First Report of Bacterial Pustule on Soybeans in North Dakota

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Bacterial pustule of soybeans (Glycine max (L.) Merr.) is found commonly in the southern United States, where environmental conditions favor disease development (5). Although bacterial pustule has been found in the Northern Great Plains (for example, Nebraska and South Dakota), it is not found as frequently as other foliar diseases.

On 10 July 2010, soybeans at the North Dakota State University Agriculture Experiment Station (NDSU-AES) in Fargo began exhibiting small, pale green spots with dark, raised pustules on either surface of the leaf (Fig. 1). The NDSU-AES is located in Cass County, whose annual planted acreage exceeds 200,000 ha, and within 2 km of commercial soybean production fields. Isolations were made by plating macerated leaf material onto nutrient glucose agar (NGA). Yellow-pigmented colonies were observed after 48-h incubation at 28°C and single colonies were transferred to fresh NGA plates. All the isolates were gram-negative, rod shaped, aerobic, oxidase-negative, catalase-positive, and amylolytic-positive. Bacteria harvested were identified as a Xanthomonas sp. (4). Further characterization of two isolates (Xag1 and Xag2) was accomplished by polymerase chain reaction (PCR) analyses of genomic DNA with the Xag-specific primers XAG-F and XAG–R (3). The primer pair amplified approximately 490-bp fragment, thus confirming the isolates as Xanthomonas axonopodis pv. glycines (Nakano) Vauterin et al. (6).

Xanthomonas axonopodis pv. phaseoli, common bacterial blight of dry bean (Phaseolus vulgaris L.) pathogen, is endemic in North Dakota. Therefore, the two Xag isolates were tested reciprocally for pathogenicity with a Xcp isolate on both soybeans (cv. RG200RR and cv. Croplan 0505) and dry beans (cv. Lariat, market class Pinto) using the method described by Athinuwat et al. (2). Briefly, the cell suspensions (10⁸ CFU/ml) were supplemented with 5 g/liter 320 mesh carborundum and 0.25 ml/liter Triton X 100, and sprayed onto three trifoliate leaves of 14-d plants using an airbrush spray inoculator at 20 psi. Inoculated plants were placed in a mist chamber for 24 h at 20°C, then moved to a greenhouse at 30–33°C under alternating fluorescent light (16 h daily). At 14-d after inoculation, the Xag isolates caused susceptible response (Fig. 2) on both soybean cultivars and none on dry bean. In contrast, Xcp caused susceptible response on dry bean, but not on the soybean cultivars. The experiment was repeated twice.

Isolates Xag1 and Xag2 were recovered from the infected soybean leaves and confirmed by polymerase chain reaction (PCR) using XAG-F and XAG–R primers (3) to complete Koch’s postulates for the soybean pathogen. A 490 bp fragment was

FIGURE 1
Bacterial pustule on the lower leaf surface as observed on soybeans at the North Dakota State University Agriculture Experiment Station in Fargo, ND, on 10 July 2010.

FIGURE 2
Bacterial pustule on a leaf of soybean cv. ‘Croplan 0505’ (A) and cv. ‘RG200RR’ (B) 14 days following infiltration with isolate Xag1.
detected in the PCR amplification of the two Xag isolates. For an alternative confirmation, sequence typing of two housekeeping genes, fusA and gyrB, was performed for Xcp and the Xag isolates recovered from the dry bean and soybean leaves respectively. Sequences were subjected to BLASTn searches in the Plant Associated and Environmental Microbes Database (1). For the two Xag isolates, the fusA and gyrB sequences aligned with those of X. axonopodis pv. glycines (Xag_ATCC_11766) with 99–100% identity to alleles 17 (fusA) and 32 (gyrB). For the Xcp isolate, the sequences aligned with those of X. axonopodis pv. phaseoli (X_phaseoli_FB1329) with 100% identity to alleles 6 (fusA) and 16 (gyrB).

Bacterial pustule has not been previously reported on soybeans in North Dakota. We hypothesize that the warm average air temperatures (2°C above the 30-year mean) and frequent days with rainfalls (eight days, 34 mm total) which occurred three weeks (20 June to 10 July) prior to the observation of bacterial pustule facilitated the establishment of the disease at the NDSU-AES. It is unlikely that bacterial pustule will pose a direct yield threat to soybeans in North Dakota, due in large part to unfavorable climatic conditions for the disease. However, the disease can be mistaken with Asian soybean rust (Phakopsora pachyrhizi Syd. & P. Syd). While soybean rust has never been reported in North Dakota, a heightened awareness of this disease exists in the state, and when combined with the recent defunding of the well-known early monitoring system (IPM-PIPE), it is conceivable that misidentification could translate into a fungicide application. Fungicide applications on soybeans in North Dakota are rarely recommended. Any fungicide application made because of misidentification between bacterial pustule and Asian soybean rust would result in economic loss to the grower.

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LITERATURE CITED