# A Bioluminescent *Xanthomonas campestris* pv. *campestris* Used to Monitor Black Rot Infections in Cabbage Seedlings Treated with Fosetyl-Al

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**ABSTRACT**

Mochizuki, G. T., and Alvarez, A. M. 1996. A bioluminescent *Xanthomonas campestris* pv. *campestris* used to monitor black rot infections in cabbage seedlings treated with fosetyl-Al. Plant Dis. 80:758-762.

Autophotography of a bioluminescent transconjugant of *Xanthomonas campestris* pv. *campestris* provided a reliable means to quantify the extent of bacterial invasion into cabbage (*Brassica campestris*). A single prophylactic treatment of fosetyl-Al at 4,800 mg $kg^{-1}$ a.i. as a spray and drench reduced the invasiveness of *Xanthomonas campestris* pv. *campestris* on inoculated cabbage seedlings. Multiple treatments did not reduce invasiveness further. The effect of fosetyl-Al was immediate (within 1 h) and persisted for at least 21 days. The efficacy of fosetyl-Al treatments was more pronounced at 20°C and decreased as temperature increased; however, the data indicated a separate effect of temperature on disease development and no direct interaction between temperature and fosetyl-Al treatment.

Black rot, caused by *Xanthomonas campestris* pv. *campestris,* is the most important disease affecting crucifers worldwide (33). Characteristic symptoms include V-shaped yellow to tan, necrotic marginal foliar lesions followed by vascular darkening, stunting, wilting, and sometimes death of the entire plant. Latent infections can occur (1,33).

Foliar infection typically occurs through stomates or hydathodes, and infested seeds are the major source of primary inoculum. Plants may become infected at any growth stage, but the wet, crowded conditions in seedbeds are highly conducive to pathogen transmission. The pathogen present in the guttation fluid of latently infected but symptomless seedlings can spread to healthy seedlings (21).

Control measures implemented at the seedling stage may significantly reduce the rate of transmission and disease development in the seedbed, thereby minimizing the potential for severe field epidemics. Very few chemical treatments effectively control bacterial diseases in the seedbed or field. Streptomycin, oxytetracycline, and copper compounds are available, but low efficacy, pathogen resistance, phytotoxicity and visible residues limit their practical use.

The systemic fungicide fosetyl-Al (Chipco Aliette, produced by Rhône-Poulenc) reduced the invasiveness of several bacterial diseases of ornamentals when applied as a prophylactic spray (4–7,22, 23,32). Weekly fosetyl-Al treatments on *Anthurium andraeanum* plants subsequently inoculated with *Xanthomonas campestris* pv. *dieffenbachiae* resulted in a significant reduction in disease severity, lesion numbers, and lesion areas (2,23). Reduction in symptom expression of vascular phytobacterial diseases by fosetyl-Al suggested the potential for controlling latent bacterial infections. Fosetyl-Al is rapidly assimilated into the vascular system and is both acropetally and basipetally translocated, resulting in rapid distribution throughout plant tissues (10,11). These properties suggest fosetyl-Al as a good candidate for controlling *X. campestris* pv. *campestris* infections on crucifer seedlings.

Recently, a genetically transformed bioluminescent *X. campestris* pv. *campestris* strain containing the *lux* gene cassette was used to monitor infections of cabbage under different nutritional regimes (20). This transconjugant was phenotypically identical to its wild-type parent and was constitutively bioluminescent, allowing the visualization of infected tissues with autophotography on X-ray film. This procedure detected the pathogen in presymptomatic tissues, suggesting that this method might be useful for evaluating the use of fosetyl-Al in reducing latent systemic infection and spread.

The objectives of this study were (i) to determine an accurate method of assessing infections with visible and latent components, and (ii) to use that method to evaluate the efficacy of fosetyl-Al in reducing disease in cabbage seedlings infected with a bioluminescent *X. campestris* pv. *campestris* transconjugant.

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Accepted for publication 9 April 1996.

Publication no. D-1996-0509-04R


## MATERIALS AND METHODS

**The pathogen.** A bioluminescent transconjugant of *X. campestris* pv. *campestris* was developed by triparental mating of a highly virulent wild-type strain, G171 (originally isolated from diseased cabbage in Hawaii) with *Escherichia coli* HB101 containing the *lux* gene cassette in pUCD607 and *E. coli* HB101 with the mobilizing plasmid pRK2013 (20). The latter two strains were kindly provided by Shaw and Kado (30). The resulting transconjugant, designated strain 171LIIH-7, contained chromosomally integrated *lux* genes and expressed constitutive bioluminescence in culture and in planta (20). This strain was identical to its wild-type parent in all aspects studied, including pathogenicity, virulence, growth rate, nutritional requirements, and serological and physiological characteristics (20).

Seedling preparation and fosetyl-Al treatment. Cabbage (*Brassica campestris* L.) seeds of cv. Early Jersey Wakefield were assayed for xanthomonads. Seeds were sown into 9.0-cm-square plastic pots containing 650 $cm^3$ of a 2:1 (vol/vol) mixture of Supersoil/vermiculite medium amended with 3 kg of Osmocote 17-6-12 (Grace-Sierra Horticultural Products Co., Milpitas, CA) and 5 kg of dolomite per cubic meter of medium, and allowed to germinate on the greenhouse bench. Plants were thinned to a single plant per pot and fertilized again with 1.35 g of Osmocote per pot 3 weeks after emergence. Plants were watered twice daily, except for 24-h periods following fosetyl-Al treatment or inoculation. Uniform 2- to 3-week-old seedlings at the three- to five-true-leaf stage were selected for all experiments.

Seedlings were sprayed and drenched with a 4,800 mg $kg^{-1}$ a.i. fosetyl-Al suspension in deionized water. Foliage surfaces were sprayed until runoff (approximately 2 ml per plant) using a hand atomizer, then 25 ml of the suspension was drenched directly onto the medium in each pot. Watering was withheld for 24 h post-treatment. Nontreated control plants were sprayed and drenched with deionized water.

**Inoculum preparation and inoculation method.** Inoculum was prepared by suspending cells from a 24-h culture of *X. campestris* pv. *campestris* strain 171LIIH-7 grown on peptone glucose agar medium (peptone 1%, glucose 0.5%, agar 1.7%) into sterile 0.01M phosphate buffered saline pH 7.2 and adjusted to a concentration

of approximately $10^8$ CFU/ml. For quantitative measurement of infection, a single 1-mm-deep incision was made with a sterile scalpel at the distal hydathode of the midvein on the second fully expanded leaf of the seedling. The wound site was dipped into inoculum for 20 s while contact with proximal hydathodes was carefully avoided. Inoculated plants were placed into plastic bags and held for 24 h at 25°C on the laboratory bench. Bags were then opened and the plants were held either on the greenhouse bench or in growth chambers. Leaves of control plants were wounded but immersed only in sterile buffer.

**Monitoring infection with autophotography.** Plants were prepared for autophotography by placing them on the laboratory bench at 22°C for 12 to 16 h to enhance bioluminescence. Inoculated leaves were excised from the stem and placed directly onto precut Fuji RX Medical X-Ray (Fuji Photo Film Co. Ltd., Tokyo, Japan) film mounted in cardboard holders. The film was exposed to the leaves in total darkness for 12 h at 25 to 28°C, then processed in Kodak GBX developer and fixer at rates and times recommended by the manufacturer (Eastman Kodak Company, Rochester, NY).

Tissue sections from symptomatic and asymptomatic areas on the inoculated leaves were macerated in sterile deionized water and dilution-plated onto modified tetrazolium chloride (TZC) medium (24) to isolate and differentiate bacteria, and onto Medium 523 (18) containing 40 ppm spectinomycin to reisolate the transconjugant and to observe bioluminescence. Noninoculated controls were also assayed.

**Area and length measurements.** Leaf area and length measurements were determined with a Kurta IS/One digitizing tablet and SigmaScan area analysis firmware (Jandel Scientific, Corte Madera, CA). Leaf outlines and visible lesions on inoculated leaves were traced onto clear acetate sheets placed over leaves to provide a permanent record of total and infected leaf areas. The tracings were then measured by means of the digitizing tablet. Bioluminescent lesions were digitized directly from exposed areas on the X-ray film autophotographs.

The total leaf area included the area circumscribed by the leaf blade and the petiole. Leaf length was the linear distance from the distal hydathode (inoculation site) along the midvein and petiole to the juncture with the stem. Visible lesion area was the chlorotic and necrotic leaf area proceeding from the inoculation site. Visible lesion length was the length of darkened vascular tissue proceeding from the inoculation site along the midrib towards the petiole. Bioluminescent lesion area was the bioluminescent leaf area proceeding from the inoculation site and detected by autophotography. Bioluminescent lesion length was the length of the bioluminescent vascular tissue extending from the inoculation site along the midrib towards the petiole and detected by autophotography. All lesion area and lesion length measurements were expressed as a percentage of the total leaf area and leaf length measurements, respectively. The mean of the replicates for each treatment was indicated as the mean proportional (percentage) value for that treatment (Fig. 1.).

**Data analysis.** To determine the best quantitative estimator of infection for evaluating treatment effects, the mean proportional lesion areas and lengths of both visible and autophotographic lesions were compared. Due to variability in leaf area and length after treatment and growth, proportional length and area measurement data were subjected to Bartlett's test for homogeneity of variances (19). Based on the results of Bartlett's test, $\log(x)$ and $\log(x + 1)$ transformations were performed to ensure the homogeneity of the compared treatment variances (16,19). Analysis of variance was performed on the transformed data to determine the significance of treatment effects, and Duncan's protected new multiple range test was used to compare treatment means. Regression analysis with temperature as the independent variable and relative lesion area as the dependent variable was used to evaluate temperature effects on fungicidal control.

**Multiple fosetyl-Al treatments.** The effect of multiple fosetyl-Al treatments on the progress of *X. campestris* pv. *campestris* infection in cabbage seedlings was determined. Eight replicates of single seedlings were treated with 1, 2, or 3 applications of 4,800 mg $kg^{-1}$ a.i. fosetyl-Al at weekly intervals prior to inoculation. Control treatments substituted deionized water for the fungicide suspension. The application dates for the treatments were staggered so that all treatments ended on the final application date.

All seedlings were inoculated 2 days after the final fosetyl-Al application, after which the plants were bagged and kept in the laboratory at 22°C for 24 h, then unbagged plants were returned to the greenhouse. The plants were maintained in the greenhouse with day and night temperatures between 24 to 35°C and 19 to 24°C, respectively, for the duration of the experiment. After 13 days, when black rot symptoms developed on the non-fosetyl-Al-treated control plants, all inoculated leaves were excised and autophotographed. All plants were 5 weeks old at the end of the experiment.

**Duration of fosetyl-Al activity.** Uniform 20-day-old cabbage seedlings were treated with 4,800 mg kg-1 a.i. fosetyl-Al or deionized water (controls) using spray and drench described earlier. Eight replicate sets containing a treated plant and a nontreated control plant were inoculated 0, 3, 7, 14, and 21 days after treatment, as described earlier. Day 0 plants were inoculated 1 h after treatment, when all of the foliage had dried. Infection was allowed to progress for 5 days after each inoculation date, then the leaves were excised and autophotographed. All plants were maintained in the greenhouse for the duration of the experiment. Greenhouse experiments were arranged in a completely randomized design, with periodic bench rerandomization to minimize proximity effects.

**Temperature effects.** The effect of temperature on the interaction between fosetyl-Al and infection was studied in growth chambers at 20, 24, and 30°C and in the greenhouse. Selection of growth chamber test temperatures included an op-

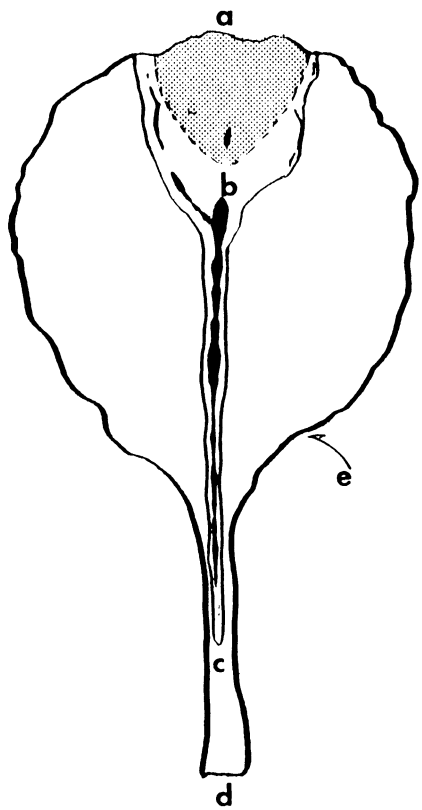


**Fig. 1.** Autophotograph trace of a cabbage leaf infected with bioluminescent *Xanthomonas campestris* pv. *campestris* strain 171LIIH-7. Leaf was excised 5 days after inoculation at the distal hydathode (a) and exposed to X-ray film for 12 h. Darkened areas on film correspond to infected areas on leaf. Each autophotograph was overlaid with an acetate film upon which visible and total leaf areas were traced. Linear distance between points (a) and (b) represents visible lesion length; between points (a) and (c) is bioluminescent lesion length; and between points (a) and (d) is total leaf length. Area circumscribed by dotted line at (b) represents a visible lesion area. Bacteria no longer multiply in the symptomatic tissue and, hence, no longer bioluminesce. Area circumscribed by thin line at (c) represents bioluminescent lesion area; heavy line designated by arrow at (e) outlines total leaf area. Length and area measurements for each inoculated leaf were measured digitally; these values were used to calculate the proportion of infected tissue.

timum for seedling growth (20°C), an optimum for pathogen growth (30°C) and an intermediate temperature optimal for disease development (24°C) (3,28).

Two-week-old seedlings from the greenhouse were transferred into growth chambers at constant 20, 24, and 30°C with a 12-h photoperiod under 660 μE $s^{-1}$ $m^{-2}$ and 75% relative humidity. The seedlings were acclimated in each growth chamber for 5 days, after which six replicate plants were treated with a single application of fosetyl-Al as described earlier. Equivalent control sets were treated with deionized water. All plants were inoculated 2 days after fosetyl-Al treatment. Eight treated and eight nontreated control plants were maintained in the greenhouse and were inoculated on the same dates as the plants in the growth chambers. Plants were arranged in a completely randomized design within chambers with periodic re-randomization to minimize location effects.

Infection was allowed to progress for 9 days following inoculation, when most (5/6) of the nontreated controls at 20°C exhibited typical black rot symptoms. All inoculated leaves were then excised and autophotographed. The plants were 5 weeks old at the end of the experiment.

## RESULTS

Isolations from tissue sections excised from inoculated plants were dilution plated onto modified TZC and Medium 523; all yielded visibly pure cultures of *X. campestris* pv. *campestris* that were bioluminescent. No pathogens were recovered from noninoculated control plants or from seeds in the lot used to produce the plants for this study.

**Data analysis.** Autophotographic measurements revealed larger estimates of the infected area than visible lesion measurements and were independent of host symptom response. In asymptomatic tissues, autophotography provided the only measure of infection severity. Values associated with autophotographic measurements exhibited heterogeneous variances that required transformation prior to analysis. Log($x$) and log($x$ + 1) transformations equalized the variances adequately, since log($x$ + 1) transformation also accommodated samples with small proportions of infected tissue resulting from fosetyl-Al treatment, All analyses were based upon log($x$ + 1) transformed autophotographic areas of infected leaves. Variance analysis of nontransformed bioluminescent lesion data also indicated highly significant differences; however, these data exhibited heterogeneous variances not conforming to the assumptions required for analysis of variance. After transformation, both area and length measurements using both visual and autophotographic methods were acceptable quantitative estimators of infected tissue severity (Table 1). The pattern of black rot infection usually results in an expanding V-shaped lesion rather than a linear vascular lesion, even when inoculation occurs at a single, marginal, foliar hydathode. Lesion area measurements included all infected foliar tissue and represented the most accurate estimate of infection severity.

**Multiple treatments.** Reductions in infection due to all treatments with fosetyl-Al were highly significant ($P$ = 0.01). A single application of fosetyl-Al reduced the mean bioluminescent lesion length by 62% and area by 61% relative to nontreated controls; two applications decreased the mean lesion length by 70% (Table 2). Infected areas in plants treated with a single application were not significantly different ($P$ = 0.05) from infected areas on plants receiving two or three weekly applications of fosetyl-Al. Plants receiving multiple weekly treatments of fosetyl-Al appeared spindly and soft, and exhibited slight yellowing and anthocyanescence consistent with early signs of nutritional or physiological stress. In contrast, plants receiving only a single treatment appeared normal. A single prophylactic treatment provided the greatest reduction in infection with no detrimental effects, and was used in tests for temperature effects and duration of activity.

**Duration of activity.** Fosetyl-Al treatments applied to seedlings less than 1 h before inoculation with *X. campestris* pv. *campestris* were effective in reducing the extent of infection (Table 3). Plants inoculated 3 to 14 days following treatment showed reduced infection compared with controls, but the differences were less striking than for plants inoculated immediately following treatment. The effect of the fungicide was still significant 21 days following treatment, however (Table 3).

**Temperature effects.** The greatest reduction in both visible and bioluminescent measurements of disease was at 20°C. For fosetyl-Al treated plants, there was a linear

**Table 1.** Calculated chi-square values for Bartlett's test for variance homogeneity

| | Calculated values for transformations | | |
|---|---|---|---|
| Parameter measured[y] | None | log($x$) | log($x$ + 1) |
| Visible lesion area | 1.84[z] | 4.06 | 2.06 |
| Bioluminescent lesion area | 7.90 | 4.81 | 4.03 |
| Visible lesion length | 4.11 | 6.69 | 5.89 |
| Bioluminescent lesion length | 13.33 | 6.24 | 6.28 |

[y] Disease parameters compared for quantifying infection were the mean proportional (percentage) values, determined visually for visible lesion length and area and determined by autophotography for bioluminescent lesion length and area.

[z] Variances were homogeneous and satisfied the assumptions for analysis of variance if calculated Bartlett's values for measurements were <7.81 (df = 3 and $P$ = 0.05). Log ($x$ + 1) transformations accomodated small values commonly observed with treatment effects.

**Table 2.** Comparison of relative lesion lengths and areas on infected cabbage seedling leaves determined by visible symptoms and autophotography following weekly applications of fosetyl-Al, then inoculation with bioluminescent *X. campestris* pv. *campestris* strain 171LIIH-7

| | Proportional (%) lesion length[x] | | | Proportional (%) lesion area | | |
|---|---|---|---|---|---|---|
| No. of fosetyl-Al applications | Visible | Bioluminescent | Reduction (%)[y] | Visible | Bioluminescent | Reduction (%)[y] |
| 0 | 31.1 a[z] | 73.8 a | | 15.4 a | 23.5 a | |
| 1 | 15.4 b | 28.2 b | 62 | 5.5 b | 9.1 b | 61 |
| 2 | 11.5 b | 22.1 b | 70 | 5.3 b | 11.2 b | 52 |
| 3 | 17.5 b | 37.0 b | 50 | 8.4 b | 14.0 b | 40 |

[x] Values represent percentage of diseased tissue relative to total leaf length or area and are the mean of eight replications. Lesion length is relative length of the V-shaped lesion measured from hydathode inoculation site toward the petiole, determined visually or by autophotography. Lesion area is relative area of infected tissue, determined visually or by autophotography.

[y] Reduction (relative) in mean bioluminescent lesion length or lesion area based on relative total leaf length or area, respectively, resulting from fosetyl-Al treatment, compared with control treatment.

[z] Means followed by same letter in same column are not significantly different ($P$ = 0.05).

**Table 3.** Duration of activity of a single fosetyl-Al treatment shown by reduction in relative lesion area on cabbage seedlings inoculated with bioluminescent *X. campestris* pv. *campestris* strain 171LIIH-7

| | Mean proportional (%) leaf area infected[x] | | |
|---|---|---|---|
| Post-treatment interval (days) | Non-treated | Fosetyl-Al | Reduction (%)[y] |
| 0 | 36.5 a[z] | 22.8 a | 37.5 a |
| 3 | 23.7 b | 20.6 a | 13.1 b |
| 7 | 26.2 b | 24.8 a | 5.3 b |
| 14 | 18.0 b | 15.4 a | 14.4 b |
| 21 | 41.4 a | 21.3 a | 48.6 a |

[x] Mean proportional lesion area is lesion area determined by autophotography divided by total leaf area × 100. Values are means of eight replicates.

[y] Reduction in disease is the relative decrease in lesion area between fosetyl-Al–treated plants and nontreated control plants for each post-treatment time interval.

[z] Means followed by same letter in same column are not significantly different (P = 0.05).

increase in the mean proportion (percentage) of bioluminescent lesion areas with increasing temperature ($r^2$ = 0.96) for the range of temperatures studied (Table 4). The efficacy of fosetyl-Al was diminished at 30°C when compared with cooler temperature treatments; however, there still was a significant reduction in disease compared with the nontreated control plants. Plants maintained under fluctuating greenhouse temperatures exhibited 25% reduction in infected area due to fosetyl-Al treatment.

## DISCUSSION

Detection of bioluminescent plant pathogens in planta by autophotography has provided a valuable tool to quantitatively estimate and monitor the extent of internal infection in diseased plants. In this study, bioluminescence was more representative of the actual infection status of the plant. Previous applications using bioluminescent plant pathogenic bacteria (8, 9,29,30), and bioluminescent symbiotic *Bradyrhizobium japonicum* on soybean (25) provided qualitative data for monitoring disease progress and locating sites of infection by means of various detection methods. Similarly, the detection and visualization of the bioluminescent rhizobacteria *Pseudomonas* sp. and *Enterobacter cloaceae* on roots indicated a linear correlation between bacterial populations and light intensity; however, large experimental variations rendered this technique useful only for qualitative estimations and location of bacterial populations (13,15). The quantification of infected areas provides a more uniform estimate of infection severity than does the use of cell populations alone, as indicated by the homogeneous treatment variances for the experimental results in this study. This aspect is critical when comparing the effects of various treatments on infection severity.

Bioluminescence is particularly useful for estimating latent infections caused by *X. campestris* pv. *campestris*, whereby internal infections may progress in the absence of any visible symptoms. Detection of the bioluminescent pathogen within host tissues may be nondestructive, can provide spatial and temporal data on disease incidence and spread, and is suitable for field experiments (9,20,29).

In every experiment, treatment with fosetyl-Al reduced the proportion of diseased tissue. A single prophylactic application of fosetyl-Al on cabbage seedlings was sufficient to significantly reduce infection caused by *X. campestris* pv. *campestris*. Additional treatments were effective in reducing disease but caused plants to exhibit symptoms of nutritional deficiency that may have resulted from the cumulative effects of the acidic fosetyl-Al solution (pH 3.7 to 4.0) on plant nutrition and growth. The planting medium pH temporarily decreased from 6.8 to 5.3 after each drench application, which is lower than the optimum pH of 6.0 to 7.5 recommended for cabbage growth (14). Many nutrients, notably P, K, Ca, Mg, S, and B, are unavailable when the medium pH is below 5.5 (14,31). Fosetyl-Al treatment also reduced the growth of maize seedlings when applied at rates recommended for *Pythium* spp. control, resulting in significantly diminished dry weight, fresh root weight, root length, and nutrient uptake (27). Treated maize plants were spindly and stunted, and exhibited symptoms consistent with multiple nutrient deficiencies (27).

Multiple prophylactic treatments of fosetyl-Al were reported to be more effective than a single application in reducing root rot of tomato seedlings infected with *Phytophthora parasitica*, indicating that the fungitoxic activity does not persist in young tomato plants (12). The effective concentration of the fungicide in the plant should be maintained with multiple applications to control disease and to compensate for dilution due to seedling growth (12). In contrast, a single soil or foliar application of fosetyl-Al on avocado seedlings planted in potting mix resulted in sufficiently high levels of direct antifungal activity to control avocado root rot caused by *Phytophthora cinnamomi* 8 weeks after treatment, suggesting extended stability in some plants (26).

Significant reduction in *X. campestris* pv. *campestris* infections on cabbage leaves following a single fosetyl-Al treatment suggests a stable and prolonged antibacterial effect in cabbage. The immediate effect of fosetyl-Al treatments (within 1 h after treatment) is consistent with the appearance of fosetyl-Al breakdown products and the expression of antifungal activity within 15 min of treatment (10,11). The prolonged effect observed in this study with the 21-day post-treatment interval was unexpected. Other studies indicated both decreased antifungal activity and decreased tissue concentrations of fosetyl-Al 3 weeks after treatment due to in vivo dilution resulting from rapid tomato seedling growth (12). Treatment of anthurium plants with fosetyl-Al was effective in controlling anthurium blight caused by *X. campestris* pv. *dieffenbachiae* if plants were treated at 7- or 14-day intervals, but was only marginally effective when plants were treated at 21-day intervals (23). The persistence of activity may be a function of the host/pathogen combination, plant growth stage, or cultural or environmental conditions. The manufacturer's recommendation for monthly application intervals thus should be empirically evaluated for efficacy for each host-pathogen combination and plant growth stage.

The effect of temperature on a host-pathogen-inhibitor disease system can only be measured as the net result of the interaction between the system's individual components. The selection of treatment temperatures based on the temperatures for optimum growth of the component organisms in this host-pathogen pathosystem produced expected relative infection severities between temperature treatments. Inoculated plants maintained at temperatures favoring host growth (20°C) were less severely infected than those maintained at temperatures favoring disease development (24°C) or pathogen growth (30°C) (Table 4). This is consistent with the general observation that conditions that optimized fungal growth reduced the observed activity of fosetyl-Al (17); however, target organisms may respond differently to fosetyl-Al and temperature. Increasing temperature increased the activity of fosetyl-Al against *Phytophthora nicotianae* var. *parasitica,* but decreased the effect against *Pythium ultimum* (17). In our experiments, more disease was observed at intermediate temperatures, since neither host nor pathogen was compromised and each was able to grow adequately as infection progressed. This trend was identical for both nontreated and fosetyl-Al–treated plants. The trend for relative disease reduction followed a similar pattern, with the greatest reduction in disease occurring at the temperature favoring the host and the least reduction at the temperature favoring the pathogen. Naturally fluctuating greenhouse environmental conditions produced the greatest proportion of infection for both nontreated and treated plants, and the least

**Table 4.** Effect of temperature on reduction of visible and bioluminescent lesion areas on cabbage seedlings treated with a single application of fosetyl-Al and inoculated with bioluminescent *X. campestris* pv. *campestris* strain 171LIIH-7

| Temperature (C)[x] | Treatment[y] | Mean proportional lesion area[z] Visible (%) | Bioluminescent (%) |
|---|---|---|---|
| 20 | Nontreated | 3.8 | 6.0 |
| | Fosetyl-Al | 1.2 | 2.1 |
| | Reduction (%) | 68.4 | 65.0 |
| 24 | Nontreated | 8.9 | 15.0 |
| | Fosetyl-Al | 3.8 | 5.8 |
| | % Reduction | 57.3 | 61.3 |
| 30 | Nontreated | 4.9 | 9.4 |
| | Fosetyl-Al | 3.8 | 7.4 |
| | Reduction (%) | 22.4 | 21.3 |
| 35 (Greenhouse) | Nontreated | 8.8 | 15.6 |
| | Fosetyl-Al | 6.6 | 11.6 |
| | Reduction (%) | 25.0 | 25.6 |

[x] Chambers were at constant temperature with 12-h photoperiod under 600 μE $s^{-1}$ $m^{-2}$ and 75% relative humidity. Mean greenhouse temperature was 35°C (daytime), with a range of 33 to 37°C.

[y] Plants were inoculated 2 days after treatment with fosetyl-Al. Infection was allowed to progress for 9 days.

[z] Infected lesion area divided by total leaf area × 100.

reduction of infection compared with constant temperature treatments.

For crucifers grown at cool (18 to 20°C), dry, high-elevation tropical climates that are optimum for production and less than optimal for disease development, fosetyl-Al may significantly reduce disease. At middle elevations, we can expect moderate levels of disease and reasonable control. In the warm (30°C), humid, low-elevation tropics where crucifers are commonly cultivated and the disease pressure is high, fosetyl-Al may have greatly diminished efficacy, but may still be useful if combined with other control measures. When integrated into a cropping system, prophylactic fosetyl-Al treatments could provide an effective and economical means of controlling *X. campestris* pv. *campestris* infections in crucifer seedlings.


## ACKNOWLEDGMENTS

We would like to thank Rhône-Poulenc Ag Company for assistance and for supplying Chipco Aliette. We are grateful to J. J. Shaw and C. I. Kado for providing *E. coli* strains used to generate a stable bioluminescent strain of *X. campestris* pv. *campestris*. We thank Rosemarie McElhaney for supplying strain 171LIIH-7, and for valuable feedback concerning its application. This research was supported in part by the U.S. Department of Agriculture under CSRS Special Grant Agreement No. 89-34135-4614, managed by the Pacific Basin Advisory Group (PBAG).


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