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NEW OR UNUSUAL DISEASE REPORTS

Neofusicoccum parvum causes stem canker of thornless blackberry in Italy

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Summary. Important damage caused by the fungus *Neofusicoccum parvum* in thornless blackberry in southern Italy is reported for the first time. The most noticeable symptoms were stem cankers and yellowing of the foliage. Cankers on stems were initially elongated, and infected tissue was darkly pigmented. The fungus was identified based on morphological characteristics and by sequencing the ITS1-5.8S-ITS2 region and part of the translation elongation factor 1-alpha (EF1-a) gene. Pathogenicity tests confirmed that *N. parvum* caused the disease on blackberry, inducing symptoms similar to those occurring under natural conditions. This is potentially a serious disease of blackberry in southern Italy, where cultivation is expanding.

Key words: Botryosphaeriaceae, *Rubus fruticosus*, blackberry canker.

Introduction

Rubus fruticosus L. (*Rosaceae*) is a rambling shrub known for its blackberry fruit, which has significant medicinal, cosmetic and nutritional values (Zia-Ul-Haq *et al.*, 2014). A large number of different cultivars are grown, many of which are without thorns. Thornless cultivars of *R. fructicosus* are more productive and produce greater quality fruit compared to wild varities. Blackberry production, although widespread, is usually on small scales, but is expected to increase in many regions. The greatest recent expansion in fresh blackberry production has occurred in North America, for consumption in the United States of America and Europe. Blackberry production in Europe is also increasing in several countries, including the United Kingdom, Germany, Romania, and Spain.

In Italy, most blackberry is cultivated in northern regions, mainly in the Piedmont, Lombardy, Emilia-Romagna, Friuli-Venezia Giulia and particularly Trentino regions, where more than 60% of the Italian production (approx. 30 ha) is concentrated (Beccaro *et al.*, 2002). Recently, the cultivation of blackberry is also expanding to the central-southern areas of Italy. This has resulted in increasing interest from farmers, who can produce fresh fruit in periods of low supply, thus expanding the period of blackberry marketing.

A canker disease was observed for the first time in thornless blackberry plants in a commercial planting of cv. Loch Ness (Nessy) in the Catania province (eastern Sicily, Italy) in 2015. Crop losses caused by the disease were estimated between 5 and 15%. Disease symptoms first appeared in early summer, and initially consisted of dark brown lesions starting at lateral buds and nodes on the affected plant stems (Figure 1). Later, cankers developed around and beneath the nodes girdling the stems. Leaf blades on the entire stems or only on the portions above the cankers turned chlorotic and then desiccated remaining attached to the petioles along the stems (Figure 2). The disease progressed, and numerous black pycnidia developed on the bark surfaces. Well-developed

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Figure 1. Stem canker, caused by *Neofusicoccum parvum*, on a thornless blackberry plant grown in southern Italy.

Figure 2. Diseased foliage on a thornless blackberry plant affected by *Neofusicoccum parvum*, grown in southern Italy.

stem cankers split longitudinally exposing the stem pith tissues. In most cases, the portions above the cankers either died or were extremely weak and did not produce marketable blackberries. These symptoms were similar to those of cane canker of blackberry, caused by *Botryosphaeria dothidea* in the United States of America (Maas and Uecker, 1984).

The aim of the present study was to identify the causal agent associated with cankers in thornless blackberry, from a commercial plantation in eastern Sicily.

Materials and methods

Fungal isolation and morphological characterization

Isolations were made from ten blackberry plants showing stem canker with different disease severity. Stems were washed with running tap water, surface sterilised with 2% sodium hypochlorite and then 70% ethanol, rinsed in sterile distilled water, and blotted dry with filter paper before being cut into 5 mm pieces. Stem tissue pieces were plated onto potato dextrose agar (PDA) with 200 mg L^{-1} of streptomycin sulfate in Petri dishes (three pieces per dish), and the dishes were then incubated for 15 d at 24±1°C in darkness. Hyphal tips of fungus colonies growing from the tissue pieces were subcultured onto fresh PDA to obtain pure single hyphae cultures. Sporulation of the isolates was induced by placing mycelium plugs from isolate cultures onto 2% water agar cultures containing double-autoclaved pieces of blackberry stems (1 cm length). After 14 d incubation in natural light at 23 ± 1°C, conidiomata growing in these cultures were sectioned vertically and mounted in 100% lactic acid. Light microscope examinations were made with an Olympus BX50 microscope for description of the morphological characteristics of the isolated fungus.

Two isolates out of five used in this study have been deposited in the culture collection of the Department of Agriculture, Food and Environment at the University of Catania, with the culture collection codes BB01-Np and BB02-Np.

Molecular identification

Genomic DNA was extracted from pure cultures grown on PDA of two representative isolates (BB01-Np and BB02-Np) recovered from blackberry, using

the DNeasy Plant Mini Kit (Qiagen GmbH). The ITS1-5.8S-ITS2 gene region and part of the translation elongation factor 1-alpha (EF1-α) gene were amplified, respectively, with primers ITS1/ITS4 (White *et al*., 1990) and EF1-688F (Alves *et al*., 2008) /EF1-986R (Carbone and Kohn, 1999). Amplifications were performed in a 25 µL reaction volume containing 1× PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate, 0.5 µM of each primer, 10 ng template DNA, and one unit of Taq DNA Polymerase (Invitrogen). PCR reactions were performed in an automated thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer Cetus) programmed as follows: 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 58°C (ITS) or 60°C (EF1-α) and 1 min at 72°C. All reactions ended with 10 min at 72°C. Amplicons were visualized by gel electrophoresis, purified (ExoSAP-IT), and sequenced in both directions. Consensus sequences from the two isolates were compared with reference sequences in GenBank using BLAST analyses.

Pathogenicity tests

Two isolates from blackberry (BB01-Np and BB02-Np) were used in pathogenicity tests. Colonized PDA mycelial plugs (2–3 mm diam.) from actively growing colonies were placed into bark wounds in the centres of young stems (4–6 mm diam) of 2-year-old potted plants of blackberry cv. Loch Ness. Inoculated wounds were wrapped with Parafilm. Control plants were inoculated with non-colonized PDA plugs. Inoculated (four per isolate) and control plants (four) were then kept in a greenhouse and watered as needed.

Results and discussion

Fungus isolates with uniform colony morphology were obtained from stems of the 10 symptomatic blackberry plants, with a mean isolation frequency of 90%. The isolates developed abundant aerial mycelium that became dark grey after 5–7 d in culture, and formed black, globular pycnidia after 2 weeks. Conidia were hyaline, aseptate, unicellular, thin walled and ellipsoidal, each with a round apex and flat base. They had dimensions of 16.3–22.5 × 4.0–4.7 µm (av. ± S.D. of 300 conidia = 19.5 ± 2.3 × 4.5 ± 0.7 µm), and a mean L/W ratio of 3.2 (SD = ±0.5). Mature conidia were each 1-2 septate and light brown in colour, with the middle cell darker than the terminal cells.

The ITS (Acc. Nos. MF279190 and MH204447) and EF1-α (Acc. Nos. MF279191 and MH220403) sequences from isolates BB01-Np and BB02-Np showed 100% homology with the *Neofusicoccum parvum* ex-type isolate CMW9081 in the NCBI database.

Based on the morphological characters and DNA analyses, the fungus was identified as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Phillips *et al.*, 2013).

All the inoculated blackberry plants showed dark brown lesions on stems 40–50 d from inoculation, with mean lesion lengths ranging from 2.5 to 3 cm. Later, these lesions developed cankers showing zonate patterns of dark and lighter discolouration. No significant differences in lesion lengths among two isolates of *N. parvum* were observed. Three months after inoculation, all blackberry plants showed low vigour and leaf chlorosis. No symptoms were observed in control plants. *Neofusicoccum parvum* was successfully re-isolated from symptomatic tissues, thus fulfilling Koch's postulates.

Neofusicoccum parvum (Botryosphaeriaceae), is emerging as a common and cosmopolitan species on a wide variety of hosts (Phillips *et al*., 2013). In Italy, this fungus has been reported as a pathogen of English oak, sycamore (Moricca *et al*., 2012), olive (Carlucci *et al*., 2013), mango (Ismail *et al*., 2013), loquat (Giambra *et al*., 2016), Australian blackwood (Sidoti, 2016) and pomegranate (Riccioni *et al*., 2017). Boyzo-Marin *et al*. (2014) reported different fungal species associated with dieback and stem canker of blackberry, including *N. parvum* as the most prevalent species in Michoacan (Mexico). To the best of our knowledge, this is the first report of *N. parvum* causing stem canker on thornless blackberry canker in Italy. Further studies are needed to evaluate the incidence of this fungus in different blackberry cultivars and local conditions, since this is potentially an important disease that is likely to harm commercial fruit production.

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