

Disease Notes



First Report of *Xanthomonas campestris* pv. *campestris* as the Causal Agent of Black Rot on Oilseed Rape (*Brassica napus*) in Serbia

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Published Online: 7 Feb 2013 | <https://doi.org/10.1094/PDIS-05-12-0506-PDN>

Abstract

In September 2010, leaves of oilseed rape (*Brassica napus* L.) with v-shaped, necrotic lesions on the leaf margins surrounded by yellow halos were collected. Symptoms were observed on the domestic cultivar Slavica (IFVC, Novi Sad) located in the Bačka region, Vojvodina, Serbia, from a 3-ha field. Average disease incidence on 3-month-old plants was 45% (15 to 75%). Diseased leaves were rinsed in sterilized distilled water (SDW) and dried at room temperature for isolations. Leaf sections taken from the margin of necrotic leaf tissue were macerated in SDW and the extract was streaked onto yeast extract-dextrose-calcium carbonate (YDC) agar. Plates were incubated at 28°C for 3 days. Colonies were yellow, translucent, circular, and raised. Ten representative strains tested further were all gram-negative, catalase-positive, and oxidase-negative. The

partial 16S rDNA sequence of a representative strain, TUr1, was amplified using primers fD1 and rD1 (2), and determined using the IMGGI SeqService facility in Belgrade. The 1,510-bp 16S rDNA sequence of TUr1 was compared to that of known strains in the NCBI GenBank database, and showed greatest similarity with that of *Xanthomonas campestris* pv. *campestris* (Xcc) strains ATCC 33913 and B100 (99% homology). Pathogenicity of 10 strains grown for 48 h on YDC at 28°C was completed using each of three methods: spraying a bacterial suspension (10^8 cfu/ml) onto the leaf surfaces of oilseed rape plants, stabbing the major veins of each of the first two true leaves with the tip of a sterile toothpick that had been dipped into a colony of the appropriate strain, and immersing cotyledons of the plants into a bacterial suspension (10^8 cfu/ml). All three tests were performed on 4-week-old oilseed rape plants of the cultivar Slavica. SDW was used for the negative control treatment for each method of inoculation. Reference strain Xcc NCPPB 1144 was used as a positive control treatment. Tests plants (two for each method of inoculation and each bacterial strain or control treatment) were maintained in a greenhouse at $25 \pm 1^\circ\text{C}$ and 80% relative humidity by keeping the plants in plastic bags. Two control plants for each of the negative and positive control treatments for each inoculation method were also enclosed in separate plastic bags. The bacterial strains and reference strain caused yellow lesions on inoculated plants that turned necrotic starting about 7 days after inoculation (DAI). The spots coalesced within 21 DAI to form necrotic areas. Plants inoculated with SDW remained symptomless. Reisolations were done onto YDC as described above. Reisolated strains showed the same colony morphology as described above. The bacterial strains grew at 35°C; produced levan from sucrose, hydrogen sulfide, and indole; did not reduce nitrate; hydrolyzed Tween 80; starch, gelatin, and aesculin; did not show tolerance to 0.10 and 0.02% triphenyl-tetrazolium chloride; and produced acid from d-arabinose, arginine, dulcitol, galactose, d-glucose, maltose, mannose, sorbitol, sucrose, and xylose (1). All strains tested by Plate Trapped Antigen-ELISAs (ADGEN Phytodiagnosics, Neogen Europe Ltd., Scotland) reacted with Xcc-specific polyclonal antibodies. Based on these tests, the strains were identified as Xcc. To our knowledge, this is the first report of this pathogen causing black rot of oilseed rape in Serbia.

References: (1) T. B. Adhikari and R. Basnyat. *Eur. J. Plant Pathol.* 105:303, 1999. (2) W. G. Weisburg et al. *J. Bacteriol.* 173:697, 1991.