Disease Notes

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Diseases Caused by Bacteria and Phytoplasmas

First Report of *Dickeya dianthicola* Causing Blackleg on Potato (*Solanum tuberosum*) in Bulgaria. S. G. Bobev, Agricultural University, Plovdiv, Bulgaria; and J. Van Vaerenbergh and M. Maes, Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium. Plant Dis. 98:275, 2014; published online as http://dx.doi.org/10.1094/PDIS-02-13-0147-PDN. Accepted for publication 29 July 2013.

Potato (*Solanum tuberosum*) is an important and widespread crop in Bulgaria. A new disease was observed on a single potato plot (Plovdiv region) without a history of potato cultivation in the spring of 2011. Initially, single lower leaves wilted on recently emerged plants (approx. 15% incidence) with subsequent desiccation of the leaf margins. The wilting progressed over time and eventually the whole stem became desiccated. A blackleg-like necrosis was noticed at the stem base when symptomatic plants were uprooted. Most diseased stems remained green above ground but pith tissue was heavily macerated and some of the stems became hollow as the pith dried out. Mother tubers were partially or entirely macerated. In most cases, the decay was initiated from the stolon end. Bacterial strains were obtained from symptomatic stems and tubers by dilution plating on King's B medium. The strains produced indigoidin pigment and induced a hypersensitive response 24 h after infiltration into tobacco and *Sedum hybridum* leaves (2). The strains were identified as *Dickeya* spp. by the production of the PCR amplicon of the pectate lyase ADE gene cluster (3) and of the pectate lyase I gene (4). The partial sequence of the *fliC* PCR amplicon (1) of strain SB2589 (GenBank Accession No. KF442436) displayed 100% homology with four whole genome shotgun sequences of *Dickeya dianthicola* in GenBank. Pectinolytic activity was demonstrated by inoculation of surface disinfested potato tubers of cv. Kondor. Conical core tissue was removed at the apical end and 100 µl bacterial suspension (10^7 CFU in sterile 10 mM phosphate buffer) was deposited in the cavity. The cap was reattached to the tuber and immobilized by Parafilm. Positive control tubers were inoculated with *D. dianthicola* reference strain GBBC 2039 (LMG 25864) and negative control tubers were inoculated with sterile 10 mM phosphate buffer. All tubers were incubated for 48 h at 28°C under micro-aerobic conditions reducing the air pressure to 90 mb in a vacuum incubator. The *D. dianthicola* reference strain and Bulgarian strains produced maceration of tuber tissue. Maceration was not observed in the negative control tubers. Potato plants cv. Kondor were grown from minitubers in sandy soil in plastic nursery containers. The plants were inoculated by root drenching (one application of cell suspension at 10^9 CFU/liter) when the stems were 15 to 20 cm high (tuber initiation stage). Plants were incubated at 25 to 28°C with regular watering. Wilting symptoms developed within 10 days of inoculation, followed by necrosis of the pith. Strains obtained from the inoculated stems were confirmed as *D. dianthicola* as described above. Based on the disease symptoms, the cultural, molecular, and pathological features of the strains, we conclude that the disease was caused by *D. dianthicola* and to our knowledge this is the first report of the pathogen on potato in Bulgaria. Furthermore, this incident warrants further surveys of pectinolytic bacteria causing blackleg-like symptoms in potato crops in Bulgaria.

References: (1) S. Diallo et al. Eur. J. Plant Pathol. 125:349, 2009. (2) Y-.A. Lee and C-.P. Yu. J. Microbiol. Methods 64:200, 2006. (3) A. Nassar et al. Appl. Environ. Microbiol. 62:2228, 1996. (4) J. Van Vaerenbergh et al. PLoS ONE 7(5):e35738, 2012.

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First Report of *Ralstonia solanacearum* Race 2 Biovar 1 Causing Moko Disease of Banana in Malaysia. D. Zulperi and K. Sijam, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Plant Dis. 98:275, 2014; published online as http://dx.doi.org/10.1094/PDIS-03-13-0321-PDN. Accepted for publication 7 August 2013.

During March 2011 to June 2012, 50 banana plants of cultivar *Musa* × *paradisiaca* 'Horn' with Moko disease symptoms were randomly sampled in 12 different locations of 5 outbreak states in Peninsular Malaysia comprising Kedah, Selangor, Pahang, Negeri Sembilan, and Johor, with disease incidence exceeding 90% in some severely affected plantations. The disease symptoms observed in the infected plants included yellowing and wilting of the oldest leaves, which became necrotic, and eventually led to their dieback or collapse. The pulp of banana fruits also became discolored and exuded bacterial ooze. Vascular tissues in pseudostems were discolored. Fragments from symptomatic plant samples were excised and cultured on Kelman's-tetrazolium salt (TZC) medium. Twenty positive samples produced fluidal colonies that were either entirely white or white with pink centers after incubation for 24 to 48 h at 28°C on Kelman's-TZC medium and appeared as gram-negative rods after Gram staining. They were also positive for potassium hydroxide (KOH), Kovacs oxidase, and catalase tests, but negative for utilization of disaccharides and hexose alcohols, which are characteristics of biovar 1 *Ralstonia solanacearum*. For the pathogenicity test, 30 µl of 10^8 CFU/ml bacterial suspension of three selected virulent strains were injected into banana (*Musa* × *paradisiaca* 'Horn') leaves explants grown in plastic pots of 1,440 cm^3 volume in a greenhouse, with temperature range from 26 to 35°C. Leaves that were infiltrated with sterile distilled water served as a negative control. Inoculations with all isolates were performed in three replications, as well as the uninoculated control leaves explants. The inoculated plants produced the same symptoms as observed on naturally diseased samples, whereas control plants remained asymptomatic. Strain cultures were re-isolated and possessed the morphological and biochemical characteristics as previously described. PCR amplification using race 2 *R. solanacearum* primers ISR*so19*-F (5′-TGGGAGAGGATGGCGGCTTT-3′) and ISR*so19*-R (5′-TGACCCGCCTTTCGGTGTTT-3′) (3) produced a 1,900-bp product from DNA of all bacterial strains. BLAST searches resulted that the sequences were 95 to 98% identical to published *R. solanacearum* strain race 2 insertion sequence ISR*so19* (GenBank Accession No. AF450275). These genes were later deposited in GenBank (KC812051, KC812052, and KC812053). Phylotype-specific multiplex PCR (Pmx-PCR) and *Musa*-specific multiplex PCR (Mmx-PCR) were performed to identify the phylotype and sequevar of all isolates (4). Pmx-PCR showed that all isolates belonged to phylotype II, whereas Mmx-PCR showed that they belonged to phylotype II sequevar 4 displaying 351-bp amplicon. Although there were previously extensive studies on *R. solanacearum* associated with bacterial wilt disease of banana crops in Malaysia, none related to Moko disease has been reported (1,2). The result has a great importance to better understand and document *R. solanacearum* race 2 biovar 1, since banana has been identified as the second most important commercial fruit crop with a high economic value in Malaysia.

References: (1) R. Khakvar et al. Plant Pathol. J. 7:162, 2008. (2) R. Khakvar et al. Am. J. Agri. Biol. Sci. 3:490, 2008. (3) Y. A. Lee and C. N. Khor. Plant Pathol. Bull. 12:57, 2003. (4) P. Prior et al. Pages 405-414 in: Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. The American Phytopathological Society, St. Paul, MN, 2005.

First Report of Bacterial Leaf Blight of Carrot Caused by *Xanthomonas hortorum* pv. *carotae* in Korea. I.-S. Myung, M.-J. Yoon, and J.-Y. Lee, Crop Protection, National Academy of Agricultural Sciences (NAAS), Suwon 441-707, Korea; G.-D. Kim and M.-H. Lee, Plant Quarantine Technology Research and Development, Animal, Plant and Fisheries Quarantine and Inspection Agency, Suwon 443-440, Korea; and E.-Y. Hwang and H. S. Shim. Crop Protection, NAAS, Suwon 441-707, Korea. Plant Dis. 98:275, 2014; published online as http://dx.doi.org/10.1094/PDIS-07-13-0724-PDN. Accepted for publication 1 August 2013.

In December 2012, symptoms of typical bacterial leaf blight were observed on carrot plants (*Daucus carota* L. subsp. *sativus*) cultivated in commercial fields in Kujwa, Jeju, Korea. The disease was detected in 40% of 50 fields surveyed with an incidence of 10% on average. The bacterial leaf blight lesions on leaf blades were elongated, dark brown to black with water-soaked edges and chlorotic halos. Lesions were also crescent-shaped to V-shaped on leaflets. Four bacterial isolates were recovered on trypticase soy agar from leaf lesions that were surface-sterilized in 70% ethyl alcohol for 20 s. Identity of the isolates was confirmed by PCR product (1,266-bp) using a specific primer set for *Xanthomonas hortorum* pv.

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