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Phytophthora root and crown rot of raspberry in Bulgaria

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Abstract

Root and crown rot of raspberry (*Rubus idaeus* L.) was observed in a plantation at the experimental station of small fruits in Kostinbrod, Bulgaria. Isolates of *Phytophthora* spp. were obtained from diseased plants. Colony morphology, growth rates, features of asexual and sexual structures were studied and as a result two *Phytophthora* species were identified: *Phytophthora citricola* Saw. and *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian. Their pathogenicity was confirmed in artificial inoculation experiments. The isozyme (α-esterase) patterns of *P. citrophthora* and *P. citricola* isolates from raspberry and from the collection of the CBS, Baarn the Netherlands were compared, using micro-gel electrophoresis. Both species are reported for the first time as pathogens of raspberry in Bulgaria. This is only the second report in phytopathological literature of *P. citrophthora* on raspberry, the first being from Chile [Latorre and Munoz, 1993].

Introduction

Disease of raspberry caused by *Phytophthora* species has been known for many years. Waterston [1937] reported *P. citricola* on red raspberry in Scotland. Since then several *Phytophthora* species have been reported as pathogens of raspberry in different countries: *P. cactorum, P. cambivora, P. cinnamomi, P. cryptogea, P. drechsleri, P. erythroseptica, P. fragariae* var. *rubi, P. megasperma* and *P. syringae* [Brien and Dingley, 1959; Converse and Schwartze, 1965, 1968; Duncan *et al.*, 1987, 1990; Nourisseau and Baudry, 1987; Seemüller *et al.*, 1986; Washington, 1988; Wilcox *et al.*, 1989, 1993].

In Bulgaria root rot and dieback of raspberry caused by *Phytophthora* spp. was observed first in 1991. The objective of this research was to identify the isolated species (by morphological characteristics as well as isozyme analysis), to confirm their pathogenicity and describe the symptoms of the disease.

The experiments were carried out partly at the Plant Protection Institute in Kostinbrod, Bulgaria and partly at the Plant Protection Service in Wageningen, the Netherlands.

Material and methods

For isolation of the fungi, small pieces (1 cm) of necrotic tissue of diseased roots and canes were washed in tap water, placed for 1–2 min. in 70% alcohol, rinsed 2 times in sterile distilled water, dried between sterile filter papers and transferred to water- and oatmeal-agar. Plates were incubated at 24 °C and emerging colonies of *Phytophthora* were subcultured and stored on slants of oatmeal agar.

The identification of isolates was done according to the criteria of Waterhouse [1963, 1970] and Stamps *et al.* [1990]. Colony morphology and growth rate was compared on different media (oatmeal agar (OA), cornmeal agar (CMA), potato dextrose agar (PDA), cherry agar (CA) and V-8 juice agar) [Gams *et al.*, 1987]. Isolates in dishes were incubated at 24 °C in the dark and colony diameter was measured after 5 days. For each isolate 5 dishes of each medium were used.

Sporangial formation and morphology were studied in water cultures, prepared from mycelial plugs from oatmeal agar cultures (6 mm), transferred to Petri dishes with sterile pieces of grass and distilled water. In addition, pepper seeds which had been incubated

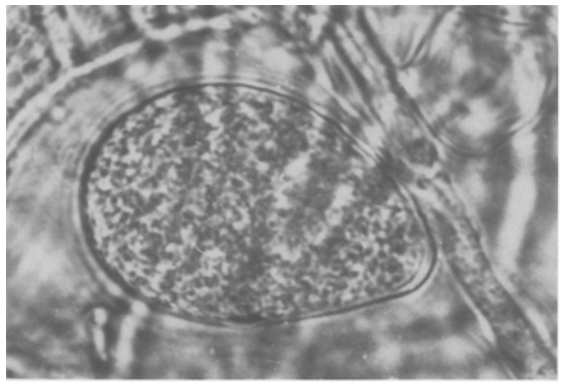

Fig. 1. Typical sporangium of *P. citricola*.

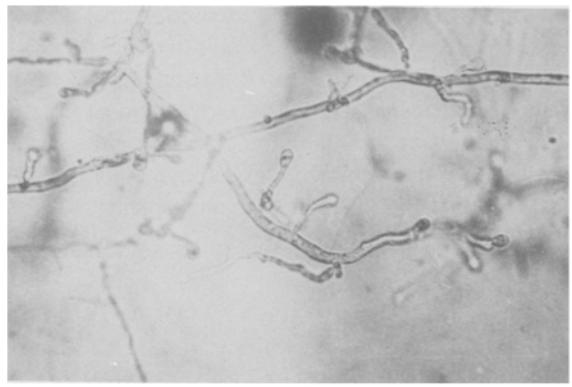

Fig. 2. Lateral hyphae of *P. citricola* with swollen tips.

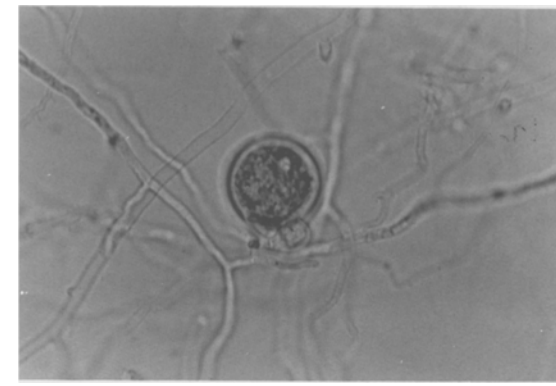

Fig. 3. Paragynous antheridium and oogonium of *P. citricola*.

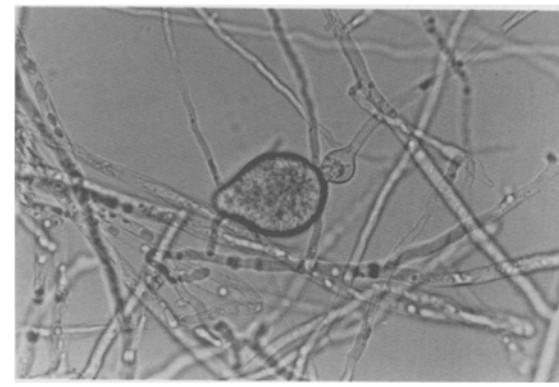

Fig. 4. Sporangium of *P. citrophthora* with basal swelling.

for 24 h on the surface of 3–4 days old agar cultures of each isolate were placed on water agar and flooded with a mixture of pond and distilled water (1:2).

The production and morphology of sexual structures were studied on cornmeal agar after growing the heterothallic isolates in the dark at 20 °C. in dual cultures with known A1 and A2 mating types of *P. nicotianae* var. *nicotianae* and *P. capsici*.

Dimensions of 50 asexual and sexual structures of each isolate were determined.

Isozyme patterns were studied by micro-gel electrophoresis according to a method developed by the Mycology Section of the Plant Protection Service [Arulappan, 1993].

Pathogenicity of the isolates was tested in artificial inoculation experiments under laboratory conditions. Seven days old mycelial plugs (6 mm) from oatmeal agar were placed on freshly made leaf scars of raspberry canes in humid chambers and resulting necrotic lesions were measured. The expansion of the lesions (in mm/day) was calculated by means of regression analysis [Verhoeff, 1965]. Two cultivars (Samodiva

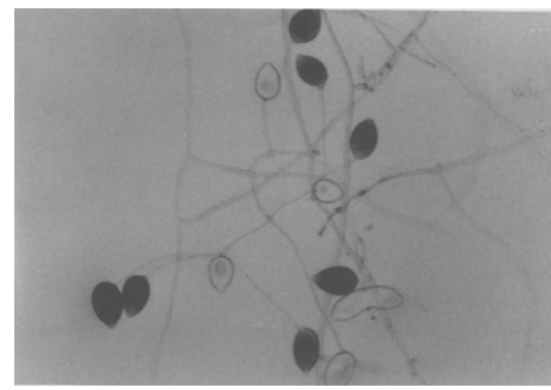

Fig. 5. Sporangia and loosely branched sporangiophores of *P. citrophthora*.

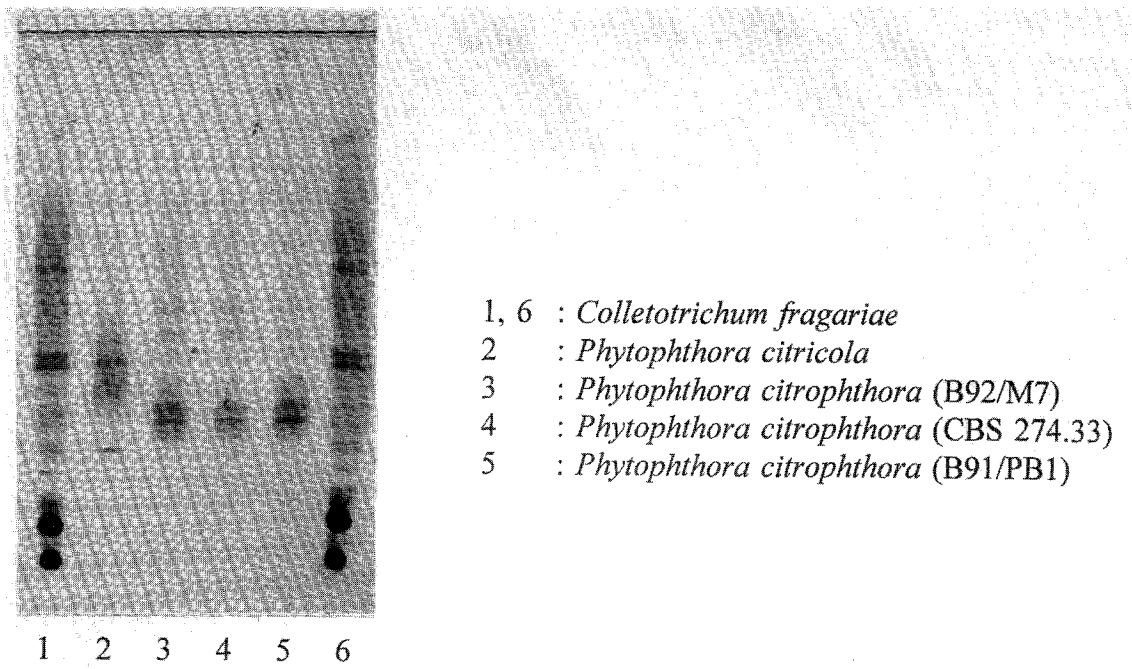

Fig. 6. α-Esterase patterns of three isolates of *P. citrophthora* (3,4,5) and one of *P. citricola* (2).

and Norna) were tested; of each five canes were inoculated with a mycelial plug.

Results and discussion

In the raspberry plantation, plants with yellow and wilted leaves were observed. At first, symptoms were visible only on the top part of the canes (both primocanes and floricanes), but later the whole cane became infected and died. At the base of diseased canes, dark brown lesions appeared and roots of affected plants started rotting. From affected roots and canes of a dozen raspberry bushes, eight isolates of *Phytophthora* were obtained. Two species could be distinguished: *P. citricola* (6 isolates) and *P. citrophthora* (2 isolates).

Isolates of *P. citricola* on agar media formed colonies of submerged mycelium with scant aerial mycelium (on all media) and a characteristic chrysanthemum pattern, which was most pronounced on PDA, CMA and V-8 agar. The mean growth rate (mean of 5 Petri dishes) of the fungus at 24 °C depended on the medium; growth was fast on OA and CMA, where colony diameter after 5 days incubation was 85 and 77.5 mm respectively. The slowest growth occurred on PDA (72.5 mm), but more aerial mycelium was produced on this medium.

Formation of sporangia was studied in liquid cultures. Sporangia were semi-papillate (Fig. 1), variable in shape, often distorted and with two papilla, typical for *P. citricola* [Waterhouse, 1963; Stamps *et al.*, 1990]. Sporangia are persistent with av. size 55.0 × 40.0 μ, av. l/b ratio 1.37. Sporangiophores were thinner than hyphae, often with swellings at the point of branching. Lateral hyphae were frequently swollen at the tip (Fig. 2). Terminal chlamydospores were also observed.

There was some difference in the size of sexual structures among isolates. Some had smaller oogonia av. 30.6 μm (24.4–34.8 μm) and oospores av. 26.5 μm (23.4–31.4 μm), others had larger oogonia with mean dimensions 35.2 μm (32.5–37.0 μm) and for oospores av. 28.7 μm (25.4–35.0 μm). In the latter group of isolates some of the oospores did not develop well.

Antheridia were mostly paragynous (Fig. 3), mono- and in some cases diclinous, but amphyginous antheridia were present also. Distinct differences in the size of antheridia between isolates was not observed (av. 12.3 × 10.2 μm and 12.9 × 11.4 μm for the groups with smaller and larger oogonia respectively).

Isolates belonging to *P. citrophthora* formed colonies with more aerial mycelium than *P. citricola* and with a slightly stellate pattern.

Sporangiophores were single and loosely branched, often with swellings (10.2–13.4 μm) (Fig. 4). Sporangia were formed on agar media but more abundantly in water culture; average dimensions 40.0 × 30.4 μm (30.3–60.0 × 20.5–35.8 μm). They were papillate (often with two papilla), occasionally caducous with pedicels of 10–24 μm long. Distorted shapes were also present (Fig. 5).

Sexual reproductive structures were not observed in single culture. They were not formed even after growing the isolates in dual cultures with A1 and A2 mating types of *P. nicotianae* var. *nicotianae* and *P. capsici*. Absence of sexual organs is characteristic of *P. citrophthora*.

In the pathogenicity experiments, all inoculated canes of the cultivars Samodiva and Norna showed lesions after inoculation with isolates of both species. These lesions did not differ significantly from naturally formed lesions, but colouration was more pronounced, dark brown to black. Isolates of *P. citrophthora* were less pathogenic (lesions up to 4 cm) than those of *P. citricola* (lesions up to 9 cm), but symptoms produced by isolates of both species were similar and could not be used to differentiate species. Disease development in the field occurred during June–July under high moisture conditions.

Three isolates of *P. citrophthora* were also compared by means of isozyme analysis. Two Bulgarian isolates; one from raspberry (B92/M7) and one from strawberry (B91/PB1), and one isolate from the CBS culture collection (citrus: CBS 274.33) showed more or less the same α-esterase patterns; 2 major bands and 5 less pronounced bands. The isolate of *P. citricola* (M41) showed an α-esterase pattern which was markedly different from isolates of *P. citrophthora*. The results also indicate that at least in the case of *P. citrophthora*, α-esterase patterns do not seem to be influenced by the host plant from which the fungus was isolated.

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