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Research Papers

Phytophthora nicotianae is the predominant *Phytophthora* species in citrus nurseries in Egypt

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Summary. Phytophthora root rot is considered to be the most destructive disease to citrus production in Egypt. *Phytophthora* species are generally present in citrus nurseries, where soil pots containing the survival propagules are considered responsible for their spread into new orchards. The goal of this study was to investigate the distribution and seasonal variation of *Phytophthora* species in soil and feeder roots in two Egyptian citrus nurseries, characterized by different management, and to identify *Phytophthora* species associated with root rot. Soil and root samples were collected at monthly intervals from Sour orange and Volkameriana lemon rootstocks during March-July period. The inoculum density of *Phytophthora* species, and the percentage of infected feeder roots, were estimated using the plate dilution method in conjunction with selective media. *Phytophthora* isolates were identified according to their morphological characteristics and on the basis of the ITS regions of the rDNA. *Phytophthora nicotianae* was the predominant isolated species, followed by *P. citrophthora* and *P. palmivora*. *Phytophthora nicotianae* was detected in both nurseries, while *P. citrophthora* and *P. palmivora* were recovered only in one nursery. Inoculum density of *Phytophthora* species fluctuated during spring and summer according to the environmental conditions, rootstock, and nursery management practices.

Key words: Phytophthora root rot, *P. citrophthora, P. palmivora,* seasonal variation, ITS.

Introduction

Citrus in Egypt is considered one of the most important horticultural crops, due to its economic export value and its local consumption and industries. Citrus crops are grown in four areas along the banks of the river Nile: Delta 59%, Middle Egypt 8%, Upper Egypt 4%, and Desert 29% (Salama *et al.*, 2007). *Phytophthora* species can infect almost every part of citrus plants causing, damping-off of seedlings, fibrous root rot, crown rot, gummosis, and brown rot of fruits in groves, and as postharvest decay during storage and transportation (Menge and Nemec, 1997).

Ten species of *Phytophthora* have been reported to attack citrus around the world, two of which cause the most serious diseases as gummosis, root and fruit rots: *Phytophthora citrophthora* R.E. Sm. & E.H. Sm.) Leonian and *P. nicotianae* Breda de Haan [syn. *P. n.* Breda de Haan var. *parasitica* (Dastur) G.M. Watherh.] (Erwin and Ribeiro, 1996). These pathogens have distinct temporal and climatic requirements; so that their relative distribution and influence vary in the different production areas. Nurseries may be contaminated through water, soil and through nursery workers and implements. In order to manage Phytophthora diseases, it is essential to raise or select pathogen-free plant material to

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avoid the introduction of possibly devastating disease. Good drainage and aeration of soil should be maintained for healthy and adequate growth of root systems. Resistant and compatible rootstock should always be used considering the diseases prevalent in the locality (Menge and Nemec, 1997).

Correct diagnosis and regular monitoring of the diseases are required for their quick and economic management. Monitoring and recording in relation to disease outbreaks in previous seasons will be helpful in developing strategies for controlling the diseases. Predisposing factors for disease development which can be averted with cultural operations should be minimized or eliminated.

Use of fungicides in young groves should be based on rootstock susceptibility, likelihood of *Phytophthora* infestation in the nurseries, and history of Phytophthora disease problems in the groves. For susceptible rootstocks, such as Cleopatra mandarin and sweet orange, fungicides may be applied to young trees on a preventive basis for foot rot. For young trees on other rootstocks, fungicide treatment should commence when rot lesions develop. In mature groves, the decision to apply fungicides for root rot control is based on yearly soil sampling to indicate whether damaging populations of *P. nicotianae* occur in successive growing seasons. Fungicide applications should coincide with periods of susceptible root flushes in the spring and fall (Ippolito *et al.*, 1992). Fosetyl-Al and Metalaxyl-M are both effective, but their alternation should be practiced to minimize the risk of the development of fungicide resistance (Graham *et al.*, 2012). This recommendation is regular with the periods of greater susceptibility of citrus trees to below-ground infections caused by *Phytophthora* spp., known to fluctuate seasonally (Dirac *et al.*, 2003).

In citrus growing-areas with Mediterranean climates, root infections caused by *P. citrophthora* are severe during May and June (spring) and less important from December to February (winter), while root infections caused by *P. nicotianae* are severe during summer and early autumn and less important in winter (Ippolito *et al.*, 1992; Dirac *et al.*, 2003). However, little information is known about the periods of citrus seedling susceptibility to *Phytophthora* spp. under nursery conditions. This information is essential to develop an appropriate programme for the control of Phytophthora infections using chemical control. This would also allow the reduction of unnecessary fungicide use, and consequently would decrease the probability of development of fungicide resistance.

In Egypt, investigations on *Phytophthora* species involved in root rot and their variability in citrus nurseries have never been carried out. The present study aimed to determine the dynamic variation of *Phytophthora* spp. in Egyptian citrus nurseries, and to detect the most common species of *Phytophthora* associated with root rot in the nurseries

Materials and methods

The investigation was carried out from March to July 2009 in two nurseries in different regions of Egypt. The first nursery was located in Delta area and the second in the desert area (Figure 1). The two nurseries varied in the system of citrus production and in plant disease management. Nursery 1 was very well managed (good irrigation and fertilization with an efficient drainage system; optimal chemical program for controlling pests and diseases). Nursery 2 showed deficiencies in nursery management.

Sour orange (*Citrus aurantium* L.) and Volkameriana lemon (*C. volkameriana* Tan. & Pasq.) rootstocks were sampled in Nursery 1 and Volkameriana lemon rootstock was sampled in Nursery 2, depending on the availability of the rootstocks in each nursery. Samples were collected from the growing media components (sand, peat moss, and/or compost), seedbed soil, and soil under the pots. Samples were collected randomly each month and analyzed for the presence of *Phytophthora* propagules.

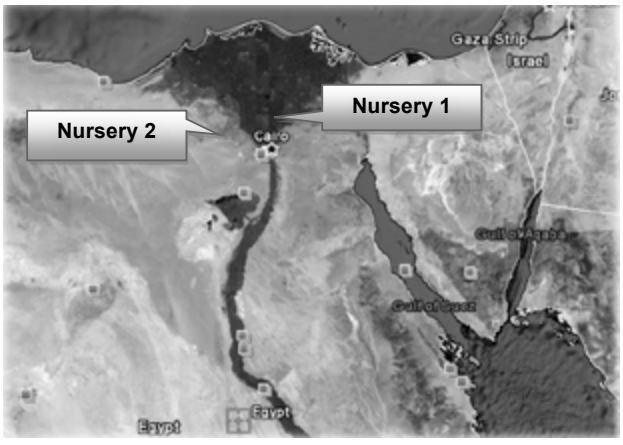

Figure 1. Map of Egypt showing the investigated nursery sites.

Ten samples per each rootstock of 200 mL soil and associated feeder roots were collected monthly at a depth of 5–10 cm from 2 L pots using soil probes, and each sample was obtained by mixing three sub samples from three different pots. Samples were each maintained in a plastic bag until processing in the laboratory. Soil samples were physically and chemically analysed for the availability of micro- and macro-elements according to standard methodology (Sparks, 1996).

The inoculum density (ID) of *Phytophthora* spp. in the soil samples, expressed as the number of propagules per gram (ppg) of dry soil, was determined by the soil dilution plate method using *Phytophthora*-selective (BNPRAH) medium (Masago *et al*., 1977; Ippolito *et al*., 2002). Each soil sample was analysed in triplicate and ten Petri dishes were seeded per replicate.

To evaluate the presence of *Phytophthora* spp. on the plant rhizospheres, citrus roots were extracted from soil samples, rinsed with tap water, dried on blotting-paper, and cut into approximately 1 cm-long segments. At least 125 feeder root segments per sample were plated in five Petri dishes containing the selective medium, and incubated for 3–6 days at 20°C. Colonies of *Phytophthora* spp. were identified on the basis of their morphology, mycelial characteristics, and morphology of sporangia (Gallegly and Hong, 2008), and used to assess the level of soil infestation in terms of ppg of dry soil and the percentage of infected root segments. *Phytophthora nicotianae* strain SCRP115 (Duncan SCRI) and *P. citrophthora* strain CBS 274-33 were used as comparative standards for species identification (Yaseen *et al*., 2010).

Phytophthora isolates obtained from soil and roots were molecularly analysed to confirm morphological identification. Colonies of 24 isolates of *Phytophthora* spp. collected from the two nurseries were transferred onto malt extract agar (MEA), covered with sterile cellophane sheets and grown for 4–5 days at 25°C to produce enough mycelium for DNA extraction. Total DNA was extracted following the method of Schena and Ippolito (2003). PCR amplification was carried out as reported by Cooke *et al.* (2000) using universal primers (ITS6–ITS4) designed on the Internal Transcribed Spacer (ITS) region. PCR products were separated by electrophoresis in 1.5% agarose gel, in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA), stained with ethidium bromide and analyzed under UV light (Sambrook *et al*., 1989). PCR products were sequenced using the primers used for amplification and *Phytophthora* isolates were identified by blasting the sequence at NCBI data Bank.

Results

Chemical and physical analyses of the plant growing media showed differences between the two nurseries. In particular, the growing mixture in Nursery 1 had less gravel, greater C/N ratio, and greater amount of total calcium, compared to the growing mixture of Nursery 2 (Table 1).

Phytophthora spp. propagules were not detected from the growing media components (sand, peat moss, and compost). In Nursery 1, no propagules of *Phytophthora* spp. were detected in soil samples of sour orange rootstock, whereas the pathogen was detected

Table 1. Chemical and physical analyses of the nursery growing mixture from two citrus nurseries.

Soil analyses	Nursery 1 mixture	Nursery 2 mixture
Bulk Density (g cm^{-3})	1.65	1.59
Gravel (> 2 mm) (g kg^{-1})	126.0	190.4
pH (H_2O) 3:50 (w/v)	8.15	7.81
pH ($CaCl_2$) 3:50 (w/v)	7.19	7.16
EC 1:10 25°C (dS m^{-1})	0.33	0.37
Organic Carbon (g kg^{-1})	7.7	5.6
Organic Matter (g kg^{-1})	14.7	10.8
Total N (g kg^{-1})	0.5	0.1
C/N	15.4	56
Total P (g kg^{-1})	0.7	0.5
Total P_2O_5 (g kg^{-1})	1.6	1.1
Total Ca (g kg^{-1})	4.6	2.7
Total Mg (g kg^{-1})	1.0	0.4
Total K (g kg^{-1})	0.2	0.1
Total Na (g kg^{-1})	0.18	0.08
Total Fe (g kg^{-1})	5.6	4.7
Total Cu (mg kg^{-1})	6.9	7.2
Total Mn (mg kg^{-1})	106	93
Total Zn (mg kg^{-1})	10	6
Total Ni (mg kg^{-1})	89	161
Total Cr (mg kg^{-1})	225	406
Total Pb (mg kg^{-1})	7.0	0.8
Total Cd (mg kg^{-1})	<0.1	<0.1

in soil samples collected from Volkameriana lemon rootstock. The inoculum density of *P. nicotianae* fluctuated slightly throughout the sampling period, with a general increase from April to July (Figure 2); the maximum density did not exceed 4 ppg of dry soil. In Nursery 2, *Phytophthora* inoculum density was higher as compared to nursery 1 with values reaching 16 ppg (Figure 3). Inoculum density increased gradually from March to reach the greatest value in May, then decreased sharply in June and increased again in July.

No *Phytophthora* spp. propagules were detected from the roots of citrus seedlings from Nursery 1, neither in sour orange nor in Volkameriana lemon. In Nursery 2, the degree of *Phytophthora* root infection fluctuated during the monitoring period, showing the same pattern of pathogen propagule density in the soil (Figure 4); in particular, the proportion of infected roots reached the maximum value in May (4%) and were less (0.8%) in June and July.

The inoculum density of *Phytophthora* spp. in samples collected from soil under pots of sour orange and Volkameriana plants was very low in Nursery 1, reaching a maximum of 4 ppg, whereas in Nursery 2 this reached about 16 ppg of dry soil (Figure 5). No *Phytophthora* spp. propagules were obtained in seedbed samples collected from Nursery 1, while in Nursery 2 very high numbers of propagules were found both in seedbeds and soil under seedbeds, reaching 12 and 30 ppg, respectively (data not shown).

Phytophthora species were identified based on colony form on different media (Table 2) and based on morphological characteristics (Table 3). Regarding molecular identification, a fragment of approximately 900 bp was amplified and the same fragment of the expected size was obtained from the positive controls. No amplification was achieved with the negative DNA control. Based on DNA sequence information, a total of three *Phytophthora* species were

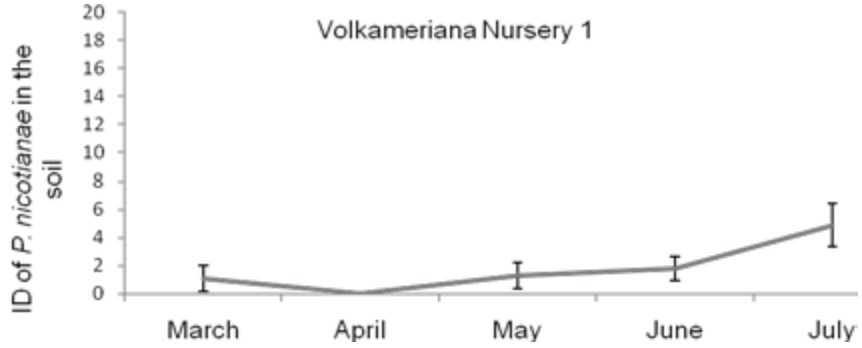

Figure 2. Seasonal variation of *Phytophthora nicotianae* inoculum density (ID) in Nursery 1, expressed as propagules/gram of dry soil.

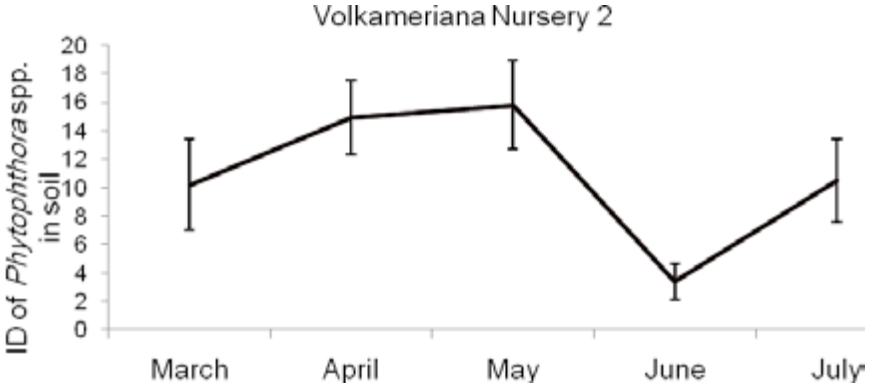

Figure 3. Seasonal variation of *Phytophthora* spp. inoculum density (ID) in Nursery 2, expressed as propagules/gram of dry soil.

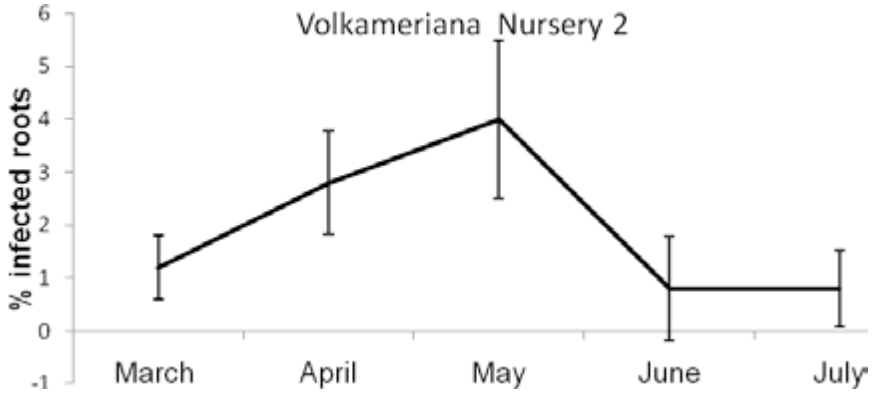

Figure 4. Percentage of feeder roots infected by *Phytophthora* spp. in Nursery 2.

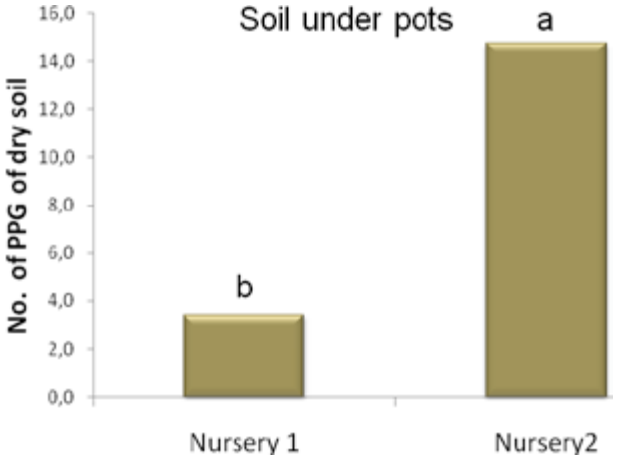

Figure 5. Average number of *Phytophthora* spp. propagules/gram (ppg) present in soil under the pots in the two monitored nurseries. Columns with different letters are statistically different according to Duncan's multiple range test ($P \leq 0.05$).

identified, being *P. nicotianae*, *P. citrophthora*, and *P. palmivora*. All the isolates collected from soil samples of sour orange and Volkameriana lemon in Nursery 1 were identified as *P. nicotianae*, whereas the three *Phytophthora* species were isolated from soil and roots samples of Volkameriana lemon in Nursery 2.

In Nursery 2 the incidence of *P. nicotianae* was high during all the monitoring months, while incidence of *P. citrophthora* reached the greatest value in April and then decreased; *P. palmivora* was found only in March with low numbers of propagules (Figure 6).

Discussion

Study of the seasonal variation of *Phytophthora* spp. in soil and on feeder roots in two nurseries showed that *Phytophthora* spp. population varied according to differences in climate, management, and rootstocks, with general increasing incidence of the pathogens starting from March. This fluctuation is similar to the pattern of seasonal variation of *Phytophthora* spp. populations in Italian citrus orchards (Ippolito *et al*., 1992) and nurseries (Salama, 2008).

The seasonal variation of *Phytophthora* spp. has important implications for the most effective timing of control measures. Knowledge of seasonal variation in the susceptibility of citrus rootstocks to *Phytophthora* make it possible to apply effective disease control measures to coincide with those periods when disease is most intense and when soil temperatures favour disease development (Matheron and Matejka, 1993).

The physical analysis of nursery growing media showed greater gravel and less calcium content in Nursery 2 compared to Nursery 1. These findings can in part explain both the high ID of the pathogen found in Nursery 2, since gravel is conducive to pathogen infection, and the low ID in Nursery 1, because calcium is a disease suppressive element (Ippolito *et al*., 1990). The large pore size present in a gravel rich soil allows high mobility of *Phytophthora* zoospores, which could swim more easily towards host roots. Calcium is an essential element that preserves the structural integrity and functionality of plant cell membranes and cell walls, increasing host resistance to invasion by certain pathogenic micro-organisms and enhanc-

Table 2. Colony patterns of *Phytophthora* species isolated from Nurseries 1 and 2 on different media. All isolates belonging to the same specie showed the same characteristics.

Media	Colony characteristics of *Phytophthora* species [a]		
	P. nicotianae **(seven isolates)**	***P. citrophthora*** **(20 isolates)**	***P. palmivora*** **(ten isolates)**
Corn meal agar	Rose color	Striate	Uniform
Potato dextrose agar	Stoloniferous	Petaloid	Stoloniferous
V 8 –juice agar	Fluffy	Stellate	Highly fluffy
Frozen Pea medium	Cottony uniform	Cottony	Radiate
Oat grain agar	Radiate	Rosaceous	Radiate
Malt extract agar	Stoloniferous	Chrysanthemum	Moderately fluffy

[a] Colony morphologies were recorded after 5 days of incubation.

Table 3. Variation in morphological characteristics of isolated *Phytophthora* spp.

Characteristic	***Phytophthora*** **species**		
	P. nicotianae	***P. citrophthora***	***P. palmivora***
Hyphae swellings	+	-	-
Chlamydospores	Present	Present	Present
shape	Globose, abundant	Globose, Rare	Globose
position	Term., Inter.[a]	Term., Inter.	Term., Inter.
Sporangia			
papilla	Papillate	Papillate	Papillate
shape	Spherical, ovoid		Elliptical
caducity	-	-	+ Short pedicel
Sporangiophore			
proliferation	-	-	-
sympodial	Simple, sympodial	Irregular, branched	Simple, sympodial
Oospores formation			
in the same culture	Not formed	Not formed	Not formed
in paired culture	Not tested	Not tested	Not tested

[a] Term., chlamydospores terminal in the mycelium; Inter., chlamydospores intercalary in the mycelium.

ing tolerance to abiotic diseases. Calcium accumulates in cell walls, giving them stability and integrity and strongly affecting pectinolytic enzymes (Biggs *et al.*, 1997; Campanella *et al.*, 2002). Calcium is also necessary for production of *Phytophthora* zoosporangia and normal motility and adhesion of zoospores. However, high levels of extracellular calcium can rapidly immobilize *Phytophthora* zoospores, induce zoospore cysts to germinate without a host, or trigger and suppress the production of further zoospores. High soil calcium levels have also been associated with suppression of disease by several zoospore-forming pathogens (von Broembsen and Deacon, 1997).

In both nurseries, no *Phytophthora* propagules were detected in the growing media mixture components, which contained clean sand, coming from new virgin land, peat moss, and coco peat fibre. All of these componeents are poor for fungal nutrition.

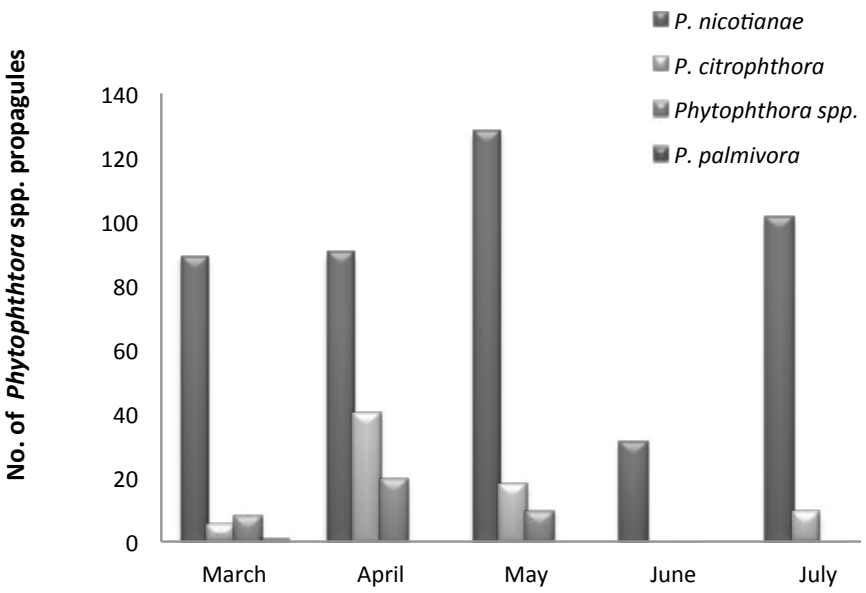

Figure 6. Number of *Phytophthora* spp. propagules/gram (ppg) of different *Phytophthora* species during the monitoring period isolated from soil and root samples in Nursery 1 and Nursery 2.

Samples from soil pots showed greater numbers of propagules and greater amounts of root infection in Nursery 2 compared to Nursery 1. In Nursery 1, no *Phytophthora* was detected in sour orange rootstock, while in Volkameriana low numbers of pathogen propagules was detected. Differences in pathogen populations between the two nurseries can be ascribed to the different management and also to the different susceptibility of the rootstocks. In Nursery 1 the best practices to prevent *Phytophthora* contamination/infection were fulfilled: i) using treated seeds; ii) planting seeds in benches at 60–80 cm from the soil level; iii) placing the pots over a layer of gravel to prevent soil from splashing into pots, to provide air circulation, and to avoid contact of the roots with the contaminated soil under the pots (Menge and Nemec, 1997). Good water and fertilization management, with efficient drainage, in addition to a regular pesticide program for controlling pests and fungal diseases, may have contributed to the absence or very low populations of *Phytophthora* spp.

In Nursery 1, the inoculum density of *Phytophthora* in Volkameriana rootstocks showed a sudden decline in April. This can be attributed to the soil application of Metalaxyl-M after the first assessment in March; the peak population observed in July may have resulted from the temperature increase.

The significant differences between the two Sour orange and Volkameriana rootstocks in the presence of *Phytophthora* propagules could have resulted from variability in resistance to the pathogens.These results are in agreement with Graham and Timmer (1994) who considered sour orange resistant to *P. nicotianae* and *P. citrophthora.* The ability of this rootstock to grow new roots subtending infected feeder roots is probably the basis of its resistance/tolerance to Phytophthora root rot (Lutz and Menge, 1986). In addition, phytoalexin production at the root tip level is reported as a possible mechanism of resistance for sour orange rootstock (Graham and Timmer, 1994), but the role of phtoalexins has not been confirmed in the case of gummosis (Menge and Nemec, 1997).

Sour orange was first discovered to be tolerant to *Phytphthora,* but it is no longer recommended as a rootstock because sweet orange trees grafted on it are highly susceptible to Tristeza virus (Bar-Joseph *et al.*, 1979). The search for rootstocks other than sour orange, that are resistant to *Phytophthora* is very active throughout the world. The use of tolerant rootstocks with desirable horticultural characteristics is the best management strategy against Phytophthora diseases in order to reduce the costly applications of fungicides. However, under conditions that favour *Phytophthora,* such as soil water logging, good control

is not achieved even with resistant or tolerant rootstocks (Erwin and Ribeiro, 1996).

In the Nursery 2, *Phytophthora* propagules in soil and feeder roots reached a peak in May with 15.8 and 4 ppg respectively, while low values of ID and root infection were detected in June (3.1 and 0.8 ppg, respectively). The increase of *Phytophthora* ID could be related to the activity of citrus root growth in May, which provides substrate for the multiplication of *Phytophthora* (Ippolito *et al*., 1992); the feeder roots release exudates (aspartic acid, glutamic acid, sugars, and other compounds) attracting and triggering zoospore infection in the root elongation zone, so root infection increased (Lutz and Menge, 1986). The sudden decrease in June could result from some nursery practices performed at that time; plants under investigation were transferred to another greenhouse with different environmental conditions.

The detection of low *Phytophthora* propagules from soil under pots in Nursery 1 (3.5 ppg) as compared to Nursery 2 (15 ppg) can be attributed to the nursery management. In the soil under the pots the pathogen should be absent, in order to avoid contamination of soil in pots. As the soil mix compounds and the irrigation water were free of *Phytophthora* spp. propagules, the only source of contamination was the soil under the pots.

In this study *P. nicotianae* was present consistently, while incidence of *P. citrophthora* was greatest in the soil during low temperature and then decreased with increasing temperatures. This behaviour confirms the hypothesis that *P. citrophthora* could attack citrus roots during cool periods, due to its ability to metabolize the stored starch in citrus roots during that period, while *P. nicotianae* can infect citrus roots during flushing when the seedlings roots contain sugar instead of starch (Goldschmidt and Golomb, 1982). In contrast, other authors have found that glucose, sucrose, and starch content of roots were not correlated with the seasonality of infection, suggesting that some other aspects of root physiology, such as the secretion of phytoalexins or rhizosphere micro-organisms, can affect the ability of these two pathogens to infect citrus roots (Dirac *et al*., 2003). Although *P. citrophthora* uses more starch at low temperatures and *P. nicotianae* uses more glucose at higher temperatures, this is thought to be more a consequence of the two organisms using the resources available in their winter and summer niches than a cause for their seasonality.

Winter soil temperatures are considered too cold for *P. nicotianae* to be active and summer soil temperatures too warm for *P. citrophthora. Phytophthora citrophthora* grows at temperatures ranging from 7 to 31°C, with an optimum at 26°C. *Phytophthora nicotianae* grows from 9 to 36°C, with an optimum at 31°C, but it grows more slowly at low temperatures than *P. citrophthora* (Fawcett, 1936). However, in California *P. citrophthora* was isolated abundantly in the middle of the summer from alternative hosts planted in an infested citrus grove, but citrus root infection by *P. citrophthora* was extremely low (Dirac *et al*., 2003). They attributed these phenomena to the micro-organisms present in the soil, which naturally suppressed *P. citrophthora*.

In the present study morphological and molecular identification of *Phytophthora* showed that three *Phytophthora* species were identified, *P. nicotianae*, *P. citrophthora,* and *P. palmivora*, the latter only occasionally found. Our results are similar to those from other studies that reported both *P. nicotianae* and *P. citrophthora* are widely distributed in citrus growing areas and cause damping off, root rot, gummosis, and brown rot (Graham and Timmer, 1992; Erwin and Ribeiro, 1996; Ippolito *et al*., 2002).

Phytophthora nicotianae was the most frequently isolated species, detected mainly from soil of the two nurseries under investigation, and these results agree with those of Salama (2008). In Syria, Yaseen *et al*., (2010) reported that *P. citrophthora* was the most isolated species in citrus orchards. Although *P. palmivora* has been reported as the main pathogen of citrus together with *P. nicotianae* in Florida (Bowman *et al*., 2007), in our study its low frequency of isolation makes it less important.

DNA-based studies have provided an alternative and reliable tool for *Phytophthora* identification. Such an approach uses a simple sequence similarity search (BLAST) of a public online sequence database. The application of this technique may be of great importance in laboratories where there is no experience in the identification of pathogens based on morphological characteristics.

Conclusions

This study demonstrates that the seasonal variation of *Phytophthora* in the two nurseries was not only related to different environmental conditions, but also to nursery management practices. The presence of *Phytophthora* spp. in nursery plants is a cru-

cial issue for the establishment of high crop production and quality commercial citrus groves. Sanitary preventive measures for the control of these pathogens, as those used in Nursery 1, should be applied on a large extent in citrus nursery management. The study of seasonal variation for these pathogens should continue for the remaining months in order to provide a clear picture on their population fluctuation during the whole year.

Literature cited

Bar-Joseph M., S.M. Garnsey, D.Gonsalves, M. Moscovitz, D.E. Purcifull, M.F. Clark and G. Loebenstein, 1979. The use of enzyme-linked immunosorbent assay for the detection of citrus tristeza virus. *Phytopathology* 69, 190–194.

Biggs A.R., M.M. El Kholi, S. El Neshawy and R. Nickerson, 1997. Effects of calcium salts on growth, polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola*. *Plant Disease* 81, 399–403.

Bowman K.D., U. Albrecht, J.H. Graham and D.B. Bright, 2007. Detection of *Phytophthora nicotianae* and *P. palmivora* in citrus roots using PCR-RFLP in comparison with other methods. *European Journal of Plant Pathology* 119, 143–158.

Campanella V., A. Ippolito and F. Nigro, 2002. Activity of calcium salts in controlling Phytophthora root rot of citrus. *Crop Protection* 21, 751–756.

Cooke D.E., A. Drenth, J.M. Duncan, G. Wagels and C.M. Brasier, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Dirac M.F., J.A. Menge and M.A. Madore, 2003. Comparison of seasonal infection of citrus roots by *Phytophthora citrophthora* and *P. nicotianae* var. *parasitica*. *Plant Disease* 87, 493–501.

Erwin D.C. and O.K. Ribeiro, 1996. *Phytophthora Diseases Worldwide*. APS Press, St. Paul, MN, USA.

Fawcett H.S., 1936. *Citrus Diseases and their Control*. McGraw-Hill book company, New York. NY, USA.

Gallegly M.E. and C. Hong (ed.), 2008. *Phytophthora*: *identifying species by morphology and DNA fingerprints*. American Phytopathological Society, St. Paul, MN, USA.

Goldschmidt E.E. and A. Golomb, 1982. The carbohydrate balance of alternate-bearing citrus trees and the significance for flowering and fruiting. *Journal of the American Society for Horticultural Science* 107, 206–208.

Graham, J.H. and Timmer L.W., 1992. *Phytophthora* diseases of citrus. In: *Plant Diseases of International Importance* . Vol. III. Diseases of Fruit Crops, 250–269. (Kumar J., Chauhe H.S., Singh U.S., Mukhopadhyay A.N., ed.), Prentice-Hall, Englewood Cliffs, NJ, USA.

Graham J.H. and L.W. Timmer, 1994. *Phytophthora Diseases of Citrus.* Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, FL, USA.

Graham J.H., L.W. Timmer and M.M. Dewdney, 2012. *Florida Citrus Pest Management Guide: Phytophthora Foot Rot and Root Rot.* Florida, University of Florida IFAS Extension, Gainesville, FL 32611, USA, Publication pp-156, 1–6.

Ippolito A., V. De Cicco, S. Vanadia, V. Mauroantonio and M. Salerno, 1990. Inoculum density and citrus root infection of *Phytophthora* spp. as related to same chemical and physical soil factors. In: *Proceedings of the Eighth Congress of the Mediterranean Phytopathological Union*, October 28–November 3, 1990, Agadir, Morocco, 99–101.

Ippolito A., V. De Cicco and M. Salerno, 1992. Seasonal variation in root infection and population levels of *Phytophthora* spp. in citrus orchards of Apulia and Basilicata, Italy. *Rivista di Patologia Vegetale* 2, 57–65.

Ippolito A., L. Schena and F. Nigro, 2002. Detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils by nested PCR. *European Journal of Plant Pathology* 108, 855–868.

Lutz A. and J.A. Menge, 1986. Citrus root health II. Phytophthora root rot. *Citrograph* 72(2), 33–36.

Masago H., M. Yoshikawa, M. Fukada and N. Nakanishi, 1977. Selective inhibition of *Pythium* spp. from soils and plants. *Phytopathology* 67, 425–428.

Matheron M.E. and J.C. Matejka, 1993. Seasonal differences in susceptibility of three citrus rootstocks to root lesions caused by *Phytophthora citrophthora* and *P. parasitica*. *Plant Disease* 77, 729–732.

Menge J.A. and S. Nemec, 1997. Citrus. In: *Soilborne Diseases of Tropical Crops*. (Hillocks R.I. and Waller J.M. ed.), CAB International, Wallingford, UK, 185–227.

Salama E.A., 2008. *Aspects of Epidemiology and Biological Control of Phytophthora Root Rot of Citrus in Nurseries of Southern Italy*. Ph.D Thesis. Università degli Studi di Catania, Catania, Italy, 55 pp.

Salama E.S.S., F.G. Latif and E.M. Hassan, 2007. EuroMed-CitrusNet. *Safe and High Quality Supply Chains and Networks for the Citrus Industry between Mediterranean Partner Countries and Europe*: *Contract No. 43146 (Food Quality and Safety), Deliverable 9: National Citrus Sector Analysis: Egypt*. 31 pp.

Sambrook J., Fritsch E.F. and T. Maniatis (ed.), 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, NY, USA.

Schena L. and A. Ippolito, 2003. Rapid and sensitive detection of *Rosellinia necatrix* in roots and soils by real time Scorpion-PCR. *Journal of Plant Pathology* 85, 15–25.

Sparks D. L., Page A. L., Helmke P. A., Loeppert R. H., Soltanpour P. N., Tabatabai M. A., Johnston C. T., and M. E. Sumner. 1996. *Methods of Soil Analysis. Part 3. Chemical Methods.* Bartels J. M. and J. M. Bigham (eds), Soil Science Society of America Book Series, No. 5. Soil Science Society of America: American Society of Agronomy, Madison, WI, USA.

Von Broembsen S.L. and J.W. Deacon, 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. *Phytopathology* 87, 522–528.

Yaseen T., L. Schena, F. Nigro and A. Ippolito, 2010. *Phytophthora citrophthora* is the predominant Phytophthora species in Syrian citrus groves. *Phytopathologia Mediterranea* 49, 205–211.

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