Aerial Dispersal of *Xanthomonas campestris* pv. *campestris* from Naturally Infected *Brassica campestris*

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**ABSTRACT**


The importance of cruciferous weeds as a reservoir of inoculum and the role of aerial dispersal of *Xanthomonas campestris* pv. *campestris* (*X. c. pv. campestris*), causal agent of black rot of crucifers, was investigated. Leaves of *Brassica campestris* and air samples were collected in a naturally infected patch of *B. campestris* weeds in a noncultivated area. Leaves were sampled for *X. c. pv. campestris* by assaying tissue removed with a cork borer and by assaying wet filter-paper disks placed on leaf surfaces. We detected *X. c. pv. campestris* on surfaces of lesion areas and nonlesion areas of leaves. As many as $6.7 \times 10^4$ cells per gram fresh weight were found on lesion areas of leaves. Leaf surface populations of *X. c. pv. campestris* on symptomless leaves decreased after periods of heavy rainfall and low nighttime temperatures of 0.6-6°C. Air samples were taken with an Andersen viable particle sampler during rain and periods of no rain. Counts of viable *X. c. pv. campestris* per cubic meter of air ranged from a high of 13.68 during rain to a low of 0.84 during dry periods. Aerosol dispersal of *X. c. pv. campestris* from cruciferous weeds could be an important primary source of inoculum.

Black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* (*X. c. pv. campestris*) is a disease of worldwide importance (26). Sometimes, primary inoculum comes from infected seed. Also, several cruciferous weeds can serve as sources of primary inoculum (13,19). The role of weeds and nonhost species as alternate sources of inoculum for disease epiphytotics has been noted for several plant-pathogenic bacteria including *X. campestris* pv. *phaseoli* on bean (2) and *X. campestris* pv. *vesicatoria* on tomato (8,9). More recently, the importance of the survival of plant-pathogenic bacteria as resident or epiphytic populations on weed host and nonhost species has been reported in relation to primary inoculum for epiphytotics (10). Survival of plant-pathogenic bacteria on the surfaces of symptomless plant tissues has been reported for a number of pathogen and host species including *X. campestris* pv. *manihotis* on cassava (3,16), *X. campestris* pv. *populi* on poplar (18, *Pseudomonas syringae* pv. *syringae* on bean (4), *P. syringae* pv. *tomato* on tomato (22), *P. syringae* pv. *glycinea* on soybean (7), and *Erwinia amylovora* on pear (12). Plant canopies are reported to serve as major sources of airborne bacteria as naturally produced aerosols (11). The importance of naturally produced aerosols in epiphytotics has been reported for three other important bacterial diseases (5,6,14,15,17,23,24). Although the black rot pathogen spreads at least 12 m from infected weeds to cabbage transplants in Georgia (19), no data are available on the survival of *X. c. pv. campestris* in association with natural weed hosts or on the dispersal of this pathogen from naturally infected weeds. Since native cruciferous weeds are abundant in the crucifer production areas of California and are commonly infected with *X. c. pv. campestris* (19), we investigated the aerial dispersal of *X. c. pv. campestris* from naturally infected plants of *Brassica campestris* L. and monitored the populations of this pathogen on surfaces of symptomless leaves of *B. campestris*.

**MATERIALS AND METHODS**

Identification of *X. c. pv. campestris* in leaves of *B. campestris*. Leaf disks 7 mm in diameter (mean fresh weight 0.1 g) were taken from leaves of symptomless plants, from nonlesion areas of leaves with symptoms, and from lesion areas. In addition, sterile filter-paper disks (7 mm in diameter) soaked in sterile distilled water were placed for 5 min on the upper surfaces of leaves adjacent to areas where the tissue samples were taken. Five leaf disks and five paper disks were taken randomly from a single leaf of 10 symptomless plants and five plants with symptoms. The plants were scattered among a stand (10-15 m) of *B. campestris*. The five disks from each leaf were comminuted in 5 ml of sterile 0.85% NaCl (saline) at 5°C, then diluted serially to $10^{-4}$ in tubes of sterile saline. Three 0.1-ml replicates of dilutions of $10^{-2}$, $10^{-4}$, $10^{-6}$, and $10^{-8}$ were spread onto plates of nutrient-starch-cycloheximide agar (NSCA) (20) and incubated at 30°C for 5 days. Colony counts were made and...
results recorded as number of viable cells per square centimeter of upper leaf surface area for the filter-paper disk samples and number of viable cells per gram fresh weight of leaf tissue for the tissue disk samples. The identity of suspected colonies of X. c. pv. campestris was confirmed by pathogenicity tests as described.

**Plant leaf assays.** Leaf samples were collected weekly for 10 wk starting in January 1982 from symptomless *B. campestris* plants. Fifteen leaves were collected at sunrise from 15 plants selected at random from the stand. Samples were taken 0.6–0.8 m above ground level, placed in separate plastic Ziploc storage bags, carried to the laboratory on ice (about 30 min), and assayed immediately. Leaves were stored at 5°C during the assay. For assay, each leaf was weighed and comminuted with a mortar and pestle in a small volume of sterile saline at 5°C. The final volume was brought to 100 ml and filtered through sterile cheesecloth. Serial 10-fold dilutions were made to $10^{-12}$ in tubes containing 9 ml sterile saline; 0.1-ml aliquots of $10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}$, and $10^{0}$ were pipetted onto triplicate plates of NSCA, spread with an alcohol-flamed, L-shaped glass rod, and incubated at 30°C for 5 days. Characteristic colonies of *X. c. pv. campestris* were counted, and pathogenicity of 15 representative colonies was determined each week. Results were recorded as the log of number of cells per gram fresh weight of leaf tissue.

**Airborne populations of X. c. pv. campestris.** Air samples were taken with Andersen viable particle samplers (Andersen Samplers, Inc., Atlanta, GA) (1) from the center of a 10-m-diameter clearing within a stand of *B. campestris* with symptoms of black rot. About 80% of the approximately 200–300 plants showed symptoms. The nearest cruciferous crop was 180 m or more southeast. No visible symptoms of black rot were observed in this crop. A six-stage and a two-stage sampler were used to collect airborne particles on plates of NSCA. An Andersen vacuum pump was battery-powered to draw air through the samplers at a rate of 0.028 m³/min as monitored with a Matheson compact flow meter (Matheson Gas Products, East Rutherford, NJ). The samplers were operated vertically with the orifice 35 cm above the soil surface. Forty-six samples of 15-min duration were collected on seven dates. The samplers and collection plates were placed inside a closed vehicle outside the sampling area for loading and unloading. After collection, plates were incubated at 30°C for 5 days and colonies of *X. c. pv.*

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**Table 1.** Actual counts of *Xanthomonas campestris pv. campestris* cells on and in leaves with and without black rot symptoms in a naturally infected stand of *Brassica campestris*.

<table>
<thead>
<tr>
<th>Leaf samples</th>
<th>Filter-paper disk samples</th>
<th>Mean cells/cm²</th>
<th>Leaf disk samples</th>
<th>Mean cells/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomless leaf (10 samples)</td>
<td>$1.3 \times 10^7, 2.6 \times 10^7,$</td>
<td>9.6 $\times 10^6, 0, 0, 0,$</td>
<td>0 $\times 10^{-2}, 0, 0, 0,$</td>
<td>NA⁴</td>
</tr>
<tr>
<td>Nonlesion area</td>
<td>$8.4 \times 10^4, 5.2 \times 10^4,$</td>
<td>1.4 $\times 10^5, 2.6 \times 10^5,$</td>
<td>2.2 $\times 10^4, 2.6 \times 10^4,$</td>
<td>3.4 $\times 10^6, 2.1 \times 10^6,$</td>
</tr>
<tr>
<td>Lesion area</td>
<td>$1.0 \times 10^3, 0.9 \times 10^3,$</td>
<td>5.6 $\times 10^6, 1.66 \times 10^7,$</td>
<td>8.2 $\times 10^6, 13.6 \times 10^6,$</td>
<td>1.3 $\times 10^9, 6.7 \times 10^7,$</td>
</tr>
</tbody>
</table>

⁴Samples obtained by placing sterilized water-soaked 7-mm filter-paper disks on the upper surface only of leaves of *B. campestris*. Five paper disks were comminuted in 5 ml of sterile 0.85% NaCl (saline) at 5°C. Figures are cells per square centimeter.

⁵Samples obtained by aseptically cutting 7-mm disks from leaves of *B. campestris*. Five disks were ground in 5 ml of sterile 0.85% NaCl at 5°C. Figures are cells per square centimeter.

⁶95% Confidence interval for the true mean.

⁷Not applicable because configuration of data confidence interval of mean not appropriate.
campestris were recorded. All suspected colonies were confirmed as X. c. pv. campestris by pathogenicity tests. Results were recorded as number of viable cells per cubic meter of air.

Pathogenicity tests. Cultures were grown in Difco nutrient broth for 18 hr at 30 C on an orbital water bath shaker and adjusted to give 10^8 cfu/ml as described (25). Plants were inoculated and observed for symptoms as described (21). Briefly, three healthy cabbage seedlings (B. oleracea var. capitata 'Headstart') at the two-leaf stage were inoculated by injecting leaf petioles with about 0.1 ml of bacterial suspension, using a 1-cc syringe and a 25-gauge needle. Plants were placed in the greenhouse at 19-32 C and observed for symptoms for 14 days.

Weather data. Rainfall, relative humidity, wind speed and direction, cloud cover, and temperature data were obtained from the flight services office of the Salinas airport about 3.2 km from the sampling area. These weather data were supplemented with independent observations on leaf surface conditions recorded at the sampling site on collection dates.

RESULTS

Plant leaf assays. X. c. pv. campestris was recovered in high numbers from symptomless leaves of B. campestris as well as from from lesion and nonlesion areas of leaves with symptoms (Table 1, Fig. 1). All 150 colonies tested for pathogenicity were positive. X. c. pv. campestris was not always detected from symptomless leaves (Table 1) by leaf-disk or paper-disk assay. However, X. c. pv. campestris was always detected in high numbers from symptomless leaves by the whole-leaf assay (Fig. 1). This difference indicated that X. c. pv. campestris did not exist evenly on or in the leaf. For leaf-disk samples, lesion areas yielded significantly greater average counts of X. c. pv. campestris than did their paired nonlesion counterparts. In four of the five paired filter-paper disk samples, counts of X. c. pv. campestris were greater in the lesion areas than in the corresponding nonlesion areas (Tables 1 and 2).

Airborne population. Expected numbers of cells of X. c. pv. campestris in the air were significantly greater (P = 0.10, 90% confidence interval) during rainy periods than during no rain/dry leaf conditions. There were also significantly (P = 0.05, 95% confidence interval) more cells during dry periods with dew than during dry periods without dew (Tables 3 and 4). All 74 colonies tested for pathogenicity were positive. The distribution of the size of cells of X. c. pv. campestris collected varied with environmental conditions. During rain, most viable cells were collected on plates of stages 03 (3.3-2.1 μm) to 05 (2.1-1.1 μm) (Fig. 2). Over all environments, most cells of X. c. pv. campestris were collected on plates of stages 03-05 (4.7-3.3 μm) (Fig. 2).

DISCUSSION

The large numbers of cells of X. c. pv. campestris on symptomless leaves and nonlesion areas of leaves with symptoms of a cruciferous weed species suggests the pathogen's epiphytic nature (10) or survival on leaves of B. campestris. However, some or all such leaves could have been infected internally without visible lesions. These results agree favorably with the report that weeds serve as a source of inoculum of X. campestris pv. phaseoli in Michigan (2). Crucifers are the dominant weed in the area, and

Table 2. Statistical analysis of data on leaves with symptoms

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Filter-paper disk samples</th>
<th>Leaf disk samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample estimate (P)</td>
<td>4/5 = 0.8</td>
<td>5/5 = 1</td>
</tr>
<tr>
<td>95% Confidence interval for true P of population</td>
<td>0.27 ≤ P ≤ 1</td>
<td>0.47 ≤ P ≤ 1</td>
</tr>
<tr>
<td>Sample mean pair difference</td>
<td>NA</td>
<td>6.5 × 10^7 cells/g</td>
</tr>
<tr>
<td>Significance level for overall mean pair difference &gt;0</td>
<td>NA</td>
<td>94%</td>
</tr>
<tr>
<td>95% Confidence interval for true mean paired difference</td>
<td>NA</td>
<td>6.7 × 10^7 ± 7.2 × 10^7</td>
</tr>
</tbody>
</table>

*From data presented in Table 1.

Table 3. Airborne population levels of Xanthomonas campestris pv. campestris collected near a naturally infected stand of flowering plants of Brassica campestris under different weather conditions

<table>
<thead>
<tr>
<th>Air and leaf-surface conditions</th>
<th>Actual counts*</th>
<th>Estimated expected value</th>
<th>Asymptotic 95% confidence interval for true expected value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain, leaves wet</td>
<td>47.62, 33.33, 4.76, 2.38, 1.74, 1.19, 2.38, 0</td>
<td>13.40 (58.02)</td>
<td>13.40 ± 14.93</td>
</tr>
<tr>
<td>No rain, leaves wet</td>
<td>4.76, 2.38, 1.74, 4.76, 19.04, 2.38, 2.38, 0, 0, 0, 0, 0</td>
<td>2.67 (0.76)</td>
<td>2.67 ± 1.71</td>
</tr>
<tr>
<td>No rain, leaves dry</td>
<td>1.19, 3.57, 3.57, 5.57, 1.19, 1.19, 0, 0, 0, 0, 0, 0, 0, 0</td>
<td>0.65 (0.06)</td>
<td>0.65 ± 0.48</td>
</tr>
</tbody>
</table>

*Each sample was taken over a period of 15 min. Figures are cells per square centimeter.

Table 4. Comparison of the overall expected value of data on airborne population levels of Xanthomonas campestris pv. campestris

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Differences between estimated expected values</th>
<th>Standard error of difference</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain vs. no rain (leaf wet)</td>
<td>10.73</td>
<td>7.667</td>
<td>&gt;0.10*</td>
</tr>
<tr>
<td>Rain vs. no rain (leaf dry)</td>
<td>12.75</td>
<td>7.621</td>
<td>&lt;0.10*</td>
</tr>
<tr>
<td>No rain (leaf wet) vs. no rain (leaf dry)</td>
<td>2.016</td>
<td>0.8202</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*From data presented in Table 3.

Not significant at 90% level.

Significant at 90% level.

Significant at 95% level.
black rot has been reported to be widespread in many different species in the Salinas area (19). Survival on cruciferous weeds may be important in the epidemiology of black rot in the Salinas Valley.

The data suggest that populations of *X. c. pv. campestris* on symptomless leaves fluctuate with changes of temperature and rainfall. For example, the first increase in population of *X. c. pv. campestris* (5–12 January) occurred during a period of no rainfall (14 days) and increased high (daytime) and low (nighttime) temperatures. A reduced population of *X. c. pv. campestris* was associated with the first measurable rain (2.23 cm) on 19 January. A further drop in counts of *X. c. pv. campestris* occurred on 26 January and could have been caused by a rapid decline in high and low temperatures during the period of 16–20 January. An unusually low temperature of 0.6 °C occurred on the night of 20 January. The increase in population on 2 February and decrease on 9 February could have been a result of a 4-day dry period of increased temperatures and a rainfall on 8 February, respectively. Had a sample been taken on 6 February, the population might have been higher. The frequent rain during 23 February to 2 March could have contributed to the low population on 2 March. Finally, the last increase in population on 9 March might have been caused by the increase and stable high and low temperatures and relatively low rainfall just before sampling. Had we sampled at closer intervals, the relationship between rain, temperature, and population of *X. c. pv. campestris* might have been much clearer.

The presence of viable and pathogenic cells of *X. c. pv. campestris* in the air during the fall appears to be most likely due to aerosol particles naturally generated by raindrops hitting wet bacteria-covered leaf surfaces. These aerosols could remain suspended for varying amounts of time, depending on particle size, wind, and rain (11). The movement of viable cells of plant-pathogenic bacteria in aerosol particles could be epidemiologically significant because aerosol particles can travel farther than splashing raindrops (5). The epidemiologic importance of aerosol dissemination of *E. carotovora* has been reported, with viable cells being carried at least 100 m from a ground-level source of inoculum (6,15). Cells of *E. amylovora* have been reported to survive in significant numbers for as long as 2 hr in aerosol particles exposed to the open air (23). Cells of *X. c. pv. campestris* collected from air during periods without rain may have been dried bacteria windblown from leaf surfaces, either singly or in masses. Although *E. carotovora* was never collected from the air during dry periods (17), rain is not essential for the generation of bacterial aerosols from plants (11). It could be possible for wind-generated aerosol particles to be formed by the shearing force of a gust of wind on a bacteria-containing droplet of water or dew. This might occur sporadically and would correlate with the lower and less consistent number of viable cells of *X. c. pv. campestris* collected during these conditions. Sudden wind changes and occasional rain-out of particles from the air have also been reported to affect aerosol dispersion.

Collection of most cells of *X. c. pv. campestris* on stages 04–05 of the Andersen sampler is similar to that reported for *P. glycinea* (24); however, the airborne population of *X. c. pv. campestris* was much smaller than that reported for *P. glycinea*. This may be due to differences in host-pathogen relationship and/or environment. It should be noted that *X. c. pv. campestris* was collected in a natural open field with naturally infected plants, whereas work with *P. glycinea* was with inoculated plants. When an Andersen sampler was used to determine the distribution of particle sizes of *E. carotovora*, it showed that more than 80% of the particles were smaller than 7 μm in diameter (15). This was generally true for our collections as well. Collections of particles on stage 01 may contain liquid droplet contamination. And precipitation or moisture would be pulsed by vacuum through the impactor inlet cone and deposited on stage 01 (Andersen Sampler Company, personal communication). Some particles collected in the sampler could have been made up of more than one bacterial cell; thus reported numbers of bacterial cells per unit volume of air may be lower than actual true numbers (24).

Collection of viable cells of *X. c. pv. campestris* from air during wet and dry conditions warrants further investigation. We have no proof that the cells of *X. c. pv. campestris* collected in the sampler originated from air dispersal from the infected plants of *B. campestris*; however, no other major source of cruciferous weeds was nearby. The closest cruciferous crop, *Brassica* 180 m south across a state highway, and moreover, no visible symptoms of black rot were observed in the brocoli. We concluded that the aerosols of *X. c. pv. campestris* collected came from our stand of *B. campestris*, which had 80% incidence of infection and was 10 m from the sampler. Further work is needed on the ecology of *X. c. pv. campestris* on *B. campestris* and cruciferous weed hosts. We consider the survival of *X. c. pv. campestris* in association with *B. campestris* and aerosol distribution important factors in the epidemiology of black rot disease.

**LITERATURE CITED**


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