The Citrus Nursery Practices In Brazil

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Preface

The exchange of information, of good or bad experiences, contributes to the improvement of people, processes and products.

The purpose of contributing to improve the Citriculture by means of its base, "***citrus nursery tree***", a group of citrus nurseries founded in October, 1988, 'Associação Paulista de Viveiros Certificados de Citros', currently referred to as 'Paulista de Viveiros de Mudas Citrícas' (Vivecitrus), located in Araraquara, State of Sao Paulo.

During the 15 years of its existence, the Vivecitrus and its members contributed in establishing citrus nursery tree production quality standards and methodologies, according to the rules of the Sanitary Plant Protection of the Office of Agriculture and Supply of São Paulo, contributing to generate healthy orchards. Within its technical purpose, Vivecitrus often holds meetings with researchers and Brazilian and foreign technicians for discussion and preparation of pilot projects related to irrigation, nutrition, prevention and control of pests and diseases.

Since 1999, Vivecitrus arranges the Nursery Growers Day, since 2010, the *Management Meetings to Production Citrus Nursery tree*, held at, respectively, Sylvio Moreira/IAC Citriculture Center and at Citriculture Experimental Station of Bebedouro. It quarterly publishes the *Vivecitrus informative*, as well as it maintains the website www.vivecitrus.com.br, which is focused on current topics of citrus production.

Its members actively attended the preparation of Rules for Production of Certification Nursery Tree in Protected Environment, as well as they worked in the preparation of funding rules for construction to protected structures in the citrus nurseries and in the preparation of insurance against citrus canker and weather conditions.

Aiming to share the knowledge acquired during 15 years of professionalism, the members of Vivecitrus presented to guide The Citrus Nursery Practices in Brazil to the citrus community, which included orientations about the best method to produce citrus nursery tree, according to current legislation. The guide encompasses infrastructure guidelines necessary to install the nursery, features of main scions and rootstocks, stages of citrus nursery production, irrigation, fertilization, pests and diseases control and production costs.

However, our proposed to intensify the exchange of ideas and promote constructive disagreements that will contribute to the improvement of "the keystone of citrus”: citrus nursery tree.

Jorgino Pompeu Junior, PhD.

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To the citrus nurseries grower's members of Vivecitrus by the shared knowledge throughout the preparation of this paper.

To the Ministry of Agriculture, Livestock and Food Supply and Office of Agriculture and Food Supply of the State of São Paulo for the information related to standards of the citrus nursery tree production.

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To Heraldo Negri de Oliveira, Ricardo Krauss and Otavio Ricardo Sempionato for images involving pests, control disease and production of citrus nursery tree in open field.

To Agronomist Leandro Fukuda for the information involving technical of production of citrus nurseries trees within protected environment.

Sumary

1. Introduction

The first reports regarding the citrus crop in Brazil are from the sixteenth century, in the States of São Paulo, Bahia and Rio Grande do Sul. From its introduction to the recognition of the advantages associated with grafting, in the early twentieth century, the multiplication of citrus was made by seeds, originating non-grafted plants, with many thorns and beginning of delayed production due to the long period of juvenility of plants obtained by this method. The production of grafted plants enabled the size reduction plants, few thorns and early production. In addition to these advantages, the grafting enabled the production of clones, genetically identical plants, better adaptation to different environments and production systems and disease tolerance, through the proper selection of the rootstock, which attracted the interest of growers. For these reasons, vegetative propagation, by bud grafting of scion varieties of commercial interest in selected rootstocks, was established as the main method of commercial multiplication of citrus plants in the world. The use of bud grafting or budding, however, may present risks related to the spread of viruses, viroid, bacteria transmitted mechanically and/or insect vectors, considering that preventative control measures are not taken. In addition, the production of rootstocks and citrus nursery trees can be affected by pathogens that are spread by the wind, substrate, irrigation water and clothing, such as citrus canker, nematodes, gummosis caused by *Phytophthora*, among others.

It is worth to note that many of the diseases that occur in nurseries have a long incubation period, with symptoms that manifest themselves long after the infection, not only compromising the health of citrus plant nurseries, but the entire investment made in the formation of a new orchard. The increasing of diversity and complexity of diseases affect citrus production in Brazil, the care needed for the production of basic propagating material and citrus nursery tree have become priority.

In the 1990s, with the report of Citrus Variegated Chlorosis (CVC), a disease caused by the bacterium *Xylella fastidiosa*, important changes occurred in the citrus plants nurseries production system, which until then were produced in the open field (Figure 1a). The discovery of the transmission of bacteria by insect vectors (Leafhopper) led the Office of Agriculture and Food Supply of São Paulo, from 1999, to publish Ordinances aiming at health of basic propagation material and São Paulo citrus nursery tree. These Ordinances induced significant changes in the production system, such as the obligatory production of budwoods and citrus nursery tree in a protected environment (Figure 1b). Thus, all the knowledge involving the selection of location, facilities, equipment, inputs and budstocks, rootstock and citrus nursery tree production techniques held in open field production system needed to be adjusted to new legal requirements.

This guide is a summary of knowledge produced and consolidated by the efforts of technicians, researchers and citrus nurseries that, since 1998, dedicated themselves to activity of citrus nurseries trees production in a protected environment.

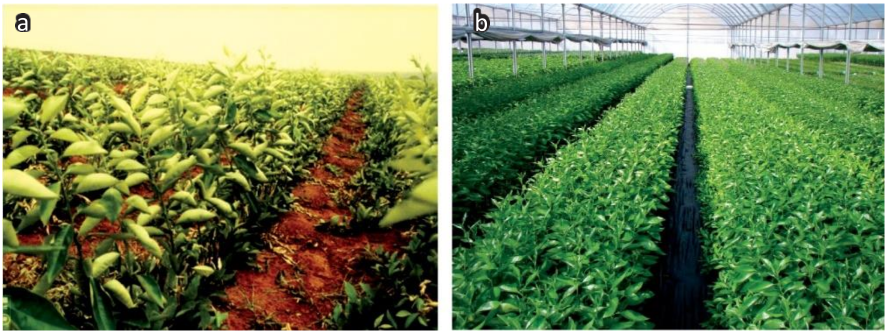

Figure 1 Production of citrus nursery tree in open field (a) and in protected environment (b). Photo: Otávio Sempionato and (b)Vivecitrus.

2. Regulating Requirements

The legislation that regulates the production of citrus nursery tree aims to ensure quality, health and genetic purity of the material multiplied and traded in Brazil. The technical and operational requirements are high and the nursery grower must be aware of each one of them, including the preparation of the technical project and in all other stages of production, under penalty of having of the nursery interdicted or have the sale of citrus nursery tree suspended. In general, federal law concerns, predominantly, aspects related to ensuring the genetic, horticultural and standardization quality of propagating material traded, while the laws of the State of São Paulo, in addition to such, encompasses phytosanitary aspects of production.

According to the standards established by the federal law, both individuals and legal entities engaged in the activities of production, processing, packaging, storage, analysis, trade, import and export of seeds and plant nurseries must be mandatorily enrolled with National Registry for Seeds and Seedlings (RENASEM) of Ministry of Agriculture, Livestock and Food Supply (MAPA), as well as having all the implementation stages of nursery and production of seedlin monitored by responsible technician, also accredited by MAPA. Moreover, all cultivars used as scions and rootstock must be previously qualified by the National Registry of Cultivars (RNC) of MAPA. The RNC ensures that the cultivars available to farmers were evaluated by Brazilian institutions and present the latest research advances in genetics and plant breeding. The list of cultivars qualified by RNC is available in the website (www.agricultura.gov.br/vegetal/sementes-mudas). Also at the federal level, the producer of citrus propagating material must be aware of the Normative Instruction No. 48 of the MAPA (IN 48), issued on September 24, 2013, providing rules for the production and trade of citrus propagating material and its hybrids, valid throughout national territory. According to IN 48, all production activities, including seed, budwoods and citrus nursery tree production must be enrolled with and inspected by MAPA, respecting terms, rules and specific technical requirements of each stage of production.

In the State of São Paulo, in addition to enrollment with MAPA, the grower and nursery growers of citrus nursery trees must be registered in the Department of Agriculture and Livestock Protection of the Agriculture and Food Supply Office (CDA). This registration involves basic information about the growers and infrastructure report of production place, used in phytosanitary inspection, control of the regulatory requirements of production and issuance of Plant Transit Permit Guide (PTV), required for the trade of citrus nursery trees. This registration must be prepared by the Agricultural Engineer qualified by the Regional Council of Engineering, Architecture and Agronomy (CREA), and must be renewed every three years. The legislation in the State of São Paulo requires that the production of rootstocks, budwoods and citrus nursery trees are carried out in an appropriate place, intended solely for the production of citrus nursery trees and protected by antiophidic screen (against aphids input and leafhoppers) and through the use of seeds and budwoods obtained by matrix plants and budstocks recorded in CDA.

After the registrations in MAPA and CDA, the growers shall submit, to CDA, the technical level of production of citrus nursery trees, prepared by the Agricultural Engineer in charge for the nursery, stating the amount of citrus nursery tree, cultivars, scions and rootstock used and the production period, up to 15 days after sowing or transplanting of rootstocks, where they are purchased from third parties. Through the registration of the technical plan of production, CDA is able to inspect all production stage.

Throughout the production period, the technician in charge for the nursery must issue three phytosanitary reports. Such report of first inspection must be issued up to 15 days after sowing or transplanting of rootstock, if such is obtained from third parties, informing the material origin, as well as the sowed or transplanted amount. The phytosanitary report of second inspection must be issued in the grafting and inform the grow crops that will be used as scions, the amount of grafting, as well as the origin of budwoods. The phytosanitary report of third inspection will be issued in the release of the seedlings and buds will contain the results of health tests that prove the absence of CVC, gummosis of Phytophthora, huanglongbing (ex-greening, HLB) and nematodes, in addition to information about other phytosanitary events. It is worth to note that laboratory tests should be carried out in laboratories accredited by MAPA and registered in the CDA. In addition to these reports, it is necessary to submit a final report that should inform the total production, seedlings destination and other information required by the CDA, ending the activities of the technical production plan. Like the nursery, the budwoods requires registration with the CDA, infrastructure report and formation plan issued by the technician in charge. Thus, along the bud grafting useful life, it shall also be issued inspection reports and inspection of bud grafting. The first report is issued at sowing or transplanting rootstock, stating the origin of the seed or budstocks; the second report, at the time of grafting, proving farming and source of budstocks to be used as scions, and the third report of inspection, in release of budwoods. Semiannually, budstocks should be subjected to inspection by CDA inspector and, annually, laboratory tests proving the health of plants (in relation to CVC and HLB or when found the presence of symptomatic plants) should be made for its intended use by nursery gardener and/or trading. After five years, counted from the grafting of plants of bud grafting, it should be discarded in the presence of CDA inspector.

Detailed information about legal matters of production of seedling may be obtained in Law 10.711/2003, Decree 5153/2004, Federal Normative Instruction N.9/2005,24/2005,42/2009,2/2010 and 48/2013, and in State Ordinances N. 5 and 23. It is worth to note that the legislation governing the activity of seedling production can be modified, aiming its adaptation to the industry needs, leaving the nursery growers and technicians the responsibility to be updated with any changes in production standards. To this end, it is recommended often access the website of CDA (www.cda.sp.gov.br) and of MAPA (www.agricultura.gov.br).In Annex 1 is included a list of records, licenses and other documents required by law and applicable to the production of seeds and citrus nursery tree in the federal and state levels.

3. Nursery installation

3.1 Area

The selection of the area where the source citrus tree bud and the citrus nursery will be installed is the first step for the health of the citrus nursery tree to be protected. In this respect, the main factor to be evaluated is the distance between the area intended for installation and commercial or domestic citrus orchards. According to legislation governing the activity in the State of São Paulo (Ordinance N. 05 of CDA), the distance between nursery and other citrus plants must be, at lease, 20 meters (Figure 3.1a). When focus of citrus canker is registered in a period less than two years in orchards nearby nursery installation area, this must be installed at a minimum distance of 1200 m. Hedges of ornamental plants such as myrtle (*Murraya paniculata*) should also be avoided, as are alternative diseases affecting citrus host, such as bacteria associated with HLB

The place where the nursery will be installed should present soil and relief that promote the drainage of rainwater, preventing the accumulation of water and possible spread of soil-borne pathogens. It is also important to observe the prevailing conditions, particularly the temperature of the air, for both low temperatures as high, may compromise the formation of changes. Thus, one can anticipate the need for additional devices in nurseries for maintaining the proper temperature for the growth of citrus nursery tree. When the purpose is to reduce the air temperature, may be used netted shade or woven meshes.

Figure 3.1 Nursery of production of citrus nursery plants insulated from commercial citrus orchards(a) and greenhouse equipped with woven meshes to control solar radiation and internal temperature (b). Photos: (a) Vivecitrus and (b) Horst Bremer Neto.

(Figure 3.1b). In order to maintain higher temperatures, curt insider may be used.

Another important aspect in the implementation of the citrus nursery is enough water available to meet the demand of irrigation plants throughout all stages of production and sanitation of the nursery. When the water is taken from rivers, lakes and dams, its treatment is mandatory, aiming to reduce the risks of biological contamination. When water is extracted from artesian wells, a sanitary treatment is not necessary. Regardless of the source of water for irrigation. the acceptable limits of concentration of salts must be respected in order to prevent salinization of the substrate and not jeopardize the proper growth of citrus nursery tree.

3.2 Infrastructure

3.2.1 Nursery and budwoods

According to Ordinance N. 05 of CDA, the physical structure and basic equipment for installation of greenhouse will compose nurseries and bud grafting include woven meshes (maximum of 0.87 x 0.30 mm), without hole or cracks; waterproof cover, without holes or cracks (usually used in a transparent polyethylene film with a thickness of 150 µm); antechamber with minimum area of 4 m^2, to reduce the risk of entry of insect vectors, with footbaths for disinfection of footwear and container for disinfection of hands and utensils containing chlorhexidine digluconate (Figures 3.2a, b, c, d); countertops, installed 40 cm above ground level in order to avoid the contact of the root system of young citrus tree with the floor and prevent possible infection by Phytophthora and nematodes;

Figure 3.2 Antechamber in nursery and protected environment for Antiophidic screen and waterproof cover (a), footbath (b,c) and reservoir for disinfection of hands and utensils(d).Photos: Horst Bremer Neto.

spaces between the countertop with the floor or with crushed stone layer, or similar, with, at least, 5cm thick (Figure 3.3a; b; c) and side short wall along the entire perimeter of the greenhouse to prevent invasive water entry (Figure3.4a). Although allowed, it is not recommended to use crushed stone for paving of the nursery, since this material may favor the incidence of weeds and hinder cleaning of the nursery. In this case, the nursery growers should give preference to concrete pavements or raffia (Figure 3.3a; b). Countertops can be made of wood (not recommended for hindering cleaning and disinfection), iron or masonry, using blocks, beams and precast grids (Figure 3.3a; b). At the federal level, the requirements of IN 48 are smaller in relation to the laws of the State of São Paulo, limited to the coverage with antiophidic screen, antechamber and hand washing devices for the production of bud grafting and certified citrus nursery tree.

The quality and strength of the materials used in the greenhouse structure cover are essential to reduce the risk of breakage of the screen, avoiding the entry of vector insects and pathogens. The use of windbreak composed by high species reduces the wind speed by protecting the structure of the greenhouse, besides acting as a physical barrier against pathogens, insect vectors and solid particles which may be adhered to the side screens and the greenhouse cover reducing the brightness (Figure 3.4b). It is recommended that the right foot of the greenhouse presents a minimum height of 4 m, enough to promote adequate air circulation, reducing the occurrence of high temperatures. The internal area must be free of pest plants, the carrier between the countertops and the screen

Figure 3.3 Bench consisting of grids and concrete blocks on raffia floor (a) e b) and corridor paved with concrete (c). Photos: Horst Bremer Neto.

and between countertop must present minimum distance of 50 cm and the outer perimeter of the greenhouse should be free of vegetation in a minimum range of 1 m. The place destined for the storage and handling of the substrate should be paved and free of invasive water.

The greenhouse used for the production of budsticks shall be used solely for this purpose. The production structure should have waterproof plastic cover and side closure antiophidic screen (maximum mesh 0.87 x 0.30 mm), antechamber, footbath, among others, with the same specifications applied to greenhouses that will produce the citrus nursery tree. It is worth to note that under the plastic cover, the installation of antiophidic screen is obligatory. The right foot should provide minimum height of 4 m and a distance between the side screen and the plants should be at least 1 m. The plants can be grown directly on the ground (floor) or vessels containing substrate, suspended in countertop (Figure 3.5). The renewal of budwoods cultivated the ground presents difficulties related to the elimination of crop residues, especially the root system and may compromise the health of the plants of the new planting. On the other hand, the budwoods grown in pots, even though they have higher costs and require greater care in the management of irrigation and nutrition, are easy to be renewed and for this reason the bud grafting production system is most widely used. In this system, bags with capacity from 3 to 20 L of substrate are used, depending on the conduction system of the plants.

In addition to the care mentioned, vehicle and person access control in the nursery, and especially within the production greenhouses, is essential to practice safety and phytosanitary guarantee the production of budwoods, rootstock and citrus nursery tree.

Figure 3.4 Greenhouse production of citrus nursery tree containing side short wall and rainwater conduction system (a) and windbreak of Casuarina (*Casuarina equisetifolia*) (b). Photos: (a) Simone R. Silva and (b) Horst Bremer Neto.

Figure 3.5 Budding of citrus grown in bags of 7 liters on countertop. Photo: Horst Bremer Neto.

Thus, it is recommended that the vehicle traffic is restricted. Vehicles should not have access to production greenhouses. Also, it is recommended that a wheel disinfection facility is installed for the disinfestation of vehicles that will need to move in the nursery. Unauthorized persons shall not have access to nursery. Additionally, it is recommended that the nurseryman provides uniforms to employees who perform activities within the greenhouse, ensuring at least two shifts per week. The uniforms should be washed in the very facilities of nursery or specialized companies, under the responsibility of nursery gardener. Discarded substrate, seedlings and debris cannot be stored inside the greenhouse and must be deposited in an independent environment to incineration or sent to sanitary landfill.

3.2.2 Mother Nursery

The structure used for phytosanitary protection of stock plants of cultivars used as scions should be composed of structure resistant to strong winds and the greenhouse should be aphid-proof, providing protection against vectors of CVC, HLB and Citrus Sudden Death (CSD). The structure can be protected by screen or by screen association of plastic coating, provided that the latter reduces the leaves wetting and risk of occurrence of citrus canker and of black spot.

The height of the greenhouse should be more than 4 meters, to facilitate air circulation and avoid problems resulting from high temperatures. It is important to note that only the physical protection of the stock plant is not enough to keep the sanity of it. Other measures should be adopted, such as the strict control of the transit of persons, disinfestations in footwear, equipment, tools and clothing, use of traps, inspections and preventive sprays to control insect vectors and fungal diseases. At the federal level, according to IN 48, basic and stock plants should be kept in a protected environment, which should contain antiophidic screen, antechamber and device for hand washing.

The stock plants used to obtain seeds, aiming at the production of rootstocks, can be grown in the open air under appropriate control disease, since diseases such as CVC, HLB, Citrus Sudden Death (CSD), *tristeza*[1] and decline are not transmitted by seeds, even when the stock plant is contaminated. The stock plants, however, that are suspected or proven carriers of these diseases should not be used for this purpose.

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[1] [**tristeza**: *Citrus tristeza virus (CTV) is a viral species of the Closterovirus genus that causes the most economically damaging disease to its namesake plant genus, Citrus. The disease has led to the death of millions of Citrus trees all over the world and has rendered other millions useless for production*].

4. Stages for production of citrus nursery

Commercial production citrus nursery tree consists of two distinct parts of citrus plants. The rootstock, or stock, comprises the root system and lower stem or trunk. It usually grown from seed. The upper part of the tree, consisting of the limbs, leaves and fruit, is known as the scion. The scion is derived by inserting tissue, it is a bud. (Bud of the desired cultivar into the rootstock in such a way that it unites with the rootstock and develops the fruiting). Rootstock is the root system of the budded plant. The development of buds and rootstock will result in the formation of the new plant scion and root system, respectively. By this method, is possible to achieve the combination scion/rootstock that combines desirable characteristics such as plant adaptation to various soil conditions, climate, disease and production systems, enabling high fruit production with the desired quality. Moreover, obtaining rootstocks by seed and grafting and bud grafting enables the formation of uniform plants, with reduced production and early bearing. In Annex 2, it is presented a flowchart showing the main steps and processes involved in the production of citrus nursery tree

4.1 Rootstocks production

The production of citrus nursery tree is obtained fruit removed from fruit produced in registered stock plants. The harvest of fruit for the removal of the seed shall be carried out in full ripening of fruits. It must be avoided the fruit from the ground and those located in the canopy of the base, close to the ground, avoiding possible contamination by *Phytophthora spp*. and other pathogens that compromise the storage of seeds and development of rootstocks.

The extraction of seeds from fruits may be performed manually or mechanically. The mechanical method has higher yields and can be conducted with corn thresher or sugarcane chopper, containing toothed blades instead of knives. After extraction, the seeds must undergo chemical treatment to the removal of mucilage (slime substance adhered to the outer integument) and of the outer integument, popularly called "shell"(Figure 4.1a). The mucilage should be performed right after the seed extraction, immersing the seeds solution containing hydrated lime (0.5%) for approximately 10 minutes. Then, the seeds are washed in water and subjected to heat treatment for 10 minutes in water at 52°C (Figure 4.1b), 15 ml of caustic soda (NaOH) and 3 ml of hydrochloric acid per liter of seeds. The solution containing the seeds must be moved every 15 minutes for 45 minutes. Later, the seeds should be washed under running water (Figure 4.1d) and subjected to further treatment, in solution containing 100 g of hydrated lime, aiming to remove chemical residues. Again, washing is made in running water, keeping the seeds in a container containing water for manual removal of the integument (rubbing the seeds between the hands) (Figure 4.1e).

Another procedure may also be adopted aiming at the removal of the outer integument after removal of the mucilage and heat treatment such as immersion of 3 kg of seeds in 6 L of water at a temperature between 35-37°C and pH between 11.5 and 12[2]. After the pH control, 3L of sodium hypochlorite is added[3](NaClO,10%). The solution containing the seeds must be stirred, by hand or mechanically, every 15-30 minutes, verifying the time that the integument begins to detach from the seed. When the integument starts to detach, treatment should be stopped and the seeds washed immediately. After washing, rub the seeds between your hands until the complete detachment of the integument. The separation of the loose integument seed can be carried out after partial drying of the material by means of blowers. It is worth to note that the treatment time varies among cultivars and maturation stages of seeds. Seeds of more acidic fruit, typically require more time. Younger seeds require less time, but are more susceptible to chemical and mechanical damage during processing.

It is not recommended seed storage after removal of the seed coat, because its removal may promote germination. Thus, the integument must be removed from seeds that will be promptly used or stored for up to 10 days. If stored without external integument, the seeds should be kept in plastic bags under 4°C. Seeds with integument can be stored in cold chamber (Figure 4.1f), for a period exceeding six months under temperature and relative humidity maintained between 6 and 8ºC and 60 and 80%, respectively.

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[2]The pH of the solution is adjusted with caustic soda (NaOH).
[3]The pH adjustment is necessary because sodium hypochlorite (NaClO) in solution with pH values equal to or lower than 7 can kill the embryos.

The loss of germination power during the storage period is variable depending on the type of the rootstock.

According to IN 48, the minimum standards for trading citrus seeds with genetic origin evidence are: 98% purity, 50% germination, absence of inert materials and maximum presence of other seeds of 0.05% and 0 07% when obtained from stock plants and clonal garden, respectively. When derived from plant base, the presence of other seeds should be zero.

Figure 4.1 Citrus seed with outer integument (left), with partially removed outer integument (center) and without outer integument (right) (a); seed under thermal treatment (b); application of a caustic soda in the solution (c); seeds washing (d); removal of the outer integument (e); citrus seeds stored in cold chamber (f). Photos: Horst Bremer Neto.

The sowing is done in substratum packed in small plastic tubes. The plastic tube volume should present between 50 and 70 ml and contain longitudinal grooves to prevent entrapment of the root system (Figure 4.2a). The plastic tubes should be placed in wire mesh divided into individual spaces for each plastic tube or trays in continuous and suspended countertop in the approximate height of 1.5 m to prevent contact of the root system with the greenhouse floor (Figures 4.2b; c).

The number of seeds per tube is set depending on the quality of seeds and rootstock, ranging from one to four seeds. Species presenting greater polyembryony rate (when more than one seedling germinates from the same seed) (Figure 4.3a) require fewer seeds, given the greater likelihood of nucellars embryos[4].In this case, it uses one or two seeds in each. On the other hand, the 'Rangpur' (*Citrus limonia Osbeck*), due to lower polyembryony rate, requires more seeds, three or four. 'Swingle' [*Citrus. paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] requires two or three seeds per plastic tube. More seeds should be adopted when the batch of those have low quality (smaller seeds with low germination rate). Seeds should be arranged in the horizontal position or with the apex facing down (Figure 4.3b), otherwise it increases the percentage of seedlings with defective root system.

Figure 4.2 Plastic tube for citrus rootstocks production containing lateral grooves (a) and suspended countertops containing trays for supporting plastic tubes (b; c). Photos: Horst Bremer Neto.

[4]The embryos originate nucellar plants of interest for the production of rootstocks, as they are genetically identical to the mother plant (clone), showing surely typical and desirable characteristics of the species. Plants from these embryos are called popularly "female". In the nucellar embryo, the seed may contain zygotic embryos originating from plants with unknown characteristics. Since they are not identical to the mother plant, it should be discarded.

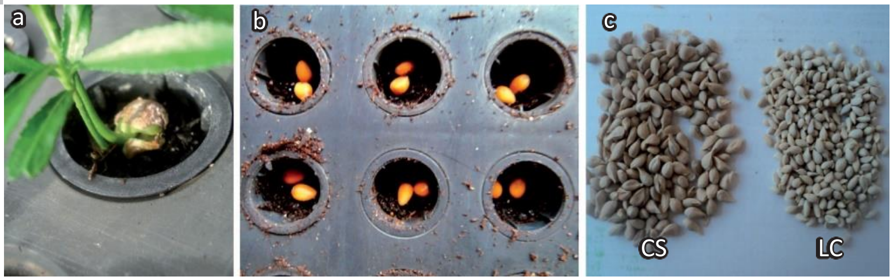

Figure 4.3 Emergence of two seed seedling of polyembryony species (a) provision of seeds in the cartridge (b) and seeds of Swingle (CS) and Rangpur (LC) (c). Photos: Horst Bremer Neto.

The depth of sowing is variable depending on the rootstock and the size of the seed. 'Swingle' citrumelo seeds presenting larger size in relation to the other rootstocks (Figure 4.3c) can be seeded up to 2.5 cm in depth, while for the other rootstock cultivars, sowing depth should not exceed 1.0 cm. The substrate should provide retention capacity and adequate water drainage, allowing the plant to have its water requirement provided without oxygenation of the root system is compromised and still provide physical conditions conducive to the growth of the root system. Moreover, according to law federal (IN 48) and São Paulo State (Ordinance CDA in 5) laws, the substrate may not contain soil and should be free of nematodes and pathogens, mainly *Phythophtora spp*., causal agent of gummosis, not contain seeds or propagules of pest plant. In general, commercial substrates are used consisting of Pine bark, coconut fiber, vermiculite or organic material, usually charcoal (Figure 4.4a). The handling of the substrate must be carried out in clean place, without contact with the ground to prevent contamination (Figure 4.4b).

Citrus Seeds emergence occurs between 15 and 45 days after sowing, due to the environmental and species conditions. Up to 15 days after sowing, the technician in charge for the nursery needs to deliver to CDA the production plan and the plant report of first inspection (Anexx 1). About three or four months after sowing, it shall be selected the most vigorous plants with typical characteristics of the species, discarding non true-to-type and less vigorous plants. In Figure 4.5 it is presented the examples of hybrid plants of Rangpur (Figures 4.5d, f) and 'Swingle' (Figure 4.5a) and plants with typical features of these species (Figures 4.5b, e). In Figure 4.5c, it is possible to note 'Swingle' with greater and lower effective. In this case, the plant with less force must be discarded.

Figure4.4 Substrates used in commercial production of rootstocks and citrus nursery tree compose of Pine bark (left) and coconut fiber (right)(a) and handling of the substrate to fill the tubes in countertop(b). Photos: Horst Bremer Neto.

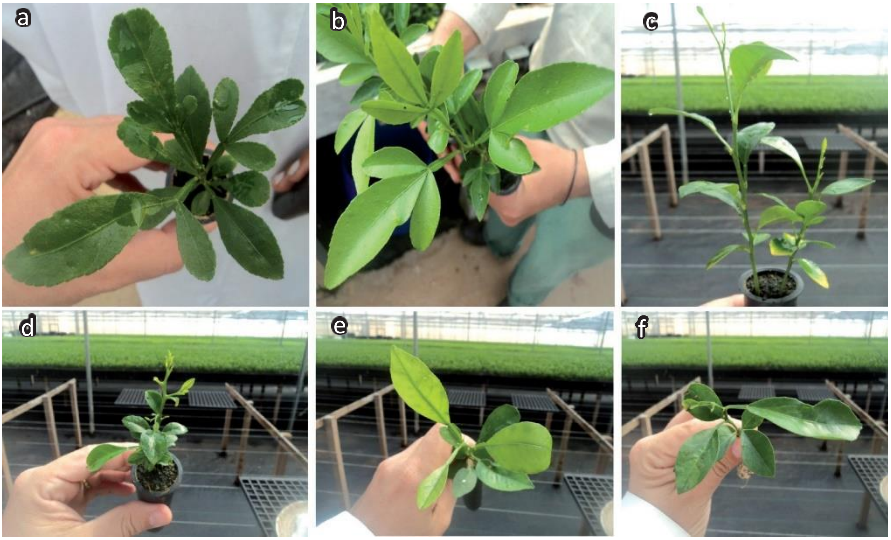

Figure 4.5 Examples of hybrid plants of 'Swingle' citrumelo (a) and Rangpur (d; f) presenting atypical leaves and growth; nucellar plants of 'Swingle' (b) and Rangpur featuring typical characteristics of the species (e); containing nucellar plant having greater force (left) and hybrid plant less vigorous (right) (c). Photos: Horst Bremer Neto.

When the plants selected present height between 15 and 30 cm, the root system sufficiently developed to maintain the clod fixed after its removal from the plastic tube and mature stem, the transplanting of rootstocks for larger containers is performed (Figure 4.6c), where they will remain until the end of the production cycle. It is recommended that the substrate is irrigated to facilitate the removal of the plastic tube plant with intact clod.

Transplanting to the final bag can be carried out with intact clod or removing a part or all of the substrate (Figure 4.6b). The removal of the substrate allows visualization of the roots and disposal of defective plants root system (Figure 4.6a).

Due rootstocks disposal due to malformation, occurrence of zygotic embryos, among others, should overestimate the amount of the rootstocks to be produced in two to three times the amount which is desired. Overall, discards are more than 30%, still higher in lime rootstocks of 'Rangpur', which reaches up to 60% of disposal. The impact of rootstocks disposal on production costs is high and nursery gardener should establish strategies to take advantage of at least 70% of tubes. The selection and sowing of more seeds per tube are valid strategies and maximize rootstocks ratio obtained by plastic tube used. In the selection of seeds, nursery gardener must remove the seeds were empty, defective, broken and blackened by attacking any pathogen. When purchased from other nurseries, the rootstocks used in the production of seedlings should be sourced from registered nurseries in CDA.

Figure4.6 Rootstocks of Swingle presenting defective root system (tortuosity in plant cervical region indicated by the red arrow) (left) and a normal root system (right) (a); Rootstocks of Rangpur ready for transplanting (b); rootstocks after transplanting in bags (c). Photos: (a) and (c) Horst Bremer Neto, (b) Simone R. Silva.

4.2 Production, harvest, processing and storage of budwoods

The budwoods used in the production of citrus nursery tree are obtained from plants grown and formed solely for this purpose (budwoods source tree). Bud grafting or Budding is a particular type of grafting with the scion consisting of a single bud attached to a piece of bark and sometimes a thin sliver of wood underneath. Budding is the method of choice for propagating young citrus trees because it works well for citrus and requires less skill than other types of grafting. The budwoods should be formed necessarily from material subject to traceability (origin proven by invoice), from budstocks plants recorded and maintained in a protected environment, in addition to having production plan and being subject to plant health inspections, as submitted in items 2 and 3.2.

The Rangpur lime is the most used rootstock in the formation of budwoods tree since it induces high force at the scions allowing large numbers of shoots flows, and consequently higher yields at harvest of bud grafting. The bud grafting can be conducted with a (single stem) (Figure 4.7), two (double stem) or more branches (in stride). The conduction system will influence the spacing and producing bud grafting during the first cut. Plants conducted in a single stem enable the spacing between pots are lower, increasing the number of plants in bud grafting and bud grafting amount of the first cuts. Furthermore, plants conducted in a single stem have greater strength, increasing the number of grafting bud per stem, which is, on average, of 12 bud grafting plant by cutting.

The adequate maturity stage for bud harvest is indicated by the degree of shoot development and maturation.

Figure 4.7 Bud grafting with four years of age, conducted in single stem (a) and cultivated in the arranged 3L bags on countertops (b). Photos: Nardélio Teixeira dos Santos.

which must present dark green color, edges, sufficient rigidity (should not break when folded) and without budburst (Figure 4.8). After harvesting, in order to prevent dehydration of the branches and to facilitate the removal of budwoods, it is carried out the removal of the leaves in clean and shaded location. In general, only the grafting in the central part of the branch is used. When bud grafting is not used immediately, the branches may be stored in plastic bags in a refrigerated environment (5 - 10ºC) for up to 2 months. After this period, the branches should be discarded. It shall only be removed from the cooling budwoods to be grafted on the same day, since the bud grafting exposure to the environment can lead to dehydration and reduction of viability thereof.

4.3 Formation of citrus nursery tree

Containers used for the formation of citrus nursery tree after transplanting of rootstocks, can be made of hard plastic or plastic bags, with minimum size of 13 cm in diameter and 30 cm high. Commercially, the most widely used containers have size of 20 cm diameter and 35 cm high, with a volume of approximately 4.5 L. The nurseries must comply with the dimensions of the container, due to their influence on the distribution of the bags in the countertop, availability of light, irrigation and aeration. Thus, there is preference for higher containers, which allow better root development in depth, without the number of bags per m² is reduced. Rigid containers have the advantage of possessing side grooves, avoiding folding of the root system. Rigid containers, however, offer a higher risk of contamination by pathogens and higher costs related to the acquisition and disinfestation every new production plan.

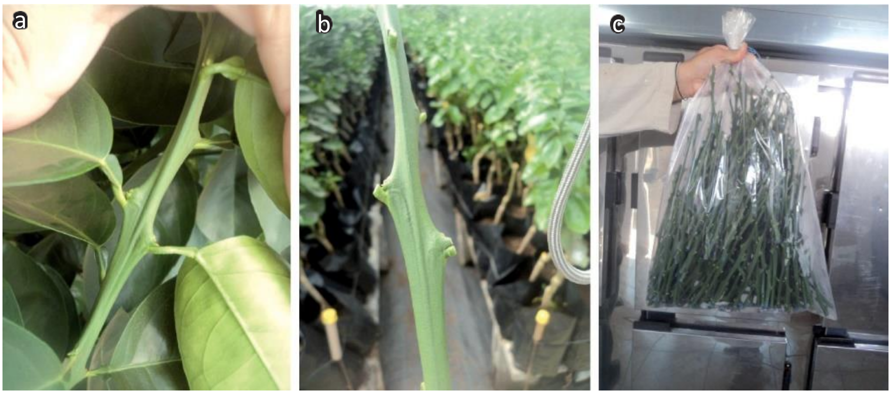

Figure 4.8 Adequate shoot development stage for bud harvest. Photos: Horst Bremer Neto.

Plastic bags do not have the grooves, but are disposable, without return, washing, disinfection, have a lower cost and enable greater agility of the planting operation. Some producers use their own bag as a protective barrier against ants and stem shading, reducing the need for bud bursting. According to the federal legislation (IN 48) and the State of São Paulo (CDA Ordinance N°. 5), the substrate for the production of changes cannot contain land from any source. The substrate used in this production step may be the same as used in the formation of rootstocks, however, with larger particle size.

The budding should be performed when the stem of the rootstocks present favorable conditions for the separation of wood bark and diameter between 0.5 and 0.8 mm (Figure 4.9). This condition is achieved from 3 to 5 months after transplanting rootstock to the bag. Grafting height depends on the scion used, adopting minimum height of 20 cm for 'Citrus limon (L.) Burm f.) and acid 'Tahiti' lime (Citrus latifolia Tanaka) and 12-15 cm for the other scion cultivars. According to IN 48, the budding should be conducted between 10 and 20 cm, measured from base plant, except when the nursery tree is from lemons or when crops intended for the production will be harvested mechanically. In these cases, the budding of time must be taken between 20 and 40 cm. In the days prior to budding, irrigation favors the detachment of the bark of the rootstock. Some days before, the budding must be performed the "cleaning", which consists of removing spikes and lateral shoots from the rootstock top region up to 10 cm above the region budding. Grafting may be accomplished by different methods, bud bursting and "T" normal or inverted "T" frequently used (Figure 4.9b). After grafting, the budwoods is tied with plastic tape (Figure 4.9c), common or biodegradable, so there is fixation and reduction of graft water loss. When used common tape, it should be taken 12 to 16 days after grafting, when fixing or healing is checked between the rootstock and the bud (Figure 4.9d). The maintenance of the common narrow ribbon after setting the bud grafting may harm the development of seedlings. Bud fixed has a typical green color, while the dead bud grafting has brown color. The bud bursting and growth of bud grafting are favored by breaking the apical dominance of the rootstock, which can be performed through different techniques, among them the decapitation (5cm above the grafting point) and the rootstock stem bending near to bud grafting (Figure 4.9c). The execution of the bending is more time consuming, difficult management of the plants and slows the onset of bud bursting, but it induces greater vigor to bud bursting, favoring the development of the stem in one vegetation flow with shorter change training, and technical most commonly used in commercial citrus nurseries.

The bud bursting should be conducted in a single stem with the help of tutors, until complete maturation of citrus nursery tree, which occurs 3-5 months after grafting (Figure 4.9d). The bud bursting staking should be performed when reaching 20 cm in length with fiber rod or metal (galvanized), thin and rigid to avoid damage to the root system when inserted into the substrate. The stem of the rootstock should be eliminated when the bud bursting the scion is with height between 20 and 30 cm, or when the first flush has ceased growth (approximately 50 days after budding), (Figure 4.9e). The final growth of citrus nursery tree, technical manager must collect and send samples of leaves, roots and substrate to the laboratory accredited by the CDA to carry out the tests of CVC, HLB, nematodes and *Phytophthora*.

When the citrus nursery tree is ready, the scion stem decapitation should be held in a minimum height of 35 cm to 45 cm for tangerines and other citrus cultivars, as from the plant's lap.

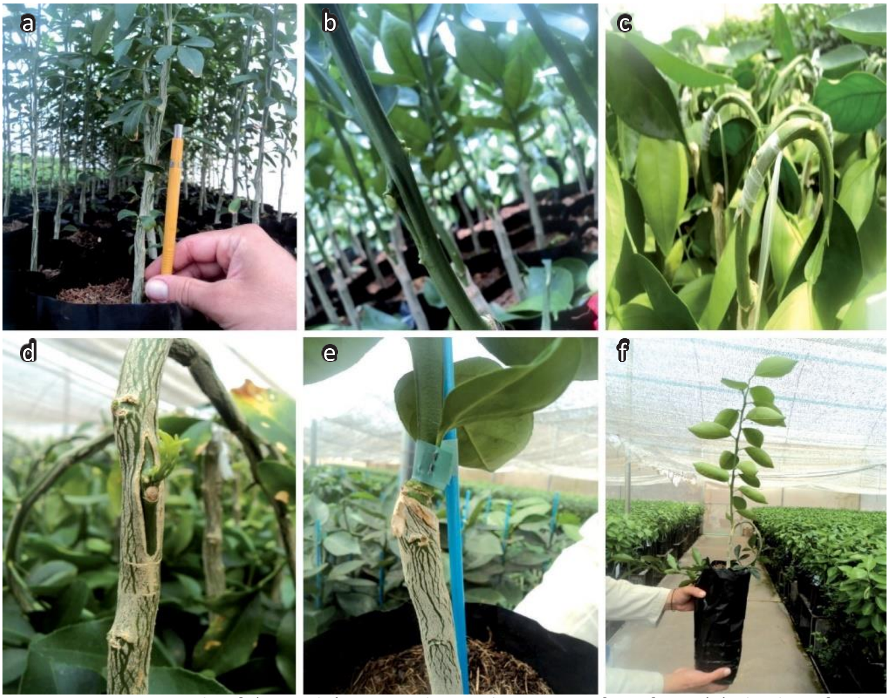

Figure 4.9 Rootstock of 'Swingle' citrumelo at the point of grafting (a); bud grafted in inverted "T" shape (b); bending and plastic tape after grafting (c); bud bursting beginning (d); staking of bud burst with plastic stem(e); in budburst stage of weaning (f). Photographs: Horst Bremer Neto.

It is desirable that the citrus nursery tree reaches the minimum of pruning height only when a growth flow, since the greater the number of flows, the greater the time of formation. The location of the pruning must be brushed with paint based on latex, to avoid possible contamination by pathogens and prevent water loss. At this stage, the citrus nursery tree is called "stick" and is ready for commercialization and planting. Some growers have adopted the planting with primary branches (with three or four budburst derived from the main stem), which requires larger containers with a volume of 6 liters and increased training time, ranging from 6 to 8 months. According to the laws of the State of São Paulo, citrus nursery formed with single stem (stick) and those formed with primary branches can remain in the nursery for at most 15 and 24 months, respectively, from the rootstock transplant. For other states, regulated exclusively by IN 48, single stem seedlings can remain in the nursery up to 24 months in the case of intergrafting plants or coming from the rootstock *Poncirus trifoliata (Poncirus trifoliata* (L.) Raf.) and its hybrids. In other cases, single stem seedlings and primary branches can remain in the nursery until 18 and 24 months, respectively. At this stage of the production cycle, discards can exceed 25%. However, careful selection of rootstock, grafting quality, nutrition, irrigation and in other cultural practices can reduce the rate of rejection for 5-10%, values considered ideal. It is recommended that after the shipment of citrus nursery tree, the countertops are cleaned and disinfected before another batch of young citrus tree is introduced.

In the nursery can also be produced tree interstocks or with two rootstocks. The nursery tree interstocks are produced in order to prevent incompatibility between the rootstock and the scion by inserting farming compatible with both scion and rootstock. Thus, for example, it is possible to form orange plants “*Pera*” (*Citrus sinensis Osbeck*) or “Murcott” tangor (*Citrus sinensis Osbeck x Citrus reticulata Blanco*) grafted on “Swingle” with the help of an orange intergrafting “Hamlin” (*Citrus sinensis Osbeck*) or “Valencia” oranges (*Citrus sinensis Osbeck*) with 5 to 10 cm in length and inserted 10 cm high of rootstock (Figure 4.10). On the other hand, the seedlings with double rootstock are those that are used more than one rootstock, to preserve the plant in case of a pathogen affecting the main rootstock. By this technique, an additional rootstock, another citrus species, is subgrafted laterally scion. This technique is often used when found the occurrence of CSD in orchards that used the Rangpur rootstock as in the North of the State of São Paulo.

According to IN 48 at the time of marketing, the citrus nursery tree with single stem and scion formed must have a minimum diameter of 0.5 cm and 0.7 cm, respectively, measured 5 cm above the grafting point. In the case of scion formed seedlings, the main stem must have been pruned between 30 and 60 cm, measured from the grafting point. Seedlings should still have mature tissue, upright branches, without physical damage, cut of rootstock scarred and difference in diameter between graft and rootstock less than or equal to 0.5 cm, measured 5 cm above and below the grafting point, constituting a single stem and upright (max. 15º) (not applicable to citrus nursery tree unterstocks). The root system should be well distributed with radicle occupying all or almost all the volume of the substrate being admitted maximum of 5% of defective seedlings (coiled above the container bottom, broken or length less than 20 cm). The methodology for sampling seedlings to root system evaluation purposes can be found in IN 48.

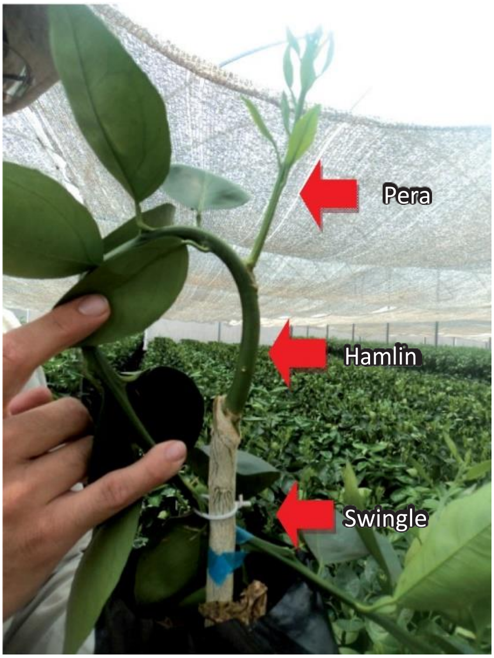

Figure 4.10 Citrus nursery tree interstocks composed of ‘Swingle’ citrumelo and 'Hamlin' orange grafting 'Pera' sweet orange. Photo: Horst Bremer Neto.

5. Irrigation

Irrigation management in citrus nursery production systems in protected environment is essential to maximize plant growth. Through proper management, it can influence the growth rate, the relation root: aerial part, discard rates of seedlings and plant performance in the post-planting phase. Moreover, the base of commercial substrates used consists of Pine bark or coconut fiber, which can undergo drying in the absence of suitable irrigation affecting the growth and seedlings quality, in some cases, an irreversible way.

Irrigation can be performed with aspersion systems (Figures 5.1a, c and d) or localized (Figure 5.1b). Aspersion system can be manual or automated. In the manual irrigation aspersion irrigation systems is performed by means "showers", or other devices (Figures 5.1c; d).

Figure 5.1 Irrigation system for automated sprinkler used in rootstock prodution (a); located irrigation system (b); irrigation systems by manual sprinkler (c; d). Photos: (a) Nardélio Teixeira dos Santos, (b) Vivecitrus, (c) Horst Bremer Neto, (d) Simone R. Silva.

Aspersion irrigation systems require greater amount of manpower and water in relation to localized irrigation systems, and promote wetting the leaves, favoring the occurrence of pathogens infections like fungus *Guignardia citricarpa*, causal agent of black spot and of *Xanthomonas citri subsp. citri* bacteria, causal agent of citrus canker. The localized irrigation is performed by means of microtubes, also called "spaghetti tube", bag by bag, directly on the substrate without aerial parts of wetting, disfavoring the occurrence of black spot, and canker of citrus, which makes this irrigation system the most recommended under the phytosanitary aspect. Due to lower waste of water, applied water volume is lower compared to irrigation by aspersion. Moreover, the need for hand labor is reduced, since the system operation can be completely automated. On the other hand, localized irrigation requires higher initial investment and greater care in the management of irrigation and fertigation.

When other factors are not limiting, the maximum growth of citrus nursery tree and rootstocks occurs when moisture from the substrate is maintained in the container capacity (maximum water content retained in the substrate). However, due to the need to obtain uniform distribution of water and nutrients throughout the volume of the substrate as well as the need to remove the residual salts resulting from the application of fertilizer, it is common practice to apply additional amount of water, called leaching fraction. Generally, the commercially applied leaching fraction is 25%. However, this value is variable depending on the water quality and the tolerance of plants to the salts present in water and fertilizers.

The weighing of the containers allows the monitoring of water consumption more often, and to establish a minimum weight, irrigation is carried when this weight is reached. By this method, it must know the weight of the bag when the substrate is at its maximum water retention capacity (container capacity) and the weight of the bag before the irrigation. Based on these values, it calculates the volume of water to be applied by means of the following equation:

$$VI = (Cc - Ps)/ (1- Fl)$$

where VI is the water volume if applied per bag (ml); Cc, is the weight of the bag in the container capacity (g);

Ps is the weight of the bag before irrigation (g); and Fl is the leaching fraction, decimal (SOARES, 2003). We point out that other methods of determining substrate moisture may be used, such as tensiometers that allow indirect determination of moisture from the substrate by means of instruments called tensiometers.

The irrigation interval (irrigation frequency) is variable depending on the seedling growth stage, local weather conditions and the type of substrate. In general, when the substrate is based on Pine bark, irrigates up to once a week in the winter months, while in summer, irrigates up to three times a week. When used comprising coconut fiber substrate, which has a higher water retention capacity, irrigates once every 10 days in winter and once or twice a week in summer.

6. Fertilization

Fertilization is fundamental practice for the quality and speed of formation of citrus nursery tree, since the substrates used are inert and do not contain the nutrients necessary for the proper development of the plants. In general, the most required nutrients in the formation of citrus plants nursery in descending order are: nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and phosphorus (P). Although these elements are found in lower concentrations, boron (B), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and molybdenum (Mo) have important roles in plant growth and must be included in the nutrition program of citrus nursery trees.

In Table 1 are presented the optimal foliar concentrations of N, P, K, Ca, Mg and S for the growth of the main rootstocks and combinations scion/rootstock used commercially during the winter and summer seasons. These values were obtained in a study published in Technical bulletin of Nutritional Standard of Citrus nursery tree and can be used as reference for assessing the nutritional status of rootstocks and citrus nursery plants at different stages of development.

Table 1. Optimum leaf concentration[1] of macronutrients estimated to rootstocks and citrus nursery tree in the end of the first bud flush and before final pruning, in two growing seasons (winter/summer).

Growth Season	Rootstock/Nursery tree	N	P	K	Ca	Mg	S
		$g\ Kg^{-1}$					
Winter	Cleopatra/Rangpur/Sunki	24.6	2.2	15.5	22.2	2.9	2.6
	Swingle	37.2	2.8	16.2	25.9	4.5	3.5
	(1st bud flush)	37.6	2.8	22.2	18.0	2.8	2.6
	(final cutting)	38.3	2.3	24.5	26.5	2.3	3.1
Summer	Cleopatra/Rangpur/Sunki	33.9	2.1	18.3	27.4	3.8	3.4
	Swingle	36.9	2.0	18.8	33.8	4.6	4.5
	(1st bud flush)	36.6	2.4	24.1	22.3	4.1	3.6
	(final cutting)	38.1	2.3	24.5	26.6	2.3	3.1

1 Sample obtained by collecting three leaves, located in the middle third of the rootstock or citrus nursery tree, in 20 plants for production countertop. Source: Bataglia et al. (2008).

Considering that irrigation is performed with high frequency and volume of containers is limited, the leaching of fertilizer applied becomes high, which reduces its availability to the plants. Thus, the gradual supply of nutrients is a recommended strategy. The use of slow release fertilizers or split application of soluble fertilizers in small doses, along with water irrigation technique called fertigation, are the most efficient means for the gradual application of fertilizers. Due to the lower cost, fertigation is the most used technique in commercial citrus nurseries.

In fertigation, the amount of water applied and the frequency of application will vary depending on the age of the nursery tree, the environmental conditions and the type of substrate used. Table 2 sources are presented in N, P, K, Ca, Mg, S and Cu commonly used and dilution suggestion for production of rootstocks and citrus nursery tree on substrate composed of Pine bark. The micronutrients Fe, B, Mn, Zn and Mo should be added to the solution so that there is nutritional deficiency that impairs the growth of nursery tree. These nutrients may be added in solution by means of commercial formulations available on the market, in dilutions recommended by the manufacturer.

The monitoring of electrical conductivity (EC, a measure of the concentration of salts) and pH (measure of acidity of the solution) are fundamental to the rational management of the application of nutrients. For the evaluation of EC and pH, it must be used two substrate parts to one-part water. It is recommended that the EC is maintained between 0.8 to 1.2 dS m^{-1} in summer and between 1.2 - 1.5 dS m^{-1} in winter and pH between 5 and 6 in both seasons.

Table 2. Fertilizers used in fertigation and dilution suggestion for nutrient solutions used in formation of rootstocks and citrus nursery tree.

Fertilizer	**Nutrient**	**Dilution (g 1000^{-1} L)**	
		Rootstock	**Nursery tree**
Calcium nitrate	N and Ca	1000	800
MAP (purified)	N and P	100	250
Potassium nitrate	N and K	100	300
Magnesium sulphate	S and Mg	500	300
Copper Sulfate	S and Cu	30	30

Source: Leandro Fukuda, 2012 (personal information).

In the production of rootstocks, the frequency of irrigation and drainage are higher, keeping the EC in low amounts, around 0.4 to 0.5 dS m^{-1}, without harming plant growth, as fertigation is conducted almost every day.

7. Nursery Pests and Diseases

In Brazil, the transport of seeds, budwoods, citrus nursery trees and rootstocks infected by pathogens has been the primary means of spread of major diseases of culture between states, municipalities and citrus properties. The production of pathogen free citrus nursery trees is the most important measure in preventing the entry and establishment of diseases in citrus orchard. All major diseases of citrus of culture can be spread by citrus nursery tree. Therefore, knowledge involving forms of infection of propagating material occurs and the preventive measures are very important for the spread of pathogens via changes will not occur, especially for areas free of disease.

In the case of pests, spread by citrus nursery trees are less common due to the easy viewing of insects and mites in frequent inspections, combined with preventive and effective control with insecticides even in nurseries. For diseases, however, the spread of pathogens by citrus nursery trees can occur without apparent symptoms. The pathogens may infect tissues of different ways: a) pathogens transmitted by grafting with contaminated budsticks and systemically colonizing the plant by phloem and xylem, as viroids of exocortis, the cachexia or xiloporose, the virus sorosis, tristeza virus and CSD and the bacteria that cause CVC (*Xylella fastidiosa*) and HLB (*Candidatus liberibacter* spp.); b) pathogens that may be present on the substrate used, whose disease can be expressed after the period of production and citrus nursery tree trade, such as *Pratylenchus semipenetrans* nematodes and *Tylenchulus jaehni* and fungi that causes gummosis caused by *Phytophthora* spp.; c) pathogens transmitted by insect vector, the disease of which can be expressed after the period of production and citrus nursery tree trade, such as CSD and bacteria causative agents of CVC and HLB; d) pathogens dispersed by wind and humans, but it is necessary water-free in tissue surface to occur to infection, such as citrus canker bacterium (*Xanthomonas citri* subsp. citri), that can express symptoms still in the nursery, and the fungus *Guignardia citricarpa* that causes black spot, a disease that does not manifest itself in leaves and branches in citrus nurseries.

Therefore, the whole process of production of the citrus nursery trees should be monitored by citrus nursery growers and preventive actions should be taken to ensure that the citrus nursery trees are produced and marketed free from harmful pathogen to citriculture.

7.1 Important citrus diseases spread by citrus nursery plants

7.1.1 Tristeza disease and Citrus Sudden Death (CSD)

The virus that causes the citrus tristeza disease is transmitted by graft propagation using infected budwood, the main propagation method used in the production of citrus nursery tree. As the transmission of the virus occurs by aphids (aphids), it is important to protect source of budwoods as well as citrus nurseries with protective insect proof structure. The main aphids citrus tristeza virus (CTV) transmitters are: the black aphid of *Toxoptera citricida* citrus, in addition to *Aphis gossypii* and *Aphis spiraecola.*

The symptoms of tristeza disease in the field are variable depending on the virus strain present and scion/rootstock combination affected. Severe strains, as opposed to mild strains, of the virus cause severe stem pitting, seedling yellows, or quick decline on sour orange rootstock which result in reduced crops or loss of trees.

In Brazil, the virus and the vector are endemic, thus, the alternative for control of tristeza disease is the use of cultivars tolerant rootstock, such as 'Caipira' sweet orange (*Citrus sinensis L. Osbeck*), the 'Rangpur' lime, the 'Rough' lemon (*Citrus jambhiri Lush*.), 'Sunki' mandarin (*Citrus sunki Hort.* ex. Tan.) and 'Cleopatra' mandarin (*Citrus reshni* Hort. ex. Tan.) and 'Swingle' citrumelo. The cultivar scion should preferably be pre-immunized with mild virus strains.

CSD, as well as *tristeza* disease, is a highly destructive disease, of easy spread, t transmitted by air vector and budsticks, which affects all cultivars of sweet oranges (*Citrus sinensis*), tangerine (*Citrus reticulate* Blanco) and "Ponkan" (*Citrus reticulate* Blanco), 'Tahiti' lime and Persian lime (*Citrus limettioides*), when grafted on 'Rangpur' and Volk (*Citrus volkameriana* Tan. & Pasq.). Due to intolerance of these rootstocks to the presence of the virus CSD, the scion sap flow to the root becomes blocked, which reduces the development of root system and the water absorption capacity and minerals, leading to death of the plants. The leaves of plants with CSD lose their luster and fall. Budburst, when they occur, are less vigorous and with thin narrow leaves. The yellowing which occurs inside the bark of the rootstock below the graft area is a characteristic symptom of the disease, visible by removing the peel or scrape the inner layers.

These symptoms are easily observed in plants from two years of age, especially in early spring, with the occurrence of rainfall. The CSD is rarely diagnosed during citrus nursery tree production phase.

The CSD is present mainly in the south of Minas Gerais Triangle and in the cities of northern and northwestern regions of the state of São Paulo, where there are high temperatures and water deficit. However, the similarities between CSD and tristeza disease, it is believed that the vector is the same, the black aphid. As the causal agent of CSD can also be transmitted by graft propagation using infected budwood, there is an imminent risk of its spread to other producing regions if preventive measures are not taken. In an attempt to minimize the spread of the CSD was prepared Rule 16, which prohibits, for all Brazilian states, trade and transit of plants and propagation material (seeds, budwoods and rootstocks) of citrus produced in nurseries without protective insect proof structure in areas with occurrence of CSD for free municipalities of the disease.

7.1.2 Citrus canker

The citrus canker is a quarantine disease in many countries, caused by the bacterium *Xanthomonas citri* subsp *citri*. The pathogen is spread over long distances by rains associated with winds and infected citrus nursery tree. Historically, the spread of canker citrus is closely related to the transit of propagative plant material contaminated.

The citrus canker causes lesions on leaves, stems and fruits and consequently leaf and fruit fall, affecting the production of infected citrus nursery trees. Symptoms start in the leaves by the appearance of yellow stains, small that gradually increase and become brown staining lesions, protruding on both sides of the leaf. In branches, the lesions are prominent, with straw and brown coloring. In fruits, the lesions are similar to those found in the leaves. With the advancement of the disease, however, the lesions enlarge and protrude, with a yellow halo around it and with brown center. The citrus canker bacterium is not systemic in the citrus plant tissue, being restricted to where the infection occurred. The bacterium penetrates into new tissues by stomata and natural openings or wounds made by thorns and insects.

The infection occurs through natural openings only in young tissues. The leaves and stems are susceptible up to 6 weeks after the beginning of its development. The fruits are susceptible to approximately 90 days after petal fall. When infection occurs after this period, the lesions are very small. The increased incidence of citrus canker in orchards is associated with the presence of leafminer *(Phyllocnistis citrella*). Lesions caused by leafminer facilitate the bacteria enter the plant allowing the installation of the disease.

Thus, the production of citrus nurseries trees covered with waterproof material, as well as the use of localized irrigation, which prevents the leaf wetness, are extremely important to minimize infections by bacteria and possible spread of the disease in citrus areas.

7.1.3 Citrus variegated chlorosis and Huanglongbing

The citrus variegated chlorosis (CVC), caused by the bacterium *Xylella fastidiosa*, was found in orchards in the Northwest region of the State of São Paulo in the 1980s. The unknown of its nature and its modes of transmission either by graft propagation using infected budwood such as several species sharpshooter leafhoppers vectors confirmed only years later, made its incidence increased rapidly in all regions of São Paulo, causing heavy losses in production.

The increase of the disease was due mainly to the use of mined accounting citrus nursery trees. In the 80 and 90, almost all the propagation material was produced in the open and as the causal agent bacterium is transmitted by several species of leafhoppers contamination of citrus nursery tree was inevitable. In open field nurseries, systematic spraying with insecticides do not prevent contamination of citrus nursey tree, because the population of insect vectors is never fully exterminated. CVC is transmitted through seed and grafting. It can also be spread via the movement of infected citrus nursery stock and plant material (budwood, cuttings, rootstock). CVC can also be spread by xylem-feeding vectors, such as the glassy-winged sharpshooter.

The bacterium that causes CVC is transmitted by 12 different species of sharpshooter. The best known are *Bucephalogonia xanthophis* and *Macugonalia leucomelas*, efficient transmission of the pathogen. Contamination occurs when sharpshooter feed on xylem sap of plants contaminated acquiring the bacteria and transmitted to healthy plants. After diagnosing the disease in the field, it is necessary to eliminate the branches of infected plants when they are in early stage or total elimination of diseased plants in the intermediate stage terminal.

The bacteria develop in the xylem clogging vessels and hampering transport of raw sap from the roots to the aerial parts of the plant. Symptoms of the disease are more evident during the dry season. Foliar symptoms first appear at the top and middle of the scion and then spread to the rest of the plant. Mature leaves are variegated leaf chlorosis, which begins by small yellow spots on its upper face, evolving to chlorosis similar to zinc deficiency. The corresponding lower face appear small scores brown color. These scores evolve into intense brown colored lesions, which can be grouped and become necrotic. Young leaves may have small size and tapered and canoe shaped. In older trees, the symptoms are localized, affecting few stems. Fruits of affected branches have their development compromised, remaining reduced size, hard and useless for trade and processing. Trees with severe CVC attacks may have their growth stalled and present death pointers. However, these trees rarely die but remain unproductive.

CVC occurs in all sweet orange cultivars, ‘P*era*’, ‘*Natal*’ ‘Hamlin’, ‘Bahia’, ‘*Baianinha*’, ‘*Valencia*’, ‘*Folha Murcha*’, ‘*Barão*, regardless of the rootstock used. They have not been displayed symptoms in ‘Ponkan’ tangerines,"Mexerica Rio" mandarin, ‘Murcott’ tangor, lemons and ‘Galego’ acid lime, which although asymptomatic, may have the bacteria in their tissues, and therefore sources the bacterial inoculum. As control measures in nurseries, both the stock plants as the citrus nursery tree should be in protective insect proof structure or greenhouses protected with anti-aphid screens.

The huanglongbing (HLB, ex-greening), associated with bacteria *Candidatus Liberibacter spp.*, is considered the most devastating disease of citrus in the world. HLB was first found in Brazil in 2004, in the region of Araraquara, São Paulo. In addition to citrus plants, the bacterium also colonizes the myrtle (*Murraya paniculata*), ornamental plant commonly used as a living fence. The handling of such infected and asymptomatic plants can often serve to increase the speed of dissemination of the disease. The bacterium associated with HLB is transmitted by insect vectors, the psyllids (*Diaphorina citri*). For the prevention of contamination of powdered citrus nursery tree *Candidatus Liberibacter spp*. transmitted by *Diaphorina citri*, nurseries throughout the production process must be protected by anti-aphid screens.

7.1.4 Citrus black spot

Black spot is a quarantine disease caused by the fungus *Guignardia citricarpa*, which produces lesions on citrus fruits. The disease prevents the export of *in natura* fruits and causes major losses in citrus production for the industry, for cause premature fruit drop before complete maturation. In Brazil, the black spot was reported in 1980 affecting commercial orchards in the state of Rio de Janeiro and its spread over long distances is related to asymptomatic infected citrus nursery tree. As in sweet orange leaf the black spot symptoms are not manifested, it becomes impossible the visual diagnosis. The pathogen to infect the citrus nursery tree needs water free of leaves and stems. The production nurseries protected anti-aphid screen does not protect the pathogen enters the nursery, because the fungal spores are much smaller and can pass through the screen. However, the coverage of the nursery with waterproof material and localized irrigation ensure that no wetting occurs in the tissue and infection of the citrus young plants by the fungus.

7.1.5 Citrus Gummosis and *Nematodes* citrus

The gummosis is a disease caused by *Phytophthora nicotianae* and *P. Citrophthora*, and is considered one of the most important diseases of citrus in Brazil. The main mechanism of this pathogen spread is through citrus nursery tree, soil, irrigation water or contaminated substrates. The pathogen can survive for up to a year in soil and substrates and can be introduced in nurseries for these materials. Another means of introduction of the pathogen in nurseries is the irrigation water. The pathogen has biflagellate zoospores that allows locomotion by water until the root system of the young trees in nurseries. Therefore, preventing the entry of *Phytophthora spp*. in nurseries, it should be clean the substrates and treating irrigation water. Container grown should be distant from the ground to prevent contamination.

When the seeds have begun to germinate, the pathogen can infect tissues of the base of hypocotyl with depressed lesions of dark color which increase in size and eventually causing the death of seedlings. This disease in nurseries is known as damping off. The pathogen can also infect and cause stem seeds before germination, compromising stand. The citrus nursery tree produced without apparent symptoms, however, the pathogen can remain on the roots of infected plants, as well as on the substrate that accompany. In order to reduce the incidence of *Phytophthora* spp. In nurseries recommended treatment with fungicides or thermotherapy treatment of the irrigation water (copper sulfate 20 ppm) nitrogen fertilization and to avoid intense.

The nematodes *Pratylenchus, semipenetrans* and *Tylenchulus jaehni* citrus possess a restricted ability of movement. The main mechanism for dissemination over long distances is the transportation of infected citrus nursery tree. The citrus nursery tree shows no apparent symptoms, however, the pathogen can remain on the roots of infected plants, as well as being present in the soil or substrate accompanying the plant nursery. The spread of citrus nursery tree was identified as primarily responsible for the wide spread of these pathogens throughout the State of São Paulo, while still producing citrus nursery tree in the open field, planted directly in the ground.

7.2 Main pests in nurseries

Ahpids

Aphids are sucking insects that can cause direct and important indirect damage. The most common species in citrus are the black aphid (*Toxoptera citricida*), green peach aphid (*Aphis spiraecola*) (Figure 7.1) and the green peach aphid-of-cotton (*Aphis gossypii*). Aphids are insects easily recognizable due to the size and formation of colonies (Parra et al., 2005).

Figure 7.1 Branches infested with black aphid (a) and green peach aphid (b); wrinkled leaves and branch atrophied by aphid attack (c); black aphid (d) and green peach aphid (e). Photos: Heraldo Negri de Oliveira.

The most important direct damages are caused by sucking sap and injection toxic substances by saliva leading to formation of wrinkled leaves and stunted shoots when aphid infestation is high. Indirect damage is related to the transmission of the *tristeza* virus and formation of sooty moldin on the affected organs (darkened cover formed by fungi of the genus *Capnodium sp.,* which feed on the rich substance sucrose excreted by aphids). The presence of sooty mold affects respiration and photosynthesis of the affected plant, reducing its growth.

Citrus leaf miner (*Phyllocnistis citrella*)

The citrus leaf miner is a small moth (4.0 mm wingspan) whose larvae feed on the new leaves, cause injuries in the form of serpentine known as mines or galleries, usually on the underside of leaves (Figure 7.2). These lesions damage the leaf tissue, reducing the photosynthetic area may lead to dryness and premature leaf fall and reduction of the affected budding growth (Parra-Pedrazzoli & Bento, 2008). Additionally, lesions favor the entry of opportunistic microorganisms, as well as the causal agent of bacteria citrus canker (Figure 7.2).

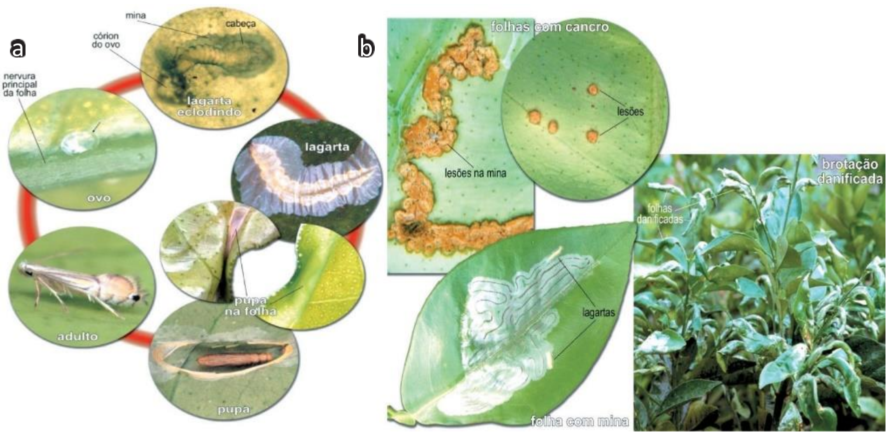

Figure 7.2 Citrus leaf miner: life cycle (a) and leaves damage (b). Photos: Heraldo Negri de Oliveira.

White Mite (*Polyphagotarsonemus latus*)

The white mite can stay in plants belonging to more than 60 families, found in several species of the Citrus genus. The mite has white to pale yellow, according to the development stage. Females are larger than males and measure, as adults, 0.17 mm in length by 0.12 mm width (Oliveira & Pattaro, 2008) (Figure 7.3).

In addition to causing damage to fruit, the toxic action of its saliva causes deformations sheets, requiring its control in nurseries. Budburst attacked originate asymmetrical leaves, lanceolate, with the edges facing down, leading to the formation of necrotic areas (dark) on the underside of leaves (Oliveira & Pattaro, 2008).

Mexican Mite (*Tetranychus mexicanus*)

Mexican mite can be found in other plant species, though citrus plants are its primary host. Its color depends on the feed. To feed on leaves and unripe fruit gets green color with dark spots on the back.

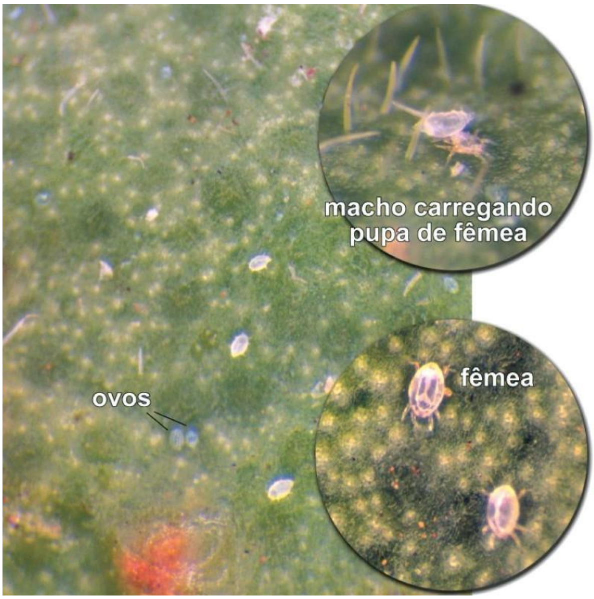

Figure 7.3 White mite: eggs and adults (male and female). Photos: Heraldo Negri de Oliveira.

Females and males reach 0.5 and 0.4 mm in length as adults, respectively. The oviposition occurs in spiderweb, produced in abundance in citrus plants grown in greenhouses (Figure 7.4).

The mexican mite infestation the leaves, cause discoloration, formation of yellow areas and intense defoliation.

Purplish Mite (*Panonychus citri*)

The female has oval body shape, length of 0.5mm and intense red color, being easily observed on the green parts of the plant (Figure 7.5). The male is smaller and tapered body (Oliveira & Pattaro, 2008). The purplish mite affects leaves, branches and fruits. When attacked, the leaves have yellowed numerous scores (mottling) and, in severe infestations, reduce plant vigor and can cause defoliation and drought pointers (Figure 7.5).

Texas Mite (*Eutetranychus banksi*)

The Texan mite has bronze-yellow-bright variable color with greenish spots to dark brown in the lateral region of the back. The female has rounded, flattened shape with 0.5 mm length. Males are smaller and triangular shape. It does not form webs, and the position is held along the ribs or on the rim of the sheets (Figure 7.6).

The Texas mite infests predominantly the upper surface of the leaves. The attacked leaves are discolored scores, loss of gloss, becoming chlorotic and may occur defoliation.

Figure 7.4 Texas mite: Eggs, nymphs and adults and damage to leaves and branches. Photos: Heraldo Negri de Oliveira.

Figure 7.5 Purplish mite: egg, larva and adult and leaves in damage. Photographs: Heraldo Negri de Oliveira.

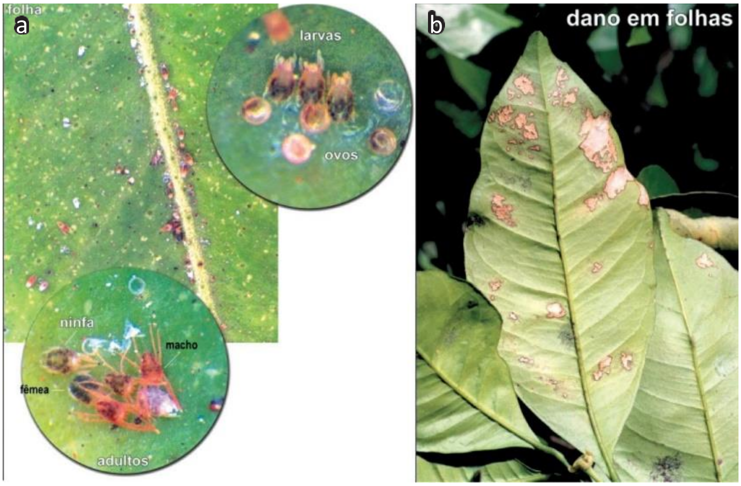

Figure 7.6 Texas mite: Eggs, nymphs, larvae and adults (male and female) and damage on leaves. Photos: Heraldo Negri de Oliveira.

Cochineal

Cochineal insects, like aphids, are sucking insects that cause direct damage by sucking sap and injection of phytotoxins, indirect, by favoring sooty mold formation. Citrus can be attacked by different species of cochineal. In citrus nurseries, the most common are green cochineal (*Coccus viridis*) (Figure 7.7), australian cochineal (*Icerya purchasi*) (Figure 7.8), white cochineal (*Planococcus citri*) (Figure 7.9) and rufous cochineal (*Selenaspidus articulatus*).

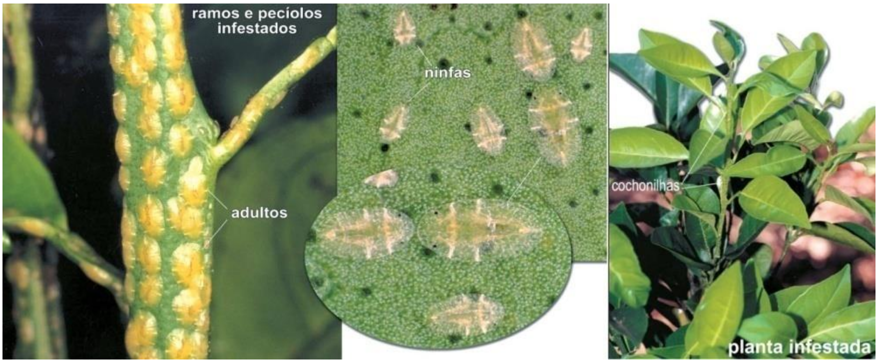

Figure 7.7 Green Cochineal: nymphs, adults, branches and petioles infested. Photographs: Heraldo Negri de Oliveira.

Figure 7.8 Australian cochineal: life cycle and branch showing high infestation.
Photos: Heraldo Negri de Oliveira.

7.3 Control of pests and diseases in nurseries

As shown in previous sections of this manual, control of insect vectors and important diseases spread by citrus seedlings is carried out preventively through adequate infrastructure and other care throughout the entire production process.

However, other pests and diseases may occur in the nursery, bringing losses for the production of rootstocks, budwoods and citrus nursery tree, requiring other preventive or control actions, with emphasis on the application of pesticides.

The application of pesticides can be performed with different equipment, depending on the nursery's production capacity, availability of skilled labor, product type and desired operating income. In large nurseries, sprayers driven by electric motor are the most used (Figure 7.10). Spraying should start by Kiln background, walking toward the front door (Figures 7.11 e 7.12).

The coverage of plants is a key aspect to the success of the control operation. On countertops with plants present height greater than 40cm, the deposition of products is impaired. In this condition, the application must be performed from both sides of the countertop (middle countertop application), requiring the transit applicator in all lanes (Figure 7.11). On countertops with plants present height of less than 40 cm, deposition is favored, allowing the application to be carried out from one side of the countertop (full countertop application) (Figure 7.12).

Figure 7.9 Cochineal white: eggs, nymphs and adults (male and female) and branch showing high infestation. Photos: Heraldo Negri de Oliveira.

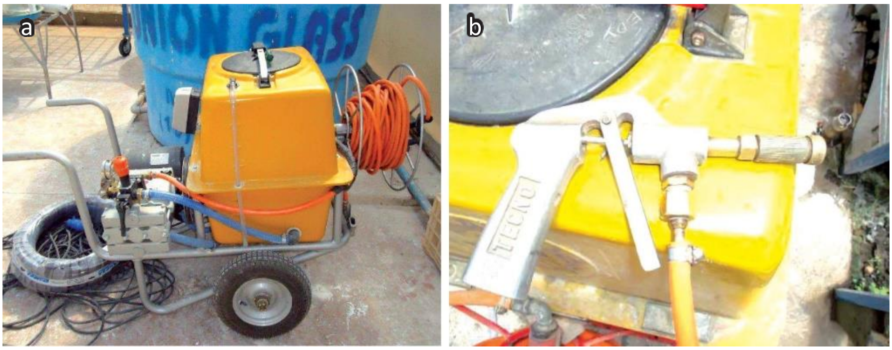

Figure 7.10 Electric sprayer with tank with a volume of 180 liters (a) and spray gun used in (b). Photos: Ricardo Krauss.

In this condition, the applicator will transition on alternate lanes, increasing the efficiency of the application. Another aspect that must be considered is the location of the target, whether internal (old leaves), external (new leaves) or the neck of the plant. In the latter, they are applied systemic products plant to plant using spray costal.

Under certain conditions are needed weekly applications for leaf miner control of citrus and mites. Every other week they can be applied fungicides and bactericides in preventive.

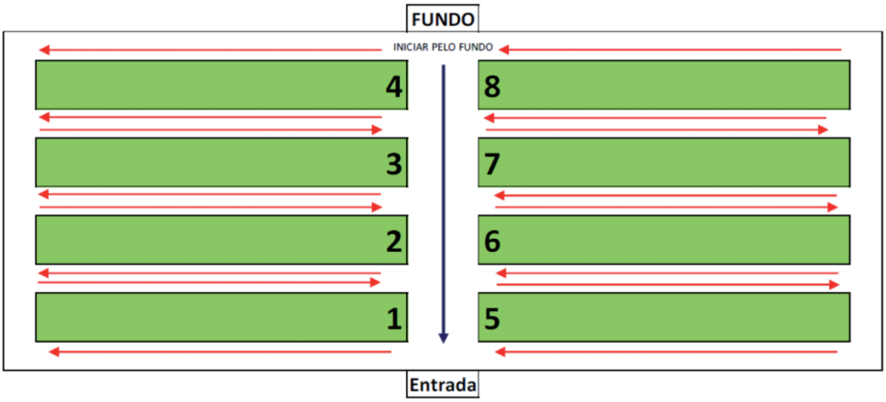

Figure 7.11 Forwarding to spray pesticides in citrus nursery tree taller than 40 cm grown in protected environment. Source: Ricardo Krauss, 2014 (personal information).

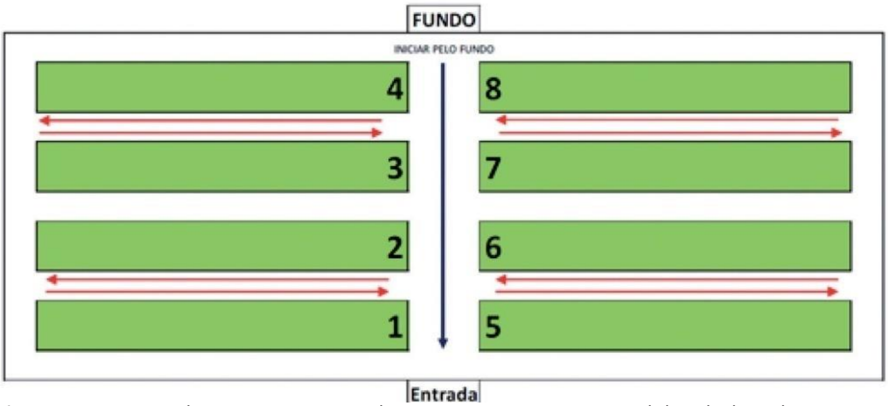

Figure 7.12 Forwarding to spray pesticides in citrus nursery tree with height less than 40 cm grown in protected environment. Source: Ricardo Krauss, 2014 (personal information).

For other pests are detected, such as cochineal and caterpillars, the nursery growers should use products with higher spectrum of action or to apply specific products as well as increasing the frequency of application. It is emphasized that preventive applications depend on the history and risk of particular pests and disease in the nursery. It is recommended to alternating active principles/mechanisms of action to prevent the emergence of resistant populations of pests.

In the event of disruption of coverage (torn plastic), should be carried out spraying insecticides preventatively on the day of realization of the damage on the cover. If the nursery remains open for longer than one day, it is recommended to perform the insecticide application at least every two days. After closing the cover, should proceed another insecticide application in preventive.

8. Weeds

The occurrence of weeds in bags and vases is common even with the use of the substrate. If not controlled, the weeds can cause direct damage such as reduced growth, and indirect, serving as host of pests and diseases and physical barrier to crop protection applications and pinching and grafting operations.

The control must be performed manually in bags and vases since rootstocks, budwoods and citrus nursery trees are very vulnerable to drift, and find themselves under development, whose damage phytotoxicity of herbicides can be severe and irreversible. When new shoots are subject to breakage, manual control should be delayed until the shoots are ripe.

In the space between the citrus plants and under the countertops, can be used contact herbicides applied post-emergence herbicides or applied pre-emergence at the end of fallowing.

9. Scions and rootstocks

The number of scions and rootstock cultivars used commercially in Brazil is reduced, although there are numerous alternatives for both groups. A greater diversification of cultivars is observed among those used as scions. Sweet oranges, tangerines, lemons and acid limes are the most cultivated, especially sweet oranges, which are present in larger area. Historically, the number of rootstocks is further restricted, predominantly only one rootstock mostly cultivated area.

The low diversification of scions/rootstocks combinations can have serious consequences for the health of orchards and the economy of the producing regions, in the case of the occurrence and aggravation of new pests and diseases. Examples of this are the occurrence of CTV and CSD in orchards grafted in Sour orange (*Citrus aurantium L.*) and Rangpur lime, in the 1940s and 2000, respectively, when millions of plants had to be eradicated. Thus, besides the production and use of citrus nursery tree that have appropriate genetic quality and health, diversification of scions and rootstocks is essential practice for the sector's sustainability.

Knowing the characteristics of each cultivar, the citrus nurseries can assist growers in choosing genetic material suitable for the market in fresh or industrial processing, and to promote the installation of orchards with high genetic diversity.

9.1 Rootstocks

The main rootstocks used in Brazilian citrus are ‘Rangpur’ lime, ‘Swingle’ citrumelo, ‘Cleopatra’ and 'Sunki' mandarins, 'Volk' lemon and *Poncirus trifoliata* (Table3). The ‘Rangpur’ lime, this is most widely used citrus rootstock in Brazil, is used in 70% of the area cultivated by inducing the canopy it budded early start of production, high fruit yield, drought tolerance and CTV, fast training in nursery and compatibility with all cultivars crown used commercially in Brazil. From the evidence of their intolerance to CSD, however, other rootstocks began to be used, notably, ‘Swingle’ citrumelo and ‘Cleopatra’ and ‘Sunki’ mandarins. Although these rootstock present disadvantages compared to ‘Rangpur’ lime, especially in relation to drought tolerance, incompatibility with important scion cultivars, early yield and vigor of the plant (higher in tangerines), benefits involving increased quality fruits, health and longevity of the orchard are evident, which made them the most widely used in the formation of new orchards, especially ‘Swingle’, which currently represents 90% of orders in nurseries associated with Vivecitrus. Other citrus species have been studied for use as rootstocks, especially the citranges' Carrizo 'and' Troyer '(*Citrus sinensis* x *Poncirus trifoliata*), citradias (*Citrus aurantium* x *Poncirus trifoliata*) and trifoliate, with focus on Flying Dragon trifoliate (*Poncirus trifoliata var. monstrosa*), which induces a reduction in the size of the plant, allowing greater number of plants per area and greater ease of harvest.

The appropriate planning of the production of seeds rootstocks after to produce citrus nursery tree, the nursery grower must know the horticultural characteristics of each rootstock, such as number of seeds produced per fruit, seed weight and the polyembryony (Table 3). These characteristics are variable, not only due to the rootstock species, but also because of environmental conditions, pollinators, the origin of the pollen and the type of fertilization (selfing or outcrossing).

Table 3. Fruits and seeds of the features recommended rootstocks for citrus cultivation in Brazil.

Rootstock	Fruit ripening season	Number of seeds per fruit	Seeds number/ kg	Polyem bryony (%)
‘Carrizo’ Citrange	March-May	15	-[1]	68
‘Troyer’ Citrange	March-May	15	5,000	67
‘Swingle’ Citrumelo	February	25	6,000	65
Source Orange	May-August	25	6,500	-
‘Caipira’ Orange	May-September	13	6,000	-
‘Rangpur’ Lime	March-August	14	16,000	43
‘Rough’ Lemon	May-July	10	12,000	96
‘Volk’ Lemon	March-July	13	12,000	53
‘Cleopatra’ Mandarin	July-November	14	9,000	-
‘Sunki’ Tangerine	May-July	4	13,000	16,8
Trifoliate	February-May	38	5,000	9,9

Source: Adaptado: Oliveira et al., 2008; [1] Unavailable data.

Furthermore, producers, technicians and citrus nurseries should be aware of the influence of rootstock on the performance of scions involving production, fruit quality, tolerance to pests and diseases and adverse weather conditions as well as the compatibility of scion cultivars and selected rootstocks, as in some cases, the combination may be incompatible. In Table 4, are presented the main characteristics of the cultivated rootstocks and those with the greatest potential for commercial use.

9.2 Scions

There are several of options scion's cultivars within the Citrus gender, among them are sweet oranges, tangerines, lemons, acid limes and grapefruits.

According to report of the Department of Agriculture and Livestock Protection of São Paulo, the most citrus varieties planted in the state in 2011 were sweet oranges 'Valencia' 29.22% *'Pera Rio'* 27.27% 'Hamlin' 12.11 % 'Natal' 11.99% *'Folha Murcha*' 3.26% and *'Valência Americana'* 1.95%, 'Tahiti' acid lime 2.94%, ‘Murcott’ 1.29% and ‘Ponkan’ tangerine 1.06%, and the other cultivars have made up 8.91%. From the reported data, the characteristics of each cultivar scion in fruit maturation function (early, mid-season and late) and the destination of production, *in natura* and/or industrial processing will be highlighted.

The sweet orange 'Hamlin' is the main cultivar early maturation of the State of São Paulo. It produces fruits of oval-shaped, almost spherical, with three to four seeds per fruit. The bark is thin and has deep orange and well defined. The juice has low commercial value, being light in color and flavor less than the cultivars 'Pera', 'Valencia' and 'Natal', usually aimed at specific customers or production mixes with juices from other cultivars. The fruit juice volume has low, below 50%. Displays total soluble solids content around 11°Brix and harvest between April and August. Despite the low quality, this cultivar allows greater industrial processing.

'Westin' orange is a cultivar highly productive and with few seeds. Its fruits are appreciated both for the fresh fruit market and for industrialization and, after completing their maturation, remain for a short period in the plant.

Table 4. Horticultural performance, adaptability to environmental conditions and compatibility of the main commercially rootstocks used.

Rootstocks		Rangpur L.	Volk L.	Rough L.	Poncirus T.	Swingle C.	Troyer C.	Carrizo C.	CleopatraT	Sunki T.	Souce Or.	Caipira Or.
Gommosis		M	M	B	A	A	M	M	B	M	M	A
Tristeza Disease		T	T	T	T	T	T	T	T	T	T	S
Exorthis		S	T	T	S	T	S	S	T	S	T	T
Sorosis		T	T	T	S	T	S	S	S	S	T	T
Xiloporose		S	S	T	T	T	T	T	T	T	T	T
Sudden Death		S	S	-[1]	T	T	-	-	T	T	-	-
Decline		S	S	S	S	T	S	S	T	T	T	T
Nematodes		S	S	S	R	R	R	R	S	S	S	S
Drainage		B	M	M	A	M	B	B	B	B	M	B
Cold Resistance		B	B	B	A	A	A	A	M	M	M	M
Water deficit		A	A	A	M	M	B	B	M	M	A	B
Soil type	Sandy	A	A	A	M	A	B	B	B	B	A	A
	Mixed	A	A	A	M	A	B	A	A	A	A	A
	Loamy	A	A	A	M	A	B	B	B	B	A	A
Roots Distribution		P	P	P	M	P	M	M	M	M	M	M
Quality of Fruits		Regular	Regular	Bad	Great	Good	Goo	Good	Good	Good	Good	Good
Vigor		High	High	High	Small	Average	Aver	Average	Average	Average	High	Averag
Canopy size		Medium	Med	Large	Small	Large	Larg	Large	Large	Large	Large	Large
Production start		Early	Early	Early	Early	Early	Aver	Average	Average	Average	Averag	Averag
Maturation of production		Early	Early	Early	Late	Late	Late	Late	Late	Average	Averag	Averag
Longevity		High	High	Short	High	High	Averag	Average	Average	Average	High	High
Incompatibility		None	Pera	Pera	Pera Murcott Sicciliano L	Pera Murcott Sicciliano L.		Pera Murcott Sicciliano L	None	None	None	None

[1]:non-existent data; Regarding diseases: S=Susceptible; T = tolerant, R = Regular; Performance and Gummosis: M = Medium; B = Low; A = High. Depth Soil: M = Average; R = Shallow; P = Deep. Adjusted: Schâferetal.,2001; Pompeu Junior,2005; Fundecitrus,2006; Oliveira et.al.,2008.

The fruit, however, has intense orange color, 2 to 3 seeds, length of 61 mm, diameter of 64 mm, average of 128 g weight, bark thickness of 4 mm, content of vitamin C, approximately 18 mg 100 mL^{-1}, infrequent navel, 45% juice percentage, total soluble solids of 11.7°Brix and acidity around 1.3%.

The ‘Ruby’ orange is made up of medium-sized trees, with an average yield of 250 kg of fruits with mass around 172 g, 49% juice, intense orange color, total soluble solids (TSS) of 9.9°Brix, 0.86% acidity and ratio 11.5, premature maturation, fruit a very recommended both for the fresh fruit market and for industrial processing.

The 'Valencia' orange produces large fruit, high percentage of juice, spherical to elliptical, with convex base and concave apex, slightly rough surface smooth, with orange pulp, 7-10 seeds, and the average height of the plant is high. It produces fruit with early maturing to mid-season, which are co-lected in a period that overlaps the oranges 'Hamlin' and 'Pera'. The orange 'Pera' is one of the most planted cultivars in the State of São Paulo dental the average maturity of oranges. Present fruits shapes varying from spherical to oblong, average weight 150g, its bark is smooth and thin with orange color, average of four seeds per fruit. The fruits of this variety are the most suitable for industrialization, because they have higher yield and quality of juice, and stay in the trees for a period long time. It has, however, an irregular flowering, an average of three per year. The plants are vigorous, large, erect, productive, little tolerant sadness and moderately resistant to canker citrus. They are incompatible with rootstock of ‘Volk’, *Poncirus trifoliata* and ‘Swingle’.

The most produced late cultivars are currently 'Natal', 'Valencia' and 'Folha Murcha'. A 'Natal' orange fruit in circle shape, with 3 to 4 seeds, average weight 140 g, thin skin, light orange color and orange pulp, SST 12°Brix, acidity 1% to ratio 12. Plants are productive, reaching on average 250 kg per plant.

The orange 'Valencia' is the most widely planted cultivar in the world. In Brazil, it is cultivated in almost all states, producing high quality fruit, with income in excess juice 50%, appropriate relationship between total soluble solids and acidity (ratio), fruit average weight 150-170 g, format approximately spherical and seeds 6 per fruits. The skin is thin and slightly rough surface.

The plants are vigorous, large, upright growth and productive. The fruits may be intended both for consumption in *natura* and for industrial processing. Another cultivar that have been used by growers is the orange 'Folha Murcha' later than oranges 'Natal' and 'Valencia'. The fruits are shape rounded, weighing between 150 to 170 g, has a high juice yield, with average SST of 13ºBrix, acidity 1%. The bark is thin and its orange color, produces 2 to 6 seeds per fruit. Although its fruits are similar to the orange 'Valencia', plants are less vigorous, productive and have rolled leaves. Meets quality requirements for industrial processing and fresh consumption.

The navel orange more widespread in Brazil and in the world is the orange 'Bahia'. It has seedless fruits, average weight of 220 g, spherical, color of pulp and intense orange peel, early maturing median, juice yield around 38%, SST 13.2°Brix, acidity less than 1% and ratio 14. The bark is thick, slightly rough and has great navel. The plants are vigorous, rounded crown with abundant foliage, dark green leaves, big and open growth habit. It produces an average of 150 to 250 kg per plant. Destined preferably in *natura* consumption.

The 'Lima' orange is one of the most cultivated low acidity cultivars in Brazil, with great acceptance in the fresh market. Produces fruit with an average weight of 130 g, with 5 to 6 seeds, early maturing, skin color and orange pulp. The fruits have an average of 45% juice yield, content SST 10°Brix, acidity around 0.12%. The trees have medium-sized too large, upright, productive crown, reaching up to 300 kg of fruit per plant. The others scions’ cultivars planted in São Paulo are 'Cravo' and ‘Ponkan’. tangerine trees present medium size, lance-shaped leaves, produce, on average, 200 to 250 kg of fruit per plant. Its fruits are flattened on the bases, with 20 to 22 seeds per fruit, average weight of 135g and color peel and intense orange pulp. It has 48% of juice yield, SST 10.8°Brix, acidity 0.8% and ratio of 13.5. The fruit can be intended both for industry and for the fresh market.

‘Ponkan’ tangerine fruit has a flattened shape, with 5 to 8 seeds, average weight of 138 g, average shell thickness and protruding oil vesicles.

Skin color and intense orange pulp. It produces 43% of juice, average SST 10.8°Brix, acidity of 0.85% and ratio of 12.7. It presents early maturing median. The fruits are destined for the fresh market. Another cultivar of the group of tangerines is the Tangerine Cravo (*Citrus deliciosa Tenore*) that produces fruit flattened with about 30 seeds, weight of 130 g, thin skin, staining pulp and orange peel. It has 40% of juice yield, SST 10.4°Brix, acidity 0.99% and 10.5 ratio. Its plants are medium sized with small and elongated leaves and can produce up to 200 kg of fruit per plant which are intended for fresh consumption and also to industry.

The 'Murcott' tangor (*Citrus sinensis Osbeck x Citrus reticulata Blanco*) also composes the group of tangerines. They present a medium-sized tree, with lanceolate leaves and spiky. The plants produce on average 200 kg of fruits per plant. The fruits have a flattened shape on average 20 seeds per fruit, weighing 140 g, with smooth and thin adhesive peel and intense orange color. The pulp has a firm texture and orange. They have juice yield of 48%, SST 12.6°Brix, acidity 0.92%, ratio 13.7 and late maturing. Its fruits are allocated preferentially to the market for fresh fruit, but have acceptance in industry.

In the group of acid limes, the most cultivated species are the ‘Galego’ acid lime (*Citrus aurantifolia*) and the ‘Tahiti’ acid lime, both designed predominantly for fresh consumption. The fruits of 'Gallego' acid lime is spherically shaped, with 5 to 6 seeds, average weight 35g, very thin skin of yellow color and light green squash, yield 50% juice, 9.7°Brix acid 6.5% and 1.5 ratio. The trees are of medium size, producing 150-200 kg. The Tahiti acid lime has large-sized trees, large leaves, large, dark green, produces more than 200 kg per plant. Its fruits are oval shaped, have no seeds, fruits with an average weight of 70 g, length 55-70 mm, average diameter of 55 mm and thin shell with coloring green skin and pale green pulp. It has 50% of juice yield, SST 9 Brix, acidity 6% and 1.5 ratio. The fruits can be used for the fresh fruit market and industry. Due to the large size of the plant, the use of dwarfing rootstocks, such as trifoliate 'Flying Dragon', have been used in the production of citrus nursery trees.

10. Production Costs

The cost of production of citrus nursery tree is variable depending on many factors such as location of the nursery, the level of technology applied in production, amortization and maintenance of facilities and equipment, quantity and qualification of manpower and scale of production.

The main costs involving the production of citrus nursery tree in a nursery associated with Vivecitrus, with annual production capacity of 360,000 citrus nursery tree, located in São Paulo, are shown in Table 5. The values used in the composition of the costs were charged in 2014. In the final price, the citrus nursery tree considered the very production of rootstocks and bud grafting, and 10% disposal of the total annual production (36,000 citrus young plants). The fixed cost considered depreciation of facilities (infrastructure) over 10 years, capital opportunity cost invested 8% per year and total production of 3,240,000 citrus young plants over this period (considering disposal of 360,000 citrus nursery tree in 10 years).

Investment in infrastructure is high, but its contribution to the cost of production is relatively low (4.8%), considering that its amortized over 10 years (Table 5). The input items and manpower are the most important in the formation of citrus nursery tree, totaling 78.5% of the production cost (Table 5). Among the inputs, substrate and rootstock are the most important, accounting for 20.7% of the production cost. Bud grafting and fertilizers represent 8.6% of the production cost. Thus, maximizing the profitability of commercial nurseries can be achieved mainly by reducing discharges of rootstocks and citrus nursery tree, as well as the efficient use of the substrate and fertilizers. The costs involving manpower are the highest, amounting to 48% of the total cost of production, since the operations are carried out predominantly manually. Thus, the proper selection of employees and service providers, training and continuous qualification of manpower, mechanisms aimed at retaining talent and the automation of processes are critical to improving the quality and efficiency of manual operations. Table 6 presents reference ranges involving operating income from main operations in the production of citrus nursery tree in greenhouse. In addition to the cost of production, the profitability of the activity is determined by the market value of citrus nursery tree. Thus, when planning the nursery, the producer must define what kind of market it wants to serve, what is the level of demand in this market, and the size of it.

Table 5. Citrus nursery tree production cost located in the State of São Paulo with an annual production capacity of 360,000 citrus nursery tree.

Description	Unit	Amount	Price (R$/unit)	Total (R$)	Cost(R$ citrus nursery tree)	Relative share in the cost of production
Infrastructure						
23 Modules (8,0 x 60 m) +						
Antechambers	m²	11185	50.00	559,250.18	0.168	2.50%
Landscaping	machine Hour	50	150.00	7,500.00	0.002	0.04%
Side screen	m²	2517	3.50	8,808.19	0.003	0.04%
Plastic Cover	m²	11380	4.50	51,210.41	0.015	0.25%
Central corridors (concrete)	m²	41	220.00	8,937.50	0.003	0.04%
Cover ground	m²	10567	2.70	28,531.51	0.009	0.14%
Grills	m²	9754	22.00	214,596.00	0.064	1.06%
Blocks	unit	24386	0.50	12,192.95	0.004	0.06%
Irrigation Equipment (spray - shower”)				81,286.36	0.024	0.40%
Total of Infrastructure				**972,313.11**	**0.292**	**4.82%**
Manpower						
Drivermachine	unit	2	22,171.05	44,342.09	0.123	2.04%
General service	unit	30	21,624.58	648,737.29	1.802	29.80%
Administrative assistant	unit	1	23,747.80	23,747.80	0.066	1.09%
Person in Charge	unit	1	110,565.77	110,565.77	0.307	5.08%
Uniform Cleaning/Washing	unit	1	22,170.69	22,170.69	0.062	1.02%
Night Watcher	unit	1	27,874.46	27,874.46	0.077	1.28%
Gummosis Analysis Laboratory	unit	1	23,747.80	23,747.80	0.066	1.09%
Administration Assistance	unit	1	23,747.80	23,747.80	0.066	1.09%
General Administration		1	124,800.00	124,800.00	0.347	5.73%
Total of Manpower				**1,049,733.71**	**2.916**	**48.22%**
Materials/Inputs						
Nitrile glove	unit	107	4.30	460.10	0.001	0.02%
White boots	unit	72	22.00	1,584.00	0.004	0.07%
Uniform set	unit	36	180.00	6,480.00	0.018	0.30%
Substrate	unit	357660	0.71	253,938.60	0.705	11.66%
Bud grafting (Own Production)	unit	357660	0.30	107,298.00	0.298	4.93%
Rootstock Certificate (Own Production)	unit	357660	0.55	196,713.00	0.546	9.04%
Liquid fertilizer	**L**	17031	2.44	41,556.62	0.115	1.91%
Slow-release fertilizer 220406	Kg	1788	16.20	28,970.46	0.080	1.33%
Fertilizer (Micronutrient)	L	180	58.32	10,497.60	0.029	0.48%
Insecticides / Fungicides / Acaricides				1,447.754	0.004	0.07%
Methidathion	L	12	33.38	400.56	0.001	0.02%
Abamectin	L	6	40.82	244.92	0.001	0.01%
Carbendazin	L	18	16.39	295.02	0.001	0.01%
Copper oxychloride	Kg	26	10.00	259.20	0.001	0.01%
Carbosulfan	L	6	38.28	248.05	0.001	0.01%
Fuel (2 tractors)	L	4000	2.40	9,600.00	0.027	0.44%
Total of Material/Inputs				**659,993.88**	**1.833**	**30.31%**
Other						
Lease	Month	12	3,500.00	42,000.00	0.117	1.93%
Employee Transport	unit	12	9,000.00	108,000.00	0.300	4.96%
Chemical analysis	unit	179	26.51	4,740.78	0.013	0.22%
Equipment maintenance		12	824.08	9,888.96	0.027	0.45%
Maintenance of plastic screen installation (33 Screens at each 2 years)	unit	17	1,200.00	19,800.00	0.055	0.91%
Electricity / Water	Month	12	1,800.00	21,600.00	0.060	0.99%
Association Class / Union	Month	12	567.00	6,804.00	0.019	0.31%
Miscellaneous expenses		12	535.92	6,431.04	0.018	0.30%
Transit Guide		179	42.69	7,634.25	0.021	0.35%
Other (Total)				**226,899.04**	**0.630**	**10.42%**
Commercial						
Vehicles (Depreciation15% p.y.)+insurance		1	35,000.00	7,000.00	0.019	0.32%
Expected fuel consumption3500km/month		350	3.10	17,360.00	0.048	0.80%
Marketing(Folders)		1	5,000.00	7,500.00	0.021	0.34%

Commercial Total		**31,860.00**	**0.089**	**1.46%**
Taxes	**5.00%**		**28.80%**	**4.76%**
Citrus nursery tree COST WITHOUT offtype disposal			**6.048**	**100%**
Citrus nursery tree COST WITH offtype disposal (10%)			**6.720**	

The more demanding, the greater should be the care and investment required in the nursery, which can result in higher production costs, reduced profitability, if there is no economy of scale of production and/or increase of added value of citrus nursery.

Table 6. Operating yield and average frequency of major manual operations to produce citrus nursery tree.

Operation	**Yield (plants or bags person^{-1} day^{-1})**
Bag fulfillment / transport / placement on the countertop	400
Rootstock transplant	1000
Irrigation / fertigation	50.000
Rootstock pinching	1000
Pulverization	150.000
Classification and reassignment of rootstocks	2000
Grafting and removing of rootstock	1.800
Removal of plastic tape	2000
Thinning after grafting	8.000
Standardization	500
Removal and delivery of citrus nursery tree	800

Source: Leandro Fukuda, 2012 (personal information).

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Anexx 1

LEGAL DOCUMENTATION

1. FEDERAL

a. Accreditation of technician in charge in RENASEM (law10.711and decree 5153).

b. Producer enrollment with RENASEM–National Register of Seeds and Seedlings (law 10.711 and decree5153).

c. Description of cultivars of which is the maintainer in National Registry of Cultivars (RNC) (law 10.711 and law 5153).

d. Registration of Plant Providing Propagation Material (Seeds and bud grafting) with validity of 5 years (Normative instruction N. 48).

e. Certification of Nursery (Normative instruction N.48).

f. Inspection Report: Budstocks: at least, in the pre-collecting of budwoods (Normative instruction N.48).

Rootstocks: to 60 days after the plant emergency and in transplanting or pre-trade of rootstock (Normative instruction N.48).

The budding: between 40 to 60 days after grafting and pre-marketing (Normative instruction N.48).

2. STATE

a. Registration of Nursery, duly revalidated every three years by the CDA – Department of Agriculture and Livestock Protection (CDA Ordinance N.5).

b. Seeds and budwoods registration at CDA (Ordinance CDA 5).

c. Technical project of the nursery, including infrastructure systems and equipment for the production, which shall include its very operational capacity or of third-parties (law 5153, article 5, item II).

d. Commitment statement of technician in charge (law 5153, article 5).

e. Technical Project of production (law 5153, article 51)

f. Nursery inspection report (law 5153, article51 and CDA5, article7)

g. Production and trade maps of seeds, budwoods, citrus nursery tree (law 5153, article5 and Ordinance CDA5, article 7)–see item7.1.

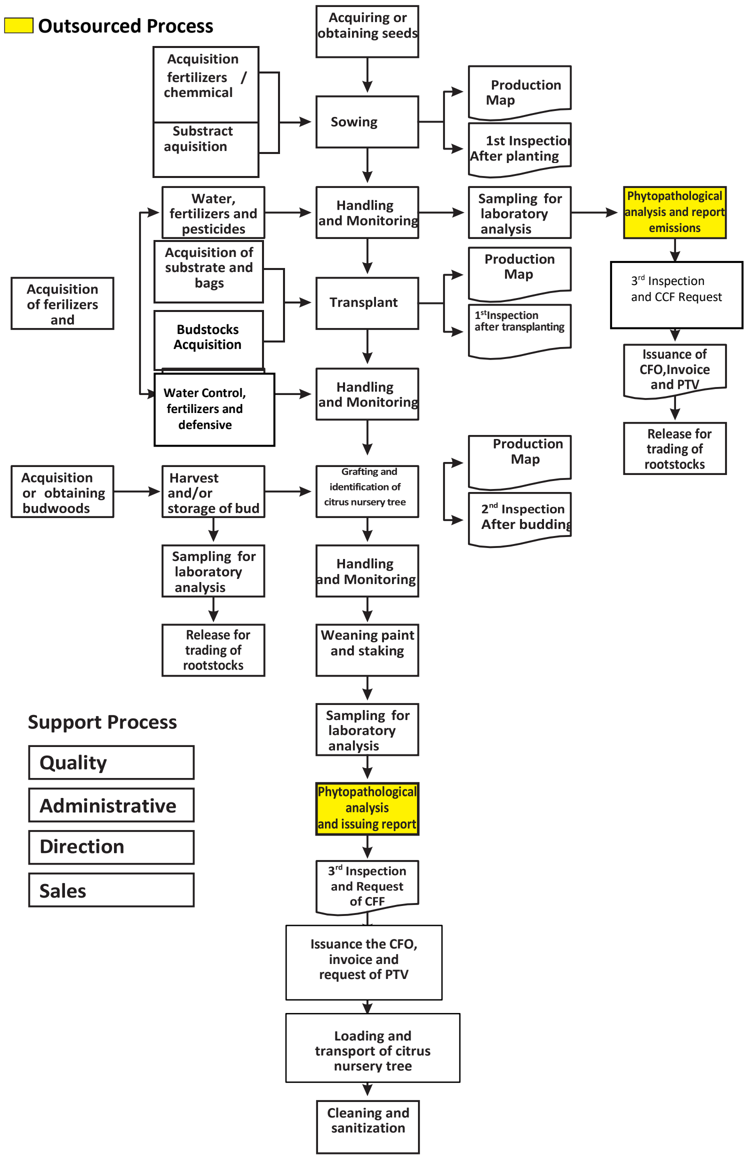
h. Visits Record Book of Technician in Charge.

The nursery growers should also maintain the documentation of the established duly regularized, according to the tax, social security and labor laws applicable at federal, state or local level.

Source:Department of Agriculture and Livestock Protection of the State of São Paulo, 2013.

Anexx 2

FLOWCHART OF PRODUCTION OF CITRUS NURSERY TREE



Source: Vivecitrus, 2012 (personal information).