microsurgery of the apical bud combined with ethylene pre-treatment.

The information of the morphological and chemical characteristics of corms and floral parts is not systematic (Craig et al., 1985; Sugimoto et al., 1986; Arifkhodzaev et al., 1986; Chrungoo and Farooq, 1985 and 1988). The presence of saponins, triterpene acids, sugars, mucilage, amino acids, sterols fatty acids, ursulic,and oleanolic acids were verified by Loukis et al. (1983). Gómez et al. (1987a) gave data on the mean weight of bulbs originated from different areas within the province of Albacete. The values ranged from 5–15 g.

4.2. Cultivation practices

Crocus sativus L. is cultivated in climatically diverse regions varying in altitude, range of temperature and humidity. It thrives best in warm subtropical climate and soils varying from sandy to well-drained free of clay soils (Sampathu et al., 1984). The land is prepared just before planting by ploughing, harrowing, and weed removal.

Effective production of cormlets (or daughter corms) is the outcome of effective shallow planting and dominance of the apical bud. Reproduction occurs in the autumn-winter period. Each mother corm results in 1–2 principal buds at its apex and several other cormlets from lateral buds. In certain places the corms remain in the earth throughout the year and for, as many consecutive years the land in use is considered suitable for the cultivation. Regular inspection of the planted corms is necessary for replanting those corms found out of the ground and for avoiding diseases that cause loss of great amounts of corms. Weeds are removed either by hand or by using herbicides.

Greece. Saffron crocus is exclusively cultivated in Kozani prefecture (West Macedonia) in Northern Greece. Most of the producers are coming from the village Krokos (Figure 12). The growers (~1500) from about 20 little villages, cultivate their own land or land they hire for several years and co-operate in trade. In this way, since 1971, Saffron Growers' Co-operative of Kozani managed to improve standardisation of the final product and achieved high prices in exports. The profits return to the producers on the basis of the dry saffron weight they presented at the co-operative within a limited defined period from February to March of each year. The area of Kozani is characterised by frequent rainfalls (precipitation >500 mm, annually) whereas temperature rarely falls below zero in winter. However, early snow in November has destroyed sometimes the production. No systematic data on soil characteristics is reported though Goliaris (1999) presents some data from four soil samples (pH ~ 7.40, K_2O ~ 7 mg/100 g) without further details. The same plantation may be kept profitable for about seven years. For a new plantation 2–3 tons of corms (22–25 mm diameter, 35–40 mm high) are used. A seven-year plantation usually yields the double amount of corms. Before transplanting use of fungicides is suggested by the responsible agronomists.


Figure 12. Harvesting of saffron flowers, Kozani, Greece.

India. Cultivation of saffron is confined to the states of Jammu and Kashmir, mainly in the Kashmir Valley at Pulwama (78% of the total cultivated area), Badgam, Anantanag and Baramulla (<1%). Saffron plantation (140 ha) is also found in Kishtwar (Doda district) in Jammu. Successful attempts to grow saffron in other areas such as Uttar Pradesh and Himachal Pradesh are also reported (Dhar and Mir, 1997). Jammu and Kashmir (32–36 °N), encompasses the western Himalayas and the Karakorum mountains, at an altitude of 1600 m above the sea level. The climate is subtemperate. Studies in the Kashmir region on the properties of the soils on which *C. sativus* thrives (or not) indicated that the former contain a somewhat greater amount of sand, available K_2O and a lower amount of exchangeable potassium. The pH ranged from 7.2 to 8.4 and higher yields coincided with higher pH values (Shinde et al., 1984). In a more detailed study (Nazir et al., 1996) the combination of properties of soils suitable for growing saffron in this area was examined using multivariate analysis. Two groups of soil properties were used as variables: a) total physical, chemical and physicochemical characteristics and b) factors of the mineralogical composition of the mud fraction. Apart for the similarities of the soils of the investigated areas, pH, content of dithionite-soluble iron and exchangeable sodium and potassium favoured saffron growing.

Iran. Behnia et al. (1999) commends on the practices followed in Iran and cites some references which indicate the interest of Iranian scientists on this issue. In Iran the spice is grown mainly in the Khorasan province in the eastern part of the country. The corms remain in the fields 6–15 years and they are removed only when the yield is not economically profitable due to overcrowding of the new corms. Based on previous studies that showed that potassium fertiliser is not suitable to increase saffron yield, the authors examined the effectiveness of nitrogen and phosphorous fertilisers as well as composed cow manure on fresh flower weight, the weight of dried styles and stigmas and leaf biomass. Their experiments conducted at two locations over three consecutive years. A total of 27 treatments arising from a factorial combination of three levels of nitrogen (0, 50 and 100 kgN ha^{-1} $year^{-1}$), three levels of phosphorous (0, 25 and 50 kgP ha^{-1} $year^{-1}$) and three levels of manure (0, 20, 40 tons ha^{-1} $year^{-1}$) were carried out. Soil characteristics before the treatments were examined (Table 7).

Table 7. Typical soil characteristics of saffron fields at Birjhand and Ghaen, Khorasan, Iran (after Behnia et al., 1999).

Soil characteristics	Birjhand fields	Ghaen fields
Potassium (mg/kg)	270	215
Phosphorous (mg/kg)	7	4.5
Total N (%)	0.05	0.06
Organic C (%)	0.51	0.71
$CaCO_3$ (%)	15.0	17.0
PH	8.0–8.1	7.5–7.9
Electrical conductivity (dS m^{-1})	2.1–3.0	2.0–3.3
Iron mg/kg	4.4–6.4	2.8–6.8
Manganese	13.2–23.0	9.6–22.0
Zinc	0.48–0.68	0.48–0.82
Copper	0.7–1.1	0.72–1.1
Clay (%)	22.6	17.6
Silt (%)	31.4	40.0
Sand (%)	46.0	42.4

Irrigation was by siphons and rains. First irrigation was three weeks before flowering (in September), the second after collection of flowers (early November) and the third in March and two more at 15 days intervals. The flowers were picked au by hand. The results of this tedious experimental work, which deserved attention, did not clarified which edaphic or climatic factors were responsible for the differences in productivity in these two locations. In any case fertilisers were found not to improve significantly the yield. This study gives evidence for the organic cultivation of saffron in the years to come.

Italy. Nowadays, cultivation is restricted to Navelli highlands and to a lesser extent in Sardinia (Cagliari province) and the Val di Taro (Parma province). Some attempts are currently made to renew interest in this cultivation in Tuscany. Sicily and Campania do not produce saffron crocus any more. Ten hectares and 100 growers are rather small figures for this otherwise profitable cultivation (Tammaro, 1994 and 1999) who reports that after experimentation in multiannual cultivation it was evidenced that by taken up the corms at the early summer and replanting at the end of August reduced significantly root rot. In the highland of Navelli rainfall is common throughout the year except for August, whereas temperatures below zero and snow are also common during late November and winter. High humidity in summer favours development of parasitic fungi that destroy corms. The use of anti-fungal agents is, thus, recommended to save the reproduction material.

Japan. Morimoto et al. (1994) referred to an ‘indoor’ cultivation system that is used for more than 80 years and that it is even mentioned in the Japanese Pharmacopoeia. The authors commended that achievement of full blooming in the room is advantageous for the homogenous quality of saffron and also for reducing labour.

Morocco. Ait-Oubahou and El-Otmani (1999) recently presented some information concerning the cultivation in the Taliouine area (1200–1400 m altitude) in the junction of High and Low Atlas. The produced annual quantity of about 1000 kg is of great importance for the locals who have rather low income. No particular evidence is found in the article that cites mainly Italian literature though it is clear that some projects are underway to expand the cultivation in other parts of this country.

Spain. In 1983 statistics indicated that 3788 ha were devoted to saffron crocus cultivation, most of them in the Castilla-la Mancha (2864 ha) region and in particular in the Albacete province (1785 ha) (Gómez et al., 1987a). 'La Mancha' is an agricultural area distributed among several provinces of the Castilla-La Mancha region (Central Spain) where the best Spanish saffron is produced (Alonso et al., 2001). Another province reported for saffron cultivation is Aragon. Some data concerning agroclimatic conditions and soil characteristics from a study in an experimental field of 90 m^2 cannot be considered as representative or typical for the Spanish saffron growing areas (Gómez et al., 1987a).

4.3. Control of pests and diseases

Since propagation of saffron plant is feasible only through annual replacement of corms, the latter are indispensable for the producers. More research seems necessary to ensure survival of the plant material. All producers have experienced sudden loss of the corms and, thus, destruction of the harvest and reduction of the yield in the following years. A cantharidine beetle damages the stigmas during its visits to the bloomed flowers. Worse is the result of attacks by rodents, which eat the corms. *Rhizoctonia crocorum* affects the corms and roots and is controlled by dipping corms in a 5% copper sulphate solution. Corm rot may be due to *Phoma crocophila* attack. Other fungi and even viruses, some of which do not cause any symptoms, know to affect other *Iridaceae* plants may also be potential enemies (Rees, 1988). A particular type of plant destruction caused by *Penicillium cyclopium* has been reported in the area of Navelli. The abnormal growth of leaves (50 cm long!) and sheaths results in loss of the one third to half of the flowers in cases of multiannual cultivation especially during the hot rainy season (Tammaro, 1999). In 1988–1989, farmers in the province of Aquila (central Italy) experienced almost 50% loss of their production due to a fungus identified to be *Penicilium corymbiferum* Westl., a known pathogen of other Crocus species and some other ornamental bulbs in this country. Plant damping off, basal stem rot and dropping and wilting of shoots was observed. Infested corms had dark lesions beneath the outer tunic and often a blue-green mould on the surface (Cappelli et al., 1991). Topsin M, captan, Sadoplon and Feunaben solutions have been used effectively against *Fusarium culorum, F. semitectum*, and *F. oxysporum orthoceras* isolated from infected *C. sativus* corms. Model experiments at growing conditions imitating early spring characteristics indicated that the four fungicides stimulated rooting of inoculated plants (Bartynska and Przytocki, 1985). Rats and moles are a major threat in Kozani (Greece).

4.4. Harvesting and stigmas separation practices

Harvesting spans four to six weeks in autumn (mid October–mid November). Each plant blossoms only for about fifteen days and harvesting, therefore, must be timely. Intact flowers are picked early in the morning to prevent withering. Bees are the competitors of the growers during this period. Spanish sources report that the flowers are picked early in the morning before the petals open. In Greece, the growers prefer to cut the stigmas directly from the bloomed plant at the base of the petals with a slight twisting supported by the fingernail. The stigmas together with parts of the flower are transferred in baskets to the processing areas. A great amount of petals remains on the soil (Figure 13). On the same day the stigmas must be removed from the harvested flowers and drying is initiated. In most cases stigmas are removed from foreign matter by hand. Some devices blowing air may assist the separation (Figure 14).

Yields of flowers vary considerably according to local site conditions. On average, however, about one hectare yields one million blooms, weighing approximately 800 kg, which provide 50 kg of fresh stigmas and 10 kg of dried saffron (Corradi

Figure 13. On the left, basket carrying floral matter and stigmas. On the right, saffron petals thrown just after harvest. Kozani, Greece.

Figure 14. On the left, hand-sorting of saffron styles, stigmas and stamens from petals. Ont the right, air-blowing as a mechanical method for the separation of petals. Kozani, Greece.

and Micheli, 1979a; Sampathu et al., 1984; Green, 1995). Larger yields are expected from the third and fourth year plants. This stage is very crucial for the overall quality of the final product. Frequently, sudden rainfall during blooming is the reason for the collection of dirty stigmas and very wet material which remaining for many hours in the baskets deteriorates. Therefore, the quantity of plant material per basket should not be great and it is urgent to remove petals, clean and dry the product as soon as possible after collection.

5. THE EFFECT OF PROCESSING ON QUALITY

Losses of desirable compounds, such as volatile constituents, are related to the drying method used for herbs and spices. Literature concerning a great number of them (thyme, sage, dill, marjoram, oregano, parsley sweet basil, etc) is useful to those that intend to adopt a drying system or technology in another case of an aromatic plant. Air drying (in shade or solar), freeze-drying, hot air drying, fluidised bed drying, microwave hot air drying, drying by means of convection, etc. (Cohen and Yang, 1995; Venskutonis, 1997) are some of the procedures used in practice by individual producers or in industrial scale. All means are not suitable for every plant material. Not to forget is the fact that smallholders, mainly in developing countries, are those who grow herbs and spices. In such cases, due to the lack of information and the absence of hygiene rules implementation, rather primitive and sometimes inappropriate drying methods for the product are employed. Moreover, pets and other 'visitors' cannot be excluded from the area where drying takes place whereas long storage period before drying or slow drying encourages the growth of microorganisms. A common process flow chart for a herb or spice involves after harvesting drying, sorting, washing or winnowing, cutting, finish drying, further cleaning, packing and storage till distribution.

5.1. Drying

Drying brings about the physical, biochemical and chemical changes necessary for imparting the desired attributes to saffron. Drying saffron is a difficult task because its principle characteristics, the crocins, are water-soluble and consequently washing is prohibited though foreign matter (dust, mud, parts of insects, etc) is not rare. On the other hand, crocins, as all carotenoids, are light sensitive so that exposure to light throughout processing should be the minimum. Picrocrocin, the bitter constituent decreases during the first drying and the subsequent treatment steps whereas safranal absent before drying and the period just after that starts to develop in the first period of storage.

The various saffron drying versions as briefly presented by Basker (1999b) show that little has been published to this direction. The practice of growers differs from country to country as a result of experience gained through trial and error. Moreover, each one of saffron growing regions or countries has devised a procedure that takes into account the available resources. Still, variations are found even among the producers of the neighbouring plots of land in the same region.

In any case, the producers should avoid drying the stigmas on the floor or under direct exposure to light (Figure 15).

Since 1989, we made many efforts to study the practices of Greek growers in Kozani area. In 2000 within the frame of a Leader project we elaborated a Manual for the Good Manufacturing Practice for Greek saffron based on scientific evidence and a considerable amount of data collected from the experience of growers who also act as processors. Saffron drying depends heavily on the temperature and the relative humidity of the drying room. Therefore, when a large quantity of fresh stigmas is dried in a small room or when the fresh stigmas is left to dry in the open air the drying period is longer than that observed under strictly controlled conditions. Late October and early November are rather humid periods of the year and the long hours required for drying can be dramatically shortened and the procedure standardised if the processors follow our suggestions. This modernisation can take place without any interference with the ownership rights of the growers; it only depends on their free will to adopt the instructions. Towards this direction, the last five years, the Co-operative of Greek Saffron Growers organised many seminars to familiarise its members with the properties of their product. In the following table there is a characteristic example from the drying conditions followed by two groups of Greek growers. The first group dried the production in their own way whereas the second group followed (partially) our suggestions to keep room temperature below 45 °C and relative humidity 45–50%. The 17 growers were volunteers and they let us make our observations and measurements for the benefit of the Co-operative.

Drying is the critical step of saffron processing. However, just after this preliminary processing saffron is not ready for the market. Two or three further treatments to remove flower waste and extraneous matter are usually needed. This procedure may last for one to two months (in winter) and it usually takes place within the houses of the producers. In Table 9, a characteristic example of the quality status of saffron samples just after drying indicates the importance of further cleaning. Indeed samples from the same producers after the third cleaning gave products ranked in the extra category (floral waste <0.1% and extraneous matter <0.5%).

The basic instructions to the Greek processors included in the Quality manual to optimise the saffron production line are the following:

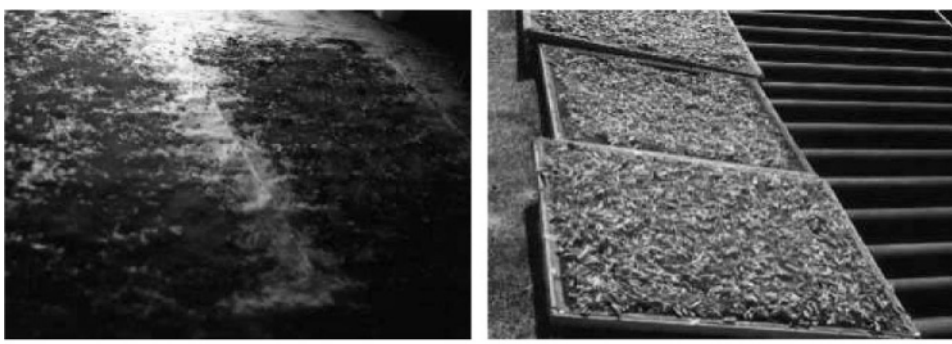


Figure 15. Drying of stigmas on the floor and under sun. Kozani, Greece.

Table 8. Drying process of saffron stigmas in Kozani, Greece (Tsimidou, unpublished data).

No.	DRYING CONDITIONS Room dimensions/type of trays and tray load/means of heating/temperature, relative moisture and total time of drying
	First group of growers
1	4.0 × 6.0 × 3.0 m/0.8 × 1.0 m, fine-mesh sieve, 300–400 g/charcoal stove, 18–20 °C, ≅4 h
2	3.5 × 0.6 × 3.0 m/shelf coated with paper/firewood stove, ≅50 °C, 18 h
3	40 m2/1.0 × 0.7 m, plastic fine-mesh sieve coated with paper, 300 g/no stove, 12 h
4	3.0 × 4.4 × 2.0 m/0.7 × 0.8 m, fine-mesh sieve/charcoal stove, 25–30 °C, ≅12 h
5	25 × 2.5 × 2.1 m/1.0 × 0.6 m, 250 g/charcoal stove, ≅40 °C, 12 h
6	2.5 × 3.0 × 2.1 m/0.7 × 1.1 m, fine mesh sieve, 250–300 g/frame/charcoal stove, ≅40 °C, 5–6 h
7	3.0 × 2.5 × 3.0 m/1.1 × 0.6 m and 1.0 × 1.0 m with a fine mesh sieve coated with cloth, 300 g/frame/charcoal stove, 25–30 °C, 14 h
8	60 m^2/1.0 × 0.5 m, 200–250 g/charcoal stove, 20 °C, 24 h
9	3.0 × 2.5 × 2.1 m/1.0 × 0.5 m, fine mesh sieve coated bottom with plastic, 250 g/charcoal stove, 40 °C, 12 h
	Second group of growers
1	4.0 × 4.0 × 2.5 m/1.0 × 0.7 m, fine mesh shieve, 250 g/charcoal sieve, ≅50 °C, 40–45%, 3 h
2	4.0 × 6.0 × 2.0 m/0.7 × 1.4 m, thick paper, 250 g/frame/charcoal stove, ≅50 °C, 35%, 3 h
3	4.0 × 4.0 × 2.5 m/1.0 × 0.7 m, coated bottom with plastic fine mesh shieve, 250 g/charcoal stove, ≅50 °C, 45%, 90 min
4	2.5 × 2.5 × 2.8 m/0.4 × 0.5 m silk-fabric bottom and 0.70.5 m fine mesh shieve, 125 g/charcoal stove, ≅50 °C, 40%, 2:30 h for frames with a shieve at bottom, 3 h for silk ones
5	3.0 × 2.5 × 1.6 m/1.8 × 0.7 m, plastic shieve and coated with paper, 250 g/charcoal stove, ≅50 °C, 50%, 1 h
6	1.9 × 3.4 × 2.2 m/1.0 × 0.8 m, plastic shieve and coated with paper, 250 g/charcoal stove, 50 °C, 40%, 4 h
7	4.0 × 2.5 m × 2.6 m/1.0 × 0.7 m, plastic fine mesh shieve, 250 g/charcoal stove/50 °C, 50%, 1 h
8	4.0 × 2.5 × 2.6 m/1.0 × 0.7 m, plastic shieve, 250 g/charcoal stove/50 °C, 40%, 1 h

Table 9. Percent floral waste and extraneous matter of saffron samples just after drying (Kozani, Greece) (after Cuko, 1997).

No.	Floral waste (%)	Extraneous matter (%)	No.	Floral waste (%)	Extraneous matter (%)
1	10.38	0.92	10	6.54	0.96
2	7.86	0.74	11	21.02	0.48
3	23.28	1.12	12	2.63	0.37
4	0.00	0.00	13	45.19	1.91
5	6.74	1.56	14	18.35	1.05
6	23.20	2.30	15	38.12	0.68
7	18.00	0.50	16	19.51	0.49
8	6.83	0.67	17	3.87	0.23
9	39.91	0.89	18	4.66	0.74

Handling of flowers. Flowers should be carried into clean baskets or sacks. Quantities per basket should be regulated carefully to let air circulation. It is suggested that processing should start as soon as possible after harvest. Arranging collection and frequent transport of flowers contributes to the product quality.

Winnowing. Removal of floral waste can be made manually or assisted mechanically. A simple apparatus for the mechanical winnowing of the flowers, shown in Figure 16, may be comprised by the following parts: a rotating table coated with an elastic cover having protuberances for holding the stigmas, a motor for the rotation of the table, a ventilator, a vessel for collecting the flowers, a slit through which the petals are moved away, a scraper for the removal of the petals. The parts of a winnowing apparatus should be constructed with materials such as wood or plastic which are friendly towards the product. Metallic segments should be painted regularly in order to avoid any contact of the product with rust. The apparatus should also be easily dismantled so that the parts can be properly cleaned and stored till the next harvest.

It is worth noticing that the space in which the flowers are winnowed should be protected from dust, insects or pests. In general, the handling of flowers should be made with clean hands or gloves.

The slit through which the petals are moved away (position 1), as well as the vessel where they are collected (position 2) seem to be of practical importance and comply with the current hygiene rules.

Drying. A special room for the drying of the flowers, preferably used only during processing of saffron is recommended. Such a room is better to be divided into a

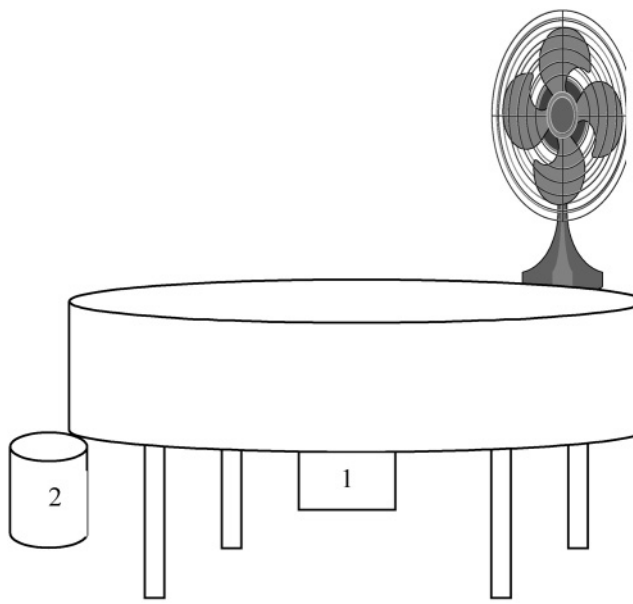


Figure 16. Vertical section of the winnowing apparatus.

space for sorting the stigmas and another one for processing where light is limited. A possible arrangement in this room is shown in Figure 17: (1) baskets for flowers, (2) a winnowing apparatus, (3) supporting bases for the frames, (4) container for collecting dried stigmas, (5) a ventilation system, (6) a heating source, (7) a light protective system (e.g. curtains).

The annual preparation of the processing room is suggested to include disinfection of the floor and walls with permitted cleaning agents and sanitizers some days before harvesting. Access to flying insects, crawling insects, rodents and birds should be strictly restricted by all means except for using chemicals. All openings should be closed and cracks repaired. Traps or ultrasonic devices, air curtains and other alternative means for the control of pests are recommended. The frames used for drying are better to have standardised dimensions and in consequence standard quantities of fresh stigmas per frame should be loaded. In case the frames are piled (Figure 18) the distance between them should be 0.5 m to allow.

Air circulation and speed drying up. The sieves used may be plastic (permitted for food use), or silk cloth. It is proposed not to use metallic sieves to avoid rust problems. The sieves are to be used only for saffron drying and then be cleaned and stored till the next harvest.

Heating. Many heating systems are acceptable. Limitations have to do with maintaining constant temperature in relation to relative humidity of the environment. It is, thus, necessary to regulate the temperature and the relative humidity in the room even with the use of a ventilator in order to avoid deterioration of the quality parameters that characterise the final product. Monitoring of the temperature and relative humidity using a simple accessory that combines a thermometer and a relative humidity meter help the control. Recording of the values of these para-

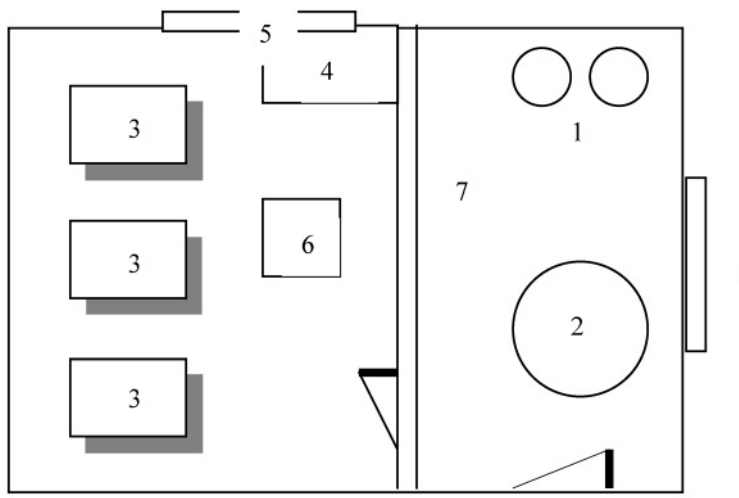


Figure 17. Winnowing and drying areas layout.

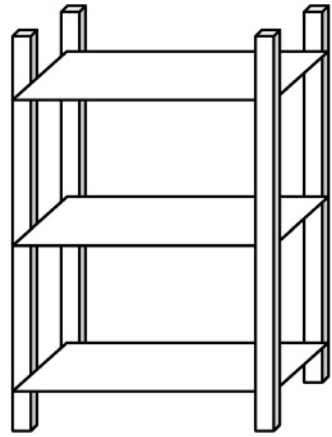


Figure 18. Illustration of piled frames for saffron drying.

meters on a piece of paper hanged on the wall is more useful than memorising them. In this way, each processor creates his/her own archives. The temperature should be kept within the region of 35–45 °C while the relative humidity should not overcome the 50%. The total time for the drying depends on the room dimensions, the conditions of temperature and relative humidity as well as the frame loading.

Sorting. The sorting of stigmas from the stamens and the remaining floral matter is a crucial stage of the processing. Producers who are also the processors of their product should always have in mind the necessity of keeping all the surfaces clean. Except for a flat surface (Figure 19), a pair of forceps could be used to assist sorting. In order to comply with ISO specifications it is a good idea to repeat sorting 2–3 times per batch.

Indian saffron is not of high quality most possibly due to the lengthy sun drying processing periods that may cause both biodegradation and oxidative destruction of the principle components (27–53 h) (Sampathu et al., 1984). Raina et al. (1996) worked on the processing conditions used in India and also elaborated some drying schemes at a laboratory scale. The authors collected samples from the two major saffron producing areas of India (Sringar and Kishtwar) for three successive years. The drying methods employed were (i) shade drying, 4–18 °C; ii) sun drying, 11 h photoperiod per day, 12–21 °C; iii) solar drying, highest interior temperature 49 °C (29 °C higher than the ambient; iv) dehumidification drying over Si-gel (blue), 40 °C; v) in a vacuum oven at 40, 50 and 65 °C and at reduced pressure of 40 mm; vi) in a cross-flow oven at 20, 40 and 50 °C and vii) in an electric oven at 40, 50, 65 and 80 °C. The optimum tray-load was found to be 1 kg m^{-2}. The findings were very close to those found for Greek saffron drying conditions. The proposed temperature for artificial drying was 40 ± 5 °C. At lower temperatures, lengthy periods of processing were experienced that resulted in pigment loss whereas at 50 or 60 °C thermal degradation of pigments was prevailed. Vacuum and cross-flow drying caused a significant reduction in safranal content but at the same time increased levels of 4-β-hydroxysafranal were observed that had as a result

Figure 19. Stigmas sorting.

unpleasant sensory characteristics (poor initial notes and intense herbal and barky end notes). Samples dried in shade or under accelerated conditions had better flavour profile that coincided with the higher amounts of safranal at the expense of isophorone derivatives. The design and development of equipment for the mechanisation of post-harvest treatment of saffron stigmas in the same areas of India is presented by Sama et al. (2000).

The use of smoke to preserve saffron, a practice met in Spain, imparts desirable flavour and may also have a bactericidal effect. However, it is not for sure that current Spanish practice is the toasting of saffron above an open fire as the documents in international literature are limited.

5.2. Decontamination

Spices may be highly contaminated with moulds, yeasts and bacteria, either as vegetative cells or spores coming from plants, soil, or the feces of birds, rodents, insects, etc. Contamination may occur during harvesting, handling, transportation or storage of the spices (Sjöberg et al., 1991). Considering their high microbial load (10^3–10^8 organisms per gram), it is obvious that when they are used untreated they may well cause several foodborne diseases. Fortunately for public health, herbs and spices are used in minor quantities and risks are, thus, eliminated.

Decontamination by chemical treatment with ethylene oxide and hot steam treatment can reduce the number of the viable cells of spices. Occasionally, other

fumigants like propylene oxide and methyl bromide are used, too (Sjöberg et al., 1991). However, fumigation with microbicidal gases has several disadvantages such as toxic residues, changes in the organoleptic properties of spices and health hazards for the workers. On the other hand, heating is restricted to low temperatures otherwise the content of the heat sensitive aroma compounds will be dramatically reduced. An alternative to these methods of decontamination, widely disputed for several years, is the treatment of spices with ionising energy. The process of irradiation produces ions, free radicals and excited molecules in the food that cause the desired effects. Conversely, in a microwave oven foods are exposed to microwaves, a type of non-ionising radiation that generates heat by increasing the molecular motion of the water molecules in moist foods. Therefore, when spices are subjected to irradiation the decrease in their volatile content is rather low, while none chemical residue is left. Legnani et al. (2001) have treated various herbs and spices with microwaves and irradiation in their attempt to examine the microbiological quality of these products. Comparing the two methods they found that only irradiation (5–10 kGy) could eliminate the faecal indicators completely. Irradiation is also believed to have an after-effect in dry spices as the number of the viable cells is reduced even during post irradiation storage depending on the irradiation dose (Sjöberg et al., 1991).

The process involves exposing food to a source of radiation such as a tightly sealed metal container of radioactive elements – cobalt 60 or caesium 137 – that produce gamma rays. The rays are directed onto the food being irradiated, with food itself never being touched by the cobalt of caesium. Another type of radiation source is an apparatus that produces X rays and high-energy electrons. Neither of these sources has enough energy to make the irradiated foods radioactive.

Radiation decontamination of dried spices with doses of 8–10 kGy is regarded as the most appropriate for killing bacteria and moulds. Irradiation at doses of 10 kGy or lower has been found to be capable of killing the most common foodborne parasites but is unlikely to kill all bacterial spores unless the initial level of contamination is low. Irradiation doses of up to 20 kGy may be required to achieve 'sterility', that is, a reduction of the total viable cell count to less than 10 per gram in natural spices. Microorganisms that survive the low and medium dose radiation treatment have lower resistance to environmental stresses or subsequent processing treatments than the microflora of untreated spices (Sjöberg et al., 1991; Farkas, 1998). The irradiation for decontaminating herbs and spices in the United States is permitted by FDA since 1983. In other countries the situation varies. A major compromise between producers and consumers concerns the packaging and labelling status of the irradiated product and their exhibition on separate selves. As regards to the legal status of food irradiation in the European Union two Directives no2 and 3/1999 became applicable on 20 September 2000. The first Directive set the framework for the introduction of this technology to food sector. It covers general and technical aspects for carrying out the process, labelling and conditions for authorising food irradiation. Then, in the second Directive, the first category in the positive list of foods and food ingredients became the dried aromatic herbs, spices and vegetable seasonings. The maximum overall average absorbed radiation dose permitted is 10 kGy.

The effect of γ-irradiation on the colour and flavour of saffron has been examined by Zareena et al. (2001). Considering the observation that Zarghami and Heinz (1971) had made upon the possible oxidative breakdown of safranal due to its exposure to ultraviolet light, the irradiation process was suspected to have a negative effect on the safranal content of saffron. No qualitative changes in the volatile oil constituents between the control and the irradiated samples were found. However, the organoleptic evaluation of the distilled oils indicated a perceptible deterioration in the oil obtained from a spice treated at doses higher than 5 kGy. The workers also noticed a substantial decrease (ca. 90%) in the crocins content of irradiated stigmas of saffron and a concomitant increase in crocetin level. They suggested a cleavage in the glucosidic linkages that may occur during irradiation and that gave rise to a twofold increase in the crocetin content whereas the loss in crocin content was 83–89%. The above findings led to the conclusion that irradiation doses for this spice should not overcome 5 kGy. Therefore, this process of decontamination can be applied to saffron only in cases of low microbial load.

Herbs and spices and also their derivatives such as, infusions, resins and extracts are precious commodities and products that require special care. Thus, other alternatives are examined to accomplish safely processing (e.g., Keith et al., 1997).

6. THE EFFECT OF STORAGE ON QUALITY

Storability of saffron is not well appreciated by producers, whole-salespersons or retailers. Saffron, as the other herbs and spices is an intermediate to low moisture content food item that is prone to changes in the relative humidity of the environment. Proper packaging could be the answer but it is well known that most of the world-wide produced saffron is transported in bulk quantities, in carton boxes, bags or tin boxes without a continuous control of the humidity and the temperature of the environment. Saffron is produced annually and the safest way is to be sold within the year of harvest. Considering that harvesting (October–November), processing and sorting takes approximately 2–3 months and that balance in the flavour compounds occurs some time after processing, transactions are expected to start from March onwards. It is urgent for each region to standardise the best time to start with selling as the customers decision rely mainly on the sensory characteristics of the product.

Saffron principle attributes do not change in the same way during storage. Although the literature on the stability of carotenoids is substantial (Britton, 1992; Rodriguez-Amaya, 1993) there is little information on the effect of storage on crocins. Depending on molecular structure carotenoid stability is affected by light, oxygen, moisture content/water activity temperature, metals and other pro-oxidants, presence of antioxidants, free radical inhibitors and the composition and physical structure of the sample. Mannino and Amelotti (1977) made the first attempt to examine the optimum storage conditions of saffron. The results indicated that color stability was greater at low relative humidity values (5–23%) and low temperatures Alonso et al. (1990) studied the kinetics of pigments and picrocrocin at

40 °C and 75% air relative humidity. Under those conditions saffron pigments bleached out within 70 days of storage whereas the bitterness loss was more than 40%. Tsimidou and Tsatsaroni (1993) studied the effect of pH and light on stability of saffron aqueous extracts. The pH values (3, 5, 7) were selected as representative for food colouring. The study was carried out in the presence of oxygen and in nitrogen atmosphere. Crocin degradation was found to be sensitive at low pHs in a way similar to that reported for annato extracts. Stability studies at different temperatures indicated that storage at 4 °C (pH 7) reduced the degradation rate by a factor of more than 3, 8 and 10 at 25, 40 and 62 °C, respectively. A temperature of 40 °C, representing a common condition of air drying of saffron, seemed to have an equally critical effect to that of light. Reduced oxygen atmosphere did not offer significantly higher protection to the pigments in the aqueous environment but as the authors suggested this factor should be further investigated. The stability of carotenoids in the presence of food ingredients or other additives is mainly studied for β-carotene but little is known on the stability and the colour modification of the more polar carotenoids such as bixin or crocetin. Knewstubb and Henry (1993) mention that sulphur dioxide at levels of 50 ppm and above causes bleaching of crocin coloured products and that a degree of protection can be afforded by the use of ascorbic acid. Orfanou and Tsimidou (1995) examined the effect of ascorbic acid on the stability of aqueous saffron extract (40 °C, pH 5) and found that it was effective only in combination with ethylenediamine tetracetic acid (EDTA). The % colour retention of the extract was three times higher after the addition of an ascorbic acid: EDTA solution (100 ppm/100 ppm). Ascorbic acid itself imparted an adverse effect on colour that from bright yellow became orange-brown within two days of storage. Extracts in tap water were less stable than those in water purified by successive application of reversed osmosis and filtration through active carbon and ion exchange resins. An adverse effect on colour retention was also reported for different pHs in the presence of preservatives.

Morimoto et al. (1994) studied the stability of carotenoid glucosides in saffron under various conditions. The investigators were not interested in the flavour and taste attributes of the spice so that the conditions of storage they propose are low temperature, low humidity and possibly nitrogen atmosphere. The results of the storage tests carried out by Raina et al. (1996) confirmed our observation that saffron should be used within the year of production.

Taking for granted that light should be excluded during storage of saffron Tsimidou and Biliaderis (1997) carried out kinetic studies on carotenoid loss and changes in other quality attributes of saffron under varying conditions of water activity and temperature. The stability of carotenoids was examined at 25, 40 and 60 °C and seven levels of water activity (0.11, 0.23, 0.33, 0.43, 0.53, 0.64 and 0.75). Loss of carotenoids, estimated by spectrometry at 440 nm, indicated that degradation followed first order kinetics. Calculation of half-life periods at the different conditions gives information that may be useful for improving saffron storability (Table 10).

Colour power retained better at lower temperature and at aw values below 0.43. For example 600 days after the start of the experiment the half-period values were

Table 10. Effect of water activity (a_w) and temperature on crocin degradation upon storage of saffron powder (after Tsimidou and Biliaderis, 1997).

Temperature					
25 °C		40 °C		60 °C	
a_w	Half-life periods (days)	a_w	Half-life periods (days)	a_w	Half-life periods (days)
0.11	*	0.11	1733	0.11	65
0.23	*	0.23	866	0.23	42
0.33	*	0.33	408	0.33	29
0.43	1733	0.43	193	0.43	25
0.53	462	0.53	48	0.53	21
0.64	31	0.64	16	0.64	6
0.75	105	0.75	15	0.75	3

not attained for samples stored at 25 °C and a_ws 0.11–0.43 and for those stored at 40 °C and a_ws 0.11–0.23. In contrast to what is reported for the oxidation of non-polar carotenoids in relation to a_w value of foods, the kinetic data for saffron carotenoid degradation showed an increasing rate with increasing a_w within the range 0.11–0.64. This behaviour may be the result of the polar character of crocins. An unexpected behaviour was observed at a_w 0.64 that concur with current concepts on the mobility of reactants in foods. This deviation was confirmed in three series of experiments and requires some further investigation.

Concerning bitterness and aroma evolution in storage it appeared that the latter is favoured in intermediate relative humidity environment. Thus, it should be cautiously taken into account that optimum conditions for aroma development are not in favour of pigment stability. Thus, a_w values of 0.33–0.43 and not very low temperatures should be used if saffron is traded as a spice. In case saffron is kept for pharmaceutical use then crocins are the active constituents that are better preserved at low temperatures and low a_ws so that the point made by Morimoto et al. (1994) for storage in a refrigerator becomes correct.

Selim et al. (2000) presented the first attempt of increasing storability of saffron carotenoids by encapsulation in amorphous polymer matrices. Encapsulation of bioactive ingredients as a means of shelf life extension is one of the popular current approaches in food technology. Spray-drying, freeze-drying, air suspension coating, extrusion, spray-cooling and chilling, rotational suspension separation, coacervation and inclusion complexes are some of the techniques to encapsulate photosensitive or thermolabile ingredients such as carotenoids (Dziezak, 1988). Pullulan, PVP40 and 360 were selected for their good water solubility and ability to form an amorphous state on dehydration. Encapsulated saffron carotenoids using freeze-drying were studied under the unfavourable relative humidity conditions reported by Tsimidou and Biliaderis (1997), i.e., 0.43–0.75. PVP 40 had the better protection effect among the polymers used because it collapses rapidly in storage and becomes in this way an effective barrier to oxygen permeation. The half-life periods in relation to control samples increased 4, 18, 7.5 and 7.7 times at aws 0.43, 0.53, 0.64 and

0.75, respectively. The potential of encapsulation is expected to be further examined using other materials and techniques.

7. EPILOGUE

Saffron is the most expensive spice in the world. Its production involves hard labour and is minimally mechanised. Considering that in some saffron growing areas the farmers have a low level of education, the need for training them to Good Agricultural Practices, Good Manufacturing Practices and storage methods is obvious. To accelerate the standardisation process of saffron quality the co-operation of growers, processors and distributors should be based not only on their financial interests but also on international requirements for product quality assurance.

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FRUIT AND VEGETABLES HARVESTING SYSTEMS

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1. INTRODUCTION

Fruits and vegetables have a high importance in world food production and human nutrition and health. Mechanical harvest of fruits and vegetables shows special problems like:

- products to be harvested are enormously variable regarding agronomic, physiological, structural characteristics, size and shape, detachment, etc.;
- harvesting machines have to be very specialized and they are used a low number of hours in a year;
- fruits and vegetables have been, and still are, harvested manually even in high developed countries, so that labour problems usually appear when trying to introduce mechanization with the aims of improving economy and quality;
- factors regarding: adequate varieties, planting systems and scheduling, soil and irrigation management, materials handling, grading and sorting, processing, and others, which in themselves need considerable know-how and technification, impose strict conditions on the viability of mechanical harvest of any fruit or vegetable species.

Most operations which are coincident with the ones used in other crop productions like: soil tillage, fertilizing, seeding or planting, spraying, etc. are generally solved using mechanical equipment, in most fruit and vegetable productions. Operations involving: cleaning, handling and transportation, which can be performed in fixed installations, are also generalized with the application of mechanization and in some cases of automation equipment (Ortiz-Cañavate and Hernanz, 1989).

Mechanical harvesting of only those fruit and vegetable products destined to processing can be considered generalized and economical in developed countries. Products for fresh market can in many cases be harvested using mechanized aids, which have attained very diverse level of sophistication for different species and locations. And in later years, robotic harvesting is being developed aimed to solve fresh fruits harvesting with the same quality as manual harvesting.

Manual harvesting of fruits and vegetables accounts for 30 to 60% of the total production costs, with a high net share in the final price of the product. Therefore, mechanization of harvest operations has a high potential for input reduction.

This section deals mainly with the existing principles and functions which make up the mechanical harvesting equipment for temperate fruits and open-air grown vegetables.

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2. HARVESTING FUNCTIONS

While detachment and removal, control, cleaning and selection, conveying and loading are the required functional operations for a harvester, the order in which these functions are achieved is determined by the requirements of the specific product or commodity (as an example, hand harvesting always begins with selection) (Srivastava et al., 1993). In mechanical harvesting systems, detachment is seldom as selective as desirable, therefore the selection function is achieved after detachment, in the form of sorting devices (or even manual sorting in the machine), or else at a later processing (sorting, cleaning, grading and packaging) inside fixed premises.

Detachment is the actual separation of the harvested portion of the plant: fruits, buds, tubers, roots, leaves, etc. Catching and control: padding of catching surfaces is required to gain or maintain product control during harvesting operations (Ryall and Lipton, 1972). Good padding materials can absorb the impact energy of the product, thus preventing its absorption and damage to the fruit; these materials have to be easy to keep and to clean, and durable.

Selection is the process in which only the ripe, correctly sized or desirable product is obtained from the entire recovered crop material, while the remainders are rejected. Size is often associated with product quality. Harvesting machines are sometimes equipped for size and color grading in the field. Product maturity requires special attention as the main property of the product which decides harvest date, and defines the product susceptibility to damage.

Transportation: whenever possible, bulk handling systems are preferred for transportation of the product from the field to the grading/marketing station. Bulk handling (trucks or tractor trailers) are used for industry products such as tomatoes, green beans, onions, potatoes, peaches, wine grapes, olives. Standard pallet containers can be handled with standard forklift equipment and are used also for fresh market products like apples, melons, cucumbers, ... Delicate fruits like strawberries need small, market-ready containers; these are also used in appropriate sizes, for example for ripe peaches and apricots. Vegetables like lettuce, cauliflower, broccoli, etc. are sometimes wrapped and packed in the field. Today, infield grading and packaging of produce is contemplated as a good solution for reducing costs and quality losses in many fresh-market products (vegetables, fruits, from these especially tropical fruits).

Damage is an important consideration (Ryall and Lipton, 1972). Product bruising, cutting, scuffing and direct damage to the remaining plant can be a consequence of mechanical harvest. Damage reduces the value of the commodity in the market; damage to the remaining plant can affect future crops or life of the plant itself (Ruiz-Altisent, 1991).

Harvesting functions often interact with each other. For example, if inertial detachment is used by interacting with the plant, the separated commodity often has an associated kinetic energy, which aggravates the problem of product control (damages) when compared to other types of detachment and removal procedures (Ryall and Lipton, 1972) (Table 1).

Table 1. Comparative damage thresholds for selected fruits.

	Drop Height onto hard surface causing visible bruise[a] (mm)	Average mass (g)	Observations
Apple	10	220	High turgidity, increased damage susceptibility
Pear	20	175	
Peach	25	150	More susceptible in compression
Apricot	150	60	
Tomato	200	75	Skin most important
Orange, lemon	400	200	Rough surface, increased damage susceptibility due to friction
Olive (table)	200	30	
Small fruits			
Rubus	50	10	
Ribes	120	10	
Strawberries	50	30	

3. PRINCIPLES AND DEVICES FOR THE DETACHMENT

The application of a well directed energy is necessary to effect detachment; the procedure in which this energy is applied is the first and basic consideration in the aim of mechanical detachment and removal, and depends on the commodity in question. Severing the attachment forces requires that the ultimate fatigue, tensile or shear strength thresholds must be exceeded (Srivastava et al., 1993) (Table 2).

3.1. Low-height herbaceous structures

Such as vegetables, strawberries. . . . We differentiate between root and surface crops.

3.1.1. *Root crops*

Two different principles are used for the harvesting of these crops: digging (potatoes, carrots, . . .) and pulling (carrots, leeks, . . .) (Ortiz-Cañavate and Hernanz, 1989; Gracia and Palau, 1995).

Digging consists of the uprooting and lifting of the crop together with considerable amounts of soil, which has to be separated, by the thrust of a digging or plowing share. Working parameters of this system are:

Table 2. Detachment forces for selected fruits.

	Detachment force (N)	Observations
Apple, pear	40[a]	
Almond	1.5[a]	
Cherries	12–16	8–10 N[a]
Grape	3–5	Single berry
	25–50	clusters
Peach, apricot	1–5	9–15 N[a]
Tomato	20–30	
Orange	20–30	
Lemon	30–40	
Strawberries	4–9	
Small fruits	0.5–2.5	
Olive	2–8	

– length and
– inclination angle of the share and working speed and cutting depth, determined by the location of the recoverable product in soil, together with the abovementioned parameters, constitute the regulations of the system.

Following the coulter(s), a vibrating rod-chain conveyor is needed to clean and to feed the product to the complementary operations of handling, cleaning and loading units. The above-ground plant parts, very fragile in comparison with the subsurface parts, may be recovered or dispersed, before or at the same time of harvesting the roots. This harvesting system is appropriate for any roots or tubers (e.g. carrots or potatoes), below-ground fruits (e.g. peanuts) or bulbs (e.g. onions and garlic).

Pulling the aerial portion of the plants is often used for the harvest of some root crops (e.g. carrots) and also some surface crops (e.g. leeks and salad greens); the structure and the strength of the plants must permit in this case the engagement of the above-ground leaves and the uprooting of the entire plant, aided by a subsurface coulter; the big advantage is that very little soil is extracted with the product (Figure 1).

Pulling force: $F_p = 2\mu N$
Pulling speed: $\bar{v}_p = \bar{v}_a + \bar{v}_r$

where

μ = friction coefficient
N = normal force exerted by the belts on the plant
$\bar{v}_a$ = working speed
$\bar{v}_r$ = speed of belts

The aerial parts have to be well aligned in the row and uniformly spaced, and they are engaged and grasped by a pair of elevating belts, which, due to the combination of the effects of: advance speed, velocity of the belts and their inclination,

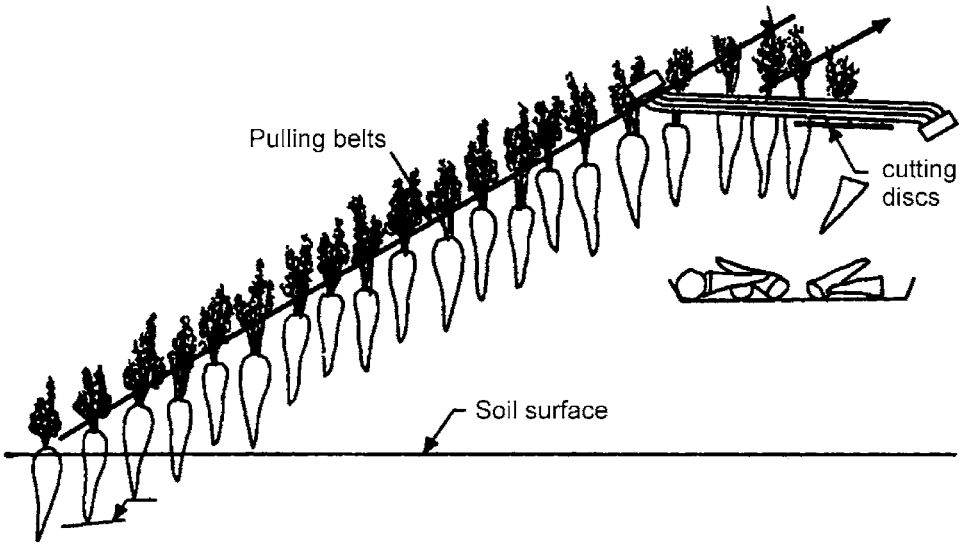

Figure 1. Pulling principle.

exert a vertical pulling force to the plant. Functional parameters of this system are then:

– belts and
– working speed,
– belt angle of inclination, and
– the pulling force exerted by the belts due to friction and compression between the belts and the plants.

At the upper end of the belts, a pair of topping implements (counter-rotating bars or rotating disks) are placed to remove the tops.

3.1.2. *Surface crops*

Cutting, combing, stripping, vibration and threshing are different functions used for the detachment of the very wide range of aerial parts found in the diverse commodities to be harvested.

Cutting action is applied mainly to the so-called leafy products: cabbage, lettuce, spinach, endive-escarole, Brussels sprouts, celery, broccoli, cauliflower, artichokes, chard, chicory, mustard, parsley, watercress, and any other so-called greens or similar plant parts. Each of these products need multiple harvests for maximum yield for fresh market, except perhaps spinach, which is mostly grown and harvested for processing. Their common properties are that they have to be cleanly removed and handled softly for minimum leaf loss. Cutting is the most effective method of removal, and it is also used in combination with other harvesting systems (like the cutting of whole plants of tomato; cutting undesired aerial parts of root crops (see above); cutting of Brussels sprouts stalks for subsequent stripping of the sprouts,

etc.). The conventional cutting devices are applied for this function: rotating discs with dented edge, flat or with some concavity; cutting bars, simple or double. In some cases the objective may be to remove entire plants, even below the surface (tomatoes, pickling cucumbers) to be subjected to further detachment of the fruits; in others, to recover the tender edible green parts (spinach, cabbage) (Figure 2). Frequently, a powered reel or rotating side cylinders are added to help feeding and further conveying the product up the band or bar-chain conveyor. Functional parameters of cutting devices are:

– cutting speed;
– advance speed;
– sharpness of the cutting edge;
– speed and position of the feeding reel or cylinders, with respect to the cutting elements.

Combing is based on particular properties of the plants:

– the plant is firmly attached to the soil by its root system and grows erect;
– the portions to be detached and removed have different size, shape and/or rigidity from the leaves and stalks (e.g. green-beans or pea pods);
– the portions to be detached possess a zone of abscission, susceptible of being severed by traction or by flexural forces;
– the parts to be recovered (e.g. strawberry fruits, green bean pods) have to be capable of resisting the action of the combing fingers.

Functional parameters of combing are:

– speed of the extreme of the combing fingers that has to be around 4 m/s for a forward speed of approx. 0.5 m/s;
– speed and position of the conveying device situated behind the combing fingers, to assure the removal of the product from further impacts;
– speed of the feeding reel, conveying band or brushes which are installed in front of the combing fingers (Figure 3).

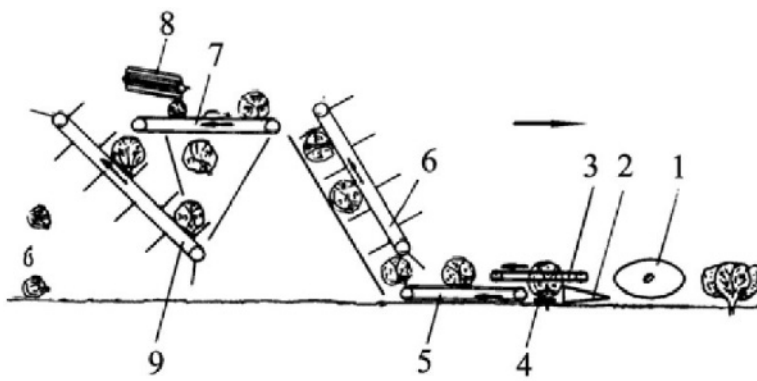

Figure 2. Cabbage harvester. (1) guiding discs, (2) guiding surfaces, (3) driving belt, (4) coulter bar, (5, 6, 7) band conveyors (8, 9) separation devices.

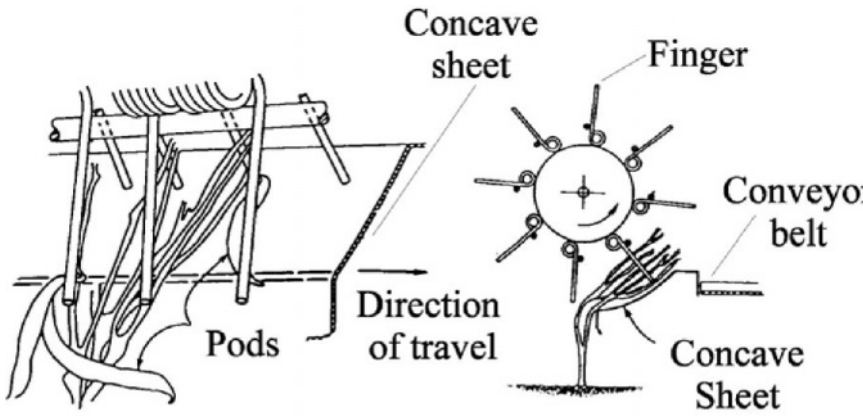

Figure 3. Cylindrical reel-type snap-bean harvester. Pods are removed by contact of fingers with the pedicle. Detachment originated by the cylindrical reel and the concave sheet in the plant.

As mentioned above, harvesters for strawberries (fruits which must be classified in this group, as aerial herbaceous commodities) may be provided with combing fingers attached to slow-moving elevating bands, and the operation is aided by air currents which elevate fruits and leaves to the fingers for easier combing (Figure 4). Instead of combing, some strawberry harvesters use a cutterbar-reel principle, which cut the entire plant and separate the fruits in the machine by again air-lifting and cutting, or by stripping The combing effect attained by two counterrotating brushes is used in harvesters for some varieties of paprika-pepper for the detachment of the fruits (the concept derives from the combers/strippers for harvesting cotton capsules).

Stripping by the action of counterrotating rollers pulling the plant down is a procedure by which the commodity (fruit) is separated from the plant, based on the differential properties between fruit and plant: size, shape and attachment strength. This principle is used to detach cucumbers (pickling) from their (already cut) supporting plants; in pepper harvesters, and in combination with other functions in selected harvesters: destemming of onions, strawberries, olives, cherries, . . . ; detachment and deleafing of (sweet) corn cobs. The principle consists of the pulling action of two counterrotating rollers, provided with some roughness condition: it can be the surface itself (rough rubber rollers), or helix-shaped structures attached to the rollers. The rollers trap the long and thin plant parts (stalks, with leaves), and severe them from the commodity by a pulling action (Figure 5, see also 4). The product is conveyed by different ways like: inclination of the rollers, or conveying by the helicoidal attachments on them; or also by falling onto separate conveyors (like in some onion harvesters). Functional parameters of this system are:

– turning speed of the rollers;
– separation between the rollers;
– engagement speed (forward speed) to the plant.

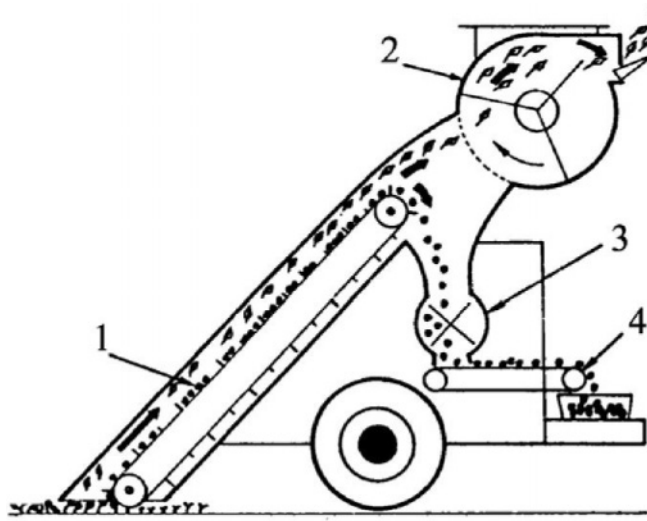

Figure 4. Strawberry harvester by the combing principle. (1) Finger band to comb the strawberry plant. (2) Fan. (3) Discharge valve. (4) Conveyor and filling device.

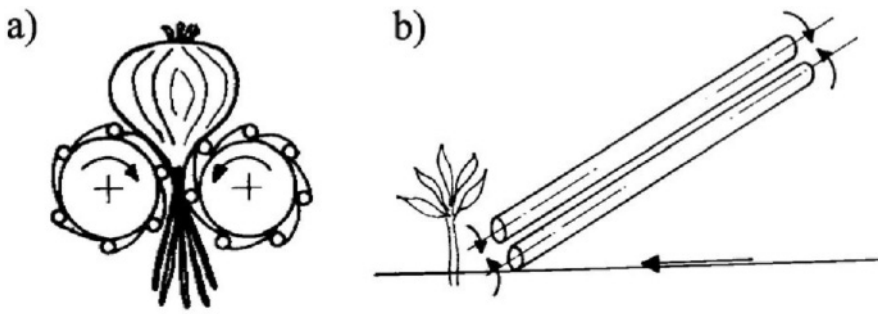

Figure 5. Stripping machine with counterrotating rollers. (a) Front view, (b) side view.

Helix-shaped open cylinders, counterrotating, have been used in paprika pepper harvesting machines, being the effect rather combing than stripping, as the helixes comb the fruits rather than strip the plant. Stripping is also used for the detachment of Brussels sprouts from the previously cut stalks. In this case, the effect is achieved by rubber cords.

Shaking is applied to plants previously cut, in tomato harvesters. Industry-tomato mechanical harvesting is the most advanced vegetable mechanical harvesting, in that there are world-wide used harvesters of numerous makes and designs which harvest a very high percentage of the industry tomato surface. Tomato plants are cut at the soil surface, plants with fruits are conveyed to the top of a vibrating platform, in which, being the tomatoes in a hanging situation, a shaking energy is exerted to them (Figure 6). Being the values of the detachment forces of tomato fruits in the range of 20–30 N, and the mass of the fruits 0.05–0.20 kg, the minimum

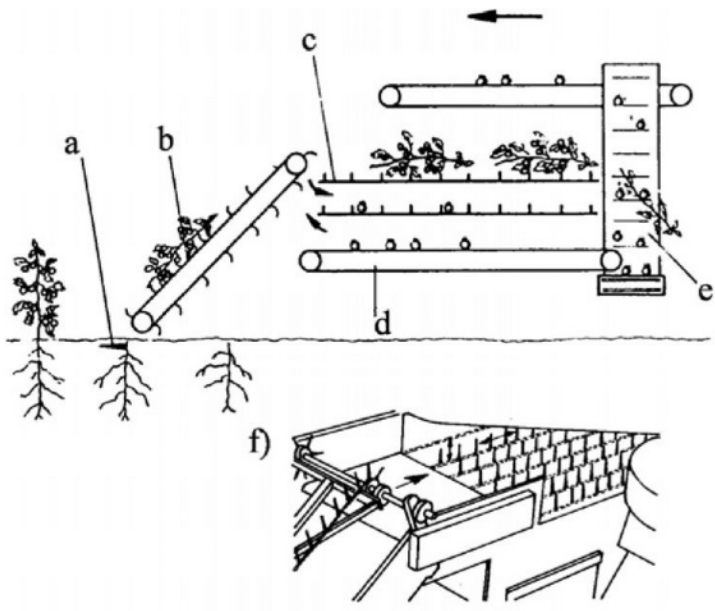

Figure 6. Tomato harvester. (a) Cutting unit, (b) elevating chain, (c) shaker, (d) selection band, (e) loading conveyor, (f) shaker unit.

applied accelerations for fruit detachment are in the order of 10–60 g's (times the acceleration of gravity) (see also Table 2)

$$\text{n.g } (\text{m/s}^2) = F_d\ (N)/m\ (\text{kg}) \qquad \text{(III.1.6.4–3)}$$

This relationship means that only those products with a relatively high mass and relatively low detachment force are easy to detach by vibration, as is the case with the present tomato varieties. In general, fruits are easy to detach by shaking if n is between 1 and 10 (Moser, 1984). Inertial application of vibratory energy for detachment results from accelerating the plant commodity with a suitable machine device to attain a pattern of vibration and a frequency which are suitable for that commodity. In this sense it has to be designated as 'the only non-contact' principle for detachment of fruits (Ortiz-Cañavate and Hernanz, 1989). In the case of tree fruits (see further ahead) the necessary acceleration has to be applied in the lowest-force abscission point of the fruit, and that is difficult for small fruits. These considerations can be applied to other vegetable crops where shaking could be a good solution for detachment.

The rest of the functions and units of tomato harvesters combine complementary operations, and manual or automatic optical VIS (visible, color) sensors or NIR (near-infrared) sensors for color grading and for soil clod separation, respectively.

Threshing: This separation procedure uses a rotating drum to remove peas (also some beans) from their pods in pea harvesters. Similarly to one of the effects used

for grain threshing, friction is used to separate the pea grain from their hulls. Friction is applied by slow-rotating rubber rollers or bands against a drum, sometimes also itself rotating, that behaves as the concave, as it sieves the peas through it (Figure 7). From there, peas are cleaned by air, conveyed and loaded to (refrigerated) bins.

3.2. Bushy structures

Such as small fruits, wine grapes. . . . As mentioned above, small fruits are at the same time:

– difficult to detach by shaking (non-contact) due to their small mass; and
– damage susceptible to be detached by combing or stripping (contact), as described for some vegetable harvesters.

Therefore, the detachment of small fruits has been solved by a combination of contact and non-contact actions applied by vibrating tools.

3.2.1. *Small fruits harvesters*

With 'small fruits', a number of species are referred: Raspberries, blackberries (*Rubus* species) and blackcurrant and redcurrant (*Ribes* species) along with gooseberries, blueberries, boysenberries, etc. They have in common their way of growing in bushy structures, their small size and their use mainly for processing. In the last ten years there has been a considerable activity in the application of mechanical harvesters to collect these fruits, which are the only fruit produced in some cold (northern) areas. Contact principles (combing and stripping) have been tested for the detachment of small fruits in bushy as well as tree structures, with little success. Present small fruit harvesters use a combination of shaking with soft combing, based on vertical, tilted or horizontal drums provided with:

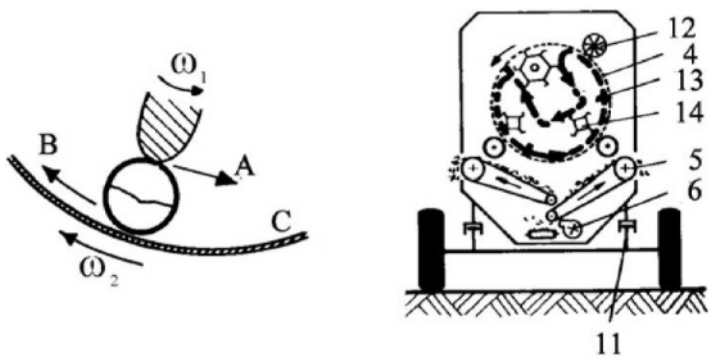

Figure 7. Pea-threshing principle (dehulling) and pea harvester. C: concave drum; ω_1 and ω_2: rotating speeds of bars and concave drum; AB: shearing effect on pod; 1; cleaning brush; 2: concave sieving drum; 3: pulling bolt; 4: threshing cylinder; 5: inclined band; 6: air cleaner; 7: slope-adjustment mechanism.

– fingers or spikes;
– oscillatory motion, which apply a shaking effect on the fruiting structures.

They work on plants trellised in different systems: 'T', 'V' or 'Y' (Figure 8) (Salamon, 1992; Peterson et al., 1989). The trellis system to be used and the shaking functional parameters depend on the fruit species and the conditions of fruiting. Drums are 50–100 cm in diameter, and the shaking is created by inertia shaking (see below, fruit shakers). The frequencies depend on the fruit species: 5–10 Hz for raspberries (*Rubus*) and much higher for currant (*Ribes*): 10–25 Hz, with amplitudes 40–75 mm. Travel speed is 1–1.7 km/h (lower speeds for lower shaking frequencies). The quality that one may expect for the mechanically harvested small fruits is highly dependent on fruit species and variety, yield, maturity, trellis system and the functional control capabilities of the harvester units.

3.2.2. *Grape harvesters*

Mechanical grape harvesters were designed in the USA during the late 50s. The fruit detachment system consists of a series of vibrating horizontal rods, that are free to move on their rear end and mechanically driven with an oscillatory motion on their front end (Figure 9). The number of rods depends on the height of the plants: 8–16 rods, for wines which are 1.2–1.8 m high. The effect of the rods on the wines

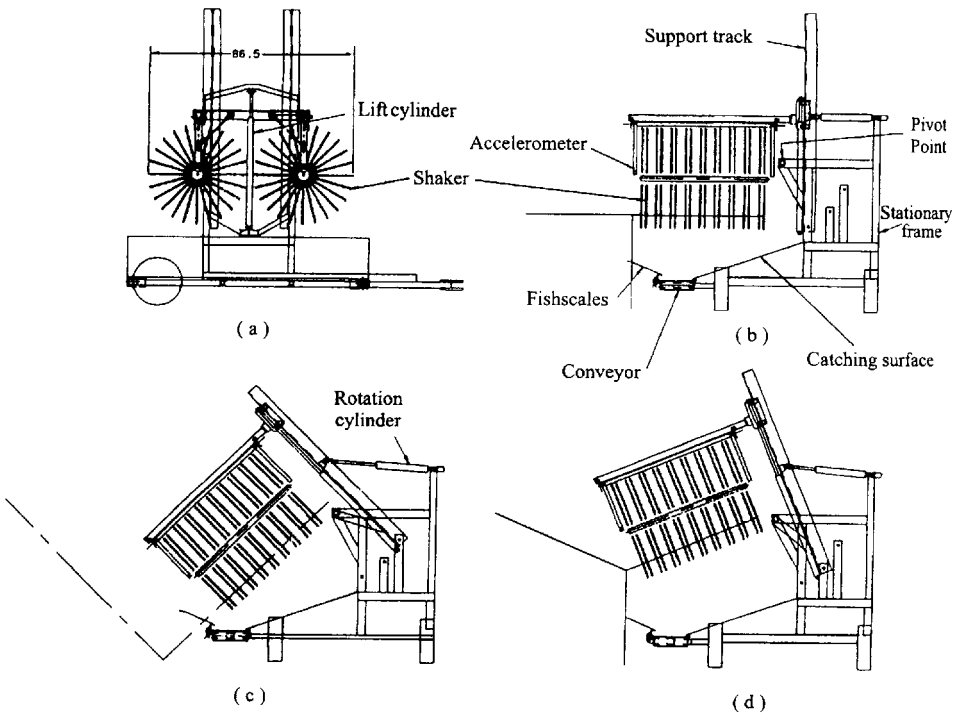

Figure 8. Blackberry harvester. Side (a) and front (b) view of 'T' trellis, (c) 'V' trellis, 'Y' trellis.

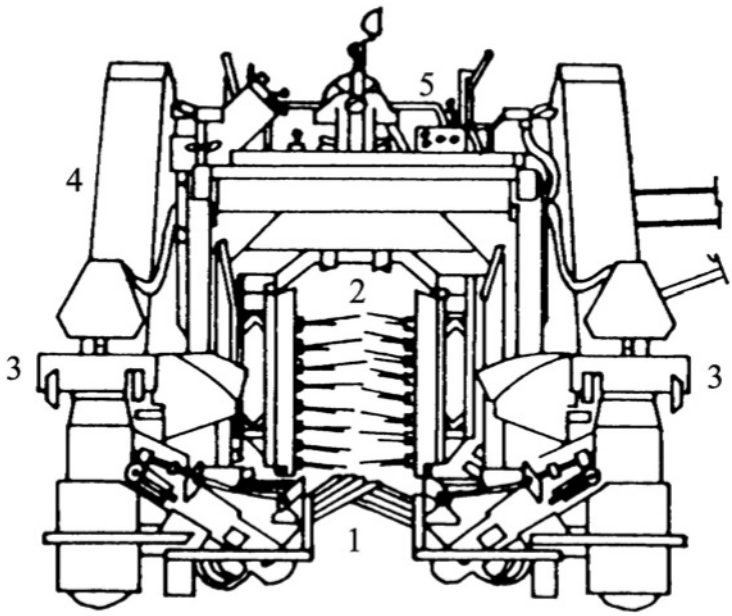

Figure 9. Over-the-row grape harvester. (1) Overlapping spring-loaded plates; (2) shaking rods (3) leaf blowers; (4) grape conveyors; (5) diving port.

is a combination of vibration and impact on the branches and on the winegrapes themselves. The frequency of the vibration is 9–10 Hz, with an amplitude of 88–140 mm (Gil Sierra, 1990).

In the USA, the latest grape harvesters have a detachment unit consisting of two sliding bars, provided with horizontal stroke. The bars engage the wine trunks at a height of 50–70 cm, which is forced to vibrate at a higher frequency (10–20 Hz), causing the detachment of clusters and also, more often, of individual grapes (Kepner et al., 1972).

In the late 80s, in France, a new design has been introduced that is capable of a more gentle handling of the vine shoots. In a similar arrangement as the former system, the rods are substituted by arched rods, which in their rear end are all fixed to the same member, that is pivotally mounted on an axis (Figure 10). The motion of these rods corresponds to the connecting bar of a bell-crank four-bar linkage. In this type of shaker, the frequency is higher: 15–23 Hz, and the amplitude smaller: 25–70 mm (Barbe et al., 1992). This means that whereas the former system applies peak accelerations of 130–180 g's, in the three perpendicular directions, on the vine trunk, the new types apply lower accelerations: 50–100 g's. The result is a reduction in deleafing, broken woods and torn woods in 50–90%, as well as a better quality of the grapes. Additionally, they can be driven at a higher speed along the row, therefore increasing their productivity. All these grape harvester are straddle type (over-the-row) (Figure 9) machines.

Functional parameters of the detachment system are therefore:

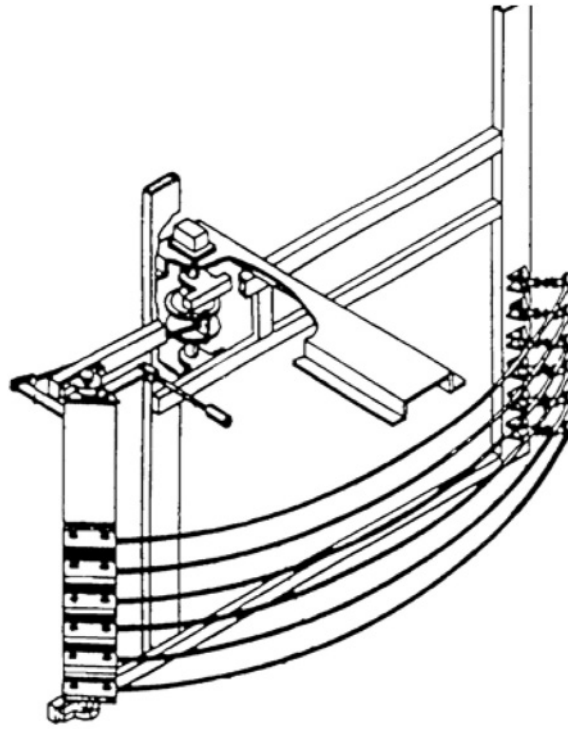

Figure 10. Oscillatory shaker with arched rods.

– frequency and
– amplitude of vibration, and
– travel speed.

In these harvesters, the shaking (detachment) unit is mounted on a hanging frame, that engages the trellised vines loosely. In the lower part of this frame, a series of overlapping plates form a catching platform. As the harvester moves forward, the retractable plates embrace the trunks, closing the catching surface tightly. The system has been developed to a very efficient level, where no more than a 5% of the crop is lost for most of the grape varieties. The machine incorporates one or two lateral conveying bands, and a loading elevator to load the grapes into the hopper, after one or two steps of air cleaning, which eliminates leaves, dust and shoot pieces. The harvesters work over-the-row and some are provided with sensing elements in front, to align it to the vine rows. Present harvesters are able to work in vineyards with narrow row spacing (down to 2 m) and are able to engage vines as low as 15–20 cm from the ground (Gil Sierra, 1990).

3.3. Tree structures

Such as fruits, nuts. . . . We differentiate between open-trained trees and high-density orchards:

3.3.1. *Tree shakers for open-trained trees*

The most extended method to harvest fruits mechanically is the use of inertial trunk or limb shakers that attach to the pertinent wood and are able to transfer large amounts of energy in the form of vibrations. Nowadays, the shake-catch method is the only mechanical harvest system used extensively in deciduous tree fruits.

The simplest shaking system appropriate for fruit trees is the tractor-mounted cable shaker. In it, the motion is generated directly by the tractor p.t.o, through an eccentric that powers the cable. Vibration is created by the returning movement of the tree branch or trunk. Frequency is 5–10 Hz and the amplitude is large: 20–60 mm. Power: 10–30 kW (Moser, 1984).

Eccentric rotating masses are the most widely used in tree shaker machines. Inertial shakers have to be isolated from the machine that carries them, so that no vibration is transferred to it. The basic principle consists of transmitting to the tree the forces generated by one or several rotating masses, or by a slider-crank mechanism.

The slider-crank shaker transmits forces in only one direction. The magnitude of the force depends on:

– the rotation speed and
– the mass of the housing of the shaker.

Slider-crank mechanisms are applied exclusively in limb-shakers. The frequency is 10–20 Hz; amplitude: 20–40 mm; power: 20–40 kW. Reciprocating housing mass: 100–200 kg; clamping force approx. 5 kN. The diameter of the branches can be maximum 30 cm, and clamping surface 2×30 cm^2.

In shakers provided with eccentric rotating masses, centrifugal forces are generated. The distribution of these forces can be varied by changing:

– the size of rotating masses;
– their eccentricity and
– their rotating speed.

Normally, forces are multidirectional, although two equal-size masses, rotating at the same speed (in opposite directions) generate a one-directional oscillating force. Multidirectional shakers are generally used as trunk shakers (Figure 11). The frequency is 20–40 Hz, amplitude: 5–20 mm; power: 30–70 kW. Eccentric masses: 20–60 kg; total mass of the shaker: 600–1000 kg, max. diam. of the trunks: 40–50 cm; clamp force 5–7 kN; clamp contact surface: 2×40 cm^2. Trunk shakers are faster and easier to operate than limb shakers (Figure 12). The structure of the trees needs to be adapted to limb shaking (3–4 main limbs at maximum). The use of trunk shakers is not well suited for too large trees (>50 cm in diameter) or for trees with hanging branches, which leads to low fruit detachment; in these cases, limb shakers are preferred.

Vibration of fruits

When a fruit vibrates, there simultaneously appear: traction, twisting, bending and shear forces, and also fatigue effects. As mentioned above for tomato shaking, an

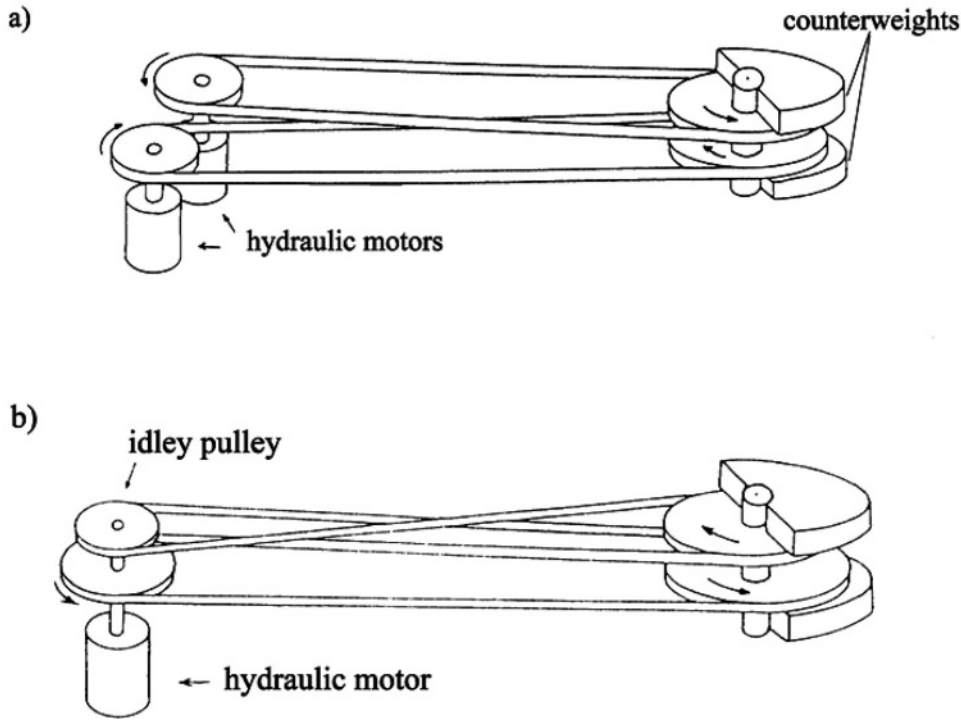

Figure 11. Drive of the rotating counterweights by two hydraulic motors with two bolts (a) and by one hydraulic motor and one idle pulley with one revolving belt.

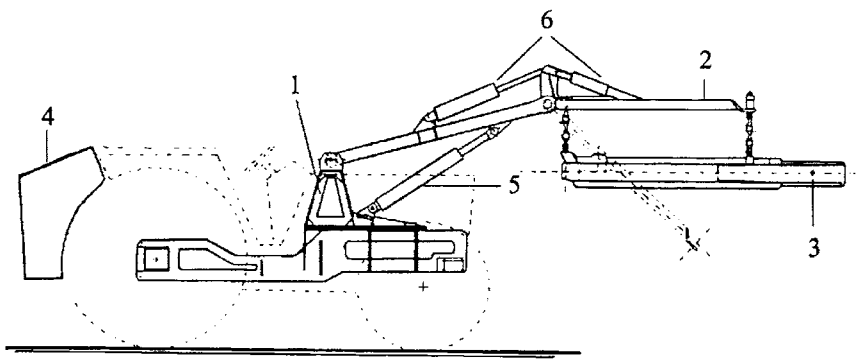

Figure 12. Tractor-mounted trunk shaker. (1) Supporting frame, (2) articulated arm, (3) shaker, (4) oil reservoir (5, 6) hydraulic system.

acceleration has to be applied to the fruit (expressed in number of 'g's') to produce its detachment from the limb or the peduncle. Table 3 summarizes vibration parameters typically used for shaker design.

Damage to the fruits is a limiting factor for the application of the shaking method, also in the case of processing fruit. The use of abscission chemicals has not wide-

Table 3. Frequencies and amplitudes used in fruit and nut harvesters.

Harvested crop	Frequency (Hz)	Amplitude (mm)	Observations
Strawberries	5–15	20–40	Mass: 6–9 g
Cherries	10–20	15–60	
Apricots	15–30	8–12	
Almonds	15–30	8–12	
Apples	15–30	8–12	
Prunes	15–30	10–14	
Peaches	15–30	12–16	
Olives	20–35	50–75	
Oranges	10–15	12–16	
Grapes	9–10/10–20	80–140	See text
15–23	25–70		
Tomatoes	5–10	30–50	
Small fruits			
Rubus	5–10	40–75	
Ribes	10–25	40–75	

spread application due to the high cost, and to problems related to timeliness of application, as well as to concerns about chemicals residues on the fruits.

Kinematics of the inertial shaker

If only one rotating mass, m, is considered (Ortiz-Cañavate and Hernanz, 1989) (Figure 13a), rotating at a constant angular speed ω, the centrifugal force has the following expression:

$$\bar{F} = mr\ \omega^2\ \exp(i\omega t)$$

where r is the eccentricity of the rotating mass. This type of orbital shaker is the appropriate for some fruits, like almonds.

If two eccentric masses are considered, m_1 and m_2 (Figure 13b), rotating at different angular speeds, ω_1 and ω_2, in the same or in opposite directions, and mounted in one or in two different axles, the forces generated by their rotations are:

$$\bar{F}_1 = m_1 r_1\ \omega_1^2\ \exp(i\omega_1 t)$$
$$\bar{F}_2 = m_2 r_2\ \omega_2^2\ \exp(i\omega_2 t)$$

The usual output of inertia shakers is a relatively high frequency of vibration (12–40 Hz), and a short stroke (5–20 mm) delivered to the tree. The resultant force $\bar{F}_1 + \bar{F}_2$ can be adjusted for magnitude and pattern in any desired way by changing the relative masses of the eccentrics and by modifying their respective rotating speeds (Figure 14).

The force generated by the shaker is (Figure 15):

$$F(t) = Mx + cx + kx$$

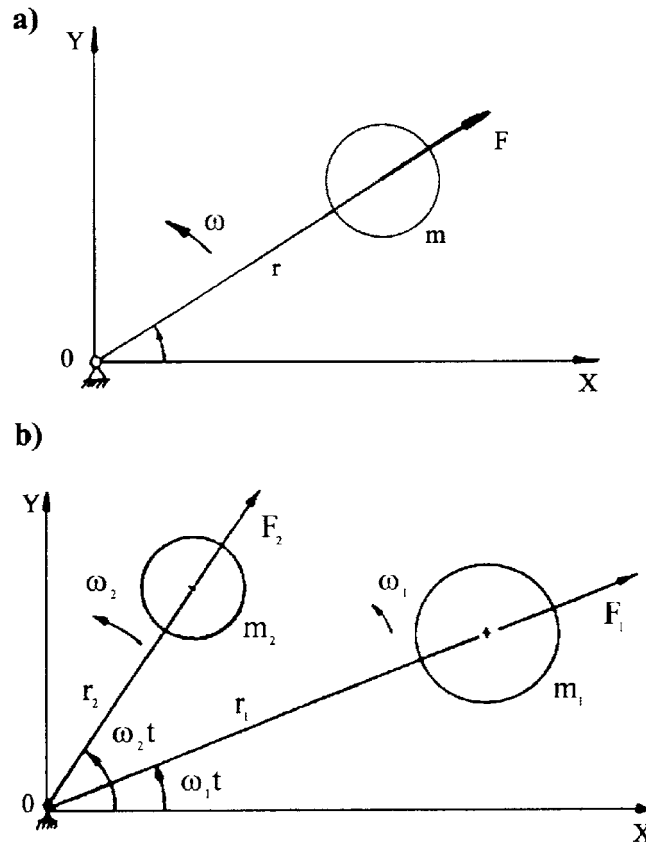

Figure 13. Multidirectional shaker with one (orbital shaker) (a) and two (b) independent rotating masses.

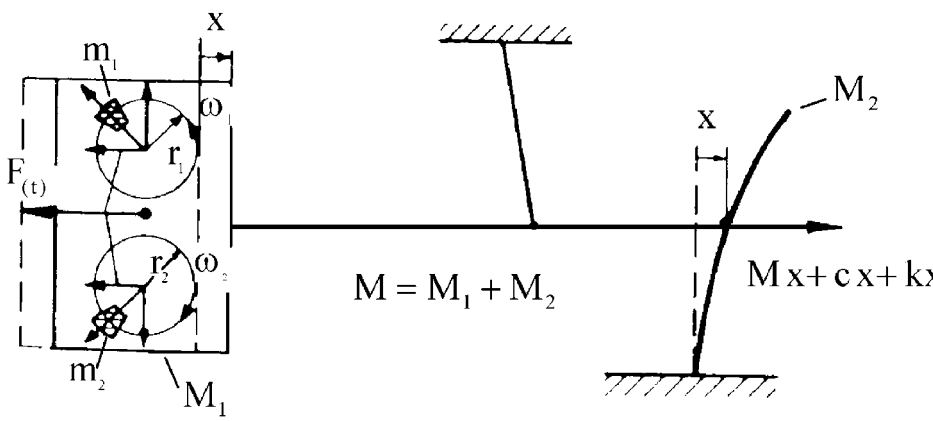

Figure 14. Forces created in a two rotating-mass inertia shaker.

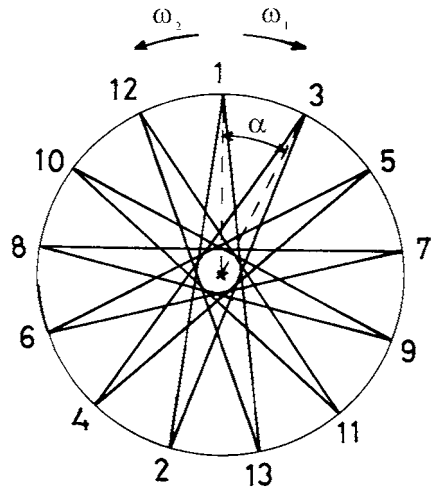

Figure 15. Directions of vibration, depending on ω_1 and ω_2.

M: total mass (equivalent mass of the tree + mass of the shaker), kg
c: damping factor, N.s/m
k: elastic constant, N/m

Being the maximum force:

$$F_{max} = 2mr\ \omega^2$$

in case where $m_1 \cong m_2$; $r_1 \cong r_2$ and $\omega_1 \cong \omega_2 = \omega$
Approximately the amplitude of vibration results [12]:

$$s = 2mr/M$$

The equivalent mass of limbs *M* can be estimated as 0.2 kg per diameter-mm and for trunks as 2 kg per diameter-mm. Natural frequency, damping factor and elastic coefficient of the trees change with time of the year, between the following approximate values:

f = 0.5–3.5 Hz
c = 0.1–0.7
$k \leq$ 2000 N/cm

Fruit detachment is, for any particular species and type of trees, the effect of a combination of:

– stroke,
– frequency and
– pattern of vibration.

To calculate the number of directions of vibration (shaking pattern) let us consider (Figure 15) that the speed ω_1 is slightly higher than ω_2, and rotating in opposite directions: $\omega_1 t = 2\pi + \alpha$; $\omega_2 t = 2\pi - \alpha$, and the number of directions of shaking is (Ortiz-Cañavate and Hernanz, 1989):

$$n = 2\pi/\alpha = (\omega_1 + \omega_2)/(\omega_1 - \omega_2)$$

Damage to the trees

The zone of attachment of the shaker to the tree (trunk or limb) has been found to be the most dangerous for tree damage. During shaking applied by the clamp to the wood, there are not only radial stresses, but also longitudinal and tangential (Moser, 1984). The admissible stresses that can be absorbed without damage under the shaker clamp are very much related to bark humidity. For fruit trees, the admissible values for tangential, radial and longitudinal stresses are, respectively: $\sigma_t \leq$ 600 kPa; $\sigma_r \leq 1700$ kPa and $\sigma_l \leq 1800$ kPa.

Figure 16 shows the comparative variation of bark resistance (N/cm^2) for different times of tree growth (times of the year) and irrigation, all which influence bark humidity.

Excessive radial stresses are likely to cause damage in selected fruit trees. The causes of excessive stress can be due to a bad design of the clamp, as well as to careless operation. For example, damage can be caused by inadequate adjustment of clamping pressure or inadequate contact area between the bark and the clamp. To avoid tangential stresses (the worst for the tree) some manufacturers place a flap on the clamp, keeping the clamp-flap contact surface well lubricated.

3.3.2. *Harvesting fruits in high-density orchards with over-the row machines*

A new possibility for fruit production is the high-density dwarf-tree plantation (Trees planted at 2×1 or 2.5×1 m^2; this means 4000–5000 trees/ha, with a higher and

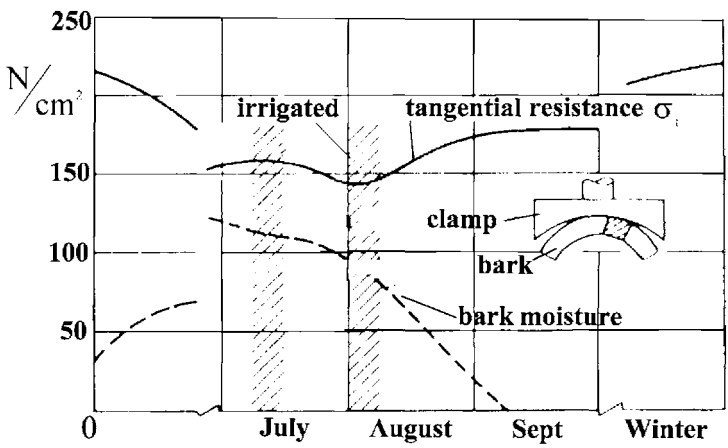

Figure 16. Bark-resistance (tangential-resistance) variation along time of the year and irrigation (plum tree).

earlier cropping). A complete mechanization process: cultivating, pruning, spraying and also harvesting is possible, implemented with over-the-row machines. Maximum height of the trees must be maintained at 2.4 m for the clearance of the machine (<2.3 m). In case the fruits are processing-oriented, adapted grape harvesters can be applied for their harvest: apples, peaches, apricots, plums, etc.

Pruning machines for hedging and topping the trees are used extensively in high density and also conventional orchards.

4. COMPLEMENTARY OPERATIONS

Operations which have to be performed by the harvesters after product detachment are very similar in all of them. A comprehensive view may include all systems designed to: catch, convey, separate, clean, grade and load the product.

These have been shown as parts of the different harvesters, and include: chain and band conveyors; aerodynamical separators; sieves and sizers; horizontal disk-roller cleaners to separate small fruits and foreign materials; manual grading platforms; electronic color and stone/soil-clods sorters; band conveyor loaders; decelerating sleeves. Specially needed in fruit harvesters based on shakers are: catching structures, frames and umbrellas; being the rest of complementary operations similar in concept to the previously mentioned for vegetable harvesters.

A common characteristic of these elements is that they have to be designed to:

- apply as low as possible energies to the fruits/products, so as to avoid damage: this objective is achieved by avoiding unnecessary accelerations and drops, unnecessary sharp turns; by adding decelerating elements: sleeves, strip courtains; and at the same time,
- they have to be covered with energy-absorbing materials (damping materials);
- sharp edges have to be avoided carefully.

Solutions applied now to fruit-handling equipment should be studied for their possible use in the development of new harvesting equipment.

Most damage to the detached fruits are caused during its fall inside the tree; to avoid this problem, fruiting branches should be located in different vertical planes whenever possible. Processing fruits should be harvested as early as possible, and be processed in the shortest time after harvest. Padding surfaces should be included on all surfaces likely to get in contact with the fruits. Padding materials are designed to absorb the kinetic energy of the falling fruit. Figure 17 shows the effect of bouncing of a fruit on any surface. The coefficient of restitution is expressed as

$$e = v_2/v_1 = [h_2/h_1]^{0.5}$$

For

$e = 1$: the impact is perfectly elastic, and for

$e = 0$: the impact is perfectly plastic. This last situation has to be obtained, *by the deformation of the padding material*, which has to be soft and resilient (i.e. slow in deformation).

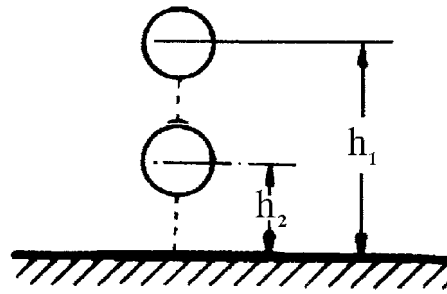

Figure 17. Bouncing of fruit onto a partially elastic surface.

For the cleaning and separation of leaves, twigs and other foreign materials of collected fruits and vegetables, blowers and vacuum blowers are mounted on the harvesters. For round type fruits, flotation air-speed can be expressed by

$$v_{fl} = [4\ \delta_{fr}\ g\ d_{fr}/3\ \delta_a\ C_r]^{0.5}$$

δ_a, δ_{fr} = density of air and of fruit, resp., kg/m^3
d_{fr} = diameter of fruit, m
C_r = air resistance coeff.
g = gravity acceleration, m/s^2

Flotation air speeds for selected fruits: peaches, apricots, plums can be found in the literature. For example, for cherries 16–19 m/s, grapes 18–20 m/s, whereas leaves 3–7 m/s.

5. MECHANICAL AIDS TO MANUAL HARVEST

Many products can be selectively (manually) detached, and the rest of operations can be subsequently performed by mechanical units. Those are: vegetables like lettuce, all cole species (cabbage, cauliflower, broccoli, Brussels sprouts), zucchini, green and bell peppers, melons; small fruits; tree fruits. Different types of carriers and conveyors have been designed and are extensively used (Ortiz-Cañavate and Hernanz, 1989).

Carts
Three-weeled carts, manually driven, are used for filling boxes in the field: celery, leeks, many types of greens. The plants are manually cut by pickers.

Carriers
Tractor pulled or self-propelled: the handpicker sits and picks the fruits, filling the boxes which are removed by a further handworker. Much used for strawberries, also for radishes, leeks and many other fresh market vegetables.

Harvesting platforms

Which can be self-propelled or tractor-pulled. They carry 8–15 workers for the wrapping, size grading and box filling, are often used for lettuce harvesting. Width: 5–14 m; speed: 0.1–1.5 km/h; power: 20–45 kW. The same type of platforms are often used for harvesting products like: cabbages, cauliflower, broccoli, melons, pineapples. Fruits can be either prepared, sized and boxed in the platform, as described for lettuce, or just conveyed to bins or trailers for bulk transportation to the plant (simple platforms, Table 4 and Figure 18). Work capacity is substantially increased with these aids (20–100%). In general, harvesting aids result in only moderate increases in productivity, as workers capacity is very interdependable (Ruiz-Altisent, 1989). Table 4 shows values of work capacity of harvest aids and harvesters for different vegetables.

Man positioners for harvesting tree fruits

These are one-man platforms, self-propelled, which can be used both for picking and for pruning fruit trees. Work capacity is not greatly improved (Ruiz-Altisent, 1989; Ortiz-Cañavate et al., 1994; Moser, 1984) as compared to the investment needed. They can be appropriate for small orchards devoted to quality fruit where no high-efficiency equipment is justified.

Table 4. Working capacities of mechanical aids and harvesters for some vegetables.

	Machine type	Capacity
Mechanical aids		
Lettuce	Platforms, simple	1.5–2 × hand-harvest capacity
	Platforms, self-propelled	25,000 heads/d
Cauliflower	Interrow platform	100–200 heads/man h
	Selective harvester	300–400 heads/man h
Artichoke	Platform, simple	300–350 heads/man h
Celery	Platform, simple	1.5–2 × hand-harvest capacity
Pepper	Platform, simple	120–210 kg/man h
Pickling cucumbers	Man-carrier 14 m-w	300 kg/h
Root crops	Platform tractor trailed	100 kg/h
Harvesters		
Cabbage	One-row	1000–1300 kg/h
Celery	One-row	3000–5000 plants/h
Green beans	Trailed, one-row	2000–2500 kg/h
	Front picker, 2–3 m wide	6000–10,000 kg/h
Green peas	Front picker, 3 m wide	1500 kg/h
Picking cucumbers	Self-propelled, 2 m wide	3500 kg/h
Green onions	Self-propelled, 1–2 m wide	2500–3500 kg/h
Root crops	Trailed, self-propelled per row	3500 kg/h
Tomato, industry	One-row, manual selection (8–10 workers	
	• Trailed	3000–4000 kg/h
	• Self-propelled	5000–6000 kg/h
	• Self-propelled, optical selection	6000–8000 kg/h
Pepper, industry	One-row, trailed	500 kg/h

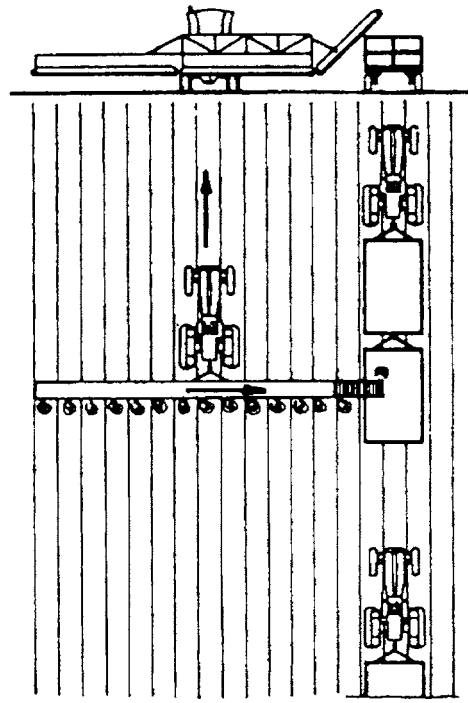

Figure 18. Tractor-trailed platform for bulk manual harvesting of produce.

Multilevel picking platforms

Picking of fresh market fruit is definitely solved by the introduction of the latest multilevel picking platforms (Various authors, 1997). Orchard must be ideally formed in hedgerows. Picking is the only operation that is performed manually, being the rest mechanized: each fruit is placed individually on soft rubber-finger conveyors, up to a soft central conveyor and to the bins. This system is able to optimally preserve the quality of the fruits, even better than totally manual ladder-and-bag procedures. Working capacities of these manual fruit-picking aids are summarized in Table 5. The fruit is placed in conveniently located conveyors, which load the fruit in bins, transported by the same unit. Travel speed is very low, harvesting capacity can be only slightly higher than manual, up to 30% higher, but the operation is greatly improved in terms of work organization and handworker comfort.

6. ELECTRONIC SENSORS AND ROBOTIC HARVESTING

Mechanic (contact) and electronic (γ rays) sensors have been developed for selective harvest, like that of lettuce by ripeness: in this case head compacity is determined as the criterium for quality.

Table 5. Working capacities of mechanical aids and harvesters for some fruits.

	Working capacity	Losses (%)
Mechanical aids		
Tree-fruits		
Single-man positioner	130 kg/man h or 0.95 ha/100 h	3
Multiple (hedgerow) platform	140–220 kg/man h or 2–10 ha/100 h[a]	3–6
Strawberries, man carriers	10–12 kg/man h	5
Small fruits, hand combs	35 kg/man h	8
Harvesters		
Grapes	2000–6500 kg/h	5–15
Nuts, ground sweepers	6000–7000 per meter width	2
Tree-fruit	40–60 trees/h	5–8
Trunk shakers		
Limb shakers	8–10 trees/h	5–15
Strawberries	200–600 kg/man h	10–20
Small fruits	500–1000 kg/man h	10–30

Optical sensors have been applied up to now for color recognition and of soil-clods separation in tomato harvesters. The development of electronic sensing has been great, but the application mainly in handling equipment.

Robotic harvesting has been oriented for soft, fresh fruit that cannot be harvested with conventional machines, because of excessive mechanical damage. Therefore, it represents an alternative to the current mechanical harvesting systems, superior from the point of view of fruit quality and with a clear projection to the future. It consist of the automated fruit picking with a robotic system which emulates the human picker.

Although much research has been oriented to this goal, no commercial robot is yet (2001) capable of replacing manual labor for picking fresh fruits. A fruit-picking robot should possess following capabilities:

- to locate fruits on the tree in three dimensions, and in any light conditions (including the dark);
- to approach and reach for the fruit;
- to detach the fruit and transfer it to a suitable container;
- to move by itself in the orchard from tree to tree and row to row without human help (Sarig, 1993).

Several up-to-date technologies are involved to achieve these goals, such as artificial vision, image processing, robot kinematics, sensors, control and computerized signal analysis. Prototypes for the robotic harvesting of commodities, different from tree fruits, like melons and grapes, are also being developed.

Looking into the future, it seems possible that the robot, equipped with the appropriate sensors, would be able to select the most suitable fruits on the tree itself (in terms of quality), to be picked concurrently with the fruit-identification process. In the case when:

– the system would be able to obtain the best quality,
– and operating in controlled spaces (e.g. high-density orchards and in greenhouses, rather than in the open) there could be commercial fruit picking robots available in some years time.

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