Evaluation of the potential of the flea beetle Phyllotreta cruciferae to transmit Xanthomonas campestris pv. campestris, causal agent of black rot of crucifers

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Evaluation of the potential of the flea beetle *Phyllotreta cruciferae* to transmit *Xanthomonas campestris* pv. *campestris*, causal agent of black rot of crucifers

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In greenhouse tests when flea beetles, *Phyllotreta cruciferae*, were allowed to feed for 24 h on broccoli plants infected with *Xanthomonas campestris* pv. *campestris* (Xcc) and then transferred to healthy plants, transmission of Xcc occurred in 5 of 32 plants (15.6%). When beetles were sprayed with a suspension of Xcc (ca. 1.25 x 10^8 colony-forming units per mL) prior to being transferred to healthy broccoli plants, transmission occurred in every case. Black rot did not develop when immigrating flea beetles were collected in cabbage fields and transferred to healthy broccoli plants in the greenhouse. These data are the first to provide evidence of insect transmission of Xcc. However we conclude that there is only a limited potential for *P. cruciferae* to act as an efficient vector for this pathogen in northern New York State.


Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), must be considered the most important disease of crucifers worldwide, occurring on all cultivated brassicas and radishes and numerous cruciferous weeds (13). This pathogen may be seedborne (2,6,12) and can survive in soil in warmer areas (1,10). From these primary sources of inoculum, secondary spread has been attributed to soaking the plants in water before transplanting (13), mechanical injury during cultivation (13), or heavy rains flooding the seedbed and spreading the inoculum (3). Cruciferous weeds have also been demonstrated to be a source of Xcc. Shaad and Dianese (10) conducted an extensive survey in Georgia and California to determine the species of weeds infected by Xcc, and they established that the bacterium is readily disseminated from infected weeds to cabbage under field conditions. Insects have been considered to be vectors of Xcc (5,13) but direct evidence is lacking.

In northern regions of New York, the flea beetle *Phyllotreta cruciferae* Goeze is an important pest of cole crops that rasps small pits in the foliage of its hosts (8). This insect disperses in the fall and overwinters on cruciferous weeds along the edges of cultivated fields (9). From these sites, adults disperse in the spring and summer and colonize cultivated host plants (11). Our observations in the field led us to hypothesize that flea beetles transmit Xcc from either wild hosts to cultivated crops or from infected cultivated plants to healthy plants, because: 1) flea beetles tend to feed on leaf margins, and this is the site where infection often occurs first; 2) there appears to be a temporal synchrony of arrival of flea beetles and disease initiation; and 3) many cruciferous weeds that are hosts for *P. cruciferae* (4,7,9) are also hosts for Xcc (1). We report herein the results of a study to determine the potential of *P. cruciferae* adults to transmit Xcc to *Brassica oleracea* var. *bostryx*.

Four separate trials were conducted in the greenhouse, with two trials occurring with flea beetle adults collected and tested in the summer of 1982, and two trials similarly conducted in the summer of 1983. *P. cruciferae* adults were collected with a D-VAC® (D-VAC Company, Riverside, CA 92506) sampler during immigration into cabbage fields near Geneva, NY. Broccoli plants, *Brassica oleracea*, cv. Waltham 29, in a 4-6-leaf stage were used as host plants in all tests. Broccoli plants were grown in 10-cm-diam pots containing Cornell soil mix. To obtain diseased plants for acquisition feeding by insects, plants were inoculated in the greenhouse by injuring the petiole with a toothpick, dipped into a colony of Xcc (Wisconsin isolate PHW117 originally obtained from Louisiana) that had been
grown for 48 h at 25°C on YDCP medium (0.25% yeast extract, 0.25% peptone, 2% dextrose, 2% calcium carbonate, 2% agar, 0.1% potassium phosphate dibasic, 0.05% sodium chloride, 0.05% magnesium chloride, pH 7.2). Symptoms developed over ca. a 2-wk period. Transmission studies were conducted in square cages (40 cm on a side) with screened sides. These cages were placed within a greenhouse maintained at 20-25°C and 50-75% relative humidity, unless stated otherwise.

The following treatments were replicated four times in a randomized complete block design: 1) three to four leaves exhibiting early to intermediate stages of black rot symptoms along the margins were supported in a flask filled with water, and 100 P. cruciferae adults were allowed to feed on the leaves for 48 h; then the flea beetles were placed in a cage with two healthy plants for 24 h, and finally the flea beetles were removed by aspiration and plants were placed on a greenhouse bench for ca. 3 weeks before symptom expression was recorded; 2) 100 P. cruciferae were placed in a 25 mL beaker, sprayed until runoff with a Devilbiss atomizer 152 (The Devilbiss Co., Somerset, PA 15501) with a suspension of Xcc (adjusted to 80% transmittance at 600 nm on a Bausch and Lomb Model 20 spectrophotometer, which resulted in a concentration of about 1.25 x 10^8 colony forming units per mL); after the suspension dried (ca. 2 h) on the beetles, the vial was transferred to a cage with two healthy plants; the beetles were allowed to emerge from the vial and feed for 24 h, after which time the beetles were removed and the plants were held for ca. 3 weeks; 3) same as treatment 1 except no flea beetles were added; 4) same as treatment 2 except no beetles were added and only the beaker was sprayed; 5) 100 P. cruciferae were allowed to feed on two healthy plants for 24 h, then removed from the plants and the plants held for ca. 3 weeks. All four trials were conducted in a similar manner except for one modification in the last two trials. In an attempt to enhance transmission in these two trials, the healthy plants were incubated for 24 h in a greenhouse chamber with intermittent mist at ca. 20-25°C during the period when the beetles were feeding on them.

In all treatments with flea beetles, substantial (ca. 25% of leaf surface injured) feeding by them occurred on all plants. In the first trial, typical black rot symptoms developed on four of eight plants on which the beetles fed after feeding on infected plants (treatment 1), and on all eight plants on which the beetles fed after being sprayed with bacteria before feeding (treatment 2). In the second and third trials only the eight plants in treatment 2 developed symptoms. In the fourth trial, only one of eight plants in treatment 1 and all eight plants in treatment 2 developed symptoms. In all four trials no transmission was observed in the controls (treatments 3 and 4) nor in treatment 5, in which immigrating flea beetles were transferred directly onto healthy plants.

Even under the intense pressure of 100 flea beetles feeding on only diseased leaves, transmission of Xcc from diseased to healthy plants (treatment 1) occurred in only 5 of 32 plants (15.6%). In contrast, the flea beetles were efficient vectors when we contaminated their body parts with the bacterium (treatment 2). This suggests that they were not efficient vectors in treatment 1 because they did not readily acquire the bacterium by feeding on infected leaves. When transmission did occur in treatment 2, we cannot differentiate whether it was initiated by contaminated mouthparts or other structures of the beetles. Regardless, such poor acquisition might have been due to the environmental conditions we used during the acquisition period, even though the last two trials were conducted under intermittent mist. The latter condition was used to enhance the development of the bacterium, but since flea beetles are not active during periods of high humidity and free moisture this may have hindered their feeding and acquisition of the bacterium. Additionally, acquisition may have been reduced because the beetles fed less on diseased tissue or veins, yet we observed the highest concentration of bacterial cells to be in these locations.

While this is the first experimental data demonstrating the ability of an insect to transmit this pathogen, our greenhouse results indicate limited potential for flea beetles to directly spread the pathogen. This low potential may account for our inability to demonstrate transmission by immigrating flea beetles in our area in either year. However in other areas, flea beetles may be able to transmit Xcc because of different environmental conditions or a high incidence of the bacterium in wild hosts.

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