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First evidence of *Itersonilia perplexans* on dill (*Anethum graveolens*) in Bulgaria

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A blight disease was detected on dill (cultivar 'Dukat') in private greenhouses in Bulgaria. The purpose of this investigation was to describe the symptoms of the disease, to identify the causal agent and to determine the pathogenicity and host range. Initial symptoms were small grey-green spots and wilting of leaf tips. Wilted leaves turned brown and collapsed as the disease developed. Necroses broadened so quickly that entire leaves dried within a short time. Foliage became a blighted making the leaves unsuitable for harvest. A fungus was consistently isolated from symptomatic leaves, petioles and stems of dill. The pathogen grew slowly on nutrient media and formed white to pale cream-colored colonies, velvety and flat with minimum aerial mycelium. The pathogenicity was confirmed on dill and other *Apiaceae* hosts. The fungus was identified as *Itersonilia perplexans* on the basis of colony morphology, hyphae with clamp connections and ballistospores. Disease caused by *I. perplexans* has not been found previously either on dill or any other host plants in Bulgaria up till now.

Key words: *Anethum graveolens*, Apiaceae, *Itersonilia perplexans.*

Introduction. The fungus *Itersonilia perplexans* Derx has been reported to be the causal agent of leaf blight of dill (*Anethum graveolens* L.) in several European countries, as Italy (Matta, Garibaldi, 1968), Germany (Geßner, 1988), Austria (Bedlan, 1988), Swiss (Usoltseva, Dahl, 2006) as well as in USA (Koike, Tjosvold, 2001) and New Zealand (Anonymous, 2001; 2002; 2004; 2005; 2007).

In the middle of June 2008 and in the end of May 2009, symptoms characteristics to *I. perplexans* infection were observed on dill grown in private greenhouses in Bulgaria.

The purpose of this investigation was to describe the symptoms of the disease, to identify the causal agent and to determine the pathogenicity and host range.

Object, methods and conditions. Infected dill plants (cultivar 'Dukat') were collected from private greenhouses in Sofia region. The causal agent was isolated from symptomatic leaves, leaf petioles and stems. Small pieces of diseased tissue were on the surface sterilized and plated on potato dextrose agar (PDA). The cultures were stored on cornmeal agar (CMA) slopes at 4 °C and subcultured every 6 months.

Three isolates obtained from leaf and stem tissue were selected to study cultural and morphological characteristics of the fungus. To determine radial growth rates of the isolates, 8 mm plugs were taken from the periphery of growing colonies and transferred to plates of PDA, CMA and 0.2 % malt extract agar (MEA). Inoculated plates were incubated at 22 °C and 12 h photoperiod. The diameter of each colony was measured 4 and 16 days after inoculation. The rate of increase in colony radius was determined as the difference in colony size between 4^{th} and 16^{th} day after inoculation. There were four replicate plates. Morphological characters, including size of ballistospores were recorded for cultures grown for 2–3 weeks on three nutrient media.

Ballistospores of each isolate were harvested by flooding the plates with sterile distilled water, scraping the plates with sterile paintbrush and filtering through double layers of sterile cheesecloth. Ballistospores were counted with a hematocytometer and adjusted to approximately 1×10^4 spores/ml.

Potted seedlings of dill, coriander, celery, parsley, fennel, caraway and carrot were inoculated by spraying with ballistospore suspension. Inoculated plants were put in a humid chamber for 48 hours. Plants sprayed with sterile distilled water served as controls. Disease symptoms were evaluated 10 days after inoculation. Disease severity was rated on a 0–4 scale: 0 = no visible symptoms; 1 = small grey-green lesions; 2 = brown lesions on leaves and petioles; 3 = leaves snapped off at the diseased lesions or 30–50 % of the leaf diseased; 4 = 50–100 % of the plant diseased. The data were processed by the McKinney's formula (McKinney, 1923), which generates a numeric disease index (DI) of the severity of the attack: $DI = (\Sigma vn)/(NV) \times 100$, where v represents the numeric value of the class, n is the number of plants assigned to the class, N is the total number of the plants in the replication and V is the numeric value of the highest class. Reisolations were made from the diseased plant parts.

Results. The symptoms on the naturally and artificially infected dill plants were characterized initially as small silvery-grey to grey-green spots distributed mainly on the leaf tips (Plate 1, a). Dark grey-green streaks were detected on leaf petioles and stems (Plate 1, b). The necroses broadened so quickly, that entire damaged leaves dried soon. Wilted leaves turned brown and collapsed (Plate 1, c). The foliage became blighted making the leaves unsuitable for harvest (Plate 1, d). The disease affected the umbel very seldom.

A fungus was consistently isolated from symptomatic leaves, petioles and stems of dill. The colonies were slow growing. Average values for growth rates (mm/day) of three representative isolates used in the study on three nutrient media are presented in Fig. CMA nutrient medium assured the fastest growth of all isolates. Among them isolate 22 manifested the best growth on all nutrient media.

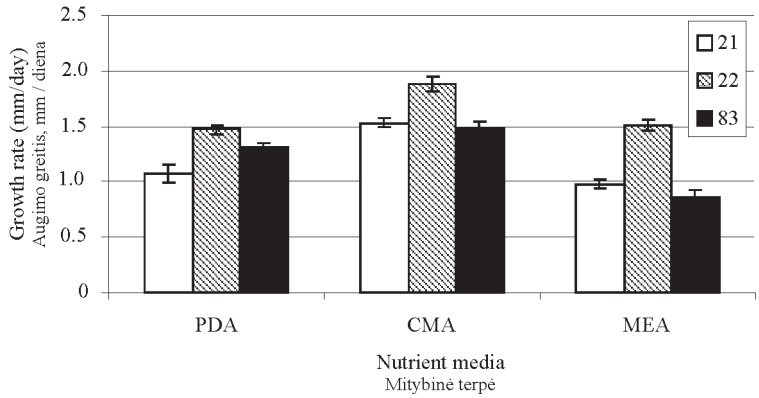

Fig. Average growth rates of *Itersonilia perplexans* (isolates 21, 22 and 83) on three nutrient media (PDA, CMA and MEA) at 22 °C and 12 h photoperiod. Bars represent standard deviations (± SD). Least significant differences are 0.05, 0.07, 0.09 for isolates and media and 0.09, 0.12, 0.16 for their interaction at P = 5 %, P = 1 %, P = 0.1 %, respectively.

Pav. Vidutiniai *Itersonilia perplexans* (izoliatai 21, 22 ir 83) augimo greitis ant trijų mitybinių terpių (PDA, CMA ir MEA), esant 22 °C temperatūrai ir 12 h fotoperiodui. Stulpeliai atspindi standartinius nukrypimus (± SN). Mažiausi izoliatų ir terpės nukrypimai yra 0,05, 0,07, 0,09, o jų sąveikos – 0,09, 0,12, 0,16, kai atitinkamai P = 5 %, P = 1 %, P = 0.1 %.

The studied isolates were all very similar, showing different colony morphology on three nutrient media (Plate II, a). The fungus grew slowly in 12 h photoperiod. In the dark the growth was still more stunted. The colony did not fill up the Petri dish in 50 days (Plate II, b, c).

On PDA the colonies were circular, more compact, pale cream-colored, velvety. The colonies on CMA and MEA were translucent, white and flat with minimal aerial mycelium (feathery margins on MEA). The mycelium was composed primarily of branched septate hyphae with prominent clamp connections at the septa (Plate III, a). Hyphae were hyaline, 2.7–4.5 µm wide and terminated in inflated sporogenous cells thin-walled, pyriform, ovoid to subglobose. These cells germinated to form hyphae or germ tubes, on which ballistospores formed. Ballistospores were lemon-shaped, broadly-lunate, ovoid to pyriform with a basal flattering and a pointed tip, smooth-walled with granular content, (10) 14.12 ± 0.25 (17) × (7) 8.95 ± 0.16 (12) µm (Plate II, b) and germinated either with hyphae (Plate III, c) or secondary ballistospores. Chlamidospores were globose to subglobose, solitary or clustered, thick-walled, 11–16 × 10–15 µm, produced mainly in old cultures (Plate III, d).

Besides the usual method of tissue plantings, the fungus was isolated readily by spore shootings from surface disinfected diseased tissue suspended on the lids of Petri dishes over PDA. White spore deposits of typical ballistospores of *I. perplexans* were observed on agar plate under the suspended diseased plant material and numerous colonies developed.

The fungus was identified as *I. perplexans* (Boekhout, 1991; Boekhout et al., 1991).

Inoculations proved the pathogenicity of the fungus. First symptoms were observed 48 hours after inoculation. All tested isolates caused typical symptoms on dill – grey-green discoloration and wilting of leaf tips and streak lesions on leaf petioles and stems (DI = 79). The symptoms on fennel were similar but less severe than on dill (DI = 61). Small (1–2 mm in diameter) circular to lens-shaped, brown necrotic spots often surrounded by yellow haloes appeared on the leaf laminae of coriander (DI = 32). On carrot, caraway and parsley only single small spots were observed on the leaf periphery (DI = 25). The celery remained healthy. The fungus *I. perplexans* was consistently reisolated from the plant parts with symptoms. Water-treated control plants did not have any symptoms of disease and were negative to *I. perplexans*.

Discussion. The causal agent was identified as *I. perplexans* based on colony morphology, hyphae with prominent clamp connections and ballistospores. The symptoms on artificially infected dill plants were similar to those on the naturally diseased ones observed in the greenhouses during June 2008 and May 2009. Some colonies of *I. perplexans*, free of contaminants, grew out from lesions but others were overrun by various fungi because of its slow growth. The fungus was isolated more readily by suspending diseased tissue on the lids of Petri dishes over PDA due to water-drop mechanism of ballistospore discharge. Lesions caused by *I. perplexans* have not been observed previously on dill plants in vegetable gardens and field experiments carried out at the Institute of Genetics, Sofia, during the last 10 years. Among the other representatives of Apiaceae family artificially inoculated fennel and coriander showed the most intensively developed disease symptoms. The fungus *I. perplexans* has been reported to be pathogenic on two main group plants belonging to Asteraceae (Sackston, 1958; McRitchie et al., 1973; McGovern, Seijo, 1999; Seijo et al., 2000; Horita, Yasuoka, 2002) and Apiaceae (Channon, 1963; Matta, Garibaldi, 1968; Channon, 1969; Geßner, 1988; Bedlan, 1988, Koike, Tjosvold, 2001; Usoltseva, Dahl, 2006). The isolates showed specificity being pathogenic to the host group they were obtained from (Channon, 1963; Koike, Tjosvold, 2001; Horita, Yasuoka, 2002).

Conclusions. The disease caused by *I. perplexans* has not been recorded previously either on dill or any other host plants in Bulgaria up till now. Since *I. perplexans* grows best at high relative humidity the way to diminish the disease appearance and development is to keep the dill plants under as dry conditions as possible. The initial symptoms of *I. perplexans* resembled those caused by lack of nutrient substances or water. For this reason it is very important to clear up the cause as the first symptoms appear and to take control measures. The infected plants have to be destroyed and do not put in the compost.

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Pirmieji *Itersonilia perplexans* požymiai ant krapų (*Anethum Graveolens*) Bulgarijoje

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Santrauka

Privačiuose Bulgarijos šiltnamiuose ant krapų (veislė ‘Dukat’) buvo aptiktos nekrozės. Šio tyrimo tikslas buvo apibūdinti ligos simptomus, išaiškinti sukėlėją ir nustatyti jo patogeniškumą bei augalus maitintojus. Pirmieji simptomai buvo mažos pilkšvai žalsvos dėmelės ir vystantys lapų galiukai. Ligai progresuojant, nuvytę lapai rudavo ir krito. Nekrozė plito taip greitai, kad per trumpą laiką nudžiūvo visi lapai ir nebetiko derliui. Grybas buvo izoliuotas nuo krapų lapų, žiedų ir stiebų su ligos požymiais. Ligos sukėlėjas iš lėto augo ant mitybinės terpės ir formavo nuo baltos iki pilkšvai kreminės spalvos kolonijas – aksomines ir plokščias, su minimaliu grybienos plotu. Patogeniškumas buvo patvirtintas krapuose ir kituose *Apiaceae* šeimos augaluose. Pagal kolonijos morfologiją, siūlinius grybus su gnybto tipo jungtimis ir balstosporas, nustatyta, jog tai *Itersonilia perplexans*. Patogeno *I. perplexans* sukeliama liga lig šiol nebuvo aptikta nei ant krapų, nei ant kokių nors kitų augalų Bulgarijoje.

Reikšminiai žodžiai: *Anethum graveolens*, Apiaceae, *Itersonilia perplexans*.