New Disease Reports

First report of root rot caused by *Phytophthora nicotianae* in avocado trees (*Persea americana*) in Cuba

M. Machado [1]*, C. Collazo [1], M. Peña [1], O. Coto [1] and M.O. López [2]

[1] *Instituto de Investigaciones en Fruticultura Tropical.7ma., No. 3005, PO 11300, La Habana, Cuba*; [2] *Instituto de Investigaciones de Sanidad Vegetal. 110 e/ 5 A y 5 F, Playa, La Habana, Cuba*

*E-mail: maylenm@gmail.com

Received: *14 Sep 2012.* ***Published:*** *17 Oct 2013.* ***Keywords:*** *PCR, Pn5B / Pn6 primers, pathogenicity*

In October 2008, avocado trees (*Persea americana*) showing decline and dieback were observed in Alquízar, western Cuba. On investigation some roots were blackened, necrotic and brittle. We conducted pathogen isolation by plating both diseased roots, and rotting material from avocado fruit previously baited with rhizosphere soil, onto selective V8 PARPH agar (Zentmyer, 1980). Growth from direct plating of roots and baited tissue was sub-cultured onto PDA. These colonies were white, light, stellate with arachnoid morphology. Hyphal growth was completely inhibited above 35°C. Single, non-caducous sporangia were formed abundantly on agar and in liquid media, between 17.5 and 65 µm (37.2 ± 8.7 µm) long and 12.5 to 45 µm (28.6 ± 6.3 µm) wide, characteristic of *P. nicotianae*. They were ellipsoid, ovoid, obpyriform or spherical, with a prominent papilla (occasionally bi-papillate) and a narrow (6.5 ± 1.6 µm) exit pore (Fig. 1). The isolates were heterothallic, although inoculum transferred from old cultures developed amphigynous and oval antheridia, with smooth and spherical oogonia and aplerotic oospores (Fig. 1) as previously reported (Brasier, 1972; Tsao et *al*., 1980). Terminal and intercalary globose chlamydospores were also observed.

Until recently, *P. cinnamomi* (non-papillate) was thought to be the main causal agent of avocado root rot in Cuba (Sánchez, 1983). However, *P. nicotianae* has also been found provoking gummosis in branches of an avocado tree (V. Zamora, IIFT Cuba, pers comm.). Mycelium of the *Phytophthora* sp. isolate was grown in potato broth for one week at 25°C, then filtered and frozen at -20°C. DNA extraction was conducted using a CTAB procedure (Ristaino *et al.*, 1998). PCR amplification using Pn5B/Pn6 primers, specific to *P. nicotianae* (Ippolito *et al.*, 2002), yielded a band corresponding to the expected size (120 bp). BLAST analysis of a nucleotide fragment of ca. 900 bp (GenBank Accession No. JN135291) amplified from this isolate using ITS5/ITS4 generic primers, shared 99% identity with *P. nicotianae* isolates from China (JX978447) and India (JF792541) and with other Cuban isolates from citrus (GU073387 and GU073388). Pathogenicity was verified by wound-inoculation of two-year-old 'Catalina' seedlings with 5 mm diameter mycelial agar plugs. Necrotic lesions around inoculation points were observed within four months (Fig. 2A). Control plants inoculated with sterile agar plugs remained healthy (Fig. 2B). The causal agent was consistently re-isolated from necrotic tissues. These results are the first report of root rot caused by *P. nicotianae* in avocado trees in Cuba.

Acknowledgements

The authors would like to thank Dr. L. Pérez and B.Sc E. de la Parte for their technical assistance measuring sporangia and Dr. E. Feichtenberger for providing the reference strains.

References

Brasier CM, 1972. Observation on the sexual mechanism in *Phytophthora palmivora* and related species. *Transactions of the British Mycological Society* **58**, 237-251. [http://dx.doi.org/10.1016/S0007-1536(72)80153-0]

Ippolito A, Schena L, Nigro F, 2002. Detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils by nested PCR. *European Journal of Plant Pathology* **108**, 855-868. [http://dx.doi.org/10.1023/A:1021208106857]

Ristaino JB, Madritch M, Trout CL, Parra G, 1998. PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora*. *Applied and Environmental Microbiology* **64**, 948-954.

Sánchez HM, 1983. Plagas y enfermedades de los frutales. La Habana, Cuba: Editorial Pueblo y Educación, La Habana, Cuba.

Tsao PH, Ugale R, Hobbs S, Farih A, 1980. Control of homothallic oospore formation in *Phytophthora parasitica* by culture manipulations. *Transactions of the British Mycological Society* **75**, 153-156. [http://dx.doi.org/10.1016/S0007-1536(80)80207-5]

Zentmyer GA, 1980. *Phytophthora cinnamomi* and the disease its causes. *Phytopathological Monograph 10*. St Paul, MN, USA: American Phytopathological Society.

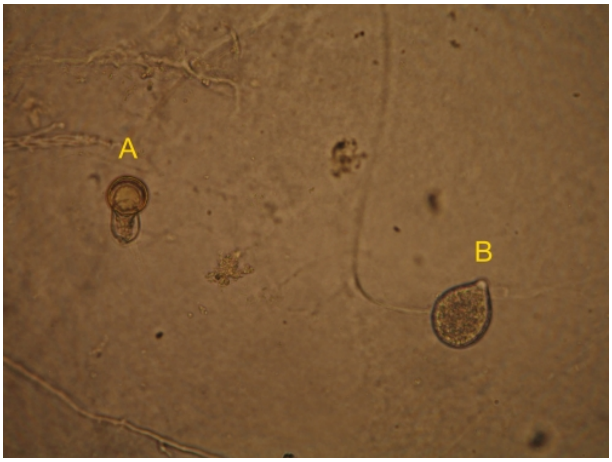

Figure 1

Figure 2

To cite this report: Machado M, Collazo C, Peña M, Coto O, López MO, 2013. First report of root rot caused by *Phytophthora nicotianae* in avocado trees (*Persea americana*) in Cuba. *New Disease Reports* **28**, 9. [http://dx.doi.org/10.5197/j.2044-0588.2013.028.009]

This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.