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Effect of soil–matric potential and phylloplanes of rotation-crops on the survival of a bioluminescent *Xanthomonas campestris* pv. *campestris*

Renée S. Arias[1], Scot C. Nelson[2] and Anne M. Alvarez[2]
[1]*Department of Biosystems Engineering, 111 Gilmore Hall, University of Hawaii, Honolulu, HI 96822, USA (Fax: +1 808 956 9269);* [2]*Department of Plant Pathology, 315 St. John, University of Hawaii, Honolulu, HI 96822, USA*

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Abstract

The survival of the bacterial pathogen, *Xanthomonas campestris* pv. *campestris (Xcc)*, was studied in plant debris-infested soil with different matric potentials (0, −10, −30, −50, −100, −200, and −900 kPa), and on the phylloplane of crops used for rotation with cabbage. Populations of cellulolytic and proteolytic microorganisms were studied in relation to soil matric potential and *Xcc*. The survival of *Xcc* was negatively correlated ($r = -0.710$; $P = 0.06$) with soil matric potential and with the abundance of cellulolytic microorganisms ($P = 0.05$). In saturated soil, *Xcc* survived for only 19–28 days, while at −900 kPa there was no significant change in the bacterial population within this period. Survival of *Xcc* on the phylloplane of cabbage (*Brassica oleracea* var. *capitata*) (host) was similar to that on mustard and lettuce (48 days), whereas the pathogen was detected for only 9 days on rice. It appears that high matric potentials reduced populations of *Xcc* in soil, whereas epiphytic survival of this pathogen depended on the plant species.

Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*) the causal agent of the black rot of crucifers, is a seed-borne pathogen (Cook et al., 1952). Once established in a cropping area, the ability of the bacterium to survive in soil within plant tissues, or to colonize non-host phylloplanes may account for recurrent epidemics of black rot. This paper addresses the effect of matric potential in reducing the survival of *Xcc* in debris-infested soil and the differential survival of this pathogen on phylloplanes of rotation crops used with cabbage.

Xcc can survive in plant residues, making a significant contribution to black rot epidemics. Infested stems of cabbage placed in soil harbored this pathogen for at least six months (Schaad and White, 1974), while in infested soil, populations of 10^6 colony forming units (cfu) g^{-1} occurred (Fukui et al., 1994). When disease-free cabbage seedlings were transplanted into infested soils, a 45% disease incidence occurred at harvest (Alvarez and Cho, 1978). In order to decrease the populations of *Xcc* in infested fields, two-year rotations were suggested (Schaad and White, 1974). However, 2-year rotations are impractical in tropical areas where growers need to transplant cabbage every 2 weeks throughout the year in order to supply the market (Collier and Davidson, 1967; Hawaii Agricultural Statistics Service, 1994).

Changes in the physical soil environment and soil microbial populations may affect the survival of *Xcc* in soils. It has been reported that *Xcc* survived for a period of 42 days during a wet winter season and only for 14 days during a dry summer (Schaad and White, 1974). However, there are only few studies about the effect of soil water content on the survival of *Xcc*. An increase in microbial diversity and microbial carrying capacity were found to suppress soil-borne fungal pathogens such as *Pythium* (Boehm and Hoitink, 1992)

and *Phytophthora* (English and Mitchell, 1988). Nevertheless, the dynamics of soil microbial populations and their possible role in the survival of *Xcc* has not been studied.

Soil particles infested with *Xcc* can reach the leaves of host and non-host plants. The contribution of the epiphytic survival of the pathogen to black rot epidemics has not been established. *Xcc* from infested soils normally reaches the leaves by wind or water splash (Shaw et al., 1992), and often is dispersed from cabbage to the phylloplane of weeds in the field (Dane and Shaw, 1996). *Xcc* was reported to live as an epiphyte on the host for at least three months (Dane and Shaw, 1994), and also can colonize non-host plants such as tomato (*Lycopersicon esculentum*) (Timmer et al., 1987) and lettuce (Alvarez et al., 1994). Epiphytic survival of *Xcc* on crops rotated with cabbage has not been studied, and this should be considered in the selection of appropriate crops.

The objectives for the present work were to determine the impact of soil–matric potential on the survival of *Xcc* and to determine the persistence of *Xcc* on the phylloplane of non-host crops. We quantified the survival of *Xcc* at different soil–matric potentials; the effect of soil–matric potential on populations of cellulolytic and proteolytic microorganisms; and analyzed the survival of *Xcc* on the phylloplane of rotation crops commonly used with cabbage.

Materials and methods

Bacterial strain

The bacterial strain 171LIIH-7 of *Xanthomonas campestris* pv. *campestris* (*Lux-Xcc*) was used for the bacterial survival studies. The plasmid pUCD607 containing a *lux* operon was inserted in the chromosome of *Lux-Xcc*. Stable bioluminescence and pathogenicity were demonstrated for *Lux-Xcc*, and the pathogenicity was comparable to the wild type strain (McElhaney, 1991). The plasmid pUCD607 confers resistance to ampicillin, kanamycin and spectinomycin (Spc) (Shaw and Kado, 1986). *Lux-Xcc* also has natural resistance to chloramphenicol.

Soil and matric potential

The soil used for the survival studies was a medial, isothermic, Typic Eutrandept (organic carbon 8.65%, total nitrogen 0.65%, pH_{water}: 6.11, pH_{KCl}: 5.58, $\Delta pH = -0.53$) from the Kula Experiment Station, island of Maui (Ikawa et al., 1985). This soil is representative of the area where most of the crucifers are produced in Hawaii. After collection, the soil was stored at 4 °C with a water content (θ) (water mass/dry mass) of 0.85 (w/w). A water retention curve was prepared using ceramic pressure plates (Klute, 1986) by applying 0, −10, −30, −50, −100, −250, −400, −600 and −900 kPa (data not shown). The curve was used to obtain different matric potentials in soil by adjusting the corresponding θ values.

Survival of Xcc in soil at different matric potentials in relation to total number of bacteria, proteolytic microorganisms and celluloytic microorganisms

To explain possible changes in the populations of *Xcc* when incubated in soil at different matric potentials, other groups of microorganisms were also traced in the same soil samples. Debris was prepared as follows: leaf-margins of 5-week-old cabbage plants (cultivar Tastie) were inoculated by clipping them with scissors infested with *Lux-Xcc*. Twelve days after inoculation, symptomatic areas were excised, dried at room temperature (23 ± 1 °C), ground to pass through a 1 mm^2 sieve and vacuum-dried for about 18 h at room temperature. Before starting each experiment, 1 kg of the soil was air dried for two days, ground to pass a 2 mm^2 sieve, and mixed with infested debris 1% (w/w). Five grams of debris-infested soil were dispensed in petri plates (4.5 cm diam) and sterile distilled water was added using the data of the water retention curve to obtain different matric potentials (ψ). The ψ values selected for the experiment were 0.0, −10, −30, −100, −200 and −900 kPa, and the experiment was repeated. Petri plates corresponding to each treatment were placed in separate plastic containers and kept at room temperature (23 ± 1 °C) during the experiment. At different sampling times (day 1, 2, 10, 21) the soil contained in each petri plate was suspended in 50 ml of phosphate buffered saline (pH: 7.4; 0.07 M) (PBS) and then placed in a rotary shaker at 180 rev min^{-1} for 15 min. Six, ten-fold dilutions were prepared in PBS and plated in microfluorplates WFB (Dynatech, Virginia, USA) using 20 µl well^{-1} and eight wells per dilution. Microfluorplates were prepared with 180 µl well^{-1} of liquid 523 medium (Kado et al., 1972) containing Cm 50 mg l^{-1}, Spc 50 mg l^{-1} and Cycloheximide 50 mg l^{-1}, and processed by the MPN-microfluorplate method (Arias et al., 1996). The total number of bacteria was determined by spread-plating on tryptic soy agar (TSA) medium. The experimental design was completely

randomized with four replicates per treatment and per sampling date, and a total of five sampling dates.

Another experiment was conducted including three ψ (0.0, −50 and −900 kPa) with samplings at various intervals during 25 days. Populations of proteolytic and cellulolytic microorganisms were enumerated using the most probable number (MPN) method with three tubes per dilution, and four replicates per sampling date. The culture media used for proteolytic and cellulolytic microorganims were Pochon and Tardieux (1962) and Krieg (1981), respectively. MPN were calculated using the program MPNES (Woomer et al., 1990). The number of *Lux-Xcc* per gram of soil was evaluated by the MPN-microfluorplate method as described above.

Survival of Lux-Xcc on the phylloplane of host and non-host plants

Epiphytic survival of *Xcc* was determined on 3-week-old seedlings of cabbage, mustard (*Brassica hirta*) and lettuce (*Lactuca sativa*). Seedlings were grown in individual (350 cm^3) pots in the greenhouse. The growth medium was steam sterilized fertile soil, vermiculite Therm-o-rock (1 : 1, v/v), $CaCO_3$ and fertilizer Osmocote 17-6-12 (Sierra Chemical Co., Milipitas, Calif.). The seedlings were sprayed to run-off with a suspension of bacteria (approx 10^8 cfu of *Lux-Xcc* ml^{-1}). To minimize splashing between plants, each pot was watered daily at the soil level with 50 ml of distilled water using a beaker. No free water was present on the leaves at any time after inoculation.

Entire leaves were collected to obtain the best representation of epiphytic populations of *Xcc*. Four basal leaves, which were present at the time of inoculation, were collected and processed from each of four individual plants per species on every sampling date. These four entire leaves were combined as one sample and placed in sterile, 250-ml Erlenmeyer-flasks containing 20 ml of PBS. Flasks were placed on a rotary shaker at 250 rev min^{-1} for 15 min for bacterial extraction. Serial 10-fold dilutions were plated in microfluorplates and processed using the MPN-microfluorplate method (Arias et al., 1996) (Figure 1). Individual leaf areas were measured using the computer

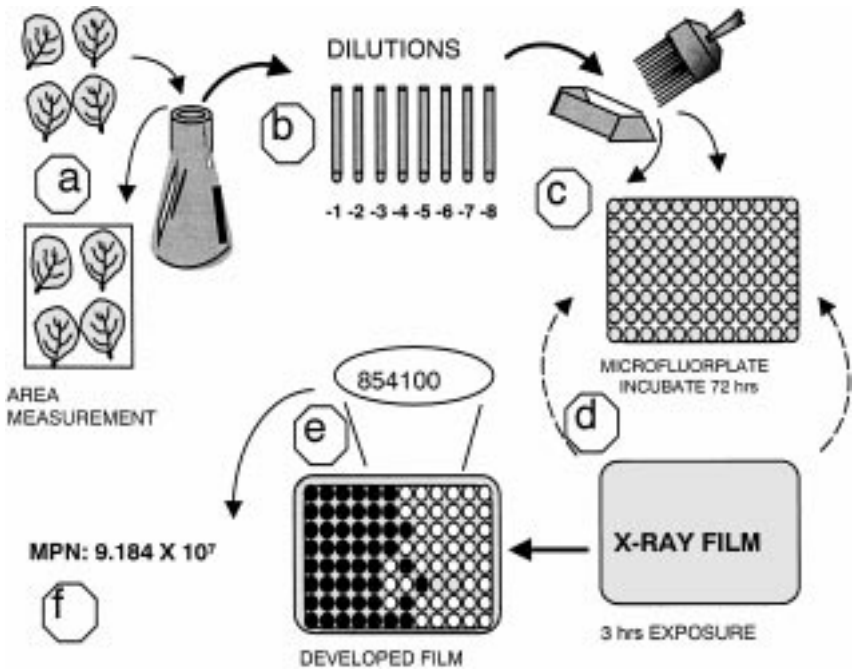

Figure 1. Schematic representation of the use of the MPN microfluorplate technique: (a) the epiphytic bioluminescent bacteria are dislodged from leaf samples by shaking in buffer; then, leaves are removed from flasks to measure leaf area, (b) the suspension is diluted in a ten-fold dilution series (in saline), (c) eight aliquots of each dilution are transferred to eight wells (one row) of the microfluorplate containing liquid medium and incubated for 72 h, (d) microfluorplates are covered with transparent plastic and exposed to X-ray film for 3 h, (e) the films are developed, and the number of wells from which light is detected is used to form a code number, and (f) the estimated number of bioluminescent cells present in the original sample is obtained using the MPNES computer program (Woomer et al., 1990).

program SIGMA-SCAN (Parker et al., 1988) after bacterial extraction. Numbers of bacteria were expressed as MPN per leaf-cm^2, each data point representing the average of four replicate samples. Plants were assayed on days 0, 3, 6, and 27, and the experiment was repeated with samplings at days 0, 3, 6, 12, 19, 27, 33, 40, and 48. Rice (*Oryza sativa*, cultivar IR20) was included in the second experiment and one additional sampling was performed for this species 9 days after inoculation. Symptomatic cabbage plants were removed and not sampled further for epiphytic populations. Lesions observed on mustard and lettuce during the experiments were monitored with X-ray film.

Statistical analysis

All data were transformed to logarithm to obtain normality for the analysis of variance (ANOVA). To avoid undefined numbers on data points equal zero, all data were transformed to $\log(x + 1)$, where x = number of bacteria per leaf-cm^2. One-way analysis of variance and Scheffé's test for comparison of means of the transformed data were performed within each sampling date. Linear regressions were calculated for the number of *Lux-Xcc* in soil vs. time for each matric potential. Analyses were performed using the computer program STATISTIX (Siegel, 1992).

Results

Survival of Xcc in soil at different matric potentials in relation to total number of bacteria, and proteolytic and cellulolytic microorganisms

The number of *Lux-Xcc* cells recovered from the soil samples decreased rapidly at high matric potentials (less negative ψ values) (Table 1). The number of *Lux-Xcc* at the beginning of the samplings was approx 10^7 cells/g dry soil and remained in the order of 10^5 to 10^7 during the first 10 days (Figure 2a). At day 10, the number of *Lux-Xcc* was significantly lower for the 0 kPa than for -30, -100, and -200 kPa treatments ($P \leq 0.05$), no significant differences in *Lux-Xcc* were found among these last three ψ (Figure 2a). *Xcc* was not detected after 21 days of incubating infested debris in saturated soil (0 kPa), while the number of *Xcc* remained nearly constant on the air-dried soil (-900 kPa). Similar results were obtained in the repeated experiment, where *Xcc* was not detected after 15 days in saturated soil, whereas at -900 kPa, no significant differences were observed in the populations of this pathogen.

The total number of bacteria within each treatment appeared to be constant during the 21-day test period (Figure 2b), (in the range of 10^5 to 10^8 cells/g dry soil) whereas smaller populations were detected for the driest treatment (-900 kPa) at every sampling date ($P \leq 0.01$).

The number of *Lux-Xcc* recovered from soil samples at different matric potentials (0, -50 and -900 kPa) was significantly lower in the 0 kPa at day 10 and thereafter ($P \leq 0.01$). *Lux-Xcc* was not detected after 15 days of incubation in debris-infested soil at 0 kPa (Figure 3a).

Proteolytic microorganisms in soil decreased in number over time in all ψ regimes (Figure 3b). The number of proteolytic microorganisms was lower in soil at -900 kPa than at 0 and -50 kPa in the first sampling ($P \leq 0.05$) and did not differ among treatments in the following sampling dates (Figure 3b). A positive correlation was observed between the number of proteolytic microorganisms and the number of *Lux-Xcc* (correlation coefficient $r_{0.05,n-2=18} = 0.4667$,

Table 1. Survival rates and average populations of *Xanthomonas campestris* pv. *campestris* (*Xcc*) in soil at different matric potentials

Matric potential (kPa)	Rates of decrease of *Xcc* populations (log of the number of *Xcc* ((g soil)$^{-1}$ day^{-1})			Range of populations at 20 days *Xcc* (g soil)$^{-1}$
	I[1]	II	III	
$0_{(\text{saturated})}$	-0.310**	-0.251**	-0.214	0–5.2×10
-30	-0.183**	-0.240*	N[2]	1.6×10–2.2×10^2
-50	N	-0.253 n.s.	-0.204*	0.9×10–8.3×10
-100	-0.261*	-0.219*	N	0.6×10–4.2×10
-200	-0.286 n.s.	-0.172*	N	0.2×10–3.6×10^2
$-900_{(\text{air dry})}$	-0.031 n.s.	-0.001 n.s.	-0.129 n.s.	2.6×10^3–9.5×10^5

Slopes of the linear regressions were calculated for the survival curves in repeated experiments. Significance level of the regressions was indicated as follows: n.s. (non significant); (*) significant at $P = 0.05$ and (**) significant at $P = 0.01$.
[1] I, II and III correspond to the results of three different experiments. [2] N: matric potentials not included in the corresponding experiment.

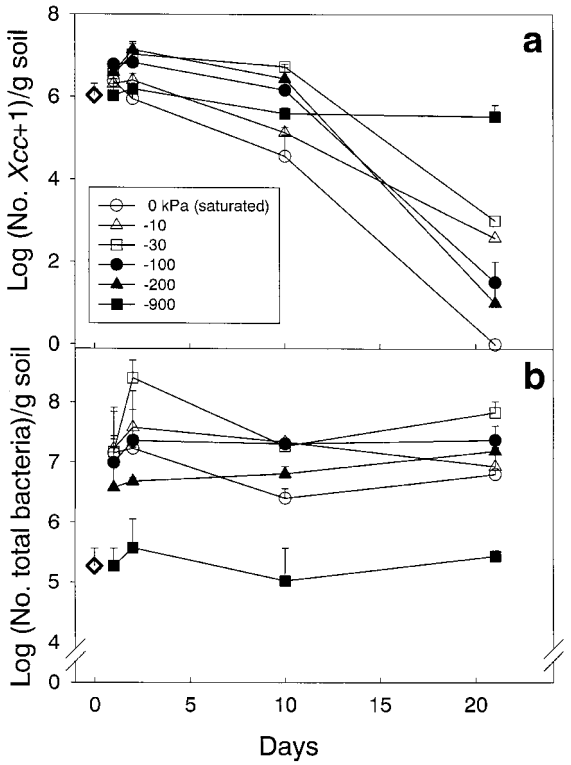

Figure 2. Survival of the bioluminescent strain of *Xanthomonas campestris* pv. *campestris* (171LIIH-7) (*Xcc*) in soil at six matric potentials, (a) number of *Xcc* at different matric potentials, (b) number of total bacteria on tryptic soy broth medium for the same soil samples. Each point represents the average of four replicas. Bars reflect the standard deviation of the mean. ◇ correspond to initial numbers of *Lux-Xcc* and total microflora respectively.

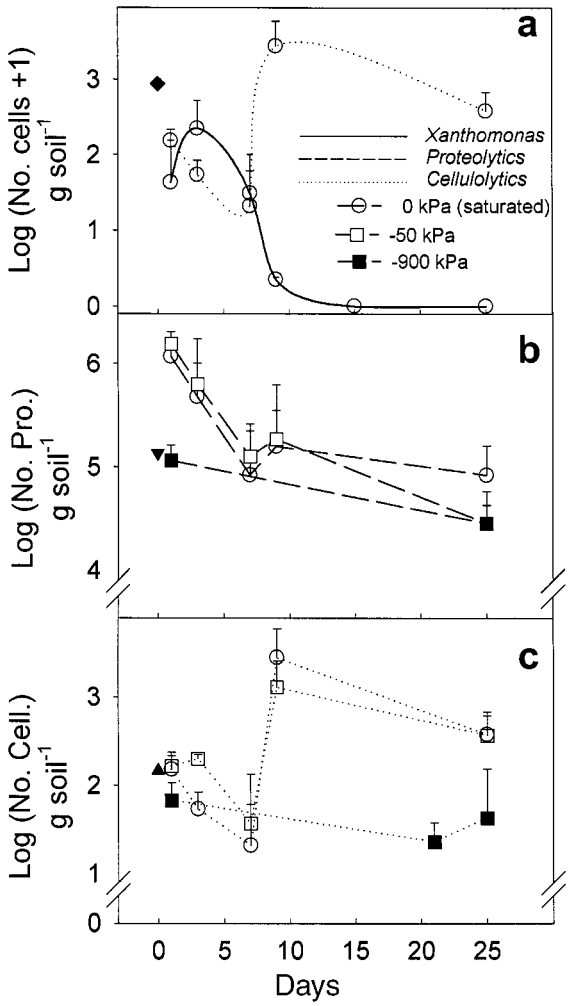

Figure 3. Numbers of: a bioluminescent *Xcc*, proteolytic microorganisms and cellulolytic microorganisms in soil at three matric potentials, (a) relationship between survival of the bioluminescent *Xcc* and number of cellulolytic microorganisms in saturated soil (0.0 kPa), (b) Proteolytic microorganisms in soil at three matric potentials, (c) number of cellulolytic microorganisms in soil at three matric potentials. Each point represents the average of four replicas. Bars indicate the standard deviation of the mean. Symbols: ♦, ▼, and ▲ correspond to initial numbers of *Lux-Xcc*, proteolytic and cellulolytic microorganisms respectively.

$P \leq 0.05$) for the −50 kPa treatment, whereas no correlation was observed between these two populations in the other treatments.

A negative correlation was observed between the number of cellulolytic microorganisms and the number of *Lux-Xcc* for the 0 kPa($r_{0.05,n-2=18} = -0.467$, $P \leq 0.05$) (Figure 3a). In general, the number of cellulolytic microorganisms in the −900 kPa soil did not change significantly over time (Figure 3c). On the contrary, for 0 and −50 kPa, a significant increase in the abundance of this group was observed at 7 days of incubation with respect to previous sampling dates ($P \leq 0.01$), and it remained high for the rest of the experiment (Figure 3c). The abundance of cellulolytic microorganisms was similar in both high matric potentials (0 and −50 kPa). Only on day 3 were more cellulolytic microorganisms detected at −50 kPa than at 0 kPa($P \leq 0.05$).

Survival of Lux-Xcc on the phylloplane of host and non-host plants

Xcc was recovered for up to 48 days on spray-inoculated cabbage, mustard and lettuce leaves, with no significant differences among these crops ($P \leq 0.05$). Initial populations of *Xcc* were 4.6×10^4 to 2×10^5 cells per leaf-cm^2, while the maximum numbers recovered

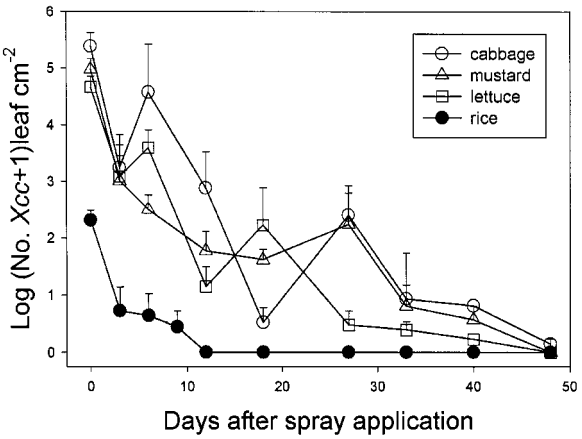

Figure 4. Epiphytic survival of a bioluminescent *Xcc* after spray inoculation of cabbage, mustard, lettuce and rice determined by the MPN-microfluorplate method. Each point represents the average of four replicas. Bars represent the standard deviation of the mean.

from these crops at 48 days were 2–6 cells per leaf-cm^2 (Figure 4). A low number of *Xcc* was detected both at day 6 (10 cells per leaf-cm^2) and day 9 (4 cells per leaf-cm^2) on rice and *Xcc* was not detected at day 12 and subsequent sampling dates on this crop (Figure 4).

The bioluminescent *Xcc* strain was consistently recovered from cabbage, mustard and lettuce by using the MPN-microfluorplate technique. Lesions observed on mustard and lettuce during the experiments were monitored with X-ray film, but no bioluminescence was detected on these species. Only one exposure (3 h) of the X-ray film, after 72 h incubation, was needed to record the total number of wells with bacteria emitting light when processing samples from the phylloplane. Leaves grew at different rates during the experiments and a significant increase in leaf area was observed in the four species ($P \leq 0.01$). Cabbage-leaves increased in size 330%, mustard 189%, lettuce 140%, and rice 47% in comparison to their initial size. These values were used to estimate the apparent reduction in the number of bacterial cells as a consequence of the leaf growth during the 48-day period.

Discussion

Increasing soil ψ resulted in reduced survival of *Xcc* in plant debris-infested soil. Twenty days after soil infestation the lowest populations of *Xcc* were detected at $\psi = 0$ kPa and the highest at $\psi = -900$ kPa. Contradictory interpretations of the possible effect of soil water content on the survival of *Xcc* have been briefly mentioned in the literature. In Georgia, *Xcc* survived for a short time during dry summer seasons (Schaad and White, 1974), while short survival times was associated with wet soil conditions in Hawaii (Alvarez et al., 1987). The present work is the first report that provides quantitative data showing that high soil matric potentials reduce the survival of *Xcc*. The effect of other factors such as temperature should also be studied in different areas and soil types to determine if matric potentials have similar effects under different environmental conditions.

Previous research has shown that *Xcc* survived longer in soil within plant tissues than as free cells. This pathogen was detected after 6 months in buried cabbage stem tissue (Schaad and White, 1974) and only up to 100 days as free living cells on mist inoculated soil (Dane and Shaw, 1994). Therefore, rapid decomposition of plant residues is desirable for reducing populations of the bacterium in soil. Our model system included only 1 mm^2 pieces of infected leaves to analyze the effect of ψ on the survival of *Xcc*. Although the total survival time may vary if different plant tissues and sizes are analyzed, we expect that the same trends will occur.

Biotic and abiotic factors may account for the more rapid extinction of *Xcc* observed in saturated soil samples. Water, by itself, could have produced an anaerobic environment detrimental for an aerobic organism such as *Xcc*. Oxygen depletion is known to result in a change from aerobic to anaerobic microflora in soil (Alexander, 1977). In activated sludge, 54% of the strictly aerobic chemoheterotrophic bacteria (including *Pseudomonas (Xanthomonas) maltophilia*) survived after 8 days of anaerobiosis (Maurines-Carboneill et al., 1998). Oxygen could have been a limiting factor because of its low solubility in water ($\cong 0.007$ kg m^{-3} at 30 °C) and/or competition by fast growing organisms as compared to *Xcc*. Low levels of dissolved oxygen were found to reduce the metabolism of *Xanthomonas campestris*, i.e., by reducing the production of xanthan gum (Flores et al., 1994). In relation to biotic factors, the number of total bacteria found in soil incubated at different ψ did not correlate with the number of *Xcc* and showed no significant temporal trends. The lowest populations of total bacteria were found at extremely low matric potentials, where *Xcc* survived for the longest time.

Xcc survived longer in soil within plant tissue than as free living cells (Shaad and White, 1974), therefore, a fast decomposition of infected plant tissue is desirable. Plant debris is mainly a source of carbon and nitrogen, therefore, cellulolytic and proteolytic microorganisms were analyzed as the first steps of its mineralization. The number of proteolytic microorganisms decreased with time at all ψ tested. This trend has also been reported for rice paddy soils, where the number of proteolytic microorganisms decreased after irrigation (Watanabe and Hayano, 1993). In our study, the abundance of these microorganisms did not explain the reduction of *Xcc* populations in soil. However, a significant negative correlation was found between the number of cellulolytic microorganisms and the number of *Xcc* in soil at relatively high matric potentials. In soils, cellulose degradation occurs mostly during anaerobiosis (Ljungdahl and Eriksson, 1985) and is accompanied by the production of several organic acids and alcohols (Leschine, 1995). These compounds are potentially detrimental to *Xcc* survival. Since cellulolytic bacteria in general are not plant pathogens, the use of cellulolytic bacteria to accelerate the decomposition of plant debris should be explored.

Considering that *Xcc*-infested soil can reach the leaves by wind or water splash (Shaw et al., 1992) and can enter through wounded roots (McElhaney, 1991), cultural practices oriented to prevent root damage and eliminate the pathogen from the superficial soil layers should be effective in reducing infection. From our data, we conclude that if plant debris-infested soil is maintained with a matric potential close to zero during 1 month, the population of *Xcc* would be drastically reduced. This practice could replace the 1- or 2-year rotations recommended by Schaad and White (1974).

We found that the MPN-microfluorplate technique was adequate for monitoring bioluminescent *Xcc* on the phylloplane, and by this method we were able to detect as few as 2 cells per leaf-cm^2. Fungal growth in these samples was minimal and the X-ray film images were generally sharp. For phylloplane samples, one exposure of the microfluorplates after 72 h incubation was sufficient to detect all the wells containing bioluminescent bacteria, whereas for soil samples two exposures (at 48 and 72 h) were necessary.

When bacteria are applied to leaf surfaces, different patterns of colonization may occur (Blakeman, 1985). In our preliminary experiments, leaf prints of spray-inoculated cabbage-plants on a selective medium and subsequent exposure of X-ray film, revealed the presence of *Lux-Xcc* as one or two small clusters of colonies located at about 2.5 cm from the leaf margins. This log normal distribution observed for *Xcc* is also characteristic of other bacterial populations on phylloplanes such as ice nucleating bacteria (Hirano and Upper, 1986). For epidemiological purposes each plant represents a unit of inoculum; hence for the survival studies the four inoculated leaves of each plant were combined in one sampling unit. Although the use of bulked samples can lead to overestimation of means (Jaques and Morris, 1995), we consider that in this case, combined samples represent each plant as a potential focus of infection in the field. The leaf growth could have contributed to underestimate the number of cells per leaf-cm^2 by up to one log unit the *Xcc* populations on cabbage and mustard, by the conclusion of the experiment. Nevertheless, the reduction in number of *Xcc* on these plant species 48 days after inoculation, was in the order of 2–3 log units (100–1000 times), which cannot be solely explained as a dilution effect of the initial inoculum on larger leaf areas, since leaves only duplicate or triplicate in size.

The survival of *Lux-Xcc* was similar on cabbage, mustard, and lettuce. Thus, a non-cruciferous crop, such as lettuce, may act as a shelter for *Xcc* in the field. On the contrary, rice leaves apparently prevented the adherence of *Xcc*-inoculum, evident as small numbers of cells per leaf-cm^2 leaf at time zero. Many bacterial pathogens have been reported to colonize leaves of non-host plants, which may represent an important means for the survival of inoculum (Blakeman, 1993; Dane and Shaw, 1996). In order to manage population sizes of bacterial pathogens on the leaves, the predominant factors that affect bacterial abundance on plants should be studied (Lindow, 1996). In the present work, survival of *Xcc* differed among various phylloplanes. Therefore, the appropriate selection of rotation crops can contribute to reduce the persistence of this pathogen in the field.

References

Alexander M (1977) Bacteria (16-35) Alexander M (ed) Introduction to Soil Microbiology, John Wiley & Sons, New York

Alvarez AM and Cho JJ (1978) Black rot of cabbage in Hawaii: inoculum source and disease incidence. Phytopathology 68: 1456–1459

Alvarez AM, Cho JJ and Hori TM (1987) Black rot of cabbage in Hawaii. Hawaii Agriculture Experiment Station, Research Service 051-08.87

Alvarez AM, Benedict AA, Mizumoto CY, Hunter JE and Gabriel DW (1994) Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. Phytopathology 84: 1449–1457

Arias RS, Mochizuki G, Fukui R and Alvarez AM (1996) Most probable number method to enumerate a bioluminescent *Xanthomonas campestris* pv. *campestris* in soil. Soil Biol Biochem 28: 1725–1728

Blakeman JP (1985) Ecological succession of leaf surface microorganisms in relation to biological control. In: Windels CE and Lindow SE (eds) Biological Control on the Phylloplane (pp 6–30) APS Press, St. Paul, MN

Blakeman JP (1993) Pathogens in the foliar environment. Plant Pathol 42: 479–493

Boehm MJ and Hoitink HAJ (1992) Sustenance of microbial activity in potting mixes and its impact on severity of *Pythium* root rot of poinsettia. Phytopathology 82: 259–264.

Collier WL and Davidson JR (1967) An economic analysis of head cabbage and dry onion production in the state of Hawaii. Agricultural Economics Report No. 77. Hawaii Agricultural Experiment Station, University of Hawaii, 52 pp.

Cook AA, Larson RH and Walker JC (1952) Relation of the black rot pathogen to cabbage seed. Phytopathology 42: 316–320

Dane F and Shaw JJ (1994) Endophytic growth of *Xanthomonas campestris* pv. *campestris* on susceptible and resistant host plants in the field environment, detection by bioluminescence. Microbiol Releas 2: 223–229

Dane F and Shaw JJ (1996) Survival and persistence of bioluminescent *Xanthomonas campestris* pv. *campestris* on host and non-host plants in the field. J Appl Bacteriol 80: 73–80

English JT and Mitchell DJ (1988) Influence of an introduced composite of microorganisms on infection of tobacco by *Phytophtora parasitica* var. *nicotianae*. Phytopathology 78: 1484–1490

Flores F, Torres LG and Galindo E (1994) Effect of the dissolved oxygen tension during cultivation of *X. campestris* on the production and quality of xanthan gum. J Biotechnol 34: 165–173

Fukui R, Arias RS and Alvarez R (1994) Efficacy of four semiselective media for recovery of *Xanthomonas campestris* pv. *campestris* from tropical soils. J Appl Bacteriol 77: 534–540

Hawaii Agricultural Statistics Service (1994) Statistics of Hawaiian agriculture, USDA National Agricultural Statistics Service, Honolulu, Hawaii, 107 p

Hirano SS and Upper CD (1986) Temporal, spatial, and genetic variability of leaf-associated bacterial populations. In: Fokkema NJ and Van den Heuvel J (eds) Microbiology of the phyllosphere pp 235–251 Cambridge University Press, Cambridge, UK

Ikawa H, Sato HH, Chang AKS, Nakamura S, Robello Jr E and Periaswamy SP (1985) Soils of the Hawaii Agricultural Experiment Station, University of Hawaii, Soil Survey, Laboratory data, and soil descriptions (BSP Technical Report 4). Research extension series 022, HITAR, College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu

Jacques MA and Morris CE (1995) A review of issues related to the quantification of bacteria from the phyllosphere. FEMS Microbiol Ecol 18: 1–14

Kado CI, Heskett MG and Langley RA (1972) Studies of *Agrobacterium tumefaciens*, characterization of strains ID135 and analysis of the bacterial chromosome, transfer RNA and ribosomes for tumor inducing ability. Physiol Plant Pathol 2: 47–57

Klute A (1986) Water retention: laboratory methods. In: Klute A (ed) Methods of Soil Analysis, Part 1. Agronomy No. 9 pp 635–662. Soil Sci Soc Am, Madison

Krieg NR (1981) Enrichment and isolation. In: Amer Soc Microbiol (ed) Manual of Methods for General Bacteriology, Chapter 8., Washington

Leschine SB (1995) Cellulose degradation in anaerobic environments. Ann Rev Microbiol 49: 399–426

Lindow SE (1996) Role of immigration and other processes in determining epiphytic bacterial populations. In: Morris CE, Nicot PC and Nguyen-The C (eds) Aerial Plant-Surface Microbiology. pp 155-168 Plenum Press, New York

Ljungdahl LG and Eriksson KE (1985) Ecology of microbial cellulose degradation. Advances in Microbial Ecology 8: 237–299

Maurines-Carboneill C, Morin L, Pernelle JJ, Derlet N, Sachon G and Leblon G (1998) Reviviscence of aerobic chemoheterotrophic bacteria in an activated sludge pilot plant after a prolonged absence of oxygen. Water Res 32: 2211–2219

McElhaney R (1991) Bacterial bioluminescence: a tool to study host–pathogen interactions between *Brassica oleracea* and the bacterial phytopathogen *Xanthomonas campestris* pv. *campestris* in black rot of cabbage. Ph.D. Dissertation, University of Hawaii, Honolulu

Parker D, Norby J and Bennington J (1988) SigmaScan program Version 1.10, Jandel Graph File Conversion Utility; Jandel Scientific, Corte Madera, CA

Pochon J and Tardieux P (1962) Techniques d'analyse et microbiologie du sol. St.-Mandé (ed) de la Tourelle pp 56–59

Schaad NW and White WC (1974) Survival of *Xanthomonas campestris* in soil. Phytopathology 64: 1518–1520

Shaw JJ, Dane F, Geiger D and Kloepper JW (1992) Use of bioluminescence for detection of genetically engineered microorganisms released into the environment. Appl Environ Microbiol 58: 267–273

Shaw JJ and Kado CI (1986) Development of a *Vibrio* bioluminescence gene set to monitor phytopathogenic bacteria during the ongoing disease process in a non-disruptive manner. Biotechnology 4: 560–564

Siegel J (1992) STATISTIX, Version 4.0. Siegel (ed), Analytical Software. St. Paul, MN

Timmer LW, Marois JJ and Achor D (1987) Growth and survival of xanthomonads under conditions nonconducive to disease development. Phytopathology 77: 1341–1345

Watanabe K and Hayano K (1993) Distribution and identification of proteolytic *Bacillus* spp. in paddy soil under rice cultivation. Can J Microbiol 39: 674–680

Woomer P, Bennet J and Yost R (1990) Overcoming the inflexibility of Most Probable-Number Procedures. Agron J 82: 349–353