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Phytophthora citrophthora is the predominant *Phytophthora* species in Syrian citrus groves

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Summary. A survey on *Phytophthora* spp. in the soils and roots of citrus groves was carried out in the main Syrian growing areas of Lattakia and Tartous. Traditional assays (selective medium with soil dilution plates) were used for pathogen detection, and molecular (PCR) assays were used for unanmbiguous identification of *P. nicotianae* and *P. citrophthora* in 38.5% of the collected samples. In both locations, *P. citrophthora* was the predominant species.

Key words: Syria, *Phytophthora* root rot, *P. nicotianae, P. citrophthora*.

Introduction

Citriculture is important among commercially-grown crops in Syria, with 30,000 hectares and 10 million trees in citrus orchards. Plantings are located primarily along the Mediterranean coast, which has a mild climate.

Phytophthora nicotianae and *P. citrophthora* cause serious soil-borne diseases of citrus in all citrus-growing regions and bring considerable yield losses worldwide (Menge and Nemec, 1997). Considering that the behaviour of these two species is quite different in relation to temperature for growth, sensitivity to fungicides, and polyphagy, generally, one of the two species prevails in a growing area. In Australia, the predominant species is *P. citrophthora*, whereas in California and Florida (USA) *P. nicotianae* prevails (Lutz and Menge, 1986). Repeated investigations in southern Italy showed a predominant presence of *P. nicotianae* in continental regions (Apulia, Basilicata, Calabria) and *P. citrophthora* in Sicily (Magnano di San Lio *et al*., 1983, 1986; Ippolito *et al*., 1991, 1992, 2004). In Egypt, a severe form of gummosis on citrus trees has been ascribed to both species of *Phytophthora* (Sawabi, 1974). Similarly, in Syria *P. nicotianae* has been reported as the casual agent of gummosis (Fadoul, 1973); however, the importance of feeder root rot due to *Phytophthora* spp. in citrus orchards has never been established.

The aim of the present study was to investigate the presence and distribution of *Phytophthora* spp. in Syrian citriculture.

Materials and methods

Sampling and isolation of *Phytophthora* spp. from soil and roots

A survey was conducted in July and August 2003, in cooperation with the Syrian Ministry of Agriculture and Agrarian Reform (Directorate of

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Plant Protection). Two hundred and twenty soil and root samples were collected from 55 orchards (four samples per orchard), 124 from Latakia and 96 from Tartus provinces (Figure 1). Each sample (about 1 kg of soil containing roots) consisted of four sub-samples collected at a depth of 5–20 cm under the canopy of plants showing slight symptoms of decline. Samples were put in plastic bags and processed on the day of sampling.

The inoculum density of *Phytophthora* spp. in soils was determined using a selective medium (Masago *et al*., 1977) as described by 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 root system, citrus roots in soil samples were extracted, 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 and used to assess the level of soil infestation in terms of propagules per gram of dry soil (ppg) and the percentage of infected root segments. Some representative colonies were transferred onto Potato Dextrose Agar (PDA), and identified following the keys of Stamps *et al*. (1990). *Phytophthora nicotianae* strain SCRP115 (Cooke D.E.L.) and *P. citrophthora* strain CBS 274-33 were used as comparative standards for species identification.

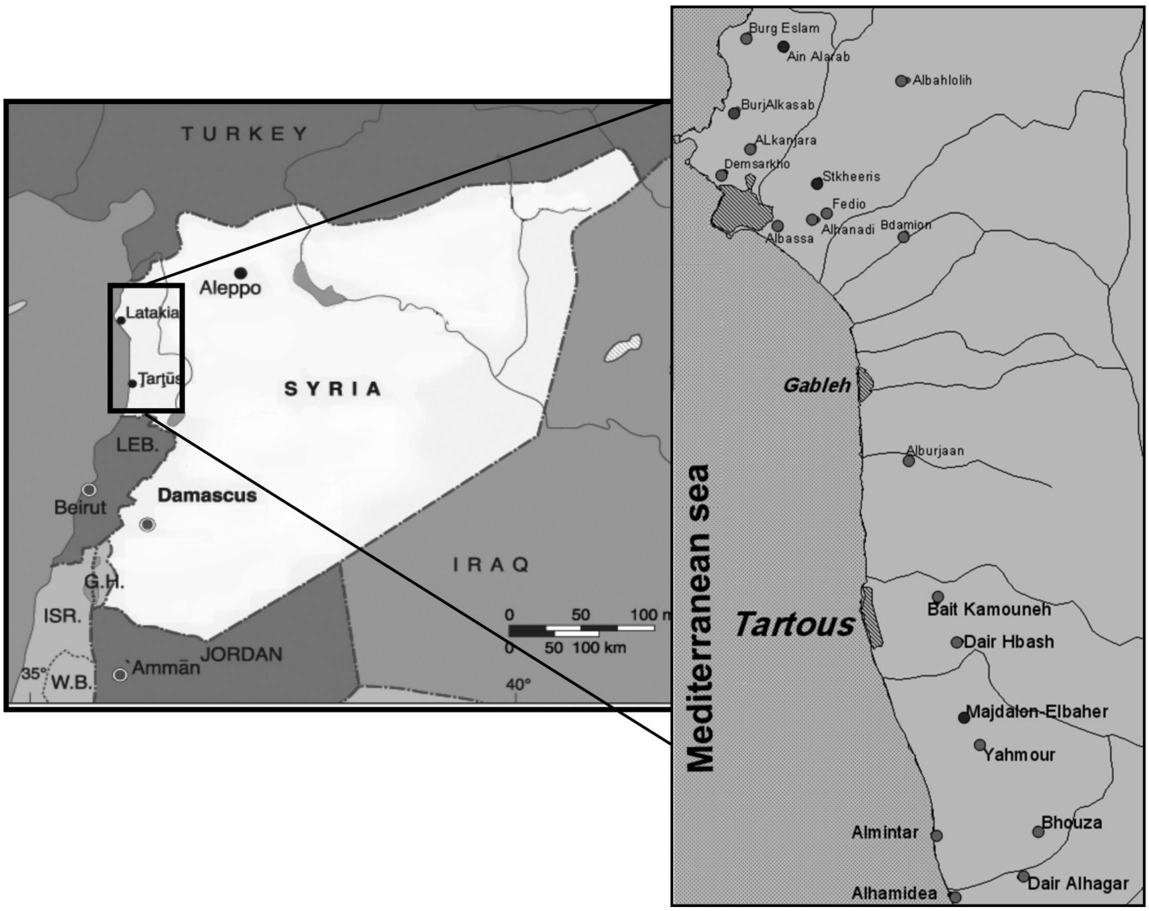

Figure 1. Map of Syria showing sampling locations in Tartus and Latakia provinces.

Molecular identification of *Phytophthora* isolates

Phytophthora isolates obtained from soil and roots were molecularly analysed to confirm morphological identifications. Colonies of *Phytophthora* spp. were transferred onto malt extract agar (MEA) 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 *et al*. (2002) using *Phytophthora* mycelium grown in Petri dishes containing MEA covered with sterile cellophane sheets. PCR amplification was carried out as reported by Ippolito *et al*. (2002), using specific primers for *P. nicotianae* (Pn5B–Pn6) and *P. citrophthora* (Pc2B–Pc7) designed on the Intergenic Transcribed Spacer (ITS) regions of the rDNA. 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).

Results

Typical symptoms of *Phytophthora* root rot ('frog eye' lesions on large roots and browning and disintegration of the cortical tissue on feeder roots) were observed during field surveys. In some cases, and in particular in the area of Borg Al-kasab, Ain Al-Arab, Yahmor, Dir Hbash and in the mountainside orchards, Bit Kamone and Set Kheres, plants with symptoms of gummosis were found.

Phytophthora citrophthora and *P. nicotianae* were the only Phytophthora species isolated. Overall, these two species were isolated from 38.5% of the collected soil samples (29 and 48% in Latakia and Tartus provinces, respectively) with an inoculum density ranging from 2 to 194 ppg (Table 1).

In both locations, *P. citrophthora* was the predominant species, being isolated from 94.4% (Latakia) and 85% (Tartus) of soil samples, whereas *P. nicotianae* was isolated from 5.6 and 15% of infested soil samples respectively (Table 1).

Phytophthora nicotianae was found only in two locations (Bait Kamona and Majdalon Albahr) in Tartus province and in one location (Al-Hinadi) in Latakia province.

In Tartus province, the average number of propagules in the sampled soil was 10.8 ppg. More than 5 ppg were detected in the area of Bait Kamona, Dair Hobach, Dir Al-Hajar, Yahmor, Al-Hamidea and Al-Borjan (Figure 2A). The average percentage of infected roots in samples was 0.99% and generally increased as the soil inoculum density increased (Figure 2B).

In Latakia province, the average number of propagules was 4.2 ppg, and only in three locations (Fedio, Ain Al-Arab and Set Kheres) were more than 5 ppg detected (Figure 3A). The percentage of root infection was low (average 0.53%) and there was no evidence of a clear-cut relationship with the inoculum density in the soil (Figure 3B).

Discussion

During the survey carried out in this study, the most important areas of Syrian citriculture

Table 1. Number and percentage of samples infested by *Phytophthora citrophthora* and *P. nicotianae* collected in Latakia and Tartus provinces.

Province	No. analyzed samples	No. infested samples	*P. citrophthora*			*P. nicotianae*		
			Root	Soil	Infection (%)	Root	Soil	Infection (%)
Latakia	124	36	26	8	94	2	0	5.6
Tartus	96	46	33	7	85	4	2	15
Total	220	82	59	15	90.5	6	2	9.5

were examined. In the visited orchards, plants with various degrees of symptom severity were observed. Whenever possible, those plants with symptoms resembling virus infection (e.g psorosis), and wood decay, were not considered for sampling. Soil and feeder root samples were generally collected from plants showing slight symptoms of decline grouped in relatively small areas of the orchards.

The molecular tool (PCR) applied in this research confirmed the morphological identification of the species of Phytophthora involved in root rot in Syrian citriculture. The primer pair Pn5b-Pn6 and Pc2b-Pc7 permitted the unequivocal identification of *P. nicotianae* and *P. citrophthora*, respectively. The application of this technique will be useful in laboratories in which there is no experience in the identification of pathogens based on morphological characteristics.

Samples from the area of Tartus showed a higher number of infested samples, a higher number of propagules and a higher proportion of in-

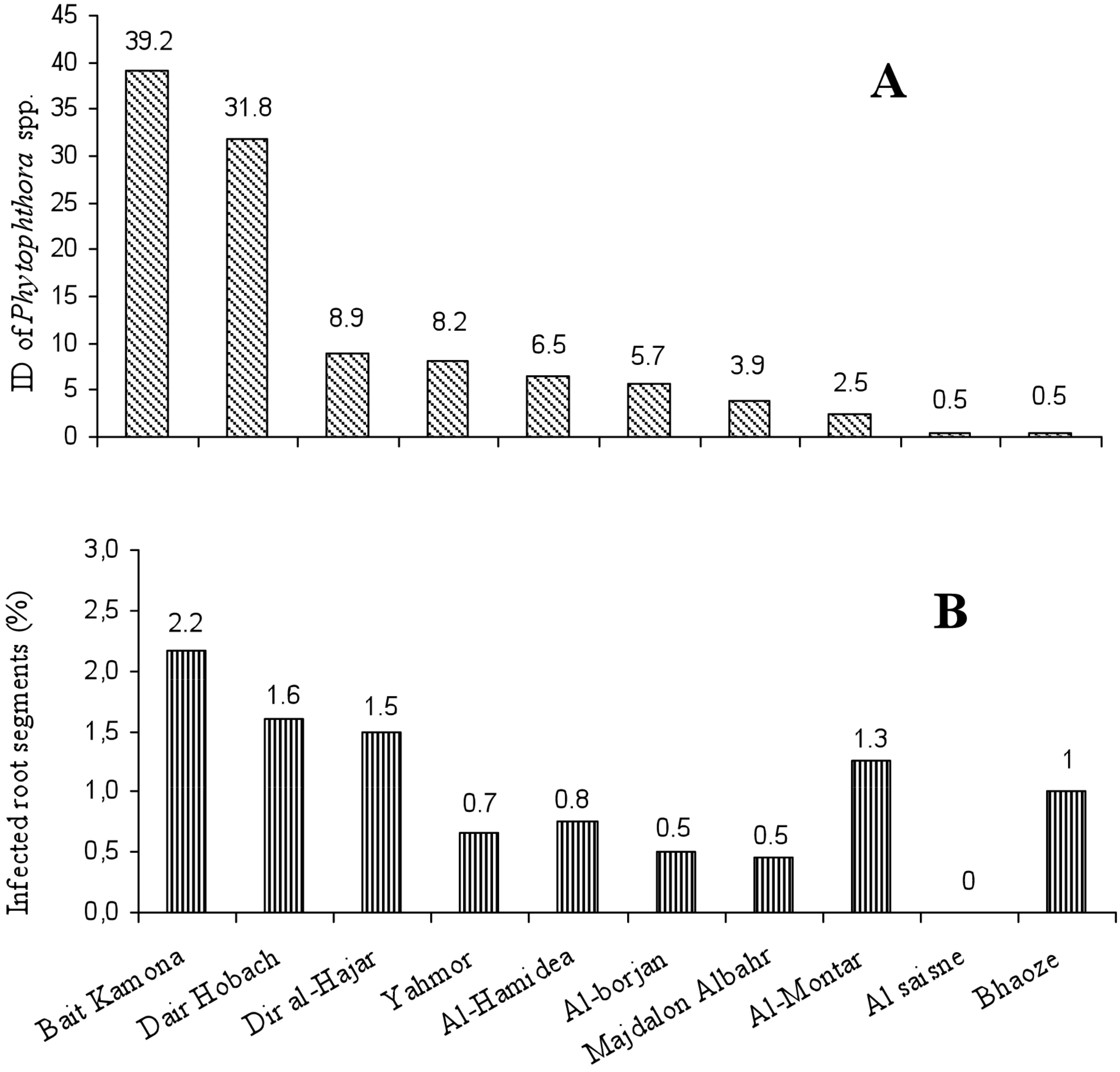

Figure 2. Inoculum density (ID) of *Phytophthora* spp. (propagules gram^{-1} of dry soil) (A), and percentage of infected roots (B) in monitored locations in Tartus province.

fected roots, compared with those from Latakia area. In addition, in six locations from Tartus province, the average inoculum density exceeded 5 ppg, with the inoculum density in Bait Kamona and Dair Hobach locations greater than 30 ppg. In Latakia, samples with more than 5 ppg were found only in three locations. A threshold inoculum density of *Phytophthora* that represents a disease risk has not been established in these soils. A population of less than 5 ppg has been considered insignificant (Graham and Timmer, 1994), whereas a population of 15–20 ppg, in the case of susceptible rootstocks (Lutz and Menge, 1986; Magnano di San Lio *et al*., 1988), or 30 ppg, in the case of resistant rootstocks, (Ippolito *et al*., 1991), can severely damage the feeder roots, leading to tree decline and yield losses. Considering that Syrian citriculture is based on the use of resistant sour orange (*Citrus aurantium* L.) rootstock, it is likely that only plants in Bait Kamona and Dair Hobach (Tartus) would be at risk of Phytophthora disease and warrant management strategies. However,

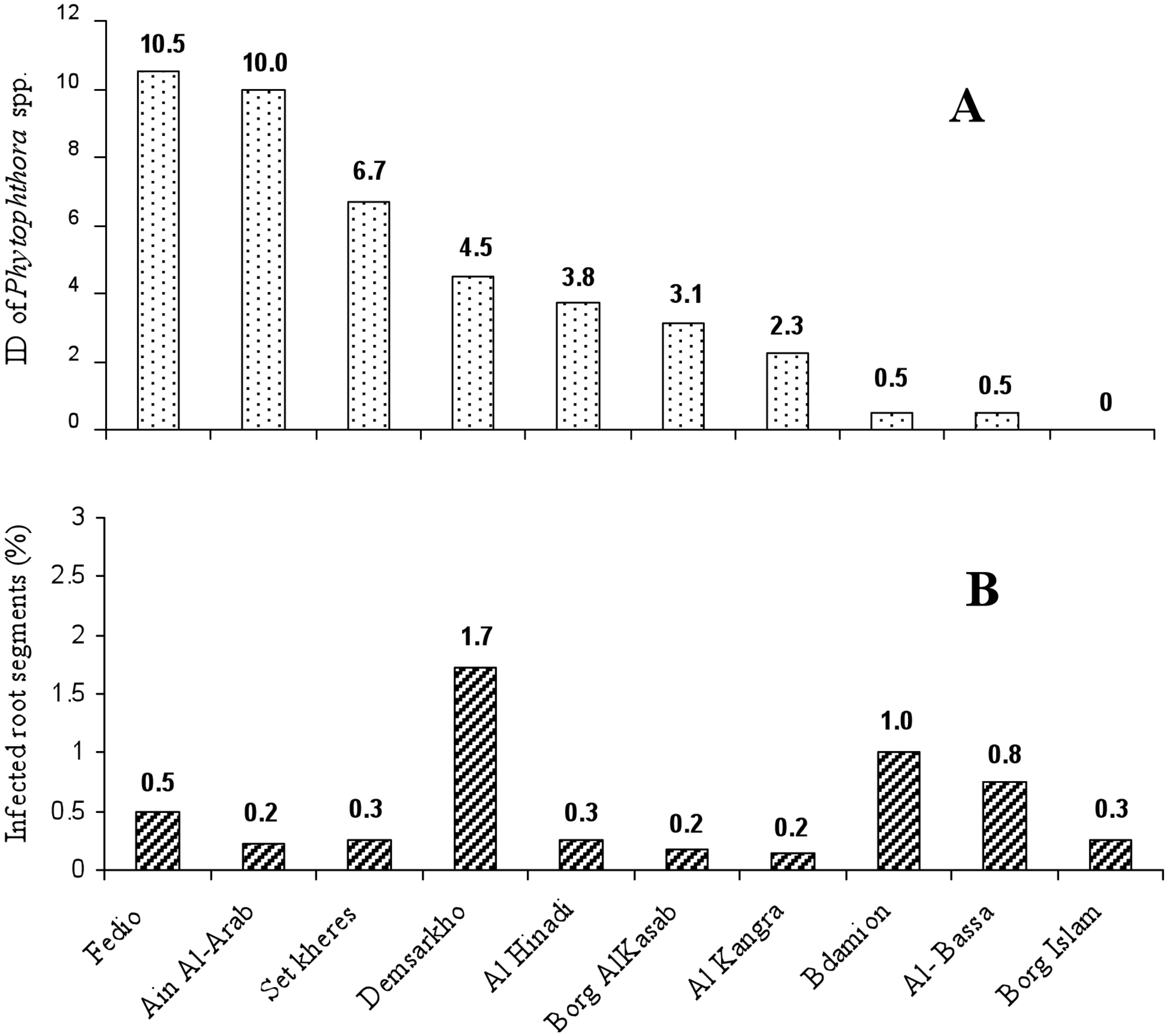

Figure 3. Inoculum density (ID) of *Phytophthora* spp. (propagules gram^{-1} of dry soil) (A), and percentage of infected roots (B) in monitored locations in Latakia province.

intrinsic factors, such as poor seed and seedling selection (Salerno and Cutuli, 1981), the incorrect applications of cultural practices, the grafted scion, and environmental conditions conducive to the disease, can reduce the natural resistance of sour orange rootstock. Therefore, even in presence of low Phytophthora inoculum density, it can be necessary to apply specific strategies, such as chemical treatments, to improve plant health. For example even though the inoculum density does not exceeds the above-mentioned threshold in Fedio and Ain Al-Arab (Latakia), a chemical treatment may be warranted because of the environmental conditions characterized by heavy soil and a flooding irrigation system that is conducive to feeder root infection by *Phytophthora* spp. (Lutz and Menge, 1986).

Water is applied to Syrian citrus groves using several irrigation systems, inducing surface (flood, basin, and furrow), sprinkler, and drip irrigation. The most common system is surface irrigation, especially in regions supported by government irrigation networks, where water arrives to the farms from dams through underground pipelines. The results obtained in the present study showed that the inoculum density of *Phytophthora* spp. was greatest in regions where surface irrigation systems were used, particularly in Fedio, Ain Al-Arab, Set-kheres, Bait-Kamona and Dair-Hobach. This can be attributed to the large quantities of water applied during each irrigation. It is generally accepted that high populations of *Phytophthora* spp. and disease incidence could be due to the number of zoospores released by sporangia when the soil becomes saturated (Lutz and Menge, 1986). Thus, the choice of appropriate irrigation systems is crucial in managing Phytophthora root rot (Thomson *et al*., 1976; Feld *et al*., 1990; Lutz and Menge, 1991). Farmers are advised to use the drip irrigation system, which applies water gradually to the soil, and only the soil directly under the dripper becomes saturated during irrigation. In addition, this method may help preventing the spread of *Phytophthora* spp., since it apparently leads to a reduction in the formation and movement of zoospores.

To date, only *P. nicotianae*-induced gummosis on citrus has been reported from Syria (Fadoul, 1973). During the survey, this species was detected in very low amount, only in two locations in Tartus and in one location in Latakia. *Phytophthora citrophthora* was the predominant species isolated from soil and roots of Syrian orchards, and this is the first report of this species in Syria. These findings are in agreement with the results reported from Sicily (Italy) (Magnano di San Lio *et al*., 1983, 1986), but contrast with the results obtained in the continental part of Italy (Calabria, Basilicata and Apulia), and in California, where *P. nicotianae* was almost exclusively isolated (Tsao and Ocana, 1969; Blaker and MacDonald, 1986; Lutz and Menge, 1986; Ippolito *et al*., 1991, 1992). The greatest incidence of *P. citrophthora* in Syrian citrus orchards is not easy to explain. Some Authors have hypothesized that in the Mediterranean-type climate *P. citrophthora* is the most important cause of gummosis and root rot because of the winter rains and the mild climate that favour the pathogen activity also during cold months (Erwin and Ribeiro, 1996). If this behaviour is confirmed by specific trials, the timing for chemical applications should be different from that suggested to combat root rot caused by to *P. nicotianae* (Menge, 1986; Ippolito *et al*., 1996).

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