

Review Article

Citrus phytophthora diseases: Management challenges and successes.

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Abstract

Phytophthora spp. are present in nearly all citrus groves in Florida and Brazil and phytophthora-induced diseases, especially foot and root rot, have the potential to cause economically important crop losses. Disease-related losses due to root rot are difficult to estimate because fibrous root damage and yield loss are not always directly proportional. Challenges from phytophthora diseases have been addressed in both countries by enacting phytosanitary requirements for production of pathogen-free nursery trees in enclosed structures, propagated from indexed and certified pathogen-free sources, in conjunction with several other cultural management practices. In Florida groves, a statewide soil sampling program provides growers with soil propagule counts to estimate the damage that *Phytophthora* spp. are causing to fibrous roots. The results can be used along with rootstock tolerance, soils, topography, irrigation, and drainage to make a decision for the need to treat with fungicides in addition to modification of cultural managements. Huanglongbing (HLB), caused by the psyllid-transmitted bacterium *Candidatus Liberibacter asiaticus* (Las), was detected in Brazil and Florida in the mid-2000s. Given the increasing incidence of HLB and deterioration of root density due to Las damage, research experiences and current phytophthora data trends suggest the need for more comprehensive management of root health by reducing the impact of abiotic and biotic stresses, including the interaction with *Phytophthora* spp.

Keywords: Florida and Brazil citrus production, *Phytophthora nicotianae*, *P. citrophthora*, *P. palmivora*, pathogen-free nursery stock, huanglongbing, *Candidatus Liberibacter asiaticus*, interaction of root damage with *Phytophthora* spp.

Introduction

Phytophthora spp. cause the most important soil and water-borne diseases of citrus (Feichtenberger 2001; Feichtenberger et al. 2005; Graham and Menge 1999, 2000). These pathogens are worldwide in distribution and cause significant citrus production losses in the high rainfall subtropics, including the first and second largest citrus production areas in the states of São Paulo, Brazil, and Florida, USA. Losses due to *Phytophthora* spp. may occur in seedbeds from damping-off; in nurseries from foot rot and root rot; in groves from foot rot, fibrous root rot, brown rot of fruit, and from further spread of the pathogen to adjacent fruit in packing boxes.

The most serious disease caused by *Phytophthora* spp. is foot rot, also known as gummosis, where in drier climates the water-soluble gum is not washed from the trunk by rainfall (Graham and Menge 1999). Infection of the scion occurs near the ground level, and produces lesions which extend down to the bud union on resistant rootstocks, or up the trunk into the major limbs of the tree. The cambium and inner bark are damaged and

lesions spread around the circumference of the trunk, girdling the cambium and killing the tree. Nursery trees and young grove trees of small trunk circumference can be rapidly girdled and killed. Large trees also may be killed, but typically the trunks are only partially girdled and the tree canopy displays leaf chlorosis, defoliation, twig dieback, and weak growth flushes. Infection of emerging seedlings by *Phytophthora* spp. causes damping-off. *Phytophthora* spp. can also infect the root cortex and cause decay of fibrous roots. Root rot can be especially severe on susceptible rootstocks in infested nursery soil or on young nursery trees planted into infested soil. In these small trees, loss of significant numbers of roots can result in death of the tree. Root rot also occurs on susceptible rootstocks in fruit-bearing groves where damage rarely kills the tree, but the tree declines in vigor and fruit production. Water and mineral nutrient uptake are impaired, and carbohydrate reserves in the roots are depleted by the repeated attacks. This damage reduces fruit size and yield due to loss of leaves and twig dieback of the canopy (Feichtenberger 1997; Sandler et al. 1989).

Infection of citrus fruit by *Phytophthora* spp. results in brown rot, in which the affected fruit rind is light brown and leathery (Feichtenberger 2001; Graham and Menge 2000). In the grove, fruit on or near the ground become infected when splashed with soil containing the pathogen. If favorable conditions continue the pathogen produces sporangia on the fruit surface. For *Phytophthora* spp. with caducous sporangia, such as *P. palmivora* (Butler) Butler and *P. hibernalis* Carne, sporangia are spread by splash or wind-blown rain to fruit throughout the canopy (Timmer et al. 2000). Most of the infected fruit soon abscise, but those that are harvested may not show symptoms until after they have been held in postharvest storage for a few days. Brown rot epidemics are usually restricted to areas where rainfall coincides with the early stages of fruit maturity. All citrus cultivars are affected, especially early season oranges and lemons.

Causal *Phytophthora* spp.

The most widespread and important *Phytophthora* spp. are *P. nicotianae* Breda de Haan (synonymous with *P. parasitica* Dast.) and *P. citrophthora* (Sm. & Sm.) Leonian (Feichtenberger 2001; Graham and Menge 2000). *P. nicotianae* is the most common species occurring in subtropical areas of the world, and causes foot rot and root rot but usually does not infect far above the ground. *P. palmivora* is highly pathogenic on roots under certain stress conditions in the subtropics and tropics, and is the common cause of brown rot epidemics in Florida (Graham et al. 1998; Zitko and Timmer 1994). *P. citrophthora* causes both gummosis and root rot; it also attacks aerial parts of the trunk and major limbs. It is the most common cause of brown rot in Brazil (Feichtenberger 2001) and in Mediterranean climates, though brown rot is also caused by *P. hibernalis* in the latter.

Estimation of crop losses from phytophthora diseases

Phytophthora-induced diseases are economically important in all citrus-growing regions, but worldwide losses due to *Phytophthora* spp. are difficult to assess accurately (Feichtenberger 2001; Graham and Menge 1999). These pathogens are present in nearly all groves in Florida and Brazil, and in Florida cause substantial root damage in an estimated 8% to 20% of the groves. Disease losses due to root rot are difficult to evaluate, because the relationship between root damage and yield loss is not strictly proportional (Graham and Kosola 2000). Nevertheless, yield losses in Florida from fibrous root rot and foot rot have been estimated to range from about 3% to 6% per year, or \$30 to 60 million, without control treatments (Graham and Menge 1999). These losses do not include yield losses due to brown rot, which varies widely with weather conditions from year to year. Overall, losses due to *Phytophthora* spp. are much more prevalent in some years in certain locations, because these

diseases are particularly damaging under wet or flooded conditions.

Citrus nursery production systems: overcoming threats from pathogens and their insect vectors

The incidence of foot rot and root rot in Florida and Brazil in new citrus plantings was high in the 1980s and 1990s due to the common use of nursery trees infected by mainly *P. nicotianae* (Feichtenberger 2001; Feichtenberger et al. 2005; Zitko et al. 1987). Contamination of field citrus nurseries by *P. nicotianae* was quite common at that time, as confirmed by a survey of Florida nurseries in the 1980s (Zitko et al. 1987) and nurseries in the state of São Paulo in the 2000s (Feichtenberger et al. 2003; Salva 2004). Very often phytophthora contamination could be attributed to surface sources of infested water used for irrigation or inundation by run-off from adjacent production groves proximal to nurseries, especially in the case of outdoor seed beds. In addition, there was widespread use of infected rootstock seedlings for budding scions in both field and container production systems in Florida (Graham and Timmer 1992; J Graham, unpublished). This was also confirmed by an extensive survey of seedbeds in the state of São Paulo in 2001, wherein *Phytophthora* spp. were detected in 18.7% of 48 seedbeds surveyed (Feichtenberger et al. 2003; Salva 2004).

In the early 1990s, 50% of the trees propagated in Florida nurseries were produced in containers, while 50% were produced in the field; the latter with minimal sanitation and heavy reliance on the fungicide metalaxyl for phytophthora control (Timmer et al. 1998). Failure to control phytophthora foot rot on recently planted trees in the fall of 1992 led to surveys of the nursery situation in 1993 (Coleman 1993; Fisher 1993). *P. nicotianae* was detected in 9 operations, and metalaxyl-resistant strains were detected in 8 of 14 nurseries surveyed. As concern for the widespread occurrence of resistance grew, a larger follow-up survey of 41 field nurseries and 22 greenhouse operations was conducted by the fungicide manufacturer (Novartis in the 1990s, now Syngenta Crop Protection). Of the field nurseries surveyed, 39 were positive for *P. nicotianae*; 21 of which harbored metalaxyl-resistant *P. nicotianae* strains. Among greenhouse operations, 17 of 22 were positive for *P. nicotianae* and 10 of these showed some level of resistance. The survey also established that metalaxyl-resistant isolates were widely disseminated into Florida citrus groves in new plantings and replants in existing groves. The relative competitive ability of resistant and sensitive isolates showed at least some metalaxyl-resistant isolates were able to compete favorably with sensitive isolates in the absence of metalaxyl (Graham et al. 1998). In a field trial, a high percentage of the population remained resistant to metalaxyl even after 2.5 years without treatment with this fungicide. Nurseries with any level of metalaxyl resistance detected by extensive survey were advised to: (1) discontinue use of metalaxyl, (2) destroy infested

nursery stock, (3) fumigate nursery beds, (4) fallow for one year, and (5) continue monitoring for metalaxyl-resistant isolates. Fosetyl-Al (Aliette; Bayer Crop Science) was recommended for use where metalaxyl-resistant populations were established in citrus groves because no cross resistance to fosetyl-Al was detected (Coleman 1993). By 1998, most of Florida's nursery tree production was in containers, and only occasional metalaxyl resistance has been detected in nurseries: less than 2% of groves surveyed have detectable resistance (Graham 2003).

In 2000, São Paulo State had 1,817 registered field seedbeds and nurseries producing 12.6 million citrus seedlings and 16.1 million nursery trees (Fundecitrus, 2000). In 267 of the field nurseries surveyed in 2000, *P. nicotianae* was detected in 43.8% (Feichtenberger et al. 2003; Salva 2004). In 1999, a new citrus nursery tree certification program was established in São Paulo, and became mandatory in 2001. The program was primarily implemented to prevent dissemination of the citrus variegated chlorosis (CVC) pathogen, *Xylella fastidiosa*, by nursery trees. The production of disease-free nursery trees was based on indexed and certified pathogen-free sources propagated in screened facilities to exclude sharpshooter vectors of *X. fastidiosa*. This program also required the production of nursery trees free of *Phytophthora* spp., nematodes, and other systemic pathogens harmful to citrus, as well as the citrus canker pathogen *Xanthomonas citri* subsp. *citri*. Later in 2004, after the detection of Huanglongbing (HLB) in São Paulo, the program was updated to include the requirement that nursery trees be free from the HLB pathogens *Candidatus Liberibacter asiaticus* (Las) and *Candidatus L. americanus* (Lam), as well as the insect vector *Diaphorina citri*.

Discovery of HLB in Florida in August 2005, led to similar rapid and dramatic changes in nursery production in 2006 to 2007. New nursery stock production regulations took effect January 2007. New citrus nurseries were required to be located at least one mile away from commercial citrus groves to give a degree of separation from disease inoculum sources. Existing citrus nurseries were not required to relocate; however, total enclosure of stock was required along with several other sanitary measures.

Nursery regulations in Florida and Brazil currently require the production of nursery trees free from Las, Lam, *X. fastidiosa*, *X. citri* subsp. *citri*, *Phytophthora* spp., parasitic nematodes, citrus viruses, viroids, and pests harmful to citrus. To achieve exclusion, several measures are mandatory including:

1. Production of rootstock seedlings and nursery trees in facilities with a plastic top cover and screened openings to prevent entry of psyllid, sharpshooter, and aphid vectors.
2. Use of propagative materials from indexed and certified pathogen-free sources maintained in screened facilities.

3. Use of phytophthora-free well water to irrigate the plants.
4. Site selection to avoid runoff from surrounding areas entering the nursery facility.
5. Fencing and copper foot baths at the entrances.
6. Personnel, clothes and shoes, vehicles, equipment, and tools are washed thoroughly and disinfested before entering the area through restricted entrances.
7. Rootstock seeds from fruits treated at 52 °C for 10 min to eliminate seed-borne phytophthora.
8. Plants grown in containers with soilless potting mix free of *Phytophthora* spp., nematodes, and other pathogens harmful to citrus.
9. Propagation carried out on benches at least 40 cm above ground level.

Other sanitary measures recommended in nursery operations included frequent washing and disinfestation of the floor, wall, and benches; as well as prompt rouging of diseased or abnormal trees.

In São Paulo, as part of a cooperative project including the State Department of Agriculture and Fundecitrus (Fundo de Defesa da Citricultura), screened nursery facilities have been surveyed for *Phytophthora* spp. yearly (Table 1). Samples are assayed for *Phytophthora* spp. by baiting with 'Siciliano' lemon (*Citrus limon* L. Burm) leaf pieces (Grimm and Alexander 1973). *P. nicotianae* was detected in 54% of the nurseries and in 25.9% of the samples collected in 2000; in 49% of the nurseries and 14.7% of the samples in 2001; in 43% of the nurseries and 9% of the samples in 2002; and in 26.1% of the nurseries and 4.9% of the samples in 2003 to 2004 (Fig. 1) (Feichtenberger et al. 2003; Salva 2004).

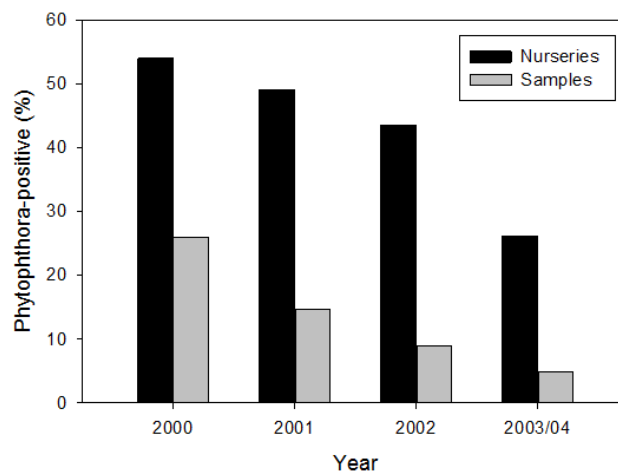


Fig.1. Percentage of phytophthora-positive citrus nurseries and samples from nurseries in São Paulo State, Brazil, from 2000 to 2004.

As expected, the nursery certification program in São Paulo has been instrumental for reducing the incidence of phytophthora diseases in new plantings (E Feichtenberger, unpublished). However, in 2008 an

increase in phytophthora incidence in citrus nurseries occurred as nurserymen were less vigilant in their sanitation practices because the inspections by the state plant protection regulatory agency were relaxed. This occurred after the detection of HLB in São Paulo in 2004. As HLB rapidly became the most important problem facing the São Paulo citrus industry, the federal rules for HLB eradication required more inspections of groves by the state regulatory agency. As a result, less attention was given to phytophthora sanitation measures required by the nursery certification program. The increase in disease incidence in nurseries observed in 2008 (Table 1) led to an increase in the incidence of phytophthora diseases in new plantings in the southern region of the state. In this region, citrus groves were being established in areas that had not been planted with citrus previously. Thus, the occurrence of *P. nicotianae* in these new plantings was probably due to the use of phytophthora-contaminated nursery trees. Based on the results of the survey program, the State Department of Agriculture strengthened exclusion measures by increasing the number of inspections of citrus nurseries. As a result, the incidence of *Phytophthora* spp. in nurseries dropped in 2012 and 2013 (Table 1), as confirmed by results from surveys conducted by the Research Laboratory of Sorocaba (E Feichtenberger, unpublished) and the Citrus Center “Sylvio Moreira” (H Coletta-Filho, unpublished).

Table 1

Incidence of samples positive for *P. nicotianae* from citrus nurseries in São Paulo State from 2003 to 2013. Samples processed in the Sorocaba Research Laboratory and the Citrus Center Sylvio Moreira at Cordeirópolis, São Paulo State Department of Agriculture.

Year	Number of samples	Number of positive samples	Positive samples (%)
2003	6672	228	3.4
2004	14595	297	2
2005	12723	309	2.4
2006	10178	243	2.4
2007	12402	221	1.8
2008	13459	433	3.2
2009	5806	133	2.3
2010	4433	101	2.3
2011	5272	118	2.3
2012	5818	64	1.1
2013	4202	28	0.77

Rootstock resistance/tolerance: the foundation for management of phytophthora diseases

Resistant rootstocks are the best solution for the control of phytophthora diseases. However, some highly resistant rootstocks are susceptible to other diseases, are incompatible with some commercial scion cultivars, are horticulturally unacceptable, or are not adapted to particular soil conditions such as high pH, calcium

bicarbonate, and poorly or excessively drained soils (Bright et al. 2004; Feichtenberger et al. 1992; Graham 1993). All commercial scion cultivars are susceptible to bark infection, but several scion-rootstock combinations are at least moderately resistant to bark infection by *Phytophthora* spp. (Feichtenberger et al. 1992). However, rootstocks vary widely in their susceptibility to root rot depending on the predominant *Phytophthora* spp. present (Table 2).

Table 2

Differential susceptibility of the major rootstocks planted in Florida and Brazil to root rot caused by *P. nicotianae* and *P. palmivora*.

Rootstock	<i>P. nicotianae</i>	<i>P. palmivora</i>
Cleopatra mandarin - <i>C. reticulata</i> Blanco	Susceptible	Tolerant
Sunki mandarin - <i>C. reticulata</i>	Susceptible	ND*
Rangpur lime - <i>C. limonia</i> Osbeck	Susceptible	ND*
Sour orange - <i>C. aurantium</i> L.	Susceptible	Tolerant
Volkamer lemon - <i>C. volkameriana</i> Pasq.	Tolerant	Tolerant
Trifoliolate orange - <i>Poncirus trifoliata</i> (L.) Raf	Resistant	Susceptible
Carrizo citrange - <i>C. sinensis</i> (L.) × <i>P. trifoliata</i>	Tolerant	Susceptible
Swingle citrumelo - <i>C. paradisi</i> Macf. × <i>P. trifoliata</i>	Moderately Resistant	Susceptible

* Not Determined

All rootstocks are affected by root rot following artificial inoculation or in grove soils. Root rot resistance to *Phytophthora* spp. has not been clearly defined (Widmer et al. 1998). Certain rootstocks are considered resistant because roots become infected but do not rot, while others are classified as tolerant because they generate new roots to maintain root mass density in phytophthora-infested soil (Graham 1995; Kosola 1995). Young fibrous roots of most rootstocks support equally high populations of *Phytophthora* spp. However, as roots of resistant or tolerant rootstocks age, the pathogen population declines in rhizosphere soil whereas populations are sustained on susceptible rootstocks (Graham 1995). Sour orange and Cleopatra mandarin are susceptible to root rot caused by *P. nicotianae*, whereas trifoliolate orange and its hybrids Swingle citrumelo and Carrizo citrange are resistant and tolerant of root rot, respectively (Table 2; Graham 1995). Conversely, trifoliolate orange, Swingle citrumelo, and Carrizo citrange are susceptible to *P. palmivora*, while sour orange and Cleopatra mandarin are tolerant (Bowman et al. 2003; Graham 1995). Volkamer lemon is tolerant of root rot caused by *P. palmivora* and *P. nicotianae*.

Tolerance to root pests and the role of rootstock resistance to *Phytophthora* spp.

Diaprepes abbreviatus (Coleoptera: Curculionidae) is a polyphagous root weevil that attacks *Citrus* spp. and other agricultural crops and was introduced into Florida in 1964 from the Caribbean Basin. Brazil has similar

Curculionidae on citrus, *Naupactus* spp. and *Parapantomorus fluctuosus*, but their impact on citrus production has not been assessed. In Florida, diapaese root weevil (DRW) has been dispersed primarily by nursery stock and now infests more than 66,000 hectares of commercial agriculture, including approximately 12,000 hectares of citrus (Hall 2000). Larvae of DRW feed on all commercial rootstocks and, at later developmental stages, can strip the bark from the taproot and structural roots causing girdling and eventual death of trees.

As DRW infestations have grown in scope over the last 4 decades, citrus production managers noted that trees at lower elevations and in wetter areas of the groves were the first to decline. Trees on rootstocks such as sour orange or Cleopatra mandarin, which are susceptible to *P. nicotianae*, declined more rapidly than in adjacent groves on rootstocks more resistant to this pathogen, like Swingle citrumelo (Graham 2000). Conversely, on the east coast of Florida in poorly drained, high pH soils with high calcium bicarbonate content, trees on Swingle citrumelo were more severely declined than those on Cleopatra mandarin and sour orange. Severity of root damage by the complex between *Phytophthora* and *Diaprepes* (PD complex; Graham et al. 1997) was not due to differences in susceptibility to larval feeding since root damage to Cleopatra mandarin and the trifoliolate hybrid rootstocks, Swingle citrumelo and Carrizo citrange, is similar (Graham et al. 2003; Rogers et al. 2000).

Greenhouse studies confirmed that larval feeding predisposed fibrous roots of seedlings of Cleopatra mandarin to more severe infection by *P. nicotianae*, and of trifoliolate orange to more severe infection by *P. palmivora* (Rogers et al. 1996; Graham et al. 2003; Rogers et al. 2000). More severe infection by these *Phytophthora* spp. resulted in greater root damage and higher populations of the pathogens in the rhizosphere. The most severe damage was encountered when *P. palmivora* was the predominant pathogen in the PD complex with DRW (Graham 2000).

The *P. palmivora*-*Diaprepes* complex was associated with fine-textured, poorly drained soils on rootstocks normally resistant or tolerant of *P. nicotianae*, i.e., Swingle citrumelo and Carrizo citrange. A field trial with Flame grapefruit was planted in May 2000 at a site affected by *P. nicotianae*, *P. palmivora*, and *D. abbreviatus* (Bowman 2003). The trial contained advanced rootstock selections from the USDA Horticultural Research Lab (USHRL) in Ft. Pierce, Florida, as well as Swingle citrumelo, Carrizo citrange, and Cleopatra mandarin. Soil types in the trial area were fine textured sands with high soil conductivity and calcareous deposits, and nearby trees were heavily infested by DRW. Trees in the trial were inoculated at the time of planting with roots showing characteristic symptoms of phytophthora infection from the nearby trees. After 24 months, a strong correlation was confirmed between tree size and the *Phytophthora* spp. populations on roots (Fig. 2). After 36 months, trees on US-802, US-

942, US-897, and Cleopatra mandarin were apparently healthy and vigorous, while trees on Swingle citrumelo, Carrizo citrange, and some other USHRL rootstocks were small and weak (Bowman 2003). Differences among the rootstocks were related to their ability to tolerate the PD complex because the poorest performing rootstocks supported the highest soil populations of *P. nicotianae* and *P. palmivora*. Thus, in this site, rootstock susceptibility to *Phytophthora* spp. was an important predictor of tree performance. The most tolerant rootstocks to PD complex were released by USDA; these are US-802 and US-897. US-897 is a hybrid of Cleopatra mandarin with trifoliolate orange that is tolerant of PD complex and provides tree-size control for higher density plantings (Bowman et al. 2008). Also tolerant of PD complex is US-802, a hybrid of pummelo (*C. grandis* L. Osbeck) and trifoliolate orange that produces large size trees with high production (Graham and Menge 2000). A larger group of pummelo or mandarin hybrids have shown the most promise as rootstocks with greater tolerance to *Phytophthora* spp. and DRW (Graham et al. 2007; Grosser 2007).

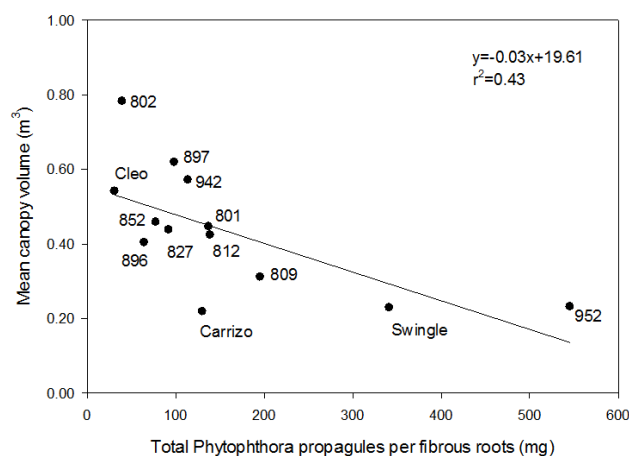


Fig. 2. Comparison of canopy volume for commercial rootstocks and USHRL hybrids, and total *Phytophthora* spp. recovered from the rhizosphere per milligram of roots 2 years after planting in a site with adverse soil types and infested with *Diaprepes abbreviatus* root weevil in Vero Beach, Florida. Swingle citrumelo (*Citrus paradisi* x *Poncirus trifoliata*), Carrizo citrange (*C. sinensis* x *P. trifoliata*), Cleopatra mandarin (*C. reticulata*), US-801 (*C. reticulata* 'Changsha' x *P. trifoliata*), US-802 (*C. grandis* 'Siamese' x *P. trifoliata*), US-809 (*C. reticulata* 'Changsha' x *P. trifoliata*), US-812 (*C. reticulata* 'Sunki' x *P. trifoliata*), US-827 (*C. limonia* Osbeck x *P. trifoliata*), US-852 (*C. reticulata* 'Changsha' x *P. trifoliata*), US-896 (*C. reticulata* 'Cleopatra' x *P. trifoliata*), US-897 (*C. reticulata* 'Cleopatra' x *P. trifoliata*), US-942 (*C. reticulata* 'Sunki' x *P. trifoliata*), US-952 (*C. paradisi* x *C. reticulata*) x *P. trifoliata*.

Biomonitoring in support of disease management decisions

Biomonitoring is necessary to know whether *Phytophthora* spp. are present and, if so, in what quantities likely to cause damage. Fruit baiting and leaf baiting are used for detection of *Phytophthora* spp.

(Grimm and Alexander 1973). These assays are relatively simple and require minimal equipment and supplies, but are qualitative. For quantitative measurement of propagule density in soil, selective culture media have been developed for the isolation of *Phytophthora* spp. (Timmer et al. 1988). Methods of sample collection and handling are standardized, so that propagule counts can be compared throughout a citrus-growing area. Propagule density is highest where the fibrous root density is greatest. Thus, populations diminish with depth and distance from the tree. For routine determinations of propagule density in soils, samples are collected at random in the grove (Timmer et al. 1989).

Small amounts of soil containing fibrous roots are collected from 20 to 40 locations within a 4 hectare area. Samples are taken under the canopy, within the drip line of the tree, or near irrigation emitters where roots are most numerous. The samples are combined in a resealable plastic bag to retain soil moisture and kept cool for transport to the laboratory. The same soil sample can also be tested for populations of pathogenic citrus nematodes, *Tylenchulus semipenetrans* and *Pratylenchus jaehni*. Propagule densities vary seasonally and from year to year. In most Florida groves the density ranges from 1 to 20 propagules/cm³ of soil, but it occasionally reaches 100 to 200 propagules/cm³. Precise thresholds for damaging populations of the pathogen are difficult to establish, but populations of less than 5 propagules/cm³ are considered insignificant, and populations in excess of 10 to 20 propagules/cm³ are considered potentially damaging (Graham and Menge 1999).

Every season for over 25 years, Syngenta, the manufacturer of Ridomil® fungicide, has conducted a soil sampling program in Florida which provides the growers with soil propagule counts to estimate the damage that *Phytophthora* spp. are causing to fibrous roots. The results can be used in conjunction with other factors including rootstock resistance, soils, topography, irrigation, and drainage to make a decision for the need to treat with fungicides in conjunction with changes in cultural managements.

Cultural management precedes the use of fungicides

Management of foot rot in young groves

In groves with a history of root rot and foot rot, resistant rootstocks are recommended for replanting. Although use of clean nursery stock is now mandatory in Florida and Brazil, sampling for pathogen detection along with inspection of the taproot and fibrous roots of nursery stock before planting is recommended to avoid root health problems in the new plantings. In existing groves, replant sites can be sampled for pathogen populations to determine disease potential. Where foot rot epidemics occur, soil samples should not be collected beneath severely declining trees, because fibrous roots supporting *Phytophthora* spp. will have died, and the pathogen population may be small or non-detectable. Except in the case of susceptible rootstocks, foot rot on young trees can

be alleviated by cultural practices including: (1) budding of rootstock seedlings well above the soil, (2) planting with the bud union well above the soil line to avoid contact between the susceptible scion bark and infested soil, and (3) providing adequate soil drainage (Feichtenberger 2000; Feichtenberger et al. 2005; Graham et al. 2011; Graham and Menge 1999).

If cultural controls are inadequate to control foot rot in young trees, chemical control may be warranted during initial stages of grove establishment. The use of post plant fungicides, i.e., metalaxyl and fungicides containing phosphite (PO₃³⁻), in young groves should be determined by: rootstock susceptibility, the likelihood of infestation in the nursery, and the history of phytophthora diseases at the site. Fungicide treatments should commence after foot rot lesions develop. Fungicides applied as trunk paints or sprays are the most effective; applications to foliage or the soil surface are less effective against foot rot. The fungicide program should be conducted for at least one growing season for tolerant rootstocks, and may continue beyond the first season for susceptible rootstocks. Both metalaxyl and phosphites are effective when applied at recommended rates, so alternating use of fungicide should be considered to minimize the risk the development of pathogen resistance (Feichtenberger 1990; Feichtenberger 1997; Graham et al. 2011).

Management of root rot in mature groves

Once a grove matures and begins to bear regular crops of fruit, foot rot is usually no longer a serious problem. Loss of fibrous roots due to *Phytophthora* spp. may still produce tree decline and reduced yields (Salva 2004). *Phytophthora* populations usually remain low on resistant rootstocks, and probably little fibrous root loss is incurred unless groves are established on soils ill-suited for the rootstocks (e.g., soils with high pH and bicarbonate, or high clay content, etc.). Damage to fibrous roots is difficult to assess directly. Where the rooting depth of citrus is limited by a high water table and restrictive soil layers, root damage occurs in saturated soil. Under conditions favoring the pathogen, *Phytophthora* spp. infect fibrous roots within hours and completely destroy roots within 4 to 6 weeks (Graham and Kosola 2000). Proper drainage and irrigation management are essential for regeneration of replacement roots. If populations of *Phytophthora* spp. are at damaging levels, i.e., more than 10 to 20 propagules/cm³ soil, they will increase in number during wet periods as a result of increased root infection (Graham and Menge 1999).

In areas with poor drainage or a high water table, the first step toward controlling the pathogen is installation of drainage tile to provide additional internal drainage in the soil profile and proper maintenance of drainage ditches (Graham and Menge 1999). If fibrous root losses are not attributable to direct damage due to wet conditions, then soil populations should be monitored prior to and after treatment with fungicides. Fungicide applications are based on periodic soil sampling to indicate whether damaging populations of *Phytophthora* spp. are present in

successive growing seasons. A fungicide program should be based on seasonal applications during susceptible root flushes in the spring (after the spring leaf flush) and fall. Application of fungicides to the soil surface should be directed at areas of highest fibrous root density. In groves where damaging populations of both the citrus nematode (*T. semipenetrans*) and *Phytophthora* spp. are present, management of both pathogens will only marginally increase yields over those obtained with management of only one of the pathogens (LW Duncan and J Graham, unpublished). The lack of additional response to treatment with nematicide plus fungicide is apparently due to the release of phytophthora activity from interference by the citrus nematode (El-Borai et al. 2002). As of 2014 in Florida, given their cost and efficacy, the use of registered fungicides is favored over nematicide treatment.

Management of brown rot in mature groves

In Florida and Brazil, brown rot is a localized problem associated with restricted air and/or water movement in the grove (Feichtenberger 2001; Graham et al. 2011). It commonly appears from late summer to early fall following periods of extended high rainfall, hence brown rot can be confused with fruit drop due to other causes at that time of the year. If caused by *P. nicotianae* or *P. citrophthora*, brown rot is limited to the lower third of the canopy because pathogen propagules are splashed onto fruit from the soil. In contrast, if caused by *P. palmivora* or *P. hibernalis*, brown rot may occur throughout the canopy due to splash and wind-blown rain dispersal of propagules and caducous sporangia (Graham et al. 1998; Timmer et al. 2000).

Early season inoculum production and spread of *Phytophthora* spp. are minimized with key modifications in cultural practices. Skirting of the trees reduces the opportunity for soil-borne inoculum to come into contact with fruit in the canopy. In Florida, boom application of herbicides and other operations dislodge low-hanging fruit. Fruit on the ground become infected and produce *P. palmivora* inoculum that can result in brown rot infection in the canopy in mid-summer while fruit are still green. The beginning stages of the epidemic are very difficult to detect before the fruit are colored and showing typical symptoms (Timmer 2014).

Usually a single application of a phosphite fungicide before the first signs of brown rot appear in the grove is sufficient to protect fruit through most of the normal infection period (Graham and Dewdney 2014). Phosphites are highly systemic, moving both acropetally and basipetally in the tree (Graham 2011). They provide 60 to 90 days control and protect against postharvest infection, but are marginally effective when applied post infection. Copper fungicides are capable of killing propagules and sporangia on the fruit surface, and may be applied after brown rot appearance and provide protection for 45 to 60 days. Because phosphites have also been reported to have effects on citrus fruit set and quality, sprays are widely used by Florida and Brazilian citrus growers even though phosphites do not act as a nutritional source of

phosphorus (Orbovic 2008). Nevertheless, many phosphite nutritional and fungicidal products are marketed throughout the world (Thao and Yamakawa 2009); hence phosphites are relatively inexpensive. Large scale use of phosphites has led to the virtual disappearance of brown rot despite the presence of *Phytophthora* spp. in grove soils.

The HLB challenge: *Phytophthora* interaction with *Candidatus Liberibacter asiaticus*

In the mid-2000s, the most destructive disease of citrus world-wide was discovered in São Paulo State, Brazil, and in Florida shortly thereafter. HLB, caused by Las in Florida and Las and Lam in Brazil, is spreading rapidly (Gottwald 2011; Gottwald and Graham 2014). No sources of HLB resistance in citrus scions or rootstocks are known and no readily applicable measures exist for control of the tree's decline once infected. The only proven approach for HLB control is an integrated program of removing diseased tree inoculum (rouging) and application of insecticides to control the vector, Asian citrus psyllid. HLB reduces yield, soluble solids, and quality of orange juice. As the Florida and Brazilian citrus industries move into the future, both face the challenge from HLB and other exotic citrus pests and diseases to maintain economical production of citrus fruit. For these citrus industries to sustain profitability, a collaborative production and marketing strategy will be essential (Timmer et al. 2011).

HLB was first found in Florida in late 2005 and is now widely distributed throughout the commercial citrus-growing regions. Survey data for 2009 indicated that the cumulative incidence of infected trees was in the range of 8% to 10% statewide, but in 2010 climbed to 18% and as of 2014 is approaching 100%. When HLB was first discovered, the Florida citrus industry adopted the recommended practices including control of the insect vector, use of disease-free planting material, and the removal of infected trees to lower the inoculum load (Gottwald and Graham 2014). However, as HLB infection increased in groves, many growers began moving to alternative treatments. Currently, most of the industry has stopped removing trees and, as an alternative practice, has adopted nutritional programs (Timmer 2014). This management scenario has heightened awareness of horticultural practices to sustain tree health.

Unfortunately, the management of HLB with such practices is more complex because Las infects all parts of the citrus tree including the roots (Tatineni et al. 2008). Root sampling of Las-infected trees in Florida demonstrated that root dieback occurs before visible HLB symptoms in the canopy (Graham et al. 2013). Asymptomatic trees with detectable Las in fibrous roots already have massive fibrous root loss (Johnson, Gerberich, et al. 2014). The order of magnitude of root loss due to HLB was equal to or greater than 30% in surveys of both young trees (3 to 4 years old) and older trees (10 to 25 years old) (Graham et al. 2013). In some

locations, higher *P. nicotianae* per root, as well as phytophthora populations per cubic centimeter of soil, were detected on Las (+) compared to Las (-) trees. Fibrous root loss from HLB damage appeared to interact with *P. nicotianae* depending on grove location and time of year.

Studies in Taiwan (Ann et al. 2004) and Florida (Wu et al. 2014) suggest that increasing incidence of HLB in citrus groves in the presence of *P. nicotianae* may have a greater impact on fibrous root health than that caused by the Las pathogen alone. Prior infection of roots by Las accelerates phytophthora infection and damage of fibrous roots of potted seedlings and trees. The Las-induced predisposition of roots to *P. nicotianae* is apparently caused by a greater attraction of swimming zoospores to roots, acceleration of infection, and less resistance to root invasion. However, the Las-phytophthora interaction may not ultimately promote more severe root damage than Las alone, but accelerate root turnover (Johnson, Wu, et al. 2014).

Survey data from Florida groves also suggests a resistance-breaking interaction of Las with *Phytophthora* spp. Because Syngenta Crop Protection’s statewide survey of *Phytophthora* spp. spans over 2 decades, covers all production areas, and is largely driven by grower requests, the results serve as an indicator of emerging disease trends. Comparison of the survey data for seasons since HLB became widespread in Florida groves shows a strong trend toward higher incidence of damaging phytophthora populations coincident with the rise in HLB disease incidence (Fig. 3A, Fig. 3B). Most recently there has been a strong downturn in the populations apparently associated with a loss of fibrous root density as trees continue to decline from HLB (Fig. 3C). The survey has created heightened concern for root health of HLB-affected trees and initiation of measures to reduce root stress as well as *Phytophthora* spp., citrus nematodes, and DRW. The most important root stress identified in Florida groves is bicarbonates in irrigation water and soil liming with dolomite which directly reduce the ability of trifoliolate hybrid rootstocks, Swingle citrumelo and Carrizo citrange, to take up important nutrients (Ca, Mg, and Fe). Bicarbonates and associated elevated pH have also been identified as a major factor predisposing roots to infection and damage by *Phytophthora* spp. and other root pests (Graham et al. 2014).

Past research experiences and current phytophthora data trends indicate a need for more comprehensive management of HLB-affected trees. Fibrous root health is fundamental to sustain soil, water, and nutrient uptake; tolerance of marginal soils; fluctuations in soil moisture; root pests; and other adverse conditions. Symptoms of stress intolerance are off-colored foliage and excessive leaf and fruit drop of HLB-affected trees, even when trees been managed under intensive nutritional programs for several seasons. Preliminary data indicate that fungicides may be reduced in their effectiveness for control of *Phytophthora* spp. and prevention of root loss because

Las infection is the major contributor to damage of co-infected roots (Johnson Gerberich, et al. 2014).

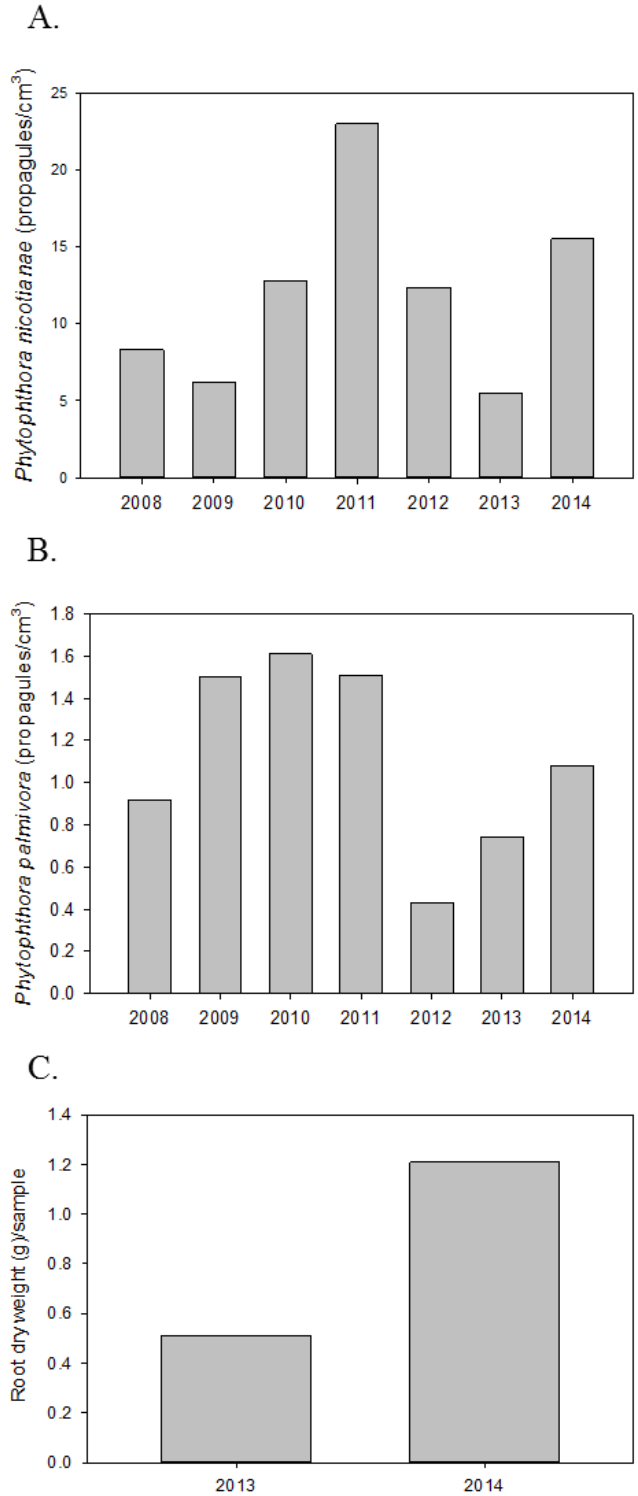


Fig.3. Florida survey for (A) propagules of *Phytophthora nicotianae* and (B) propagules of *P. palmivora* in rhizosphere soil samples collected in Florida groves between May and December from 2008 to 2014. (C) Dry weight of fibrous roots in soil samples collected between May and December 2013 and 2014 (data courtesy of JB Taylor, Syngenta Crop Protection).

Conclusions

Phytophthora spp. are present in nearly all groves in Florida and Brazil, and phytophthora-induced foot and root rot diseases have the potential to cause economically important crop losses. *Phytophthora* spp. are much more prevalent in some years in certain locations, because these diseases are particularly damaging under wet or flooded conditions. Disease losses due to root rot are difficult to estimate as fibrous root damage and yield loss are not always directly proportional. Florida and Brazil have successfully addressed challenges from phytophthora diseases primarily by enacting phytosanitary requirements for the production of pathogen-free nursery trees free of systemic pathogens and their vectors that pose considerable threats to the citrus industries. In the process of implementing mandatory requirements, *Phytophthora* spp. have been greatly reduced and the production of clean stock has resulted in a low prevalence of phytophthora disease in newly established groves.

The best solution for endemic *Phytophthora* spp. has been and will continue to be resistant rootstocks for minimizing risk of phytophthora disease-related losses. Most rootstocks are at least moderately resistant to bark infection, but vary widely in their susceptibility to root rot depending on the predominant *Phytophthora* sp. present. New rootstocks based on trifoliate orange hybrids with pummelo and mandarin have been screened under the adverse soil, climate, disease, and pest conditions in Florida and are demonstrated to have resistance/tolerance to *P. nicotianae* and *P. palmivora*. An objective is to use rootstock resistance to *Phytophthora* spp. as the basis for tolerance to the complex that *Phytophthora* spp. forms after root damage by DRW.

To assess areas where the risk of phytophthora diseases are suspected, soil sampling for propagule counts can be used to estimate the damage that *Phytophthora* spp. are causing to fibrous roots. The results, in conjunction with other site factors including rootstock tolerance, soils, topography, irrigation, drainage, and presence of DRW, can be used to make a decision for the need to treat with fungicides in addition to cultural and pest management. Currently, several phosphite products are marketed for citrus as fungicides or nutritionals. Phosphites are relatively inexpensive and used on a large scale in Florida and Brazil groves. This practice has led to the virtual disappearance of brown rot of fruit despite the presence of *Phytophthora* spp. in grove soils.

HLB, the most destructive disease of citrus worldwide, was detected in Brazil and Florida in the mid-2000s. When HLB was first discovered, the citrus industries adopted the recommended practices including control of the insect vector, use of disease-free planting material, and the removal of infected trees to lower the inoculum load. However, as HLB infection has increased in groves, many growers stopped removing trees and, as an alternative practice, adopted nutritional programs. Given the increasing incidence of HLB and deterioration of root density due to Las damage, research experiences

and current phytophthora data trends suggest the need for much more comprehensive management of root health by reducing the impact of abiotic stresses, such as excess bicarbonates in irrigation water and soil, and the interaction with prevalent biotic stresses, including *Phytophthora* spp.

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