Dissemination and Transmission of *Xanthomonas campestris* pv. *begoniae* in an Ebb and Flow Irrigation System

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ABSTRACT


Low populations of *Xanthomonas campestris* pv. *begoniae* were disseminated from infected roots of *Begonia hiemalis* into a recycled ebb and flow irrigation solution developed for the production of potted horticultural crops. Plants irrigated with the infested solution developed low levels (2.5% incidence) of the disease. The low level of transmission appeared to be attributable to high death rates of the pathogen in the irrigation solution. We conclude that transmission of this pathogen through this solution does not pose a great threat to producers of flowering begonias but could be important in production of pathogen-free begonia stock plants.

During the past decade, a new irrigation method for potted greenhouse crops has been developed in Denmark, Germany, and The Netherlands to abate groundwater pollution (3,4). In this system, known as ebb and flow, a plant nutrient solution is pumped from a storage reservoir or tank onto a water-tight table supporting potted plants. After a short flooding period, the pump is deactivated and the irrigation solution drains back into the tank. The potential for dissemination of plant pathogens through this recirculating irrigation system under conditions prevailing in the United States is unknown but of concern.

In The Netherlands, losses caused by soilborne plant pathogens in floricultural crops produced in the ebb and flow system appear similar to those in the overhead irrigation system (10,11). Dissemination and transmission of *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cyclaminis* Gerlach and *Phytophthora cryptogea* Pethybr. & Lafferty was not severe (10) in this system. Propagules of *F. o. cyclaminis* settled out on the bottom of the storage reservoir (11). Storage tanks with special traps were developed to enhance settling.

In the United States, *Fusarium* wilt of cyclamen has been a problem in the ebb and flow system during summer months only. This problem may be attributable to the high temperature requirements for the disease (9). During the summer of 1989, severe outbreaks of bacterial leaf spot and blight of begonia (*Begonia hiemalis* Fotch) occurred in Ohio and Illinois in greenhouses with the ebb and flow system. Cells of *Xanthomonas campestris* pv. *begoniae* (Takimoto) Dye, the causal organism of the blight, would remain suspended in the irrigation solution because of Brownian movement. Moreover, the pathogen infects roots and causes systemic infection (5,15,16). Thus, the
irrigation solution could be a source of inoculum to blight outbreaks. In this paper, we report the potential for despersal and transmission of *X. c. begoniae* through the recirculated solution of an ebb and flow system.

**MATERIALS AND METHODS**

**Isolation of *X. c. begoniae* and selective media.** Cultures of *X. c. begoniae* were obtained from diseased begonia plants produced at commercial growers as well as in the laboratory in an ebb and flow production system and from samples of irrigation solution using a semiselective medium (BS) developed for the isolation of a *Xanthomonas* sp. from a walnut (8). All colonies positive for starch hydrolysis were transferred to Difeco sucrose nutrient agar (SNA). Cultures were screened for pathogenicity and aggressiveness by injecting stems of pathogen-free *B. hiemalis* (cv. Connie) with 0.5 ml of an aqueous cell suspension (10^7 cfu/ml) washed in dilution buffer (7.0 g of K_HPO4, 3.0 g of KH2PO4, and 0.2 MgSO4·7H2O per liter). Alternatively, roots were dipped into the same suspension. The presumptive cultures of *X. c. begoniae* were identified with a Microbial Identification System (Microbial ID, Inc., Newark, DE) (12).

A rifampicin-resistant strain of *X. c. begoniae* (XcbR1) was isolated from wild type strain Xcb2 using a procedure similar to that described by Weller and Saettler (18). The SNA was amended with rifampicin (99.9% active) and chlorothalonil (50WP) to 60 and 30 µg/ml, respectively. Resistant strains of Xcb2 were isolated by placing 0.5 ml of a 10^7 cfu/ml suspension on the amended medium (RCSNA). The plates were allowed to dry and then incubated for 3 days at 29 C. Colonies were subcultured on RCSNA, transferred to SNA, purified, and tested for pathogenicity. The most aggressive strain (XcbR1) was kept for further work and stored in a glycerol water suspension at -70 C (14). The identity of this strain was confirmed with the Microbial Identification System.

The aggressiveness of wild type strain Xcb2 was compared with that of XcbR1 in 4-wk-old pathogen-free *B. hiemalis* ‘Renaissance.’ Various populations (0, 10^2, 10^3, 10^4, and 10^5 cfu/ml) of the pathogen harvested from 24-hr-old Dife sucrose nutrient broth (SNB) cultures and washed in dilution buffer were injected into petioles. This highly susceptible cultivar (16,17), was supplied by Mikkelsons Inc., Ashtabula, OH. Control plants were injected with sterile buffer solution. Plants were incubated in a greenhouse with day and night temperatures of 28–35 C and 20–24 C, respectively. Leaf disease severity was rated at 7, 10, 14, 18, and 25 days after inoculation with a five-tier foliar disease severity rating scale where 1 = symptomless, 2 = symptoms covering <25, 3 = 25–75, 4 = 75% of the leaf surface area, and 5 = the entire leaf (5). There were eight replicates per treatment in a completely randomized design. The effects of strain and inoculum density on severity was evaluated with an analysis of variance. The relationship between inoculum density and disease severity after various incubation periods for each bacterial strain was determined by linear regression.

To determine the ability of isolates Xcb2 and XcbR1 to induce systemic infection, we isolated from stem cross sections at the crown level (five plants per treatment) and from root tips (seven per plant) from the surface of the root ball 35 days after inoculation of petioles. Stem sections (3 cm long) were cut from the crown of each plant and then surface-sterilized for 1 min in a 1% sodium hypochlorite solution. Thin (1 mm) cross sections of the stem of each plant were plated on BS or RCSNA (three sections per medium) to isolate Xcb2 or XcbR1, respectively. Root tips were washed in sterile tap water, dried, and plated on the selective media as described earlier. The plates were incubated for 3 days at 29 C. The mean percentages of infected root tips, crowns, or plants (both root and crown infection) were then calculated for each treatment. Effects of strain and inoculum density on proportion of root, crown, or plant infections were analyzed with ANOVA.

**Potting mix, ebb and flow system, and fertility.** A sphagnum peat-perlite potting mix was used throughout this study. The peat was fibrous in nature, H4 to H5 on the Von Post decomposition scale (1). The potting mix consisted of 60% Canadian sphagnum peat and 40% perlite (v/v). The pH was adjusted to 3.5–6.0 with calcium carbonate (all particles <100 mesh). Slow-release Osmocote fertilizer (15-10-12 plus minor, a 3- to 4-mo release formulation) was incorporated into the mix just before planting at a rate of 2.4 g/L of potting mix. The air-filled pore space in this container medium was 15% at a matric tension of 0 kPa. Microbial activity in the potting mix at planting, based on the rate of hydrolysis of fluorescein diacetate, was 1.1 µg/g of dry weight (1,6). Therefore, this potting mix was low in microbial activity and conducive to Pythium damping off and root rots (1,2). Ebb and flow benches were provided by Midwest GRoMaster, St. Charles, IL. Each bench consisted of a horizontal 125 x 110 cm table with a 10-cm-tall edge. The bench surface contained a grooved channel system that sloped toward a drain at one side of the bench. The irrigation solution was pumped onto the bench to a depth of 2 cm from a 68-L storage tank located underneath each bench. Each bench had a separate tank. The solution drained freely back into the tank at the completion of each flooding period. A second storage tank under each bench contained tap water only and was used to irrigate crops late during the production cycle, which prevented the accumulation of excessive nutrient concentrations. All tanks were covered with black polyethylene to reduce growth of algae.

Each pump was activated for 6 min by a Q-Com Gem II environmental control computer through a timer commander (Q-Com Corp., Irvine, CA). The flooding period, the actual length of time that the nutrient solution was in contact with the container medium in the base of all pots on the bench, was 20 min. The number of irrigation cycles ranged from three times per week for freshly potted rooted cuttings to twice a day for large flowering plants. The irrigation solution contained Peters 20-20-20 soluble fertilizer to a final concentration of 100 µg of NPK per milliliter of solution. Additional fertilizer was added during the production cycle of each crop to maintain the concentration. Benches, pumps, irrigation lines, and tanks were cleaned between crops and surface-disinfested with a solution of 2% sodium hypochlorite.

In some experiments, disease severity was also monitored during a postharvest period. Plants in full bloom were watered with a Chapin watermatics tube irrigation system. Fertilizer that had accumulated near the surface in the potting mix during production in the ebb and flow system (4) served as a nutrient source during the first 2 wk of the postharvest period. Thereafter, a low rate of slow-release fertilizer (2.4 g/L of potting mix) was applied as a topdressing to supply additional nutrients. Typically, plants were produced for 60–90 days in the ebb and flow system and then held 4–6 wk in the open production system to determine postharvest disease severity.

**Dissemination of *X. c. begoniae* from stem- or root-inoculated to pathogen-free plants.** Stems of pathogen-free Renaissance (one plant per pot, four plants per bench) were inoculated by injection with a washed suspension of strain XcbR1 as described earlier and placed in a row at the drain side of each bench. Three rows of four pathogen-free plants were placed away from this location on each bench. There were two benches (= blocks) per treatment in a randomized complete block. Care was taken not to contaminate any of the pathogen-free control plants. Plants were spaced 30 cm apart, stem to stem, to avoid leaf-to-leaf contact.

Both the nutrient solution and the tap water used periodically as an alternate solution were sampled to quantify populations of *X. c. begoniae* in the irrigation water. Three 20-ml samples of the solutions were collected weekly immediately after an irrigation cycle from a
depth halfway to the bottom of the tanks. Duplicate subsamples (0.2 ml) were plated on RCSNA. Colonies were counted after 3 days at 29 C. Benches with pathogen-free plants not inoculated with XcbR1 served as controls.

Stem cross sections (crown level) and root tips were plated on selective media as described earlier to monitor movement of the pathogen among plants on a bench. Root tips (five per plant) were Excised biweekly with sterilized forceps from the surface of the root ball at the bottom of the pot and adjacent to drain holes. After sampling, pots were returned to the same location on the bench. Plants were harvested after 10 wk. The entire experiment was repeated with both Xcb2 and XcbR1. In the second test, the entire lower 3-cm section of the root ball (four plants per replicate) was digged (10 s) into 10^5 cfu of Xcb2 per milliliter. Mean percentages of infected root tips and plants (root or crown infected) were calculated for each treatment.

Transmission of X. c. begoniae from infested irrigation solutions to begonia plants. Various populations of XcbR1 cultured in SNA and washed as described earlier were added to the nutrient solution and tap water in storage tanks. Final populations in the solutions were determined by dilution plating on RCSNA, as described earlier. Irrigation solutions not infested with the pathogen were the controls. Two benches (blocks) were used per inoculum level in a completely randomized design. Temperature and other culture conditions were as described earlier.

Begonias were moved from the ebb and flow benches to overhead irrigated benches after 14 wk and kept there for 7 wk. Root tips (seven per plant) were collected biweekly from the root ball in the bottom of each pot during both the ebb and flow production and postharvest periods and plated on RCSNA. After the postharvest period, a thin cross section of the basal stem portion (crown) of each plant was harvested, surface-sterilized, and plated as described earlier.

The mean percentages of infected root tips and plants, based on isolation of the pathogen from roots or crowns, were then calculated. Effects of inoculum on proportion of root tips and crowns with infections were evaluated with ANOVA. The entire experiment was performed three times.

Survival of X. c. begoniae in the irrigation solution. Cells of XcbR1 washed as described earlier, or as natural inoculum in crushed, dried, infested begonia leaves were added to either the nutrient solution or tap water. Infested leaves had been harvested from plants inoculated with XcbR1 that had developed systemic symptoms as described earlier. The pathogen can survive for years in dried leaves (5,7). Washed cells of XcbR1 were added just before flooding of benches to establish populations ranging from 10^3 to 10^5 cfu/ml of solution. The crushed infested leaves were added to tanks to establish an initial population of 10^5 cfu/ml. Control tanks were not infested. The actual population was verified by duplicate plating on RCSNA. Two replicates were used per treatment in a completely randomized design. Immediately after the first flooding period and daily for 10 days thereafter, samples (20 ml) of the solution were plated on RCSNA as described earlier. Day and night temperatures ranged from 28 to 34 C and from 19 to 25 C, respectively. This experiment was repeated once.

The effect of fertilizer concentration on survival of Xcb2 and XcbR1 was determined in a laboratory assay by measuring bacterial populations in Erlenmeyer flasks incubated at 25 C (two replicates per treatment) in the dark. Concentrations of fertilizer at 0, 100, and 200 µg of NPK per milliliter were supplied as Peters 20-20-20 water-soluble fertilizer (W. R. Grace & Co., Fogelsville, PA). Solutions were transferred daily to another sterile flask to mimic conditions in ebb and flow tanks. Survival was determined as described earlier. This experiment was repeated once.

Various concentrations of NaCl and Peters 20-20-20 soluble fertilizer were added to yeast salt broth (YSB), a medium developed for evaluation of growth of X. c. begoniae, to determine the mechanism of cell death observed for X. c. begoniae in tap water and in the nutrient solution. Tubes inoculated with 10^3 cfu/ml of the pathogen were scored for good growth (+ +), slight growth (+), or no growth (-) of the pathogen after 48 hr of incubation at 26 C. The experiment was repeated twice.

The relationship between log of population and time of incubation in tanks, flasks, or tubes containing various solutions was evaluated by regression analysis, which represented death rates. A t test was used to compare differences.

RESULTS
Isolation of X. c. begoniae from the nutrient solution in a commercial greenhouse. Presumptive colonies of X. c. begoniae on the BS medium were isolated on two occasions from the ebb and flow nutrient solution collected at a commercial greenhouse where an epidemic of bacterial leaf spot and blight of begonia was in progress. The grower had propagated plants from rooted cuttings that had not been indexed for the pathogen. The population in the irrigation solution ranged from 0.5 × 10^4 to 2.0 × 10^5 cfu/ml. Samples of the colonies caused symptoms of bacterial leaf spot and blight in begonia and were identified as X. c. begoniae with the Microbial Identification System.

Aggressiveness of XcbR1. Virulence of XcbR1 to foliage of B. hiemalis 'Renaissance' was not significantly different (P > 0.05) from that of the wild type strain Xcb2 (Fig. 1). However, XcbR1 caused significantly (P < 0.05) more systemic infections than wild type strain Xcb2 (Table 1). At 35 days after inoculation of petioles, XcbR1 was recovered from the crowns of all plants that had been inoculated with 10^5 cfu/ml of this mutant compared with only 20% from the crowns of the wild type strain treatment. Percent recovery of XcbR1 from root tips was significantly higher (P < 0.05) than that for Xcb2 at the high inoculum levels only.

Dissemination of X. c. begoniae from stem- or root-inoculated plants to pathogen-free plants. Stem-inoculated begonia plants died from systemic infections within 4–8 wk. Leaves of infected plants did not become large enough to touch the ebb and flow nutrient solution during flooding. In all cases, however, roots ramified the potting mix and reached the bottom of pots before plants died. XcbR1 was isolated from roots of all inoculated plants. Five attempts at biweekly intervals to isolate the pathogen from roots or crowns of pathogen-free control plants placed on the same bench with the inoculated plants failed. No evidence for plant-to-plant transmission was found in this or a second experiment.

In all two experiments, the pathogen was isolated from the irrigation solution 9 wk after inoculation of plants. At that time, the mean population of XcbR1 in the irrigation solution was 5.0 × 10^6 cfu/ml. The recovered isolates of XcbR1 were pathogenic to begonia. All other attempts to isolate the pathogen from the solution failed. It was not recovered at any time from the irrigation solution used in control benches.

In a similar experiment, with wild type strain Xcb2, evidence for dissemination

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![Fig. 1. Comparative virulence of Xanthomonas campestris pv. begoniae (Xcb2) and a rifampicin-resistant mutant (XcbR1) to foliage of Begonia hiemalis 'Renaissance'. Disease severity scale, in which 1 = symptomless, 2 = symptoms covering <25, 3 = 25–75, 4 = >75% of the leaf surface area, and 5 = the entire leaf, determined 10 days after injection of petioles. Vertical bars indicate standard errors. (Solid line = Xcb2; broken line = XcbR1).](http://example.com/fig1.png)
Table 1. Systemic colonization of *Begonia hiealis* ‘Renaissance’ by *Xanthomonas campestris pv. begoniae* (Xcb2) and a rifampicin-resistant mutant (XcbR1)

<table>
<thead>
<tr>
<th>Inoculum density* (cfu/ml)</th>
<th>0 × 10^6</th>
<th>1 × 10^7</th>
<th>1 × 10^8</th>
<th>1 × 10^9</th>
<th>1 × 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crows</strong></td>
<td>0.0</td>
<td>0.0</td>
<td>2.8</td>
<td>2.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Xcb2</td>
<td>0.0</td>
<td>0.0</td>
<td>8.4</td>
<td>17.0</td>
<td>51.2</td>
</tr>
<tr>
<td>XcbR1</td>
<td>0.0</td>
<td>8.4</td>
<td>20.0</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td><strong>Roots</strong></td>
<td>0.0</td>
<td>0.0</td>
<td>8.4</td>
<td>20.0</td>
<td>8.4</td>
</tr>
</tbody>
</table>

*Injected (1 ml) into the petiole of two leaves of each of five plants.

*Mean of seven root tips of each of five plants per treatment, determined 35 days after inoculation.

*Mean of five plants per treatment, determined 35 days after inoculation.

*Standard error of the percentage in parentheses under mean.

Fig. 2. Linear regression describing the proportional survival of a rifampicin-resistant mutant of *Xanthomonas campestris pv. begoniae* (XcbR1) added as washed cell suspensions to the ebb and flow irrigation solution (100 µg N/ml) to tap water (dashes), or as infused dried crushed leaf debris in tap water (dotted line). All regressions were significant ($P < 0.05$); $r^2$ is the coefficient of determination.

Survival in the ebb and flow irrigation solution. The death rates of washed cells of XcbR1 in the ebb and flow nutrient solution and in tap water did not differ significantly ($P > 0.05$) based on a $t$ test comparison of regression slopes (Fig. 2). A high population of the pathogen was released into the irrigation solution at 24 hr after ground, infested, dried leaves were added to the tanks filled with tap water. The death rate of this natural inoculum of XcbR1 in tap water was lower than that of washed cells (Fig. 2). Ten days after infestation with washed cells, the pathogen could no longer be isolated from either the nutrient solution or tap water. However, it was still isolated at a very low population ($≤20$ cfu/ml) from tap water to which natural inoculum had been added. In the second experiment, similar trends in population decline were observed.

Survival was not affected by the concentration of Peters 20-20-20 soluble fertilizer in the irrigation solution. Death rates of Xcb2 and XcbR1 in tap water or in 100 and 200 µg NPK per milliliter of nutrient solutions, estimated as the slopes in a regression analysis, did not differ significantly ($P > 0.05$). Death rates for both strains in 100 µg of NPK per milliliter of nutrient solution are shown in Figure 3.

Both strains (Xcb2 and XcbR1) of the pathogen grew profusely in YSB to which 0.5 or 1.0% NaCl had been added. Slight inhibition of growth of both was observed in 2% NaCl-YSB. Added concentrations of 5 and 10% NaCl inhibited growth in YSB. None of the concentrations of Peters 20-20-20 water-soluble fertilizer (0, 50, 100, 150, or 200 µg of NPK per milliliter) added to YSB inhibited growth.

**DISCUSSION**

A short-term flooding period was used in our work to maintain optimum conditions for plant growth (3). After this short period, when the irrigation solution had drained back into storage tanks, water was still moving up into the potting mix through capillary activity. Therefore, little opportunity existed for irrigation solution harboring pathogens to drain out of pots at the conclusion of a flooding period. Yet, enough inoculum presumably disseminated from infected roots out of pots to contaminate the nutrient solution.

The low number of plants (one out of 40) that developed foliar symptoms after irrigation with a solution infected with $5.2 \times 10^9$ colony-forming units of XcbR1 per milliliter revealed that it was not disseminated readily in the ebb and flow nutrient solution. However, the low number (2.5%) of plants that developed latent systemic infections, after irrigation with a solution harboring a population of Xcb ($2.2 \times 10^5$ cfu/ml) less than one order above that present in the

Transmission of *X. c. begoniae* through an infested irrigation solution.
irrigation solution at a commercial grower, showed that dissemination of the pathogen through the ebb and flow irrigation solution could occur. The low percentage of systemic latent infections and the lack of a consistent relationship between inoculum density in the irrigation solution and amount of disease induced suggest that very little opportunity existed for transmission. Levels of dissemination were high enough, however, to yield significant levels of infection at the conclusion of a postharvest period. The foregoing suggests that transmission of Xc through the ebb and flow nutrient solution poses a threat to producers of pathogen-free stock plants but not to florists who use pathogen-free stock for the production of finished flowering plants.

The high rate of population death of Xc2 and XcR1 in both the nutrient solution and the tap water (Fig. 2) used as irrigation solutions in the ebb and flow system may explain the low frequency of transmission. Rates of decline of the population of X. c. begoniae in irrigation solutions prepared with a range of fertilizer concentrations in laboratory tests further support this. Growth of both Xc2 and XcR1 in broth or irrigation solution to which YSB had been added as a carbon source, as well as in 1% NaCl-YSB, reveals that salinity of the nutrient solution did not cause cell death. Possibly, the lack of nutrients in the irrigation solution or osmotic shock resulted in cell death. Finally, only two strains have been examined for their ability to be disseminated in the ebb and flow system. Possibly, strains better adapted to survive and be transmitted in this system of irrigation will appear as this approach to the production of potted plants becomes used more widely.

X. c. begonia survived better if introduced into the irrigation solution as crushed, dried, infested leaves. This pathogen survives for a very long time in dried leaves (5,7). Plant debris in the form of absced leaves and flowers eventually enters the irrigation solution after it falls on the ebb and flow bench. The pathogen easily could be introduced into the system via this pathway and, therefore, could be recirculated along with the irrigation solution. Screens installed in the drain end of ebb and flow benches used in our work reduced recirculation of large debris particles. Routine sanitation procedures that include filtration and crop debris removal, therefore, could possibly reduce this potential source of inoculum.

The quantity of irrigation solution kept in tanks in our work was slightly higher than that required to flood benches to the appropriate depth. The solution was not used to irrigate other benches. It was used from three times a week to twice daily, depending upon the water and nutrient needs of the crop. Florists may irrigate entire crops and often multiple crops with the same solution, and sometimes more than once per day. Even though our strains of the pathogen died rapidly in the irrigation solution, much higher frequencies of transmission than observed in this work possibly could occur in commercial production.

Some commercial producers in the United States have installed concrete floors, rather than benches that are flooded to irrigate crops. The flooding depth is often greater than 2 cm along the edges of such individual sections. The total flooding period in such a system, particularly for plants near gutter drains in the floor, is much greater than the period we used. Water and some potting mix ingredients actually drain out of pots in such long-term flooded systems. Much greater opportunities for dissemination of plant pathogens appear to exist there. Observations made at one grower supports this. Highest plant losses were along the drains in the floor.

In the system used in this work, the nutrient solution was pumped through a hole onto the bench surface. Some growers have installed automated bench systems. In those systems, the solution is pumped on top of the bench through a nozzle. Droplets have been observed to splash onto foliage for up to a 25 cm distance away from an inlet (H. A. Hoitink, unpublished). In this type of commercial system, bacterial leaf spot and blight was observed to occur as a semicircular area of losses around inlet ports. It is possible, therefore, that splash dispersal could contribute to foliage infection in some ebb and flow systems, unless precautions are taken in the design of entry ports to reduce droplet formation.

Based on information available as far, effective transmission of X. c. begoniae in the ebb and flow system does not appear to pose a great threat to producers of flowering plants if appropriate precautions are taken. Rapid death of the pathogen in the recirculated irrigation solution appears to be responsible for the low level of transmission. The system actually may reduce losses below those in overhead irrigation because splash dispersal can be avoided in ebb and flow.

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LITERATURE CITED