Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Blue Mold Caused by *Penicillium crustosum* on Nectarine Fruit in Serbia

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Penicillium crustosum Thom. is a fungus commonly found on cheese and nuts but is also a postharvest pathogen that causes blue mold disease of pome and stone fruits including plum and nectarine (Louw and Korsten 2016; Restuccia et al. 2006). The fungus produces mycotoxins (penitrem A, roquefortine C, terrestric acid, and cyclophenol), which are of concern for human health (Frisvad and Samson 2004). In Serbia, *P. crustosum* has been previously described on apple fruit (Vico et al. 2014). On nectarine fruit (*Prunus persica* var. *nucipersica*), after 6 weeks of cold storage, symptoms of blue mold developed in a fruit market in Belgrade, Serbia. The fruit was collected and isolations performed in November 2017. Decayed areas on infected fruit were soft, light to medium brown, with blue-green sporulation on the fruit surface. Two isolates were obtained (N2AS and N2BS) and cultured on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), yeast extract sucrose agar (YES), and potato dextrose agar (PDA) at 25°C for 7 days. Isolates were identified as *P. crustosum* based on morphological features (Frisvad and Samson 2004; Pitt and Hocking 2009). On all media, mycelia were white, and colonies turned blue-green with abundant sporulation. Colonies of both isolates were radially sulcate on MEA and YES, and plane with a granular texture on CYA and PDA, and were yellow to orange on the reverse side on YES. Mean colony diameter on PDA was 29.2 ± 1.2 mm for N2AS and 31.3 ± 1.4 mm for N2BS; on CYA 30.8 ± 1.2 mm for N2AS and 30.9 ± 1.1 mm for N2BS; on YES 40.7 ± 3.6 mm for N2AS and 43.6 ± 1.4 mm for N2BS; and on MEA 330.4 ± 10.2 mm for N2AS and 34 ± 2.5 mm for N2BS. Crusts of conidial masses formed on MEA and PDA after 10 days. Conidiophores of both isolates were

terverticillate, stipes were septate with rough walls, and conidia, borne in columns, were smooth and spherical to subglobose. Conidial diameter for N2AS was 2.32 to 3.95 (average 3.13) μ m and for N2BS was 2.34 to 3.98 (average 3.27) μ m ($n = 50$). Isolates formed a yellow ring, using Ehrlich's reagent, indicating lack of cyclopiazonic acid, but production of other alkaloids. Morphological identification was confirmed by isolating genomic DNA, PCR amplification of the partial β -tubulin gene using Bt2a/Bt2b (Glass and Donaldson 1995), and sequencing. BLAST analysis revealed that N2AS sequence (MT799805) was 99% similar and N2BS (MT799806) was identical to sequences AY674351 (strain CBS 101025) and KJ775121 (strain DTO_244H8) of *P. crustosum* in GenBank. Sequences (2 $\times$ consensus) of the two isolates differed in one nucleotide, showing the existence of single-nucleotide polymorphism among *P. crustosum* isolates. Pathogenicity was tested on nectarine, peach, and apple fruit (four fruit per isolate and the control). Fruit were washed, surface sanitized with 70% ethanol, and wound (10 $\times$ 4 mm) inoculated on two sides with 40 μ l of a 10^5 /ml conidial suspension in sterile distilled water containing 0.1% Tween 20 (TSDW). Control fruit were inoculated with TSDW. Inoculated and control fruit were stored at 25°C for 7 days. Inoculated fruit developed light brown decay with cracks in the epidermis that spread from the inoculation point on nectarines and peaches. Blue-green sporulation was present on all inoculated fruit. Control fruit remained symptomless. The fungus was reisolated and was morphologically identical to the original isolates, thus completing Koch's postulates. This is the first report of *P. crustosum* causing postharvest blue mold decay on nectarine fruit in Serbia. Results show that *P. crustosum* is present not only as a postharvest pathogen of apple fruit but of nectarine as well and may pose a threat in storage of both pome and stone fruits in Serbia.

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