# First report of *Xanthomonas campestris* pv. *campestris* causing black rot on oilseed rape (*Brassica napus L.*) in France

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## Abstract

In October 2022, v-shaped necrotic lesions were observed on the leaf margins of field-grown winter oilseed rape (WOSR), *Brassica napus* L., in western France (Ille-et-Vilaine (35) and Maine-et-Loire (49) departments). Disease incidence on volunteers and cultivated WOSR was generally low (5-10 %) but occasionally up to 80% in some fields. Leaf sections sampled from the margin of necrotic leaf tissue were dilacerated in sterile deionized water and the extract was spread onto tryptone soya agar (TSA) with cycloheximide (100 $mg.L^{-1}$) and Polyflor (Syngenta, France) (2 $ml.L^{-1}$, containing 5 $mg.L^{-1}$ propiconazole) then incubated at 28°C for 2 days. Colonies were yellow-pigmented, mucoid, and convex, which are morphological characteristics of *Xanthomonas* spp. colonies. The partial *fyuA* and *gyrB* gene sequences were amplified for eight isolated strains (CFBP 9155, CFBP 9156, CFBP 9157, CFBP 9158, CFBP 9159, CFBP 9161, CFBP 9162, and CFBP 9163) using primers of Fargier et al. (2011), and sequenced (Genoscreen, France). The sequences were deposited under numbers OR232891 to OR232898 for *fyuA* and OR634932 to OR634939 for *gyrB*. BLASTN analysis of the sequenced *fyuA* amplicon showed 100% identity and query coverage with the *fyuA* fragment of *Xanthomonas campestris* pv. *campestris* (*Xcc*) CFBP 6865R (Bellenot et al., 2022). BLASTN analysis of the sequenced *gyrB* amplicon showed two allelic forms: one showed 100% identity and query coverage with the *gyrB* fragment of *Xcc* strain CFBP 6865R (Bellenot et al., 2022), the other one showed 100% identity and query coverage with the type strain *Xcc* CFBP 5241 (ATCC33913) (Vorhölter et al., 2003). Moreover, two qPCR tools were used to identify the strains successfully as *Xcc* (Köhl et al., 2011; Rezki et al., 2016) which target the same gene encoding a hypothetical protein and whose primers overlap. The pathogenicity of the eight isolated strains was validated using a bacterial suspension ($10^8$ $CFU.ml^{-1}$) for *i*) leaf spraying until runoff onto the leaf surfaces of WOSR plants previously maintained at saturated humidity for 48 hours, *ii*) wound-leaf inoculation of the two youngest true leaves with scissors that had been dipped into the bacterial suspension. Both tests were performed on 3-week-old WOSR plants of the Aviso (INRAE) genotype. Deionized water was used as negative control. Strains CFBP 5241 and CFBP 4954 (Fargier et al., 2007) were used as positive controls for disease expression. Tested plants (seven for spray inoculation and four for wound-leaf inoculation per strain and control condition) were incubated in a greenhouse at 20°C/24°C (night/day). Isolated strains and the strain CFBP 4954 caused yellow lesions with both inoculation methods that necrotized starting about 10 days post inoculation (dpi). The lesion spots coalesced within 14 dpi to form necrotic areas. The type strain CFBP 5241 caused mild symptoms, with only yellow lesions that did not coalesce. Plants inoculated with water remained symptomless. To complete Koch's postulate, re-isolations were achieved. Re-isolated strains on TSA showed the same colony morphology as described above. All re-isolated strains were identified as *Xcc* based on partial *gyrB* sequencing and *Xcc* specific qPCR test (Rezki et al., 2016). This first report in France and the recent identification in Serbia (Popović et al., 2013) may illustrate the emergence

of the disease on this crop in Europe. The prevalence and consequences of this disease should be evaluated over a wider geographic area.

References :

Bellenot, C., Carrère, S., Gris, C., Noël, L. D., & Arlat, M. (2022). Genome Sequences of 17 Strains from Eight Races of Xanthomonas campestris pv. campestris. *Microbiology Resource Announcements*, *11*(7), e00279-22.

Fargier, E., & Manceau, C. (2007). Pathogenicity assays restrict the species Xanthomonas campestris into three pathovars and reveal nine races within X. campestris pv. campestris. *Plant Pathology*, *56*(5), 805-818.

Fargier, E., Fischer-Le Saux, M., & Manceau, C. (2011). A multilocus sequence analysis of Xanthomonas campestris reveals a complex structure within crucifer-attacking pathovars of this species. *Systematic and Applied Microbiology*, *34*(2), 156-165.

Köhl, J., Vlaswinkel, M., Groenenboom-de Haas, B. H., Kastelein, P., Van Hoof, R. A., Van der Wolf, J. M., & Krijger, M. (2011). Survival of pathogens of Brussels sprouts (Brassica oleracea Gemmifera Group) in crop residues. *Plant pathology*, *60*(4), 661-670.

Popović, T., Balaž, J., Starović, M., Trkulja, N., Ivanović, Ž., Ignjatov, M., & Jošić, D. (2013). First report of Xanthomonas campestris pv. campestris as the causal agent of black rot on oilseed rape (Brassica napus) in Serbia. *Plant Disease*, *97*(3), 418-418.

Rezki, S., Campion, C., Iacomi-Vasilescu, B., Preveaux, A., Toualbia, Y., Bonneau, S., ... & Barret, M. (2016). Differences in stability of seed-associated microbial assemblages in response to invasion by phytopathogenic microorganisms. *PeerJ*, *4*, e1923.

Vorhölter, F. J., Thias, T., Meyer, F., Bekel, T., Kaiser, O., Pühler, A., & Niehaus, K. (2003). Comparison of two Xanthomonas campestris pathovar campestris genomes revealed differences in their gene composition. *Journal of biotechnology*, *106*(2-3), 193-202.

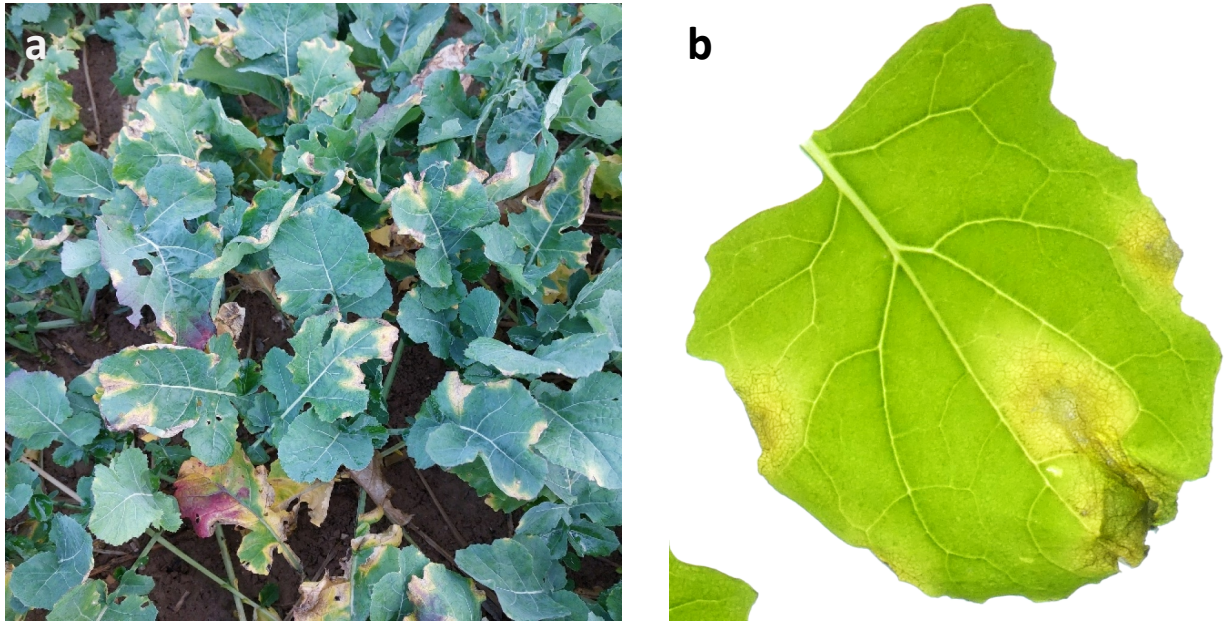


Figure S1 : typical symptoms (v-shaped yellowing areas and necrotic lesions) observed on the leaf margins, caused by *Xcc* on WOSR. a : natural field infections; b : spray inoculation of young leaves with strain CFBP 9157 in greenhouse.