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CANES WILTING WITH COLLAR AND ROOT ROT OF RASPBERRY CAUSED BY *PHYTOPHTHORA PSEUDOCRYPTOGEA* IN BULGARIA

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Abstract

A wide range of herbaceous and woody plant species are known as host plants of Phytophthora pseudocryptogea, a relatively newly described species. Recently P. pseudocryptogea was isolated from raspberry plants in Bulgaria. Diseases plants were found in the 4 to 5 years old variety Ljulin plants on the biological production orchards in Kostenets region. Canes suddenly wilt and turn brown at the onset of warm temperatures. The plants manifested disease symptoms such as collar and root rot. The precise species determination of obtained isolate was done on the basis of the colony and asexual spores morphology and sequence analyses of the ITS region of the nuclear DNA. The pathogenicity of the fungus was tested by detached leaf bioassay on several raspberry cultivars in the laboratory.

Key words*: raspberry, collar and root rot, Phytophthora pseudocryptogea, Bulgaria.*

INTRODUCTION

Phytophthora pseudocryptogea was distinguished from *Phytophthora cryptogea sensu stricto* and designated as a new oomycete species several years ago (Safaiefarahani et al., 2015). Both of plant pathogens possess a wide host range, but recently was found that they differ in their host range. Both of them are pathogenic to the same plant species as some solanaceous (potato, tomato, eggplant), pistachio, spinach, and clover. But other plant species express different responses to inoculation of both pathogens and thus are able to demonstrate the difference among their host range. The cucumber, melon, watermelon, alfalfa, soybean, cabbage, green pepper and rice, are found to be susceptible to *P. pseudocryptogea*, but are not infected by *P. cryptogea* (Delshad et al., 2020). *P. pseudocryptogea* was reported as a part of the *Phytophthora* pathogen complex responsible of declining of some plants as common alder (*Alnus glutinosa*) trees (Seddaiu & Linaldeddu, 2020) and pomegranate (Ghaderi & Habibi, 2021). In a few years the host range of *P. pseudocryptogea* expands to include more plant species.

In this study, an isolate of *P. pseudocryptogea* was obtained from diseased raspberry plants in Bulgaria and characterized.

MATERIALS AND METHODS

Isolation of *P. pseudocryptogea*

The four and five years old wilting raspberry plants of cultivar Ljulin, grown in the biological production orchards demonstrated collar and root rot. An isolate of *P. pseudocryptogea* was obtained by baiting from a soil sample, taken from the root area of diseased plants. The collected soil sample was distributed on the bottom of plastic boxes and covered by a water layer (1 cm depth). *Rhododendron* leaves were placed on the surface of the water (Themann & Werres, 2000). After developing necrotic areas on the leaves and common surface sterilization, the causal agent of the spots wasisolated on water agar and then transferred on a selective for *Phytophthora* species PARNHB medium (carrot agar supplemented with 10 mg Pimaricin, 250 mg Ampicillin, 10 mg Rifampicin, 50 mg Nystatin, 50 mg Hymexazol and 15 mg Benomyl/1,000 ml) at 25ºC. The developed mycelium then was used for DNA extraction and in pathogenicity tests.

Determination of *P. pseudocryptogea* specie

The determination of the exact species of obtained *Phytophthora* isolate was done by DNA sequence analysis. DNA extraction was done from 10-days old mycelia using DNeasy Plant Mini Kit (QIAGEN GmbH), followed by PCR amplification of the ITS region with primers ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') using PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare Life Sciences), according to the manufacturer's instructions and the following PCR thermal program: 96ºC - 2 min, 35 cycles of 96ºC - 1 min, 55ºC - 1 min, 72ºC - 2 min, and final elongation at 72ºC - 10 min. The purified PCR product was sequenced in GATC Biotech AG (Germany). The obtained ITS sequene was compared with others by performing a BLAST search in the NCBI (National Center for Biotechnology Information) database.

Morphology of colonies and asexual structures

Phytophthora pseudocryptogea isolate was grown on V8 juice agar (16 g agar, 3 g $CaCO_3$, 100 ml Campbell's V8 juice and 900 ml distilled water), for 10 days in the dark at 20°C. Colony morphology patterns were characterized according to Erwin and Ribeiro (1996). The sporangia production was stimulated according to Jung and Burgess (2009), by flooding cultured V8 agar sections of approximately 20 × 15 mm with non-sterile pond water in 90 mm Petri dishes. After 36-48 hours of incubation at ambient conditions, the dimensions and the characteristic features of 50 mature sporangia chosen at random were determined under microscope at ×400 magnification (ZEISS Axio Imager A2) with a digital camera (AxioCam ERs 5S) and a biometric software (AxioVision LE).

Pathogenicity test

The pathogenicity of the isolate was checked by detached leaf assay on several plant species (perennial woody and shrubby plants: Turkey oak (*Quercus cerris*), sweet cherry (*Prunus avium*), linden (*Tilia tomentosa*), blackberry (*Rubus fruticosus*), cherry laurel (*Prunus laurocerasus*), Japanese knotweed (*Fallopia japonica*). Turkey oak and linden are trees widely spread in forest areas in Bulgaria, as well as in urban green areas. Cherry laurel is also broadly grown as a decorative plant in city parks. Sweet cherry is often found in private yards and in orchard gardens. Japanese knotweed is an invasive plant in the country that is easy to be seen in many different habitats, often including human settlements.

Four fully developed detached leaves of each plant species were placed separately in a Petri dish each on moist filter paper. Pieces of PDA (4 x 4 mm in size) with mycelium of tested isolate of *P. pseudocryptogea* on it developed for 7 days were placed with mycelium surface on the upper sides of the leaf lamina. The Petri dishes were covered and maintained at room temperature. In additional detached leaves assay five raspberry cultivars were tested for their reaction in inoculation with mycelia mats of *Phytophthora pseudocryptogea*. The assay was maintained in the same way as it was done with detached leaves of different plant species described above. Five leaves of each raspberry cultivar were used in the test. The diameter of developed spots on the leaves was measured 7 days after inoculation.

RESULTS AND DISCUSSIONS

Determination of *P. pseudocryptogea* specie

The obtained *Phytophthora* isolate was identified based on the sequence homology of the ITS region and determined as *Phytophthora pseudocryptogea* (100% of homology).

Morphology of colonies and asexual structures

The obtained isolate of *Phytophthora pseudocryptogea* formed stellate colonies with abundant aerial mycelium on V8 juice agar medium (Figure 1, a). The species is with heterothallic mating system and did not produced sexual structures in single culture. In non-sterile pond water *Phytophthora pseudocryptogea* formed small non-papillate predominantly ovoid (Figure 1 b, c) and more rarely obpyriform sporangia (Figure 1, d, e). Their dimensions were as follows: average length of 36.61±0.62 µm, average width of 26.91±0.40 µm, and length to width ratio 1.36±0.01.

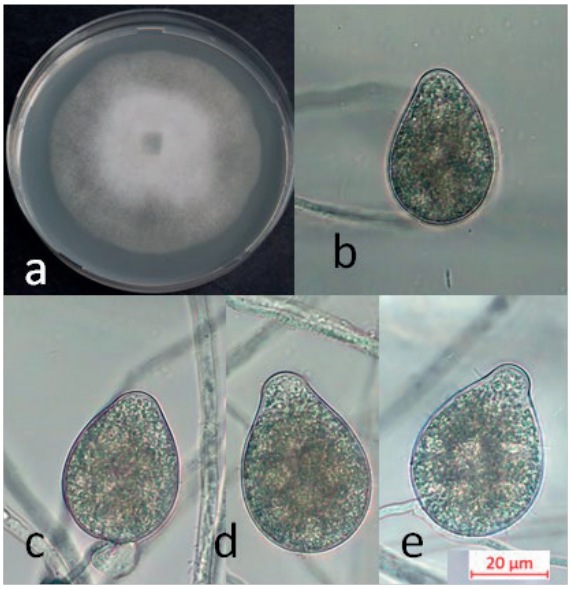

Figure 1. Morphology of the colonies and asexual structures of *P. pseudocryptogea*: a) 6 days culture of *P. pseudocryptogea* on V8 agar media; b)-e) sporangia formed in non sterile pond water after 36–48 h

Pathogenicity of *P. pseudocryptogea*

The leaves of the tested woody and shrubby plants show different responses on the inoculation with mycelium of *Phytophthora pseudocryptogea*.

The leaves of blackberry, linden and Japanese knotweed were not infected and there were no leaf spots observed (Figure 2).

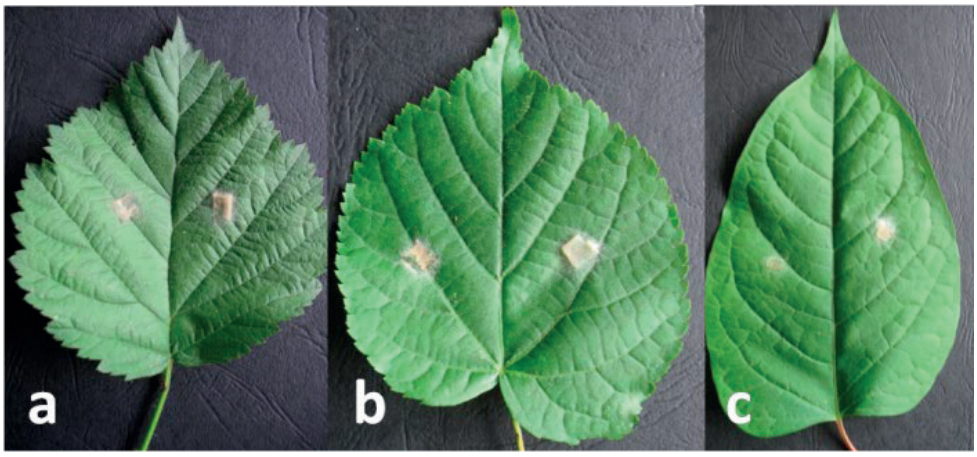

Figure 2. Inoculated detached leaves of blackberry (a), linden (b) and Japanese knotweed (c), inoculated with mycelium of *P. pseudocryptogea* 10 days after inoculation. No leaf spots developed

On the contrary very well formed large necrotic lesions developed on the inoculation points on the leaves of Turkey oak, sweet cherry and cherry laurel (Figure 3).

The successful inoculation of the leaves of the three plant species supposed that these plant species are to great extent potential hosts of the pathogen. The fact that the plants are broadly spread in ature and in the living areas amplified the risk of the distribution of *P. pseudocryptogea* and increased damages and losses in city green areas, nurseries and forestry.

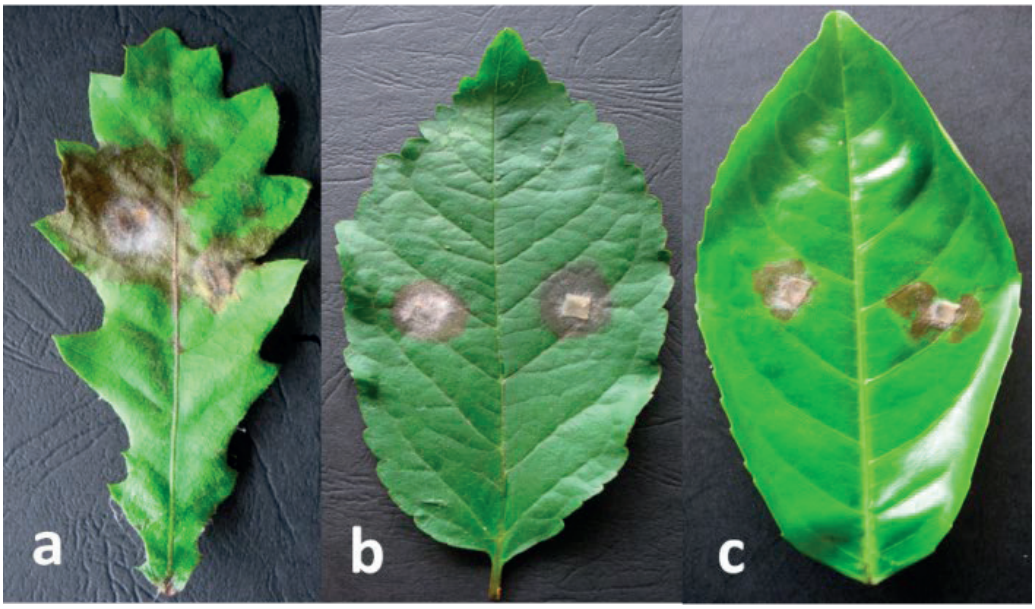

Figure 3. Inoculated detached leaves of Turkey oak (a), sweet cherry (b) and cherry laurel (c), inoculated with mycelium of *P. pseudocryptogea* 10 days after inoculation. Necrotic lesions appeared on the points of inoculation

The host range of *P. pseudocryptogea* is quite extensive. Among the plant species that are proven as hosts of *P. pseudocryptogea* are the following woody and annual plants: Pacific silver fir (*Abies amabilis*), kiwi fruit (*Actinidia chinensis*), strawberry tree (*Arbutus marina*) African daisy (*Arctotis acaulis*) thistle dyandra (*Banksia cirsioides*), sugar beet (*Beta vulgaris*), marri tree (*Corymbia calophylla*), cucumber (*Cucumis sativus*), squash (*Curcurbita pepo*), carrot (*Daucus* sp.), *Isopogon buxifolius*, walnut (*Juglans regia*), tomato (*Lycopersicon esculentum*), apple (*Malus pumila*), purple African nightshade (*Solanum marginatum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*), Western Australian grass tree (*Xanthorrhoea preissii*) (Khaliq et al., 2019; Dalshed et al., 2020; Farr & Rossman, 2020; California Pest Rating Proposal for *Phytophthora pseudocryptogea*, 2020). The above mentioned list includes trees, annual plants, wild and endemic plants, and a large number of cultivated crops. *P. pseudocryptogea*, together with *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, and *P.* sp. *kelmania* are reported to cause disease in pomegranate orchards of Iran. *P. pseudocryptogea* and *P.* sp. *kelmania* were isolated from root crowns of infected trees (Ghaderi & Habibi, 2021). *P. pseudocryptogea, P. acerina*, and *P. plurivora* are reported as associated with declining of common alder trees (*A. glutinosa*) in Italy. All three *Phytophthora* species are assumed as a serious threat to riparian alder ecosystems in Sardinia, based on their widespread occurrence and virulence (Seddaiu & Linaldeddu, 2020).

P. plurivora and *P. pseudocryptogea* were obtained using baiting and selective media from soil samples taken around symptomatic oak trees (*Quercus robur*) in Emirgan Grove, İstanbul. Both *Phytophthora* species were

proven as pathogenic in inoculation tests on 2- to 3-year-old *Q. robur* and *Q. suber* seedlings. Thus it is accepted that *P. plurivora* and *P. pseudocryptogea* may play roles in the dieback of the oaks, including *Q. robur* and *Q. suber* (Kurbetli et al., 2022). *P. pseudocryptogea* is also supposed to play a role in decline of Holm oak (*Quercus ilex*) in Europe (Mora-Sala et al., 2018). According to us, the Turkey oak (*Quercus cerris*), sweet cherry (*Prunus avium*) and cherry laurel (*Prunus laurocerasus*) are reported as potential hosts of this pathogen for the first time.

Detached leaf bioassays of raspberry cultivars

Five raspberry cultivars were tested for their response to the inoculation of *P. pseudocryptogea* in detached leaf assays. Three of them are Bulgarian raspberry cultivars - Ljulin, Shopska alena and Balgarski rubin, and two of them are introduced - Meeker and Heritage. All five raspberry cultivars appeared to be infected by the pathogen. However, there was a slight difference in the response of the cultivars (Figure 4).

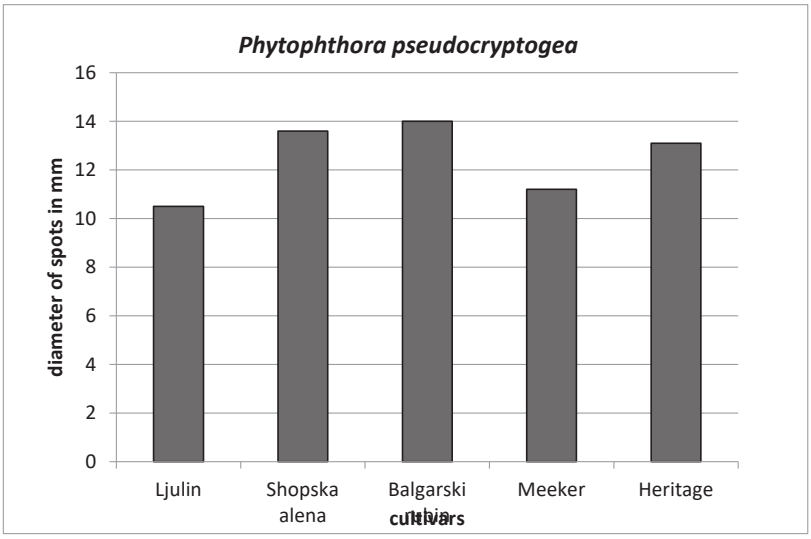

Figure 4. Response of raspberry cultivars to inoculation with *P. pseudocryptogea* in detached leaf assay

CONCLUSIONS

Phytophthora pseudocryptogea was isolated from a soil sample and was associated with the collar and root rot of raspberry in Bulgaria. It was shown to be pathogenic to Turkey oak, sweet cherry, and cherry laurel plants. Among the tested raspberry cultivars there were not found resistant ones, although a difference in the response to the infection was noticed among the cultivars.

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