

## ***Phytophthora boehmeriae* Boll Rot: A New Threat to Cotton Cultivation in the Mediterranean Region**

K. Elena and E.J. Paplomatas<sup>1</sup>

A boll rot of cotton (*Gossypium hirsutum* L.) was observed for the first time in Greece in August 1993 in Larissa and Volos counties, and in August and September 1995 in Trikala and Phthiotis counties. Fungi of the genus *Phytophthora* were isolated from diseased plants. Morphological characteristics of the pathogen were recorded on mounts made directly from the infected tissues or after growth of the isolated fungus on corn meal agar or sterile distilled water. Colony morphology, growth rates, features of asexual and sexual structures and maximum growth temperatures were examined. A *Phytophthora* species new to Europe, *Phytophthora boehmeriae* Sawada, attacking cotton bolls, was identified. The pathogenicity of the isolates was confirmed by artificial inoculations of detached cotton bolls. Analysis of  $\alpha$ -esterase isozymes revealed unique banding patterns for isolates of *P. boehmeriae* compared with those of *P. cactorum* and *P. parasitica*, which are *Phytophthora* species with similar morphology.

KEY WORDS: *Phytophthora boehmeriae*; boll rot; *Gossypium hirsutum*; cotton.

### INTRODUCTION

Fungi of the genus *Phytophthora* are important plant pathogens causing severe losses to a wide range of crops, especially when soil moisture is high. Species identification is often difficult, since morphology and growth of isolates can be variable. A number of researchers worldwide have dealt with the taxonomy of the genus (16,20,21). In Greece, many species of *Phytophthora* from several hosts have been identified (9,10,11).

Cotton is grown on a large acreage in Greece, being an important crop for the economy of the country. Moreover, cotton is a widespread industrial crop among the countries of western Asia and North Africa surrounding the Mediterranean sea. Limiting factors of cotton cultivation in Greece are mainly soilborne plant pathogens causing either seed and seedling diseases such as *Pythium* spp., *Rhizoctonia solani* and *Thielaviopsis basicola*, or wilt of grown plants, i.e., *Verticillium dahliae*.

In August 1993 in Larissa and Volos counties, in August 1995 in Trikala county and in September 1995 in Phthiotis county, a severe boll rot of cotton was observed (14). In August and September 1996 and 1997 in Larissa and Phthiotis counties, the same disease was observed. Initially, localized spots appeared on the bolls that progressively tended to join and cover the whole boll surface. Infected tissues turned almost black. Infection was restricted to the lower half to two-thirds of cotton plants. Fruiting bodies of fungi of the genus *Phytophthora* were found on the infected tissues. Sporangia were present on the surface of the rotten bolls and there were oospores on the cotton lint and internal

---

Received March 9, 1997; received in final form July 29, 1997; accepted Sept. 14, 1997.

<sup>1</sup>Benaki Phytopathological Institute, 14561 Kifissia, Athens, Greece [Fax: +30-1-8077506; e-mail: ppsall@leon.nrps.ariadne-t.gr].

carpel surface of the infected bolls. In Larissa county, where the disease appeared for the first time, the infection was localized in an area of ~20 ha in a field with a particular cropping practice followed by certain weather conditions. The year before the onset of the disease, the field was also cropped with cotton and the residue was buried by plowing. The following cropping season, 1993, the crop was irrigated 2 to 3 days before a heavy rainfall. The plants were found to be infected on the lower bolls, suggesting that the inoculum was splash-dispersed from the soil as a result of these particular conditions. A fungus of the genus *Phytophthora* was isolated from all infected tissues. Identification of the species was based on colony morphology, growth rates on culture media, production and characteristics of asexual and sexual structures, and maximum growth temperatures of the isolates (21). Furthermore, the profiles of  $\alpha$ -esterase isozymes were compared with those of other *Phytophthora* species with similar morphology.

## MATERIALS AND METHODS

The fungus was isolated on CMA (corn meal agar) or CMA+A (mycostatin 100 mg, polymixin 50 mg and penicillin 20 mg l<sup>-1</sup> CMA).

Morphological characteristics of the isolates were observed on mounts made directly from the infected tissues or after growth of the fungus on CMA or sterile distilled water. Isolates were maintained on CMA, which was also used for maximum growth temperature studies. At 22°C, oospores were formed on CMA in 4–5 days while only a few sporangia were found after 10 days of growth. However, when small pieces of CMA from the growing edge of the culture were transferred to sterile distilled water, sporangia were formed at 22°C within 48 h.

The mean size of oospores and sporangia was estimated by measuring the diameter of 100 oospores and the length and width of 100 sporangia after growth of the fungus on CMA or H<sub>2</sub>O. Moreover, for sporangia the ratio of length to width was calculated, since this is a particularly important character for species identification (21).

Finally, the maximum growth temperature of the isolates was determined by examining the radial mycelial growth for 2 days at several temperatures.

For isozyme analysis, the fungal isolates *Phytophthora cactorum* BPIC 1168 (Benaki Phytopathological Institute Collection), *Phytophthora boehmeriae* BPIC 1901 and 1909, and *Phytophthora parasitica* BPIC 1210 and 1239 were grown for one week in 100 ml liquid pea-broth medium in 200 ml Erlenmeyer flasks at 22°C. Subsequently, each sample was suction-filtered through Whatman No. 1 filter paper and the mycelia were lyophilized. For each isolate, 30 mg of freeze-dried mycelium was ground in an Eppendorf tube with 240  $\mu$ l STEB extraction buffer (MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.2 g; NaHCO<sub>3</sub>, 0.084 g; NaEDTA, 0.034 g; Tris, 0.24 g; Triton X-100, 100  $\mu$ l; sucrose, 10 g;  $\beta$ -mercaptoethanol, 78.2  $\mu$ l; bromophenol blue, 2 mg; and distilled H<sub>2</sub>O up to 100 ml; pH was adjusted to 8.0 with HCl) using a glass rod (17). Tubes were maintained on ice during the processing of the samples. Ground mycelia were centrifuged at 10,000 rpm for 20 min at 4°C. Supernatants were collected and stored at -20°C until needed.

Electrophoresis of the samples was conducted in non-denaturing polyacrylamide gels using the Mini-Protean II electrophoresis apparatus (Bio-Rad, California, USA). For each sample, 20  $\mu$ l was loaded per well and the electrophoresis was run at 100 V until the dyefront reached the bottom of the gels.  $\alpha$ -Esterase (EC 3.1.1.1) isozyme visualization was performed as described previously (17).

For pathogenicity tests, detached cotton bolls were artificially inoculated under the carpel with a 7-mm-diameter mycelial plug. Inoculum was taken from the margin of a 3-day-old *P. boehmeriae* culture grown on petri dishes with CMA medium at 22°C. For each of the two isolates tested, ten cotton bolls were inoculated. Ten cotton bolls inoculated with a CMA plug were included as controls.

## RESULTS

In all cases, sporangia on the infected tissues were almost spherical, with papilla. Moreover, a few chlamydospores and abundant oospores with amphigynous antheridia were found. The fungus was isolated from rotten bolls from Larissa, Trikala and Volos counties but not from those from Phthiotis county. Sporangia formed on culture media were broadly ellipsoid to nearly spherical, papillate with sometimes more than one papilla (Fig. 1). Sporangioophores were sympodial and sporangia were canducous, with a pedicel length of 3–6  $\mu\text{m}$ .



Fig. 1. Sporangia of *Phytophthora boehmeriae* formed on corn meal agar.

The mean size of sporangia formed in  $\text{H}_2\text{O}$  for the Larissa isolates was  $31\text{--}54 \times 24\text{--}42 \mu\text{m}$ , mostly  $32\text{--}48 \times 29\text{--}35 \mu\text{m}$ , with a ratio of 1.2–1.5, mostly 1.3–1.4. The mean size of sporangia formed on CMA for the Trikala isolates was in the range of  $35\text{--}68 \times 29\text{--}54 \mu\text{m}$ , mostly  $47\text{--}59 \times 34\text{--}49 \mu\text{m}$ , with a ratio of 1.1–1.4, mostly 1.2–1.3.

Oogonia on culture media were initially colorless, becoming light yellow. Oospores almost filled the oogonium. The diameter of oogonia formed on CMA for the Larissa isolates ranged from 29 to 40  $\mu\text{m}$ , while that for the Trikala isolates ranged from 21 to 37  $\mu\text{m}$ . Antheridia were in general amphigynous (Fig. 2), and ranged from 14 to 20  $\mu\text{m}$  and 12 to 17  $\mu\text{m}$  for the Larissa and Trikala isolates, respectively. Spore dimensions of both isolates are summarized in Table 1.

The fungus grew regularly on CMA with a maximum growth temperature of 33°C for the Larissa isolate and 32.5°C for the Trikala isolate.

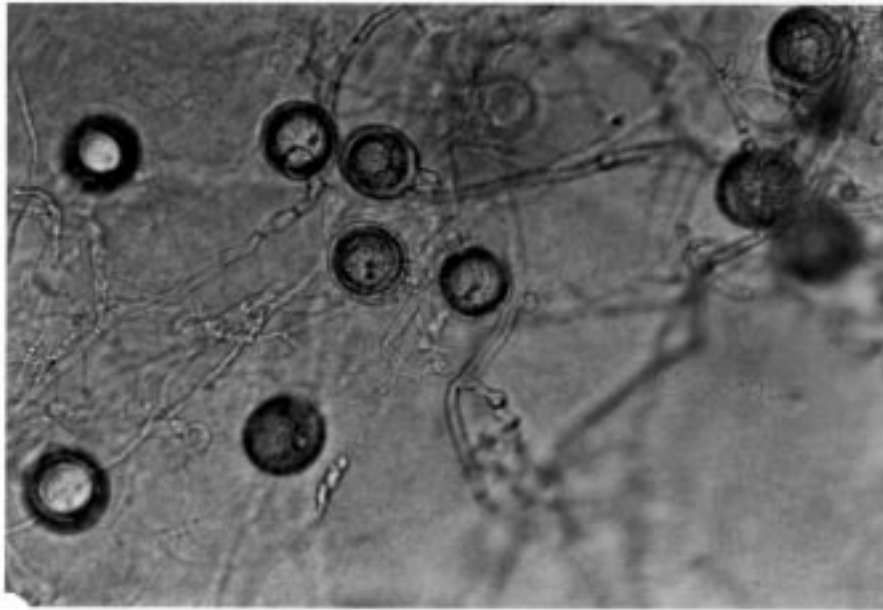


Fig. 2. Oospores of *Phytophthora boehmeriae* with amphigynous antheridia formed on corn meal agar.

TABLE 1. Spore dimensions of *Phytophthora boehmeriae* isolates from infected cotton bolls

Origin of isolate	Spore dimensions ( $\mu\text{m}$ )				
	Sporangia			Oogonia	Antheridia
	length	width	ratio	diameter	width $\times$ length
Larissa	31-54 (39-48) <sup>z</sup>	24-42 (29-35)	1.2-1.4 (1.3-1.4)	29-40 (32-38)	14 $\times$ 20
Trikala	35-68 (47-59)	29-54 (34-49)	1.1-1.4 (1.2-1.3)	21-37 (24-32)	12 $\times$ 17

<sup>z</sup>Figures in parentheses indicate the most commonly encountered measurements.

The  $\alpha$ -esterase isozyme analysis of the two *P. boehmeriae* isolates showed that they had similar patterns (three bands), which differed markedly from those of the *P. cactorum* (two bands) and *P. parasitica* (one band) isolates (Fig. 3). Isolates of *P. boehmeriae* were compared with those of two other *Phytophthora* species, because they bear similar characteristics.

Artificially inoculated cotton bolls developed disease symptoms 6 days after inoculation. *P. boehmeriae* was re-isolated from infected bolls. No symptoms were observed on the control cotton bolls.

## DISCUSSION

A new disease of cotton bolls appeared for the first time in 1993 in Larissa and Volos counties, Greece. The disease, which was observed in two additional counties (Trikala

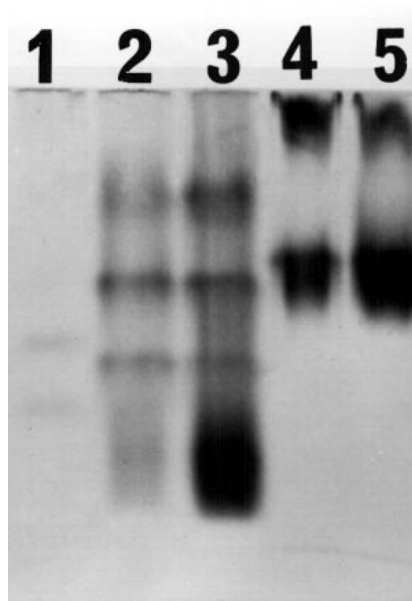


Fig. 3.  $\alpha$ -Esterase isozyme banding pattern of *Phytophthora cactorum* (BPIC 1168; lane 1), *P. boehmeriae* (BPIC 1901 and BPIC 1909, lanes 2,3) and *P. parasitica* (BPIC 1210 and BPIC 1239, lanes 4,5).

and Phthiotis) in 1995 and also in Larissa and Phthiotis in 1996 and 1997, appeared as a severe boll decay restricted to the lower parts of the cotton plants. A fungus of the genus *Phytophthora* was observed and isolated from infected tissues.

For all isolates from infected cotton bolls, sporangia were papillate and oospores had amphigynous antheridia. Based on those characters, the isolated *Phytophthora* species was assigned to group II (4,20,21). The ratio of length to width being less than 1:1.4, the maximum temperature 33°C or 32.5°C, and the abundance of oospores both on the host and the culture media, assigned the isolates to *P. boehmeriae*. The classification was supported further by the sporangia dimensions, the presence of more than one papilla and the way sporangia are released (with a short stem), together with the oogonia dimensions and the lack of hyphal swellings. Moreover, analysis of  $\alpha$ -esterase isozymes showed that both isolates of *P. boehmeriae* had a similar pattern of three distinct bands that was clearly different from the isozyme profile of isolates of *P. cactorum* or *P. parasitica*, two species that have morphology of some characters that overlap with those of *P. boehmeriae*. Isozyme analysis of  $\alpha$ -esterase has been used as a tool for supporting evidence of *Phytophthora* species identification (8).

In the literature, soft rot of cotton bolls was first reported in the Indies in 1921 and it was attributed to an unidentified *Phytophthora* species (1). To the best of our knowledge, the presence of *P. boehmeriae* in Europe is reported here for the first time. The fungus was first described by Sawada in 1927 as a new species on *Boehmeria nivea* (19). It is known to cause cotton boll blight in North China (12,22,23) and to attack *B. nivea* leaves in Formosa (3). According to the Distribution Maps of Plant Diseases (3), *P. boehmeriae*

has been found in East Asia, Australia and South America to infect *B. nivea* as well as other hosts, viz., *Pinus patula* (13), citrus trees – on which it causes orange rot (2,5,6,18) and gummosis disease (18), and young cocoa trees by artificial inoculations (15). In China, oospores, which are the resting structures of the fungus in the soil, are considered as the inoculum source for infections in the following growing season (24).

The cotton field in Greece where the boll rot was first observed was irrigated 2–3 days before a heavy rainfall. The fungus presumably formed its sporangia which were subsequently disseminated by the rain drops. This could explain why infection occurred only on the bolls closer to the ground (7). In Trikala county, the field where the disease appeared was sprinkler irrigated, whereas surrounding fields with drip irrigation were not affected.

Since cotton is widely grown in Greece, and plowing under of crop residue as well as field irrigation are common cultural practices, the fungus could be a serious threat to cotton production. If the drip method of irrigation is employed, the soil with the spores of the fungus is not splashed on the cotton fruits. Moreover, important cotton-growing countries surrounding the Mediterranean basin should be aware of this potential new threat to cotton cultivation.

#### REFERENCES

1. Anon. (1921) Report on the prevalence of some pests and diseases in the West Indies during 1919: Diseases of Economic Plants. *West Ind. Bull.* 19(1):31-37 [*Rev. Appl. Mycol.* 1 (1922):155].
2. Anon. (1941) Primera Reunion Argentina de Agronomia, Abril 1941. Resoluciones y resúmenes de los trabajos presentados. [First Argentine Congress of Agronomy, April 1941. Resolutions and summaries of the transactions presented.] [*Rev. Appl. Mycol.* 21(1942):184].
3. CMI (1980) Distribution Maps of Plant Diseases, Map 203. Issued by the Commonwealth Mycological Institute, Surrey, UK.
4. Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora* Diseases Worldwide. APS Press, St. Paul, MN, USA.
5. Frezzi, M.J. (1941) *Phytophthora boehmeriae* causante de la podredumbre morena de los frutos cítricos, en la República Argentina. [*Phytophthora boehmeriae*, the agent of brown rot of citrus fruits in the Argentine Republic.] *Rev. Argent. Agron.* 8:200-205 [*Rev. Appl. Mycol.* 23(1944):296].
6. Frezzi, M.J. (1942) Podredumbre morena de los frutos cítricos y parasitos que la producen en Corrientes, Argentina. [Brown rot of citrus fruits and the parasites that produce it in Corrientes, Argentina.] *Rev. Argent. Agron.* 9:216-220 [*Rev. Appl. Mycol.* 23(1944):294].
7. Hillocks, R.V. (1992) Cotton diseases. CAB International, Wallingford, UK.
8. Ilieva, E., Arulappan, F.X. and Pieters, R. (1995) *Phytophthora* root and crown rot of raspberry in Bulgaria. *Eur. J. Plant Pathol.* 101:623-626.
9. Kouyeas, H. (1971) On the apoplexy of stone fruit trees caused by *Phytophthora* spp. *Ann. Inst. Phytopathol. Benaki* 10:163-170.
10. Kouyeas, H. (1977) Stone tree apoplexy caused by *Phytophthora* collar rot. *EPPO Bull.* 7:117-124.
11. Kouyeas, H. and Chitzanidis, A. (1968) Notes on Greek species of *Phytophthora*. *Ann. Inst. Phytopathol. Benaki N.S.* 8:175-192.
12. Liang, P.-V. (1964) Identification of *Phytophthora* species causing cotton boll blight and cotton bean blight in North China. *Acta Phytopathol. Sin.* 7:11-20 [*Rev. Appl. Mycol.* 44(1965):195].
13. Oxenham, B.L. and Winks, B.L. (1963) *Phytophthora* root rot of *Pinus* in Queensland. *Queensland J. Agric. Sci.* 20:355-366 [*Rev. Appl. Mycol.* 43(1964):328].

14. Paplomatas, E.J., Elena, K. and Lascaris, D. (1995) First report of *Phytophthora boehmeriae* causing boll rot of cotton. *Plant Dis.* 79:860 (abstr.)
15. Ravise, A. (1970) Modalités du parasitisme de souches de *Phytophthora* de Bary sur jeunes Cacaoyers. [Details of the parasitism of strains of *Phytophthora* on young cacao trees.] *Café – Cacao – Thé* 14:295-302 [*Rev. Appl. Mycol.* 51(1972):44].
16. Ribeiro, O.K. (1978) A source book of the genus *Phytophthora*. Cramer, Vaduz, Lichtenstein.
17. Rivillas, C. and Dodd, J.C. (1996) The effects of arbuscular mycorrhizal fungi on two different coffee varieties from Colombia and their biochemical detection in roots. *Proc. 4th European Symp. on Mycorrhizas* (Granada, Spain, 1994), pp. 47-50.
18. Rosetti, V. (1947) Porta – enxertos de Citrus resistentes a “gomose” de *Phytophthora* e a “tristeza”. [Citrus stocks – resistant to *Phytophthora* “gummosis” and “tristeza”.] *Biologico* 13(5):89-90 [*Rev. Appl. Mycol.* 27(1948):129].
19. Sawada, K. (1931) List of fungi found in Formosa. Gov. Res. Inst., Taihoku, Formosa [*Rev. Appl. Mycol.* 11(1932):205].
20. Stamps, D.J., Waterhouse, G.M., Newhook, F.J. and Hall, G.S. (1990) Revised tabular key to the species of *Phytophthora*. *CAB Int. Mycol. Pap.* No. 162.
21. Waterhouse, G.M. (1963) Key to the species of *Phytophthora* de Bary. *CAB Int. Mycol. Pap.* No. 92.
22. Wu, X.Z., Li, Q.J. and Wang, F.R. (1993) Effects of exudates of cotton boll of different age on *Phytophthora boehmeriae* and *Colletotrichum gossypii*. *Rev. Plant Pathol.* 73:210 (abstr.).
23. Zheng, X.B., Lu, J.Y., He, H., Wang, T.L. and Wang, H.Y. (1992) Oospores of *Phytophthora boehmeriae* overwintered in soil as an infection source of cotton boll rot disease. *Rev. Plant Pathol.* 73:64 (abstr.).