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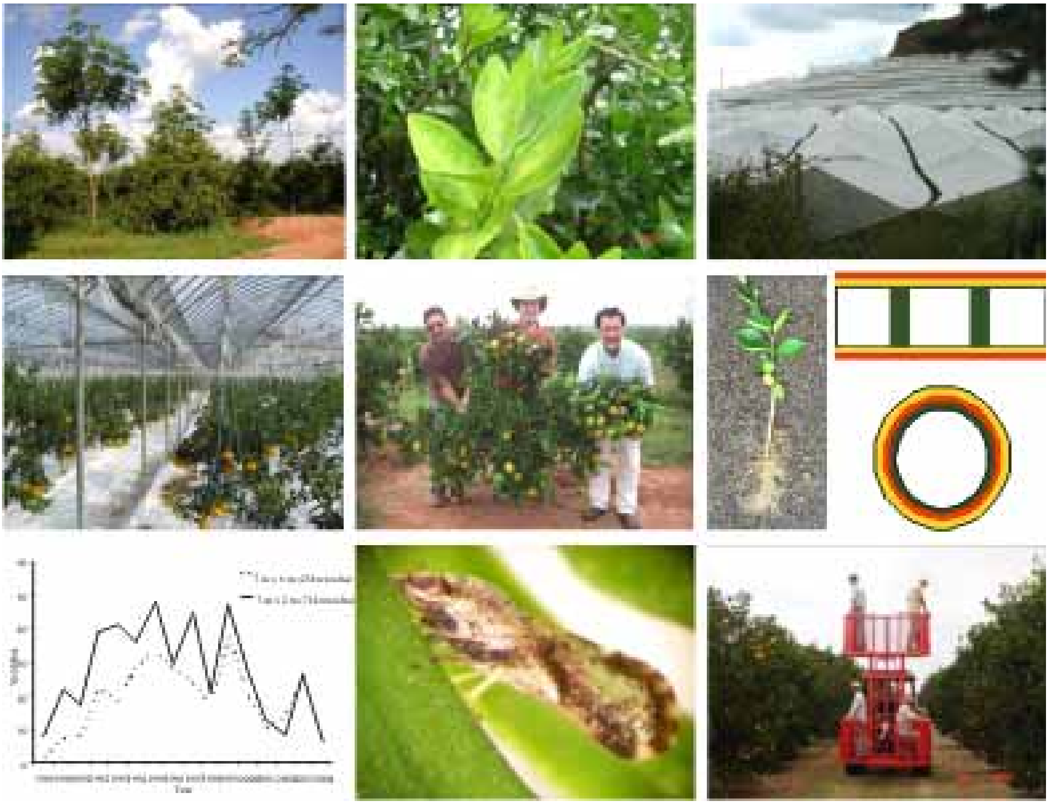

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Presentation

The citrus industry is one of the most relevant segments of the Brazilian agribusiness. This activity has irrefutable economic and social importance throughout the entire country. Nonetheless, this legacy is currently threatened by the huanglongbing (HLB) disease, identified in São Paulo State in 2004.

The present document derives from a long period of reflection, study and discussion that resulted in a series of presentations, starting from the I Roda Viva da Citricultura, in Cordeirópolis, São Paulo State, in October 2009.

Subsequently, a second version focused on more effective practices that are more likely to survive HLB in the medium term. This version was presented in several events and in a home seminar at Embrapa Cassava and Fruits.

Compiled as a text, we are pleased to introduce them as the DOCUMENTOS 189 of Embrapa Cassava and Fruits, which is available at www.cnpmf.embrapa.br. The authors proposed several horticultural practices to be evaluated by the citrus industry. As a result, it is intended to stimulate the actors of citriculture to face this challenge by developing new production systems.

Domingo Haroldo Reinhardt
Director-General
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Summary

Use of horticultural practices in citriculture to survive *Huanglongbing*

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Eduardo Augusto Girardi

Abstract

Citriculture is one of the major activities of the Brazilian agribusiness found throughout the entire country in different production environments. The presence of the Huanglongbing (HLB), reported in Brazil in 2004, has led to severe losses in important producing regions, challenging the sustainability of the citrus chain. Current control strategies are based on the use of healthy nursery trees, scouting and systematic eradication of symptomatic plants and chemical control of the insect vector. Research is being carried out at Embrapa and other Brazilian institutions aiming HLB resistance through genetic breeding via biotechnology. However, the inherent limitations to these actions determine that non-excluding and additional propositions could be considered in order to mitigate the effects of the HLB. Horticultural practices for immediate use in the citriculture are presented in order to complement the confrontation to this disease. The following propositions are discussed: selection of naturally occurring tolerant materials, evaluation of new regions for citrus production, unusual concepts of screened nursery trees, repellent and attractive plants, low-input production systems, use of resistance elicitors, protected cultivation, intercropping and ultra high density.

Indexation Terms: *Citrus* spp., Liberibacter spp., greening, crop management, genetic breeding.

Introduction

Importance of citriculture in Brazil

Brazil detains approximately 30% of the worldwide production of sweet orange [*Citrus sinensis* (L.) Osbeck], 60% of juice extraction and 82% of the international market of this commodity; moving approximately 5 billion dollars annually in all segments (Neves et al., 2007; FAO, 2009). The juice industry represents about 2% of Brazilian exports, generating around US$ 1.5 billion a year with frozen concentrated orange juice only. The State of São Paulo takes up 79% of the Brazilian orange production, which is present in more than half of its counties and generates more than 400 thousand direct and indirect jobs. This State is responsible for 95% of juice exports; a product that ranks the second position in the export market of São Paulo.

In August 2009, the harvested area of sweet orange in São Paulo corresponded to 560 thousand ha, with a total production of 360 million boxes of 40.8 kg in the 08/09 harvest season (AGRIANUAL, 2010). Minas Gerais and Paraná States also participated in the industrial production. Furthermore, there is an expressive orange cultivation in other States of the Union, such as: Sergipe (63703 ha), Bahia (53721 ha), Rio Grande do Sul (28354 ha) and Pará (11785 ha), and other citrus commercial areas are found throughout the entire country, reaching a total surface area of 921432 ha. The fresh fruit market for home consumption and export is supplied by sweet oranges (a production corresponding to 105000 ha), mandarins and hybrids (61200 ha), acid limes and lemons (47500 ha) and remaining citruses (5000 ha) (FAO, 2009). Citrus crops stands out as one of the main activities of the Brazilian agribusiness, with a structured chain in segments represented by: nurseries, citrus growers, consultants, fertilizer and implement suppliers, service assistance managers, frozen concentrated orange juice (FCOJ) and not from concentrate (NFC) processing facilities, fresh fruit market chain, essential oils companies, fine chemistry industries, and pelletized citric pulp market, among other segments.

The presence of Huanglongbing and its impacts

Huanglongbing (HLB) or greening is a devastating disease caused by the phloem bacterias *Candidatus* Liberibacter asiaticus and *Ca*. L. americanus and it is transmitted by the psyllid *Diaphorina citri* Kuwayama. HLB was reported in Brazil in 2004 (Coletta Filho et al., 2004), and the disease also occurs in producing countries in North and Central America, Africa and Asia, leading to irreparable damages. It is present in the main producing regions of the State of São Paulo, Triângulo Mineiro and North of Paraná State. Its incidence was higher in the Central and South regions of São Paulo State, respectively of 61.7 and 44.0% of grove blocks in July 2010 (Fundecitrus, 2010). Until January 2010, approximately 790 thousand symptomatic trees were eradicated only in São Paulo State, with an estimate of more than 4 million diseased plants (Fundecitrus, 2010).

HLB is a difficult disease to manage due to the non-specific nature of its symptoms, prolonged latency period in the field, irregular distribution of the pathogen in the plant, environmental effects (especially temperature) on the expression of symptoms and, possibly, on the multiplication of the bacteria; potential variations of the resistance to the bacteria by the citrus species as well as by the insect vector, and finally, due to the fastidious nature of the bacteria (Bové, 2006; Manjunath et al., 2008). The economic sustainability of the crop is seriously affected in regions where the disease becomes endemic (Roistacher, 1996).

The disease is of main importance to Brazilian citriculture, because it causes profuse damages to most citrus varieties and its vector, the psyllid *Diaphorina citri* Kuwayama, can be found throughout the entire country (Figure 1). The two main forms of dissemination of the disease are transmissions by the vector and through contaminated plant materials (budwood and seedlings). Current control strategy of the disease combines the use of healthy nursery trees, reduction of the inocula (eradication of diseased plants) and chemical control of the vector (Van den Berg, 1994; Brlansky et al., 2008; Belasque Junior et al., 2009, 2010).

Photographs: Eduardo Toller Reiff

Figure 1. Sweet orange tree presenting the initial symptoms of huanglongbing (HLB), with yellow shoots (A); Detail of shoot and leaves presenting the HLB symptoms (B); psyllid *Diaphorina citri* Kuwayama, vector of the HLB causing bacterias (C); implemented platform used for scouting of symptomatic plants in orchards (D).

Healthy nursery trees produced under protected environment have been used in the main citrus producing regions in Brazil for a few years to efficiently prevent other diseases (Carvalho et al., 2005). Scouting for symptomatic trees followed by their eradication reduces the source of inoculum and enables maintaining reduced levels of HLB incidence, especially where the disease is not endemic (Gottwald et al., 2006; Iwanami et al., 2006). The Federal government edited the Normative Instruction N°53 from 10/16/2008, regulating the subject in such a way that grove blocks that present incidence higher than 28% of symptomatic plants must be totally eradicated, because almost the totality of plants would be contaminated at this point (MAPA, 2008; Belasque Junior et al., 2009). However, this strategy is not very well accepted by many producers, because of the direct fruit loss and technical difficulties for the establishment of control measures at regional level and for the anticipated diagnosis in asymptomatic trees, whose proportion is, in general, twice as high as in comparison to symptomatic plants (Irey et al., 2006).

Finally, the chemical control of the vector is considered essential for the success of the other two strategies mentioned, contributing significantly to its reduction in managed areas besides being an accessible tool to the grower (Shivankar et al., 2000; Dewdney & Graham, 2009). On the other hand, despite the noticeable increase in the knowledge regarding the pathosystem in the last five years, there is not much information available for a more adequate management of the psyllid; consequently, the chemical control has been characterized by the indiscriminate use of insecticides, significantly increasing its application costs in orchards. As a result, the environmental, social, and economic impacts of this procedure do not suggest its sustainability in the long run. The difficulty for the ultimate control of the vector by insecticide application programs has stimulated new research approaches, such as the study of the mechanism of acquisition of the *Candidatus* Liberibacter spp. and antagonistic microorganisms by *D. citri*, aiming the elimination or restriction of the colonization of the pathogen in the vector itself (Gatineau, 2006). Alternatives such as biological control were only successful under special conditions like infestations in islands with the absence of hyperparasitism (Aubert, 2008).

Limitations to the ultimate control of HLB

The obtainment of commercial varieties resistant to HLB would consist in the definitive solution for this disease (De Lange et al., 1985). Since there is no evidence of natural resistance in the Citrus and related genus, biotechnological tools involving genetic reengineering represent a major success probability to reach this objective (Bové, 2006). Remarkable research effort has been carried out by worldwide research institutions working with citrus, with the evaluation of many candidate control mechanisms (Gottwald et al., 2008). On the other hand, time needed for the obtainment, validation, multiplication and implementation of a transformed variety may take 10 to 20 years (Machado et al., 2005).

Other limitations can be added to the relatively long period required for this strategy, including: restricted knowledge of the genetic and molecular aspects of the pathogen – vector – host relationship in the HLB pathosystem; the non definition of the most probable technical approaches for the reengineering success; difficulties in the transformation and regeneration of varieties of commercial interest; financial restrictions for transformation programs of neglected species and varieties with local social-economic importance; low probability for obtaining total resistance to the pathogen; consumer market restrictions to transgenic materials; discussions regarding patents and royalties involved in the process, and others.

Therefore, it is possible to realize that the combined triple action (healthy nursery trees + scouting and tree eradication + vector control) and the search for resistant materials constitute priority themes for the research institutions and productive sector (Belasque Junior et al., 2010). Nonetheless, the limitations of this strategy and the severity of HLB effects in the short, medium and long term, implicate in the need to develop other disease control methods.

Given this scenario, additional and non-excluding propositions can be established to collaborate with this discussion (Bar-Joseph, 2009). Scientific and empirical results observed in different producing regions indicate that crop management may contribute to HLB mitigation and, therefore, integrate sustainability into the citrus industry. These practices can be considered additional since they are based on crop management alternatives that can be incorporated to the control methods listed above. Also, can be considered as non-excluding, since they do not restrict the concomitant use with other technologies, neither suppress the need for major investigations. Actually, these propositions represent previously expected horticultural advances for citriculture that should be accelerated by the presence of devastating diseases such as HLB. After all, the search for higher yield is the main objective of competitive production systems, and particularly in the case of citrus, it can represent an important complementation to HLB management.

From the analysis of two vertices which compose the HLB pathosystem (host and managed environment), suggestions and challenges are presented to researchers, horticulturists, citrus producers and all those involved and worried about the future of citruses. Emphasis is applied to crop management and genetic materials currently available, aspects that can be accessible to commercial production and whose implications exceed the control of the disease. Scientific evaluations, economic studies and legislation attendance must be considered for any new proposition in debate, without, however, underestimating the capacity to come up with innovative and practicable solutions regardless of their origin.

Horticultural propositions for the citriculture

A – Intervening in the host

Search for natural tolerance/resistance

All commercial citrus species are considered susceptible to HLB, even though disease severity may vary according to the genotype (Gonzáles et al., 1972; Nariani et al., 1973; Miyakawa, 1980; Koizumi et al., 1993; Hong et al., 2002; Halbert & Manjunath, 2004; Subandiyah et al., 2006; Batool et al., 2007; Lopes & Frare, 2008; Shokrollah et al., 2009). The mechanism of tolerance to HLB may be of genetic origin (inherent to the genotype), epigenetic (resulting from gene expression, such as physiological age) or acquired (by the presence of endophytic microorganisms, for instance). Its manifestation can also result from the distinct predilection of the vector leading to field resistance (Halbert & Manjunath, 2004; Beattie et al., 2006; Nava et al., 2007), or even from different bacteria strains with differentiated virulence.

The symptoms are severe in sweet orange [*Citrus sinensis* (L.) Osbeck] (Brlansky et al., 2008), but little is known about the different levels of tolerance among the different varieties and germplasm accessions of this species (Van Vuuren, 1993; Folimonova et al., 2009). Regarding commercial clones used in Brazil, it is known that early-season varieties present fewer damages, as a result of the shorter period that the fruits stay in the tree reducing competition for photoassimilates whose availability is even more reduced in contaminated plants (Bassanezi et al., 2009).

It is possible, though not probable, to find resistance or tolerance to HLB available for immediate use in commercial orchards inside Germplasm Banks, which nowadays are usually exposed to the disease in the field. In the literature there are reports on less than three hundred varieties evaluated, whereas thousands of accessions are available. Plant selection is an efficient strategy for genetic breeding and has been used for centuries, especially in perennial crops such as citrus (Machado et al., 2005). The obtainment of resistant or tolerant plants by the selection of naturally existing materials would constitute the most efficient and cheapest way to control HLB (Gmitter, 2009).

Citrus trees have a long juvenile period limiting conventional breeding based on crosses, besides the difficulties observed for the manipulation of adult tissue in genetic engineering. There are at least two reasons for screening for this resistance: 1) potential existence of clones or accessions with unknown natural mutations which confer resistance to HLB; 2) clones from different environmental conditions may contain endophytic microorganisms antagonistic to the causal agent of HLB in the phloem. These materials could then be evaluated for direct use in commercial orchards or be included in breeding programs of the remaining species, slightly broadening their chance of success.

Relationship between HLB and scion/rootstock combination

Reports regarding the role of the scion/rootstock combination in the incidence and severity of HLB are controversial (Abdullah et al., 2009). Primarily, all rootstock and scion varieties are considered susceptible and therefore, it is not possible to control the disease by the combination of varieties. Seed-derived plants of *Poncirus trifoliata* (L.) Raf. are classified as the most tolerant material to the *Candidatus* Liberibacter spp. bacteria and they rarely present symptoms (Folimonova et al., 2009). Other rootstock species present symptoms at different intensities.

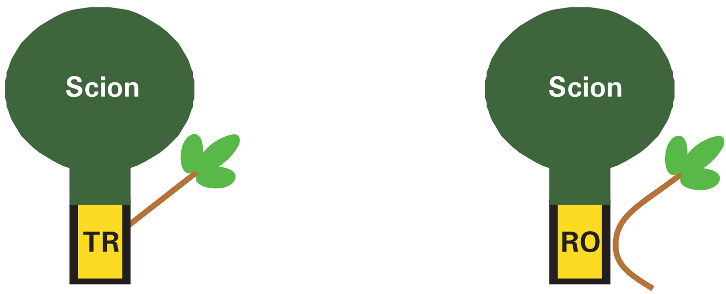
In the United States, higher incidence of the disease was reported in groves of several scions grafted on Volkamer lemon (*Citrus volkameriana* V. Ten. & Pasq.), when compared to the same clones grafted on rough lemon (*Citrus jambhiri* Lush.) and Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. x *Citrus paradisi* Macfad.). On the other hand, in South Africa, higher incidence was observed on sweet oranges grafted on *Poncirus trifoliata* in relation to other rootstock species (Van Vuuren & Moll, 1985). No differences among the clones of *P. trifoliata* were observed. In Brazil, there are still no consistent reports which indicate the effects of the rootstock on HLB occurrence.

At the Estação Experimental de Citricultura de Bebedouro (EECB), HLB progress was observed on 'Natal' sweet orange grafted onto three rootstocks, Rangpur lime (*Citrus limonia* Osbeck), Swingle citrumelo and Sunki mandarin (*Citrus sunki* Hort. ex Tanaka). Planting was carried out in April 2002 at 7 m x 4 m spacing, and the soil was a typical red oxysoil. A total of 674 plants were evaluated, with the trees being equally grouped by the rootstock. This orchard was fenced by a native forest area to the west, a sweet orange grove grafted on Sunki mandarin to the south (plantation in 2001) and a seedling-derived Cleopatra mandarin grove (*Citrus reshni* Hort. ex Tanaka) to the north (plantation in 1985). The scouting followed by systematic eradication of HLB symptomatic plants in the orchard without chemical control of the vector was adopted. Scouting was carried out with the help of implemented platforms since 2009. In May 2009, the percentage of symptomatic plants was 13.51; 2.25 and 3.15%, on Rangpur lime, Swingle citrumelo and Sunki mandarin, respectively. In October 2009, the incidence increased to 18.47; 6.76 and 5.41%. In January 2010, a new scouting determined 44.14; 17.57 and 12.61% of diseased plants whereas trees on Rangpur lime were totally eradicated because of exceeding 28% of HLB incidence. It was considered that the area with the native forest was lodging the psyllid, because plants on Rangpur lime were closer. However, symptomatic plants were not observed in the land plot with seedling-derived Cleopatra mandarin trees, whereas the average incidence of the sweet oranges grafted on Sunki mandarin was 29.33%. More recently, it was observed that 'Folha Murcha' sweet orange, 'Okitsu' satsuma mandarin (*Citrus unshiu* Marc.) and 'Persian' lime (*Citrus latifolia* Tanaka) grafted onto Flying Dragon *Poncirus trifoliata* var. *monstrosa* did not present HLB symptoms even when surrounded by severely affected experimental plots. This is a condition that has been under constant observation in Bebedouro-SP. Cantuarias-Avilés (2009) described lower incidence of citrus variegated chlorosis in plants of 'Folha Murcha' sweet orange budded on Flying Dragon trifoliate orange and justified it by the lower availability of shoot flushes.

A possible explanation for these differences could be that the shoot flushes of the scion variety are influenced by the rootstock vigor. More vigorous species induce in general higher sprouting frequency and vigor to the scion (Pompeu Junior, 2005). Consequently, there is greater availability of the preferential food for the psyllid, resulting in higher dissemination of the bacteria. This vegetative dynamic is also influenced by climate and crop management aspects related to the location of the grove, intensifying or reducing the effect of the rootstock. The selection of rootstocks which induce low vigor to the scions, due to the lower shooting frequency and growth, accompanied by high yield efficiency, could indirectly contribute to decrease the source of food to the vector and somehow facilitate its management, possibly reducing HLB presence.

The impact of HLB on the rootstock is little known. When the canopy presents disease symptoms, the bacteria is already present in practically all the organs, including the root system, in uneven titers (Tatineni et al., 2008; Li et al., 2009). The typical symptoms (yellowing, mottled leaves and branches, and deformation of fruits and seeds) indirectly derive from the accumulation of starch in the chloroplasts, because of the phloem interruption in branches with higher titer of Liberibacter spp. (Etxeberria et al., 2009). In these branches, calosis and accumulation of substances are observed interrupting the phloem and, as result, starch transport and accumulation in the roots are reduced. Although the typical damages attributed to HLB basically consist of effects on the scion and its fruits, overall decline including of the root system is observed in plants with advanced symptoms. The effects of HLB on rootstocks in asymptomatic trees and in plants with initial symptoms are unknown, thus not allowing the determination of impacts on the root system and its importance in the manifestation of the disease.

Since the sap flow from the canopy to the rootstock probably will be altered in symptomatic as well as asymptomatic plants, a possible tool to decrease the effects of HLB on the rootstock would be the use of plants with double scions or an auxiliary scion. In Spain, this practice was carried out with success in orchards on sour orange rootstock (*Citrus aurantium* L.) as a palliative measure to the citrus tristeza disease (Carrero, 1981). This disease is caused by a virus (CTV) and it represents a classical example of a disease controlled by the scion/rootstock combination. The sour orange is resistant to the tristeza virus; however, it is intolerant to its presence and, therefore, seedling-derived plants are not affected. When used as rootstock of susceptible varieties, the sour orange ends up declining. The use of an auxiliary scion consists on the partial growth of the canopy of the resistant rootstock which continues the normal supply of photoassimilates to the root system resulting in longer survival of the whole plant. This technique could be evaluated in analogous form using rootstocks more tolerant to HLB as the auxiliary canopy (Figure 2).



Poncirus trifoliata rootstock budded in the nursery without cutting-off of its canopy

Tolerant rootstock by approach grafting or 2nd budding if other rootstocks are used

Figure 2. Examples of double or auxiliary scions for citrus trees aiming a possible mitigation of HLB effects.

B – Intervening in the environment by grove management

The three ideal conditions to confront HLB

Considering a scenario in which HLB is present and there is unavailability of resistant materials in the short and medium terms, the establishment of new citrus orchards depends on solid planning that contemplates control measures currently recommended for the disease. In order to configure a friendly environment to these measures and in order to attain greater initial yield and, therefore, partially compensate the losses from HLB, three ideal conditions of grove management are suggested for setting up an orchard: high density groves, use of irrigation and planting blocks of a unique variety or varieties with similar phenology.

Higher planting densities of new plantations (600 to 1500 trees ha^{-1}) are a strategy adopted for broadening yield (Castle et al., 2007; Negri et al., 2005). Citrus are responsive to higher densities and this system is one of the explanations for the increase in yield; as observed in the last eight years in São Paulo (moving from 400 to 600 boxes of 40.8 kg ha^{-1}), besides being a factor of prognosis for greater gains in the near future due to narrowing of spacing. Traditionally, the recommended spacing until the beginning of the 90´s was approximately 7 to 9 m between rows and 4 to 6 m in the planting row, but sweet oranges presented excellent productions in spacings of 5 to 7 m in between rows and 2 to 4 m in the planting row (Donadio et al., 2002; Teófilo Sobrinho et al. 2000, 2002). In these studies, it was verified that, in average, increasing density allowed harvests 50% higher in the first eight years after planting in relation to traditional spacings (Figures 3 to 5).

High planting density is a simple tool for increasing yield which enables greater competitiveness and consequently, favors the permanence in the activity for small and medium growers (less than 100 thousand trees), which nowadays represent approximately 60% of citrus producers in Brazil. Its use can also compensate the reduction in the population of plants by eradication of HLB infected plants and therefore, has been consistently used in China (Aubert, 1989, 1990) and South Africa. Resetting following eradication can be eliminated in high density plantations, which results in the reduction of costs and also avoids the presence of young plants between bearing trees. This last condition is a highly favorable factor for HLB dissemination.

The anticipation of the financial return in high density orchards was demonstrated in experiments in different regions of the world, including of tropical climate (Muraro et al., 1995; Rabe et al., 1996; Mademba-Sy et al., 1999). As disadvantages of high density plantings, we can highlight the need for regular trimming depending on the scion/rootstock combination vigor (Wheaton et al., 1995) and also the obligatoriness of dwarfing rootstocks in order to reduce vigor.

In the case of trimmings which result in intense subsequent sprouting, additional measures of chemical control of the vector should be expected. On the other hand, the perspective of shorter longevity of the orchard and the gradual reduction of tree number can lead to discussions about the need for trimming.

Irrigation is another grove management that can increase yield in approximately 40 to 60% in citrus (Zanini et al., 1998), and, therefore, is a desirable management to reduce losses due to HLB. The irrigated citrus area in Brazil grew significantly in the last ten years, especially in the juice enterprise (Pires et al., 2005). Implementation costs of a pressurized irrigation system are mitigated as yield in the initial years is broadened by the correct use of the technique, which also favors the extension to other practices such as fertirrigation and chemigation. Also, other factors such as rootstock vigor should be taken into consideration in order to avoid excessive vegetation which in turn could favor the presence of the psyllid *D. citri*.

In conclusion, planting a single variety in blocks, or also varieties with similar phenology, that is, presenting synchronized phenological stages is recommended for HLB management in South Africa (Van den Berg, 1994). By this condition, a more uniform production cycle is attained aiming for the coincidence among the critical phases of psyllid control as spring and summer shoot flushes. This behavior favors the chemical control of the vector inside the property and also in larger areas, and also hinders the availability of more apt hosts to the psyllid. In general, planning of the everyday activities and orchard management is facilitated.

Briefly, high planting densities, irrigation and blocks of unique or uniform varieties represent, in this order, the main grove management procedures readily available to mitigate the impacts of HLB in citrus enterprises in Brazil. In the USA, all these practices have been evaluated as advanced citrus production system or "open hydroponics" with the same objectives (Morgan et al., 2009; Roka et al., 2009).

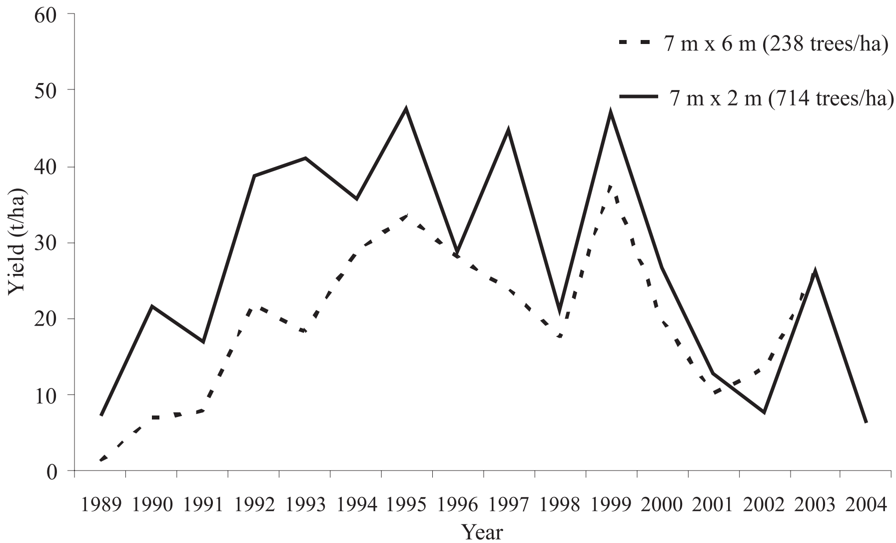


Figure 3. Yield of 'Pera' sweet orange grafted onto Cleopatra mandarin without irrigation related to the planting spacing. Bebedouro, 1989-2004 (Stuchi et al., unpublished data).

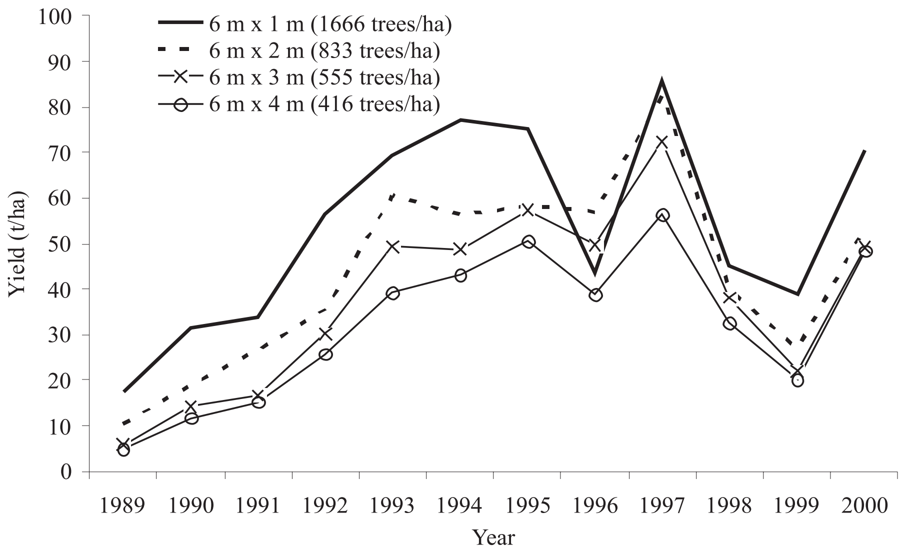


Figure 4. Yield of 'Hamlin' sweet orange grafted onto Rangpur lime related to the planting spacing. Cordeirópolis, SP, 1989-2000 (adapted from Teófilo Sobrinho et al., 2002).

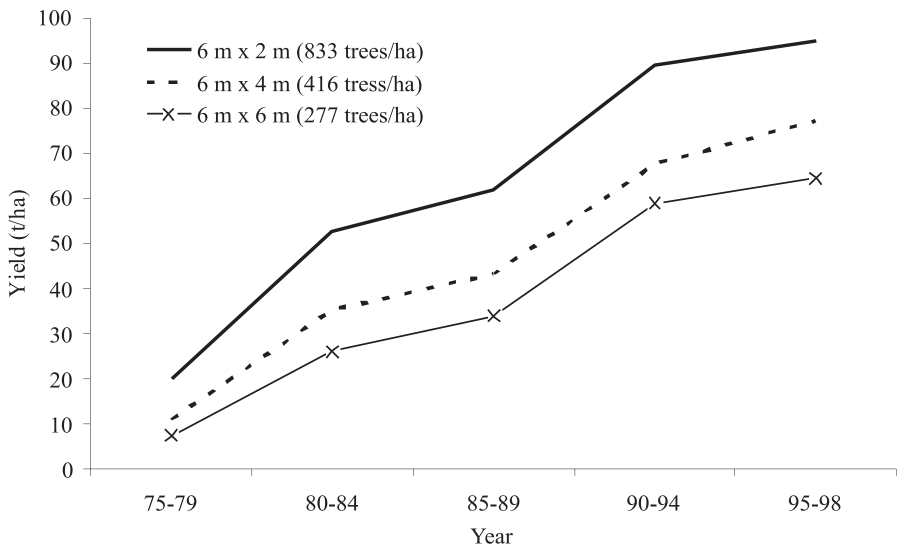


Figure 5. Yield of 'Valencia' sweet orange grafted onto Limeira trifoliate orange related to tree spacing. Cordeirópolis, SP, 1975-1998 (adapted from Teófilo Sobrinho et al., 2000).

New regions for citrus production

Regions characterized by endemic levels of HLB present intense limitation to the industrial citriculture due to low orchard yield (Aubert et al., 1996; Roistacher, 1996; Batool et al., 2007). Therefore, preventing the entrance of the vector/ and or causal agent is the main measure of control, with the pathogenic bacteria being quarantined in many countries. In regions where the HLB is present, the maintenance of reduced regional incidence is fundamental for the success of the control strategies (Gottwald et al., 2006, 2008). These reports indicate the potential of new cultivation regions as a way to enable citrus crop activity. This practice was adopted by many countries that co-exist with HLB for decades, such as China and South Africa (Zhao, 1981; Van den Berg, 1994).

In the case of Brazil, HLB is present in the main orange producing State, São Paulo, and it also affects the States of Minas Gerais and Paraná. The region of Araraquara, in the centre of São Paulo, is a traditional citrus area, however, is one of the most affected areas, estimating 45% of block contamination in November 2009. These levels of infestation hinder commercial activity. The possibility of migrating to other regions that present reduced incidence or absence of the disease would allow growing citrus under more adequate conditions for profit; although implying in new investments and eventual additional costs for the adaptation and for prevention of HLB occurrence.

Any initiative of moving the production to new areas must consider, nonetheless, other conditions required for commercial production of citrus, besides HLB incidence. Therefore, edaphoclimatic characteristics, juice and fruit quality, absence of other limiting diseases, labor availability, logistic aspects, among others, should be analyzed in order to attribute most advantages to the new production region and thus contribute to the viability of the enterprise. Therefore, proper citrus producing regions should be prioritized in the choice.

A third factor that fundaments the geographic change, besides the presence of HLB and adequate environment for citrus production, is a climate condition which will not favor the vector and/or the Liberibacter spp. In the case of African HLB, the disease is caused by the bacteria *Candidatus* Liberibacter africanus, and transmitted by the winged vector *Tryoza eritrae* (Bové, 2006). Both organisms are restricted by high temperatures, so that orchards situated in warmer regions present a more facilitated and cheaper management (Schwarz & Green, 1970; Bové, 2006). On the other hand, in China, regions of milder climates disfavor the multiplication of the psyllid, *D. citri*, the HLB vector in that country, whereas places with a humid climate are favorable to this psyllid (Zhao, 1981; Shivankar et al., 2000). In the United States, studies predict that frosts in the North and Central regions of Florida would contribute to the temporary reduction of the *D. citri* population in the producing regions (Hall, 2008), whereas areas of the South that is free of this phenomenon present elevated incidence of HLB and its vector.

In Brazil, HLB is caused by the *Candidatus* Liberibacter americanus and asiaticus bacteria, with multiplication of the former being disfavored by temperatures higher than 28°C (Lopes et al., 2009). The Asian form is more resistant to both low and high temperatures and it prevails nowadays in the country. Although the life cycle of the vector, *D. citri*, is optimum in a range of temperature between 25 and 30°C (Nava et al, 2007), other local climatic characteristics, such as low relative humidity during the winter, can be directly (by lower survival of nymphs) and indirectly (by lower tree shooting) detrimental to the development of the vector, with positive implications in the control of the disease (Nava et al., 2008).

Therefore, regions characterized by low relative humidity during the winter and prolonged drought would combine disfavoring factors to the multiplication of the vector. Under drier climate, there is the additional possibility of interference in greater or lower degree on the vegetative flushes of citrus via irrigation control. This practice allows indirect management of the psyllid by partial shooting control; for instance, the vector could be controlled previously to new shoots with less impacting products such as vegetal and mineral oils (Rae et al., 1997; Beattie et al., 2006).

The role of changing citrus production areas, if by one hand presents limitations regarding industrial citriculture for juice processing, is fundamental for the establishment and growth of local citrus productions as food security for home market. This situation would favor regions that traditionally import fruits from São Paulo and that could become potential exporters in the future.

The North and Northwest of São Paulo State, the Triangle of Minas Gerais and the Northwest of Paraná State, are highlighted as very favorable regions for the industrial citrus crop, despites HLB presence. Regions with similar edaphoclimatic characteristics and absence of the HLB nowadays, are the East and South region of Mato Grosso do Sul State and South region of Goíás State, making up the geographic continuity of the existing orange production regions. The region of Tabuleiros Costeiros in the Northeast of Brazil has enormous potential for citrus production, highlighting the boundary region between the States of Sergipe and Bahia. There are approximately 100 thousand hectares of orange groves and some juice extractors operating (Passos et al., 2004); not to mention the absence of HLB and citrus canker. The Northern region of Espírito Santo State also has similar edaphoclimatic conditions. Finally, Embrapa in partnership with other companies, promoted a detailed study about the possibility of installing a citrus industry in the Vale do Rio São Francisco, emphasizing the irrigated perimeters in the semiarid area of the Médio Vale (Neves, 2006). It was concluded that the region is apt for a sustainable citriculture with industrial purposes, whereas up until now, this region is free of the main citrus diseases, including HLB. The strategy of transferring citrus production should not be discredited, since it represents opportunity of economic diversity for regional growth and promotes a matter of food security.

Use of repellent or attractive species

Interplanting cultivated species with repellent or attractive species to specific plagues has been reported as an auxiliary measure of control for centuries in Asian countries, and it is recommended in modern production systems such as organic and biodynamic. Particularly in the case of citrus, studies involving species such as white guava (*Psidium guajava* L.) indicate the presence of sulphur derived substances (DMDS) in its leaves acting as repellent to the psyllid *D. citri* (Rouseff et al., 2008; Zaka et al., 2009). Such compounds, non synthesized by citrus, can explain the lower presence of the psyllid in citrus plants interplanted with guavas and, consequently, less incidence of HLB in groves located in Asian regions severely affected by the disease (Beattie et al., 2006). Other studies indicated mangoes (*Mangifera indica* L.) as a non preferred species by the psyllid, whereas orange jasmine (*Murraya paniculata* (L.) Jack) is the preferred and the most attractive host to the insect (Nesumi et al., 2002). Three years after grove establishment, interplanting citrus with these last two species resulted in 3 to 20% of HLB symptomatic trees, whereas 76.3% of symptomatic trees were observed in citrus monoculture.

Although interplanting alone does not represent an efficient control method, it diminishes the presence or even favors the control of the target-insect by concentration of the individuals on attractive plants or baits. The effect of wind-breakers is well known for reducing the dissemination of pathogens and diseases in citrus (Gravena, 2005). In the HLB pathosystem, the primary infection is the most damaging form of dissemination and the most difficult to control, because it is continuously caused by the initial entrance of contaminated vectors from outside areas coming into the grove (Bassanezi & Gottwald, 2009). This fact is aggravated on the border rows, as higher HLB incidence is observed in marginal areas of the orchard, probably resulting from its characteristic of transition between the external environment and the orchard (Gottwald & Irey, 2008). Therefore, planting repellent and attractive species around all grove may diminish the border effect. It is pointed out that the role of native Rutaceae species is unknown in the HLB pathosystem in Brazil, since many wild Rutaceae species are alternative hosts in Asia and Africa (Halbert & Manjunath, 2004). In Brazil there are approximately 150 native Rutaceae species grouped into 32 genera (Faria et al., 2007).

The evaluation of repellent or attractive species and their management for interplanting still needs more investigation in the country in regard to the facility of use and potential of repellency from underutilized native Myrtaceae species and commercial Lillyaceae species (Figure 6). Works have been developed in order to isolate and synthesize natural repellent and attractive compounds, constituting an alternative to control the citrus psyllid (Jing et al., 2005; Weathersbee & McKenzie, 2005).

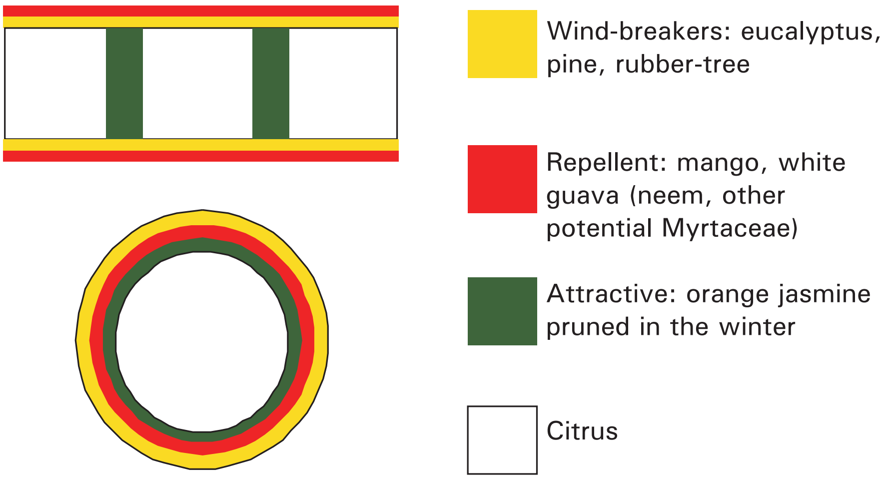


Figure 6. Examples of interplanting citrus with potentially repellent and attractive species to the vector of HLB, the psyllid, *D. citri*.

Use of intercropping in citriculture

Intercropping involves the plantation of two or more species in the same area, in such a way that one of the crops co-habitats with the other (or others), during its entire cycle, or, at least, part of it (Portes, 1984). Intercropping is a crop management which allows broadening the productivity of agricultural areas, increases biologic diversity, reduces the risks of the agricultural activity and increases the income of the grower throughout time.

Although broadly used in agriculture, intercropping is not common in the citrus production system. Eventually, maize is cultivated in the in between rows during the first two years after planting, or sometimes common bean is sowed in areas of the Tabuleiros Costeiros region. In general, citrus is cultivated as a monoculture and there is use of cover crops in the in between rows for crop management and nutrient cycling (Bremer Neto et al., 2008). The use of intercropping in citrus groves has major viability in the presence of HLB, since it can reduce the risks of the activity and leads to higher income if well executed.

Two approaches can be considered for intercropping in citriculture. In the first one, the cultivation of secondary crops in the in between rows is recommended before citrus trees reach higher size. Beside food crops, cash crops could be evaluated to anticipate the financial return. Among these, soft fruits, vegetables and ornamentals are suggested, as long as they do not include host or potentially host species such as solanaceous vegetables and potato (Dias et al., 2008). Interesting crops are: pineapple, passion-fruit (in which the trellising structure can be used for the installation of physical barriers to *D. citri*, as will be further mentioned in the text), cucurbits and vegetables in general and tropical foliages, among others.

A second approach considers citrus as a temporary crop; in this case, its cultivation is an additional and anticipated income during the exploration of another species of interest. In this system, the gradual elimination of citrus by HLB is expected until its total substitution by the second crop in a few years. In perennial crops cultivation in Brazil, such as coffee and cocoa, there are many examples of successful intercropping in agroforestry systems (Da Matta, 2004). Perennial crops candidates for intercropping with citrus are rubber-tree (*Hevea* spp.), timber forests, woody species for energetic means and cellulose, besides many other fruit and nut trees, including guavas and mangoes (Figure 7). It is important to highlight that both intercropping approaches can be carried out in the same orchard, and this production system is particularly indicated for small producers.

Photographs: Eduardo Toller Reiff

Figure 7. Intercropping of sweet orange with rubber-tree in Bebedouro, São Paulo State.

Citrus production under protected and semi-protected cultivation

Screen houses are largely used for the production of citrus nursery trees, leading to healthy propagation material by the exclusion of vectors of diseases such as HLB, citrus variegated chlorosis (CVC) and citrus sudden death. The high infrastructure investment and production costs can be paid off with the commercialization of nursery trees, while the same will not occur with fruits for industry in Brazil. In other countries like Japan, the climatic limitations, higher labor costs and the higher market value of the citrus fresh fruits, on the other hand, enable the cultivation of premium cultivars under completely protected greenhouses (Iwagaki, 1997). This condition indirectly reduces the dissemination of diseases by certain vectors (Figure 8).

If on one hand the use of permanent covered structures may represent an economic option only for certain citrus cultivars in Brazil, such as seedless mandarins and out-of-season ‘Persian’ acid lime, the temporary protected cultivation could be adopted for orchards in general. Screens, tunnels or quonset houses are maintained up to the 3rd or 4th years after planting. This period corresponds to the grove formation and is characterized by intense plant growth, being considered critical for infection by HLB (Bassanezi & Gottwald, 2009). Therefore, temporary coverage of young trees would dispense intensive chemical control during this phase, later exposing bearing trees to the environment when disease dissemination and its impacts are less severe. Materials for this semi-protected cultivation are cheaper and more concise than a permanent structure. Furthermore, individual screen houses could be used for resetting; a practice that otherwise, is not recommended in the presence of HLB (Irey et al., 2008).

Photograph: Freddie Gmitter Jr.

Figure 8. Protected cultivation of mandarins in Japan. Photographs: Eduardo Toller Reiff

Both forms of protected and semi-protected cultivation require improved practices such as drip irrigation, mulching for the control of weed, fertirrigation, among others. A simpler practice would be the use of physical barriers or netting in the orchards. In this case, there is only the horizontal coverage of the orchard with appropriate screens to reduce light intensity. In Brazil, netting is commercially used in fruit cultivation, such as apples, to protect trees from hail and animals (Figure 9). In arid climate areas, shading also improved the performance of the physiological apparatus, vegetative growth and yield of citrus (Raveh et al., 2003). The need for materials is low and therefore, less expensive.

Photograph: Eduardo Augusto Girardi

Figure 9. Screened cultivation of apple in São Joaquim, Santa Catarina State, Brazil, in order to avoid hail.

These barriers, screens, shades or nets are not used to avoid the entrance of insect vectors, but to affect their capacity of movement and, consequently, of feeding on host plants. The citrus psyllid is less attracted by the white color (Kawamura & Uchida, 2006), which could be preferentially used in screen barriers. The psyllid is a day time insect which has its flight activity and host finding behavior orientated by light (Sétamou et al., 2008). Therefore, such barriers could affect the psyllid activity by modifying light spectrum. Additionally, HLB symptoms are intensified under more intense and continuous light (Dawson et al., 2009), a phenomenon whose relationships with the citrus production environment are still not well comprehended.

The use of nets and physical barriers such as whitewash spray reduced the incidence of several diseases transmitted by winged vectors in citrus and other crops (Bar-Joseph & Frenkel, 1983; Franck & Bar-Joseph, 1992). The psyllid, *D. citri,* ability to grasp, move and oviposit was limited by monthly applications of kaolin suspension resulting in a particle film on the citrus leaves (Hall et al., 2007). Reduction in the number of eggs, nymphs and adults was, 85, 78 and 60%, respectively, in plants treated for 12 months compared with untreated trees. The insects were repelled, even though, the particle film was degraded by rain requiring constant applications. Finally, the potential effects of horizontal and vertical netting on primary and secondary disseminations of HLB are unknown at field conditions.

Symptoms attenuators

A rationale that completely goes against the other propositions presented is the evaluation of HLB symptom attenuators, since it implies in the co-existence with the disease and disuse of the systematic eradication. Symptom attenuators are all treatments whose objective is not to avoid the HLB dissemination (by controlling the vector or limiting acquisition of the bacteria by the host); but to attenuate or even eliminate the symptoms caused by the presence of the bacteria, aiming for the maintenance of economic yield for a longer period.

The mode of action of the attenuators depends on the nature of the treatments applied, isolated or in association, whereas micronutrients, plant growth regulators, elicitors of systemic acquired resistance (SAR), bioactivators, endophytic microorganisms such as *Bacillus subtilis*, antibiotics, homeopathy, among others, are the most common (Bathia et al., 1999). In general, the treatments are continuously applied via sprays, trunk injections or root absorption. In Brazilian conditions, there are no scientific data available, whereas the most promising results were reported in the USA and China, being, however, empirically conducted (Arevalo et al., 2009; Su, 2008).

Some HLB symptoms are similar to intense nutrient deficiency of iron and zinc, suggesting their temporary deficiency (Bové, 2006; Lopes & Frare, 2008). HLB symptomatic trees have, in general, leaf concentrations of nitrogen, potassium, calcium, sulphur and zinc inferior to healthy trees (Malavolta et al., 2005). The possible involvement of nickel deficiency on the development of symptoms was suggested in Brazil (Wood, 2007). In this approach, the depletion of nutrients levels is reversed. On the other hand, when SAR elicitors are used, the strategy is to conduct the metabolism of the infected plant to intensify its natural reaction to *Candidatus* Liberibactar spp. and, therefore, increase the systemic resistance of the organism. SAR elicitors usually consist in new plant growth regulators such as salicylic acid, brassinosteroids and jasmonates. Finally, antibiotic substances such as tetracyclines (acromycine) and penicillin have bacteriostatic action.

The use of symptom attenuators is controversial, since it does not suppress sources of inocula and consequently, exposes other trees to contamination by HLB (Mattos Junior et al., 2010). In addition, regardless of the nature of the treatment, the mechanisms of action involved imply invariably in temporary effects that also depend on the endogenous levels in the citrus tree. This could possibly explain why its effectiveness is variable among individuals and in time (Arevalo et al., 2009). Trees are “heterotrophized” since metabolism promoting factors are continuously supplied to diseased trees.

Nonetheless, the observation of longer lifespan of citrus groves submitted to sprays with various cocktails, resulting in reversion of advanced symptoms and satisfactory yield, is a new concept so that symptom attenuators should be further evaluated under controlled conditions in Brazil. If they will not lead to a sustainable co-existence, these studies might contribute to the comprehension of HLB development in citrus. Therefore, a better understanding of the interruption/alteration of sugar translocation in symptomatic and asymptomatic trees may support the establishment of new control strategies from metabolism promoters.

Low input production systems

Low input production systems of citrus are practiced in some Asian regions where the HLB occurs endemically for many decades limiting the presence of a well developed citrus industry (Aubert, 1990; Roistacher, 1996). In these areas, eradication is not mandatory and usually is carried out only for symptomatic plants in more advanced stages of the disease. The control of the vector by chemical means is restricted and systematic resetting with healthy nursery trees is practiced in high density orchards. Such circumstances are not applicable to the citrus juice industry in Brazil. However, its refinement may be used when other interests are considered such as certified enterprises for organic production, citrus agroforestry systems and small fresh fruit producers for home or local market (Figure 10). The high costs deriving from HLB dissemination should be compensated inside the system by higher planting density, continuous eradication and trimming and girdling of symptomatic branches in adult trees for inoculum reduction (Bar-Joseph, 2009; Van den Berg, 1994), and by the development of biological control, which was directly responsible for the eradication of HLB in some regions such as Reunion Island (Aubert et al., 1996).

Photograph: Eduardo Augusto Girardi

Figure10. Agroforestry system for citrus cultivation with low input of external chemicals.

The main goal of low input production systems is decreasing chemical inputs, especially fertilizers and agrochemicals, which are major costs of citrus production after harvesting (AGRIANUAL, 2010). Besides grove management considering soil fertility and biological diversity of the orchards under more ecological principles, such a decrease fundamentally depends on the use of tolerant or resistant varieties to other diseases that affect citrus. As a result, more resources will be available for HLB management because of economy with other phytosanitary treatments. Among these options, are: species tolerant to CVC (navel oranges, mandarins, lemons and limes); to citrus leprosis (mandarins, limes and lemons); to alternaria brown spot (14 selections of mandarins and hybrids); to citrus black spot (acid limes and sour orange); rootstocks tolerant to drought, blight and rotting of the root system, among other species and varieties (Laranjeira et al., 1998; Donadio et al., 1999; Spósito et al., 2004; Pompeu Junior, 2005; Souza et al., 2006; Freitas-Astúa et al., 2008; Souza et al., 2009).

Unusual concepts of protected nursery trees

Healthy nursery trees are the basis of HLB and other important citrus diseases management (Carvalho et al., 2005; Bové & Ayres, 2007). Nowadays, in order to certify biosecurity, nursery trees and seedling rootstocks must be grown under protected environments in major Brazilian citrus regions. Budwood increasing blocks must be cultivated under the same conditions, resulting in higher nursery tree production costs that limit the use of higher planting densities. This system also limits the selection and multiplication of elite plants and spontaneous mutations observed in the field, which are responsible for most of the diversity found in commercial species, according to Machado et al. (2005). Alternative production systems of protected citrus nursery trees which result in substantial reduction of production costs may contribute indirectly to the confrontation of HLB by enabling ultra high density of new orchards.

a. Seedlings of scion varieties

Citrus are propagated in Brazil through grafting of scion varieties onto seedlings rootstocks. However, the production of seedling-derived nursery trees of scion varieties is the cheapest and simplest method for woody plants multiplication (Hartmann et al., 2002). Citrus usually present polyembriony and nucellar apomixis (Carvalho et al., 2005), allowing to establish and multiply scion varieties directly by sowing. The limiting factor for this procedure is the accentuated juvenile period of citrus, whereas in average, 5 to 8 years are necessary to the beginning of flowering and fruit set in most species. The reduction of the juvenile period by horticultural practices is onerous and usually not persistent (Hackett, 1985), without commercial application until now.

Therefore, two approaches are considered for the production of seedling-derived citrus nursery trees without undesirable juvenile traits. The first one consists on the selection of naturally short-juvenile materials. ‘Tobias’ sweet orange was selected in Rio Grande do Sul State and it blooms and set fruits approximately one year after sowing, being classified as short-juvenile (Donadio et al., 1995). This selection also has horticultural characteristics of commercial interest, and is actually under evaluation at the Instituto Agronômico de Campinas, for the breeding of superior short-juvenile sweet oranges. The second approach aims genetic transformation of commercial cultivars for the early expression of flowering, shortening the juvenile period. Citrus transformation protocols are available from the APETALA1 gene regulation which is involved in the flowering mechanism, enabling its expression prior to one year of age (Cervera et al., 2009). Regardless of the approach, it is suggested that scion materials multiplied by direct sowing should be tested for drought and *Phythophthora* spp. gomosis, because these stresses could be considered major limiting factors to the use of seedling-derived citrus nursery trees (Pompeu Junior, 2005).

b. Intensive nursery tree production systems

The production of smaller citrus nursery trees in intensive systems implies in accentuated decrease of the dimensions of the recipients used for propagation. This condition results in less consumption of potting mix, an important component of production cost. Additionally, higher density of plants and shorter production cycle are attainable in the nursery (Girardi et al., 2007). The availability of less expensive nursery trees enables ultra high planting densities, reduces direct losses from the elimination of trees either by HLB or eradication and still favors resetting of plants in affected areas.

Vegetative propagation methods seem to be the most indicated to attain the intensive system of citrus nursery tree production. In Brazil, mother plants for seed production are usually maintained in open field, exposing the trees to contamination and elimination by the HLB. The transmission of the causal agent of HLB through seeds was reported in low frequency in some species, as well as the decrease in the seed quality of affected mother plants (Albrecht & Bowman, 2009). The direct loss of mother plants and the prevention to possible seed transmission implies in their protected cultivation. Since maintaining adult trees for seed production at high amounts in closed locations is potentially onerous, the establishment of multiplication blocks to obtain cuttings may be a more interesting alternative.

Among the available techniques for the multiplication of commercial nursery trees, it could be pointed out the direct transplanting of scion varieties cuttings (Prati et al, 1999); production of rootstock cuttings previously budded with the scion variety (Mourão Filho et al., 2009; Koller et al., 2000); twig grafting; micrografting and adapted bench grafting (Cruz, 2009). Previous works reported normal development and yield of trees propagated by such methods. An alternative method to be evaluated is micropropagation, especially of rootstocks and clones of short-juvenile scions, considering the need to improve economic viability for commercial use (Murkute et al., 2008) (Figure 11).

Photographs: Eduardo Sanches Stuchi

Figure 11. Sweet orange nursery trees obtained from budded rootstocks cuttings (left), bench micrografting (centre) and rootstock multiplied by micropropagation (right).

c. Large headed nursery trees

All previous concepts of nursery tree production mostly aim at production costs, while growing larger headed nursery trees results in higher costs and individual value of the tree. What would be the advantage of this system? In high density plantings, the implementation of the orchard with larger nursery trees with scaffold and branches anticipates the closing of the trees in the row, leading to a high bearing volume formation at the end of the first year after outplanting (Figure 12). Consequently, commercial yields can be observed in less than two years after planting, anticipating the revenue of the citrus activity. In South Africa, field experiments demonstrated that larger nursery trees accumulated greater amounts of reserve carbohydrates, especially due to the higher leaf area at transplantation, resulting in more intense growth and anticipation of production (Rabe, 2000).

Photograph: Eduardo Augusto Girardi

Figure 12. 'Valencia' sweet orange grafted on Rangpur lime six months after transplantation to the field. Headed nursery trees were 20-months-old and 1 m high with 3 to 5 branches, Piracicaba, SP, 2009.

The critical phase for HLB contamination corresponds to young orchards up to 3rd or 4th year of plantation (Bassanezi & Gottwald, 2009), when commercial yields usually begin in the traditional production system. The use of large headed nursery trees contributes to transpose this critical phase, since its trees will continue to grow in the screen house, under a protected environment until final transplantation to field (Valdés, 2009). During the screened cultivation, there is direct saving with insecticides which would be applied in the plants if they were transplanted to the field instead. However, larger headed nursery trees require additional operations in the nursery, such as change of position to allow their growth, complementary formation prunings, greater consumption of fertilizers during its permanence in the greenhouse and usually recipients with higher capacity; factors which result in higher production costs as mentioned earlier.

Ultra High Density

Nowadays, high density citrus groves in Brazil are established at spacing which varies from 4 to 6 m in the in between rows and 3 to 4 m between trees, corresponding to the interval of 600 to 1250 trees ha^{-1} (Negri et al., 2005; Stuchi, 2007). In ultra high density orchards, plant population is superior to 2000 trees ha^{-1}, reaching sometimes several tens of thousand trees per hectare (Golomb, 1988). The main objective of this system is to anticipate the production (30 to 50 t ha^{-1} $year^{-1}$ in the first harvest periods), determining high yield averages (80 to 120 t ha^{-1} $year^{-1}$) until the tenth harvest (Rabe, 2000).

Ultra high density systems (UHD) evaluated in Israel, United States, Mexico and South Africa presented promising results in arid and subtropical climates and are reference for the ultra high density in Brazil (Rodriguez et al., 1981; Golomb, 1988; Piner, 1988; Wheaton et al., 1990). Also, other fruit crops, such as pears, apples and grapevines, are commercially cultivated with success in other countries under planting densities 3 to 4 times higher in comparison to the beginning of the XIX century (Robinson, 2007). These high density plantings could be carried out due to new technologies of plant training and horticultural practices based on a greater understanding of the morphophysiological aspects of the crops (Costes et al., 2006). Olive orchards, for instance, presented an expressive increase of the yield in ultra-high density systems, moving from 5 t ha^{-1} to 20 t ha^{-1} with about 2500 plants ha^{-1} (Leon et al., 2007; Connor et al., 2009).

Considering that the lifespan of a HLB free orchard in Brazil ranges from 18 to 25 years, reducing from seven to ten years in the presence of HLB even with implemented control measures, the anticipation of high yields may contribute to low risks in citriculture under intense pressure of the disease. Moreover, systematic eradication in ultra high density orchards leads to lower yield losses due to the minor participation of the individual tree production, completely dispensing resetting. For example, a citrus grove with 2000 trees ha^{-1} suffering 15% of eradication until the sixth year of age will result in 1700 plants ha^{-1}. Under adequate management of the psyllid, there are horticultural conditions to record high yields for a longer period of time.

In high density systems, pressurized irrigation either via centre-or lateral-pivot systems may reduce costs of this grove management. Fertigation and especially chemigation becomes interesting, with other options for the psyllid management. Mechanical harvest is also facilitated, on one hand, reducing labor costs in industrial cultivations; on the other hand, demanding the development of new adapted harvesters (Sarig et al., 1988). Finally, under this system, protected cultivation of citrus becomes more cost competitive. Disadvantages related to ultra-high density orchards, besides higher initial investments and adaptation costs to the system, include a probable reduction of lifespan of the orchard due to elevated intraspecific competition for water and other resources (Whitney et al., 1991) and the lack of knowledge regarding the influence of higher planting densities on the epidemiology of the HLB.

In other countries where ultra-high density of citrus was evaluated, the main limiting factor for its implementation was the final size of trees. From the moment in which trees grow and start interfering with each other (an earlier competition as the tree density is higher), an accentuated reduction of individual yield starts and a decrease of total production per area is observed (Wheaton et al., 1984; Muraro et al., 1995; Roka et al., 1997). It is considered that an ideal citrus tree, besides being productive and healthy, should present a reduced final size or suitable to the spacing. Therefore, the application of ultra high densities in citriculture depends on some conditions, as follows:

a. Small-sized scion varieties

The use of small-sized or less vigorous scion varieties favors high density of the orchard. Some commercial scion varieties are naturally small (canopy volume inferior to 25 m^3 in bearing trees), among them, the sweet oranges cultivars 'Folha Murcha', 'Pineapple' and 'Natal Murcha', besides satsuma mandarins like 'Okitsu' (*Citrus unshiu* Marc.) (Pio et al., 2005). The Brazilian 'Cipó' sweet orange variety presents prostrated growth habit, which is an interesting characteristic for plant training, besides good juice quality (Donadio et al., 1995). However, the only dwarf sweet orange variety truly reported is 'Fuya Meñuda', which is originated from the Balearic Islands, Spain (Hodgson, 1967). This last one has potential for processing in spite of its small fruits.

In Bebedouro, São Paulo State, experiments underway at EECB suggest that old clones of sweet orange varieties are smaller than the corresponding nucellar clones and also bear good productions after pathogens indexing. Therefore, cultivation of commercial cultivars would be facilitated under higher planting densities. Dwarf 'Pera' sweet orange clones obtained after induced mutation by gamma rays are under evaluation at Embrapa CNPMF and CENA/USP in the State of São Paulo (Tulmann Neto et al., 1996; Latado et al., 2001). This variety is the main material for juice processing and the most cultivated citrus in Brazil.

b. Dwarfing rootstocks

The use of dwarfing rootstocks, which induce dwarfism to the scion varieties grafted on them, is a method used in the citriculture to propagate commercial scion varieties with intense reduction in the canopy size without affecting other horticultural traits (Oliveira et al., 2008).

The Flying Dragon trifoliate orange [*Poncirus trifoliata* (L.) Raf. var *monstrosa*] is a naturally occurring clone of *P. trifoliata* which induces accentuated dwarfism to most scion varieties (Hodgson, 1967). Scion reduction can reach 300% and canopy volume of bearing trees ranges 4 to 15 m^3, depending on the variety and edaphoclimatic conditions. Consequently, individual yield is decreased, even though high density groves become more practical (> 1000 plants ha^{-1}) leading to elevated annual yield in the orchard lifespan (> 45 t ha^{-1}). Another relevant trait of the Flying Dragon trifoliate is the high yield efficiency induced to the scion varieties, around 8 to 12 kg of fruits m^{-3} of canopy, whereas the same scion varieties present, in average, 3 to 6 kg of fruits m^{-3} of canopy when grafted on more vigorous rootstocks (Castle et al., 2007; Cantuarias-Avilés et al., 2010).

In New Caledonia, in a tropical climate, the dwarfing effects of the Flying Dragon trifoliate were documented on several citrus species, especially on the 'Persian' acid lime (Mademba-Sy et al., 1999). In Brazil, in the Bebedouro region, São Paulo State, Flying Dragon was recommended as rootstock for the 'IAC-5 Persian' lime, leading to smaller size and higher yield in high density orchards in relation to the commonly used rootstocks, *P. trifoliata*, Rangpur lime and Swingle citrumelo (Stuchi et al., 2003; Stuchi & Silva, 2005) (Figure 13). In the same region, research indicated that 'Folha Murcha', 'Hamlin' and 'Natal' sweet oranges and the 'Okitsu' mandarin performed well on Flying Dragon trifoliate orange (Cantuarias-Avilés, 2009; Stuchi et al., 2008) (Figure 14). Since it is a *Poncirus trifoliata* clone, the incompatibility of this rootstock species with certain scion varieties such as 'Pera' sweet orange, should be considered before the graftage (Pompeu Junior, 2005), and the use of interstocks could be evaluated in these cases.

No other dwarfing citrus rootstock varieties of natural occurrence are reported, even though there are differences in the vigor of the scions induced by traditional rootstocks. However, dwarfing rootstocks can be obtained through genetic breeding. Materials developed by conventional crosses, usually with *P. trifoliata* as one of the parents, are highlighted

(Pompeu Junior et al., 2002). Citrandarins [*Poncirus trifoliata* (L.) Raf. x *Citrus reticulata* Blanco] selections and other hybrids controlled size and induced high yield efficiency to 'Valencia' sweet orange in the State of Sao Paulo (Blumer & Pompeu Junior, 2005).

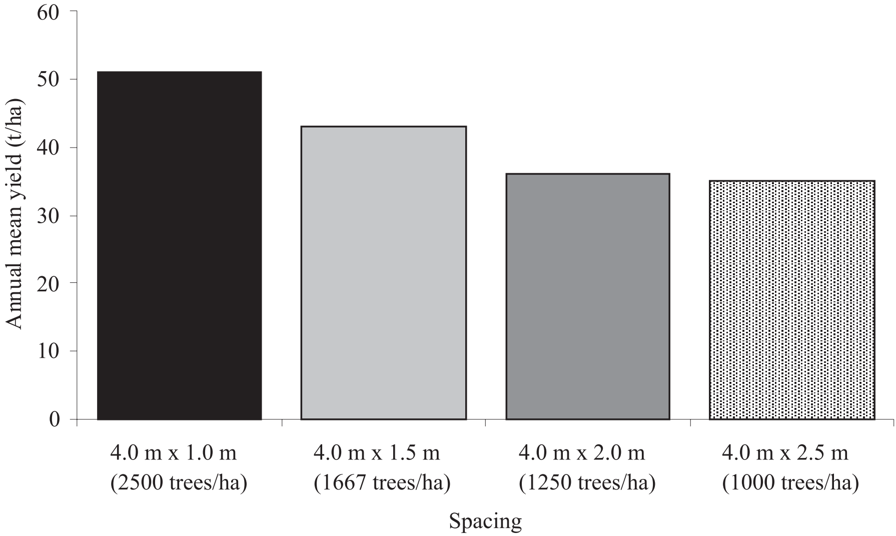


Figure 13. Annual mean yield of 'Persian' lime grafted onto Flying Dragon trifoliate orange in the 2001-2007 period, under four high density plantings. Orchard established in 1994 in Bebedouro, SP, with irrigation initiated in 2001.

Another breeding method for dwarfing rootstocks searches for autotetraploids of commercial rootstocks, such as Rangpur lime and Sunki and Cleopatra mandarins (Silva Júnior, 2008). These materials are promising because the usual rootstock traits (fruit quality and tolerance to biotic and abiotic stresses, precociousness, etc) are preserved while tetraploidy usually confers dwarfism to the clone. Somatic hybridization via protoplast fusion also can be used in the establishment of new dwarfing rootstocks (Grosser et al., 1988), whereas some materials are being evaluated in Bebedouro-SP in the Estação Experimental de Citricultura de Bebedouro - EECB, in a partnership with the University of São Paulo/ESALQ.

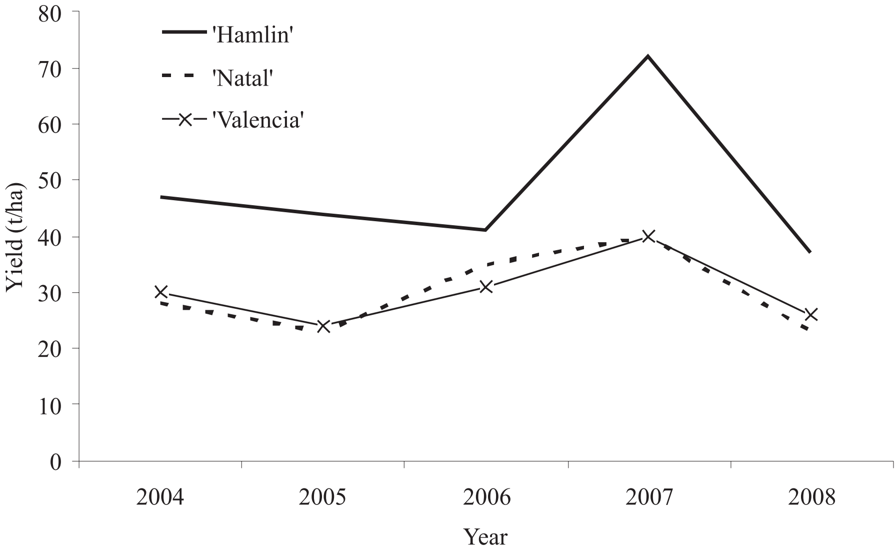


Figure 14. Annual yield of 'Hamlin', 'Valencia' and 'Natal' sweet oranges grafted onto Flying Dragon trifoliate orange in the 2004-2008 period. Orchard was established in 1994 at 4 m x 2 m (1250 trees ha^{-1}), in Bebedouro, SP, and irrigation started in 2002.

Finally, the Citrus Genetic Breeding Program (GBP Citrus) is undergoing, a partnership between Embrapa Cassava & Fruits (CNPMF) and the Instituto Agronômico de Campinas (IAC), with the development of hundreds of new citrus varieties, among them promising dwarfing rootstocks under field evaluation (Figure 15). Besides the use of ultra-high densities of new plantings, the efficacy of systemic insecticides applied on dwarfed trees could be evaluated on these new rootstocks, with possibility to improve chemical control of the vector in the field.

Photograph: Walter dos Santos Soares Filho

Figure 15. 'Valencia' sweet orange grafted on the dwarfing rootstock hybrid *Citrus sunki* x (*Poncirus trifoliata* x *Citrus limonia*), 2.5 years after transplanting in Colombia, SP. Material obtained by the Citrus Genetic Breeding Program at Embrapa Cassava & Fruits in partnership with the Instituto Agronômico de Campinas.

c. Inoculation of viroids

The inoculation of viroids is a method recommended to induce dwarfism in citrus cultivars (Eiras et al., 2009b). Its investigation was carried out mainly in Australia, Israel and Spain, where canopy size reduction ranged 20 to 60 %, without affecting the yield efficiency (Gillings et al., 1991; Semancik et al., 1997; Hutton et al., 2000). The *Citrus Dwarfing Viroid* (CDVd or CVd-III) was isolated from dwarf trees and it is directly associated to this effect without causing any other symptoms in citrus and not even colonizing other hosts. Therefore, this strategy would not represent biological risk to other species or even citrus. On the other hand, there are limitations inherent to the method, such as technical difficulties for isolation, inoculation and establishment of the viroid in certain varieties or species. In Brazil, studies involving the CDVd and other citrus viroids demonstrated the viability of its inoculation to induce dwarfism in ‘Marsh Seedless’ grapefruit (*Citrus paradisi* Macf.) and ‘Persian’ acid lime (*Citrus latifolia* Tanaka) (Stuchi et al., 2007; Eiras et al., 2009a). In South Africa, low incidence of HLB was observed in productive orchards whose trees were previously inoculated with strains of dwarfing viroids, suggesting the possible interference of these microorganisms in the manifestation of the disease (Van Vuuren & da Graça, 2000).

d. Conditioning of nursery trees prior to outplantation

Conditioning of nursery trees is the application of techniques or methods which confer interesting traits to the trees in the nursery, with the possibility to temporarily repeat the treatment after outplantation. When ultra-high density is aimed, desired traits consist basically on promoting precocious flowering and fruit set instead of excessive vegetative growth. This new physiological equilibrium leads to reduced vigor and harvest anticipation.

Techniques recommended to the proposed conditioning include restriction of the root system, use of growth regulators, tree training and deficit irrigation. These practices can be carried out alone or in association.

Restriction of the root system growth is somewhat effective in reducing overall plant vigor, being one of the principles for the bonsai technique (Geisler & Ferree, 1983). It can be achieved by constant pruning of roots or by cultivation in containers, which permanently limit the root system. Such applications are extreme and difficult to manage at field, whereas the restriction via localized irrigation is more appropriate for this situation. Drip irrigation was reported in Israel to concentrate and limit the citrus root system to the wetted volume decreasing the final size of trees (Golomb, 1988). However, similar results should be expected only in arid areas with prolonged drought periods because it is necessary to maintain the dimensioned wetted volume. Another technique which also can be evaluated in tropical regions is the root system twisting of citrus nursery trees prior to outplantation. This technique is practiced in some regions of China where citrus is cultivated intercropped with rice (Aubert, 1989, 1990; Aubert & Vullin, 1998). However, severe twisting and root-binding may lead to deformity of the root system and, consequently, accentuated delay in tree growth (Davies & Ferguson, 2000).

The use of plant growth regulators which inhibit or delay plant growth in citrus has been investigated, although results are inconclusive. Many growth inhibitors are commercially used to reduce growth of ornamental plants in containers, however, in citrus, more satisfactory results were reported with the use of paclobutrazol (PBZ), an inhibitor of gibberellins biosynthesis. Root absorption is more efficient and, therefore, PBZ is applied in the soil or potting mix. Since the effects of the growth regulators are temporary, continuous application is necessary after outplantation in order to control the size of trees. Another disadvantage is the decrease of the fruit size related to the application of PBZ on several citrus species, even though there are no reports of decrease in the total yield (Swietlik & Fucik, 1988).

Tree training is used to alter and artificially conduct the growth habit of the tree, generally with the objective to regulate the balance between vegetative and reproductive growth. It is a usual horticultural practice for many temperate and tropical fruits, but rarely used for citrus. Tree training consists basically on arching the branches and trunks with stakes, wires or even trellising structures in the orchard. Additionally, angle plantation, in which the trunk of the nursery tree delimitates an angle of 30º to 45º with the planting row, can be carried out to lower vigor (Sousa, 1983). Training mandarins on trellising structures just after outplantation led to more precocious and higher yield of high quality fruits (96.3 t ha^{-1} accumulated up to five years of age), in comparison to the absence of tree training (85.4 t ha^{-1} accumulated up to five years of age), besides facilitating the management of ultra high density orchards (2500 to 5000 trees ha^{-1}) (Rabe, 2000). On the other hand, in South Africa, angle plantation of sweet orange nursery trees did not decrease vigor of the trees and the trunk re-established its vertical orientation a few years after outplantation (Piner, 1988).

Finally, citrus nursery trees submitted to deficit irrigation in the nursery were induced to bloom before outplantation. Using decomposed pine bark as substrate, irrigation was suspended until the water potential of the substrate reached the threshold of -25 kPa, when the substrate capacity were restored (Girardi, 2008). The flowering of the scion variety could be observed after deficit irrigation was carried out for four to six months, with successive cycles of irrigation suspension and restoring (Figure 16). Nursery trees which flower earlier can set fruits and yield normally, as long as there is enough leaf area for translocating carbohydrates to the fruits. Early yields contribute to decrease the tree vigor after outplantation resulting in partial size control, as long as deficit irrigation is continued. If desired, vigorous vegetative growth can be restored after adequate management like optimal irrigation, fruit thinning and increased nitrogen fertilization.

Photographs: Eduardo Augusto Girardi

Figure 16. Conditioning of nursery trees for precocious flowering by deficit irrigation in the nursery. To the left, fruit set of 'Persian' lime just after budding on Flying Dragon trifoliate orange rootstock, using a bud eye obtained from the mother plant submitted to controlled water deficit. To the right, 'Valencia' sweet orange budded on Rangpur lime and flowering after deficit irrigation management.

Final comments

Citriculture is one of the main economic activities in Brazil and probably represents a single case of Brazilian incontestable leadership in the agribusiness. It is definitely a legacy of the nation. This position comes from a differentiated competitiveness, which is consequence of a combination of factors such as edaphoclimatic aptitudes, professionalism of growers and of industry entrepreneurs, and excellence of the technical-scientific institutions. The genetic breeding programs and the pest and disease integrated management always surpassed the limitations inflicted on the citriculture in the last century. Perhaps because of this success, studies regarding alternative citrus production systems did not present the same dynamism in Brazil or in comparison to other cultivated species. Nowadays, the great challenge represented by the *Huanglongbing* constrains the viability of citriculture as it is practiced, and demonstrates the need to aggregate different strategies for this disease confrontation: genetic advances, control of both the vector and the pathogen, and new horticultural practices. The solution will not be isolated.

The purpose of this publication is to provoke reflections, instigate discussions and provide citrus researchers that are not very familiarized with the HLB problem with the implications from the pathosystem and, perhaps, stimulate them to develop research based on the information hereby presented.

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