Distribution of *Phytophthora* spp. in Southern Texas Citrus Grove Soils

M. RoyChowdhury, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843-21362; **M. Saini**, Syngenta Crop Protection, Greensboro, NC 27409; **T. Driver**, Syngenta Crop Protection, Crawford, TX 76638; and **B. Minton**, Syngenta Crop Protection, Cypress, TX 77433

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Phytophthora nicotianae and *P. palmivora* are common soil-borne oomycete pathogens that affect tree health in citrus groves by infecting fibrous roots. Recent evidence indicates that trees affected by huanglongbing (HLB), caused by ‘*Candidatus Liberibacter asiaticus*’, have higher chances of increased propagule counts of *Phytophthora* spp. (Graham et al. 2003). In response to the discovery of HLB in Texas in January 2012, we initiated this study on the distribution of *Phytophthora* spp. in soils of South Texas citrus orchards. Soil samples were collected from 61 citrus groves (Fig.1), from four quadrants of a single tree selected arbitrarily. The sample area was about a 3-m-diameter circle under the canopy of the tree and in the root zone of each tree. Five replicate samples were collected from each quadrant. Those samples were pooled prior to analysis and the propagule density determined for each of the four quadrants and then averaged for the site. *Phytophthora* spp. was first identified by following a standard soil plating protocol (Timmer et al. 1988). The medium used was supplemented with 10 mg/liter of rifampicin. DNA was extracted from all 61 soil samples using PowerSoil DNA Isolation Kit (Catalog No. 12888-50, MO BIO Laboratories, Inc. DNA), and was amplified by PCR using primers common to 16 species of *Phytophthora* (Drenth et al. 2006) and also species-specific primers (Drenth et al. 2006; Ippolito et al. 2002). No *Phytophthora* spp. was detected at Sites 60 and 41 using universal primers, indicating the absence of the 16 species. *Phytophthora citrophthora* was restricted to 13 sites (Fig. 1; Table.1). *Phytophthora citrophthora* usually occurs in cooler sites near coastal areas (Graham and Timmer 1992). *Phytophthora nicotianae* was identified from most of the sites. *Phytophthora* spp. isolates from Site 11 and Site 15 showed morphological features resembling *P. ramorum*, but that species was not detected by PCR. *Phytophthora palmivora* was not identified in any soil sample; *P. palmivora* is a pathogen of citrus and many fruit crops primarily in the humid tropical areas (Zitko et al. 1991). Data were analyzed by calculating the proportion of orchards that had soil populations above or below 10 to 20 propagules/cm³, a value that is considered of concern (Graham et al. 2003). Since our data were not normally distributed, a non-parametric statistical test, signed rank, was used. The tests were one-sided and conducted against baseline values of 10 or 20

propagules/cm³ ($\alpha \leq 0.05$). Twenty-four sites had a *P* value of ≤ 0.05 . Our results indicated that 18 sites were significantly higher than the baseline of 20 propagules/cm³. Though counts of 10 to 20 propagules/cm³ are considered important, establishment of firm threshold levels are not simple or easy because of the complex relationship between fungal populations, fibrous root densities, and yield (Zitko et al. 1991). Prior evaluations of presence of *Phytophthora* spp in the Lower Rio Grande Valley (Timmer 1973) identified only *P. parasitica* (now *P. nicotianae*). In our study, we have now established the presence of *P. citrophthora* in the Valley. In addition, we have quantified populations in that area for the first time. Growers with orchards with propagule counts greater than 10 to 20 propagules/cm³ should consider treatment with an appropriate fungicide such as mefenoxam or fosetyl-Al (Zitko et al 1991).

TABLE 1
Distribution of *Phytophthora* spp. in different sites.

Location	<i>Phytophthora</i> spp.	<i>P. nicotianae</i>	<i>P. citrophthora</i>
Sites 1*, 2*, 5*, 6, 7*, 8, 9, 12*, 13, 14*, 16, 21, 22*, 23, 25, 26*, 27*, 29, 30*, 31, 32, 33, 37, 38, 39, 40, 42, 43*, 44, 45*, 47*, 48, 49, 50, 51, 52, 53, 54, 55, 59*	+	+	–
Sites 3*, 4, 24, 28*, 34, 35, 46	+	–	+
Sites 10, 17, 18, 19, 20, 58	+	–	–
Sites 11*, 15*, 36, 56, 57, 61*	+	+	+
Sites 60, 41	–	–	–

*Sites with more than 20 propagules/cm³ ($P \leq 0.05$); + tested positive and – tested negative. Samples were collected from four quadrants at each site. Five replicates were taken from each quadrant and samples were pooled prior to analysis and the propagule density determined for each of the four quadrants and then averaged for the site. Sample site selection was random.

Corresponding author: M. RoyChowdhury. Email: roycmoyt@isu.edu.

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FIGURE 1

Distribution of soil samples collected from 61 locations in South Texas. Numbers (1 to 61) indicate the locations where soil samples were collected. Samples nos. 14, 15, 23, 26, 36, 38, 40, 43, and 47 have not been listed because of confidentiality agreements.

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LITERATURE CITED

- Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., Irvine, G., and Irwin, J. A. G. 2006. Development of a DNA based method for detection and identification of *Phytophthora* species. *Aust. Plant Pathol* 35:147-159.
- Graham, J. H., Johnson, E. G., Gottwald, T. R., and Irey, M. E. 2013. Presymptomatic fibrous root decline in citrus trees caused by Huanglongbing and potential interaction with *Phytophthora* spp. *Plant Dis.* 97:1195-1199.
- Graham, J. H., and Timmer, L. W. 1992. *Phytophthora* diseases of citrus. Pages 250-269 in: *Plant Diseases of International Importance. Vol III. Diseases of Fruit Crops.* J. Kumar, H. S. Chaube, U. S. Singh, and A. N. Mukhopadhyay, eds. Prentice-Hall, Englewood Cliffs, NJ.
- Ippolito, A., Schena, L., and Nigro, F. 2002. Detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils by nested PCR. *Eur. J. Plant Pathol.* 108:855-868.
- Timmer, L. W. 1973. Characteristics of *Phytophthora* isolates from Texas citrus orchards. *J. Rio Grande Valley Hortic. Soc.* 27: 44-49.
- Timmer, L. W., Sandler, H. A., Graham, J. H., and Zitko, S. E. 1988. Sampling citrus orchards in Florida to estimate populations of *Phytophthora parasitica*. *Phytopathology* 78:940-944.
- Zitko, S. E., Timmer, L. W., and Sandler, H. A. 1991. Isolation of *Phytophthora palmivora* pathogenic to citrus in Florida. *Plant Dis.* 75:532-535.