Protozoa as Agents Responsible for the Decline of *Xanthomonas campestris* in Soil

MITIKU HABTE AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14850

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A streptomycin-resistant mutant of *Xanthomonas campestris* was used to assess the persistence of the plant pathogen in soil and the changes in populations that might be important for its survival. In soil into which large numbers of the organism were introduced, a marked decline in its abundance occurred, but after about 1 week its population density reached a level of about $10^8$ and did not continue to fall during the test period. No such marked decline was evident in sterile soil inoculated with *X. campestris*. The bacterium did not lose viability if starved for carbon or inorganic nitrogen. Although abundant in soil, the numbers of propagules capable of producing antibiotics or lytic enzymes active against *X. campestris* did not increase coincident with the pathogen's decline, and no increase in tartrate-extractable toxins was observed. Neither bdellovibrios nor bacteriophages active against the xanthomonad were found in the soil, but a marked increase in the frequency of protozoa paralleled the phase of rapid diminution in the *X. campestris* population. In actidione-treated soil, in which protozoan activity was severely limited, the high cell density of the pathogen was maintained. On the basis of these data, it is concluded that predation by protozoa is responsible for the abrupt fall in frequency of the bacterium in natural soil.

Plant pathogenic bacteria enter the soil as free propagules or in association with dead and dying plant tissues, although the soil may be the natural habitat of a few species that have the ability to grow in soil saprophytically. After their introduction into soil, however, most species of plant pathogenic bacteria decline rapidly, but almost all of these populations endure for long periods in sterile soil (1, 7, 10); thus, the lack of survival in soil appears to be a function of the prevailing antagonistic microbial relationships. These deleterious interactions may be competition, amensalism, parasitism, or predation.

That amensalism might be of some importance is supported by the findings of Patrick (12), who showed that members of all genera of plant pathogenic bacteria are susceptible to antibiotic activity of soil microorganisms in vitro. Other investigators believe that bacteriophages are of considerable importance in reducing the abundance of pathogens in soil, and the ability of phages to lyse the pathogens in vitro has been demonstrated (11, 21). The possible role of bdellovibrios in the ecology of the pathogens in soil has yet to be studied, but Starr and Baigent (15) showed that *Bdellovibrio bacteriovorus* could parasitize species of *Erwinia* and *Pseudomonas* in culture, and Scherff (13) reported that inoculating infected soybean plants with *B. bacteriovorus* led to control of the blight caused by *Pseudomonas glycinea*.

The present study was designed to investigate why the population of a particular pathogen, *Xanthomonas campestris*, declines rapidly in soil and to establish which type of microorganisms is responsible for the decline.

**MATERIALS AND METHODS**

A streptomycin-resistant mutant derived from a culture of *X. campestris* C-3 (5) was used, and the bacterium was grown in tryptic soy broth (Difco) with and without 1.0 mg of streptomycin per ml. To provide inocula for the various experiments, a 24-h culture was harvested aseptically by centrifugation, and the cells were washed three successive times in sterile, distilled water. A 1.0-ml portion of a dilution of the bacterial suspension in carbon-free solution (3) was usually used, and the cultures were incubated at 30 C.

The survival of *X. campestris* in nonsterile Valois silt loam, pH 6.5, was determined by inoculating 1.0 ml of a suspension containing about $10^{14}$ *X. campestris* cells into 150-ml capped dilution bottles containing 10.0 g of soil at a moisture content of 25%. The bottles were gently agitated to insure mixing of the inoculum with the soil, and the bottles were incu-
bated at 30°C. Counts were made of three of the dilution bottles at regular intervals by diluting the soil samples in the carbon-free solution and plating on nutrient agar (Difco) containing 1.0 mg of streptomycin and 0.50 mg of actidione per ml. Counts were made after the plates were incubated for 6 days. The persistence of _X. campestris_ in sterile soil was determined in the same manner, except that the water lost during autoclaving was replaced with sterile, distilled water before the pathogen was added. To sterilize the soil, 10-g portions of moist soil were autoclaved for 2 h, incubated at room temperature for 5 days, and then autoclaved again for 2 h.

The response of _X. campestris_ to carbon starvation was determined by employing the carbon-free solution, and this solution minus KNO₃ was used for investigating the response of the bacterium to the combined lack of carbon and nitrogen. To 100-ml portions of these sterilized solutions containing 250-ml Erlenmeyer flasks were added 1.0-ml samples of _X. campestris_ cells washed by centrifugation and suspended in distilled water. The flasks were incubated at 30°C on a rotary shaker operating at 120 rpm, and triplicate samples were withdrawn regularly for plate counts on nutrient agar containing 1.0 mg of streptomycin and 0.50 mg of actidione per ml.

Dilution bottles containing 10-, 50-, or 30-g portions of soil, respectively, were used for: (i) counting of antibiotic producers, lytic microorganisms, and protozoa; (ii) enumeration of bdellovibrios and bacteriophages; and (iii) the measurement of toxin production. Washed _X. campestris_ cells suspended in the carbon-free solution were introduced into the bottles, each receiving 1.0 ml of the inoculum per 10 g of soil. After the soil and bacteria were well mixed, the bottles were incubated at 30°C.

Counts of antibiotic producers were made by using Patrick’s (12) glucose-rich agar medium. Dilutions of the soil samples were placed in petri dishes, and the molten agar medium, containing 5% (vol/vol) tryptic soy broth in which _X. campestris_ was grown for 24 h, was poured into the plates and mixed with the soil dilutions. Antibiotic-producing colonies were considered to be those having clearing zones around them after a 5-day incubation period.

The numbers of bdellovibrios and bacteriophages were determined by employing the double-layer technique, using the procedures and media of Stolp and Starr (16) for bdellovibrios and the technique of Cook and Quadding (5) for bacteriophages. Soil fungi were enumerated on peptone-glucose-rose-bengal agar (8) after an incubation period of 5 days.

Lytic microorganisms were enumerated on agar containing live and heat-killed _X. campestris_. Cells for making xanthomonas agar were obtained by growing the bacterium for 48 h in tryptic soy broth containing 1.0 mg of streptomycin per ml and then washing the cells by centrifugation using sterile, distilled water. The washed cells were resuspended in distilled water to yield approximately 10^12 cells/ml. Either 20 ml of live cells or a bacterial suspension that had been autoclaved for 30 min was added to each 200 ml of melted and cooled water agar just before it was poured into petri dishes containing dilutions of soil samples. The number of colonies of bacteria, fungi, and actinomycetes that were surrounded by clearing zones was recorded after a 3-day incubation.

The procedure for counting protozoa was a modification of the Singh ring method (14). Melted water-agar (1.5%) was added to petri dishes, and five sterile glass rings (20 mm wide by 10 mm high) were placed in each plate immediately afterward. When the agar was cool and well set, 0.1 ml of a thick cell suspension (derived from a 24-h culture of _X. campestris_) in sterile, distilled water and 0.5 ml of a soil solution were added to each ring. The plates were incubated for 3 to 6 days, and then the growth in each ring was examined under a microscope. Quantification was achieved by means of the most-probable-number technique (9). To obtain the 95% confidence limits for the counts, the values obtained are multiplied by 0.30 and 3.3 (4).

To ascertain whether toxins were present in the soil, 50 ml of a 0.1 M sodium tartrate solution was added to a 30-g soil sample. The mixture was shaken vigorously and subsequently allowed to stand for 24 h. The particulate matter was then removed by centrifuging the suspension for 10 min at 15,000 × _g_, and the supernatant fluid was passed through Whatman no. 1 filter paper. The resulting filtrate was sterilized by passing it through a 0.45-μm membrane filter (Millipore Corp.). To assess the toxicity of this extract, 30 ml of tryptic soy broth, 5.0 ml of the extract, and 0.2 ml of a 24-h-old _X. campestris_ culture were added to a 300-ml Erlenmeyer flask fitted with a side arm. The flasks were incubated on a rotary shaker operating at 120 rpm, and the growth rate was assessed by recording regularly the change in optical density at a wavelength of 550 nm using a Bausch and Lomb Spectronic 20 spectrophotometer.

**RESULTS**

The changes occurring when _X. campestris_ was added to natural and sterile soil are shown in Fig. 1. In sterile soil, the abundance of the pathogen did not change appreciably in the first 2 days and then declined to a level of 4.5 × 10^4 cells/g on day 6, after which the cell density remained essentially constant for the test period. In marked contrast, the abundance of the pathogen in nonsterile, inoculated soil fell rapidly after day 2, declining from an initial value of 2 × 10^6/g to a level about five orders of magnitude lower, in the vicinity of 7 × 10^4/g, before the period of rapid decline terminated. The dramatic fall in numbers in the sample containing the natural soil community as compared with the slight change in the soil that had its indigenous populations eliminated strongly suggests that biological agencies are responsible for the significant elimination of _X. campestris_ cells.

The stabilization in numbers of survivors in nonsterile soil at about 10^4/g indicates that the
agent or mechanism of destroying the pathogen was effective at high but not at low bacterial densities. To assess whether the extent of elimination was, in fact, dependent on pathogen abundance, three different *X. campestris* densities were introduced into nonsterile soil, and the survivors were enumerated at regular intervals. The pathogen died at a reasonably rapid rate, but the final density of survivors at the end of the test period was the same whether the inoculum was $3.9 \times 10^8$, $3.8 \times 10^8$, or $1.6 \times 10^{10}/g$, about $10^8$ *X. campestris* surviving per g of soil (Fig. 2).

The death of the xanthomonad may have been the consequence of competition between it and soil heterotrophs, the former dying because it could not cope with the more vigorous utilization by other heterotrophs of some limiting resource in the environment. Inasmuch as carbon is usually the chief limiting nutrient in soil for heterotrophs, followed by nitrogen, a study was made of the ability of *X. campestris* to withstand carbon and nitrogen starvation, assuming that starvation for the nutrient in inadequate supply is the probable reason that an organism, initially present in large numbers, loses viability when the mechanism of elimination is competition. When $2.1 \times 10^4$ cells/ml were added to the carbon-free solution, the maximum degree of change observed in the

12-day test period was less than one order of magnitude, and the number of survivors was never less than $1.5 \times 10^7$/ml. A slightly greater decline in *X. campestris* numbers took place if $3.2 \times 10^4$ cells/ml were introduced into the carbon- and nitrogen-free solution, but at no time did the count fall much below $10^7$/ml and, indeed, a subsequent rise occurred so that the density at 12 days was almost the same as in the original inoculum. These data support the view that the bacteria did not lose their viability in nonsterile soil owing to a competition for carbon or nitrogen.

The possible significance of antibiotic producers during the pathogen’s decline was assessed by counting the numbers of antibiotic formers, as well as *X. campestris*, in Valois silt loam inoculated with the xanthomonad. Plate counts made every second day revealed that the pathogen density fell from an initial value of $1.6 \times 10^{10}$ to $2.0 \times 10^8$ at day 8 but was still $8.7 \times 10^7$/g on day 12. There were initially $6.7 \times 10^4$ antibiotic formers, but after 12 days their abundance was still only $5.3 \times 10^9$/g; moreover, the counts made every second day revealed no value above 6.7 and none below $3.7 \times 10^9$/g. In a parallel test of the frequency of antibiotic formers in uninoculated soil, the numbers ranged from $4.0 \times 10^4$ to $7.3 \times 10^4$, with a mean of $5.7 \times 10^4$/g. Hence, no response is evident among these potential toxin-synthesizing microorganisms.
Similarly, sodium tartrate extracts made every other day of a soil inoculated with *X. campestris*, as well as from an uninoculated sample, failed to depress the rate of growth of the bacterium in tryptic soy broth. The extracts were taken for a 12-day incubation period, during which time the bacterial population had gone from $1.6 \times 10^{10}$ to $8.3 \times 10^{9}/g$. Admittedly, the inhibitory principle may not have been extracted by the tartrate, but the present study provides preliminary data suggesting that amensalism is not the cause of the loss of bacterial viability.

The possible role of lytic inhabitants in inactivating the pathogen was assessed by enumerating the propagules possessing lytic activity. Counts were made every second day for 12 days in a soil receiving $4.9 \times 10^{4}$ *X. campestris* cells/g and in uninoculated soil. The xanthomonad had declined to $3.0 \times 10^{8}$ in 2 days, $4.0 \times 10^{8}$ in 4 days, $2.7 \times 10^{9}$ in 6 days, and $4.7 \times 10^{9}/g$ at 8 days, and later counts revealed no appreciable further decline. By contrast, the abundance of lytic bacteria did not change during the course of the incubation in either the inoculated or uninoculated soil, maintaining a level of $2.1 \times 10^{7}$ to $3.7 \times 10^{7}$ in the former and $2.2 \times 10^{7}$ to $3.7 \times 10^{7}/g$ in the latter. Similarly, the density of lytic actinomycetes did not vary significantly during the 12 days, ranging from $4.3 \times 10^{7}$ to $5.7 \times 10^{7}$ in the inoculated and $4.0 \times 10^{7}$ to $6.0 \times 10^{7}/g$ in the uninoculated soil. Thus, although the frequency of cells possessing the potential for lysing *X. campestris* is high, their abundance is not modified as a result of the introduction of this species.

In addition to bacteria and actinomycetes, occasional colonies of lytic fungi developed on the plates. The occurrence of these fungi was so irregular that it was difficult to assess their importance. However, counts of total fungi were made to determine whether fungi in general responded to *X. campestris* inoculation. In this instance, the bacterial density fell from $3.8 \times 10^{10}$ to $3.1 \times 10^{9}/g$, whereas the fungi showed no appreciable change in abundance, the counts of the latter ranging from $0.96 \times 10^{7}$ to $4.7 \times 10^{7}$ and from $2.1 \times 10^{6}$ to $4.7 \times 10^{6}/g$ of the inoculated and uninoculated soil, respectively.

Many yellow colonies grew on the agar medium containing a soil dilution and heat-killed cells of *X. campestris*. These colonies, which caused lysis of the pathogen and clearing of the agar, were observed during the first 4 days after the soil was inoculated with viable *X. campestris*, and the number of such lysis-inducing colonies was comparable with the numbers of viable cells of the pathogen remaining in the soil. No such colonies were apparent when dilutions from uninoculated soil were plated on agar amended with heat-killed xanthomonads. These results suggest that viable *X. campestris* lysed heat-killed cells of the same species. In support of this hypothesis, it was found that dilutions of a pure culture of the pathogen plated on the same solid medium lysed the heat-killed cells.

Repeated attempts to isolate bdellovibrios and bacteriophages from soils inoculated with *X. campestris* yielded negative results, even when large quantities of soil were used for the attempted isolations. Four bdellovibrio isolates which were able to parasitize members of the genus *Rhizobium* were without effect on *X. campestris*. The lytic counts discussed above were performed on media with dead *X. campestris* cells, but no lytic colonies were noted if soil dilutions were plated on agar containing viable cells.

By contrast with the lack of changes in the abundance of the previous microbial groups, protozoa responded to inoculation of soil with *X. campestris*. A marked difference in the protozoan density was evident between inoculated and uninoculated soil (Fig. 3). The counts

![Fig. 3. Numbers of protozoa in soil inoculated with X. campestris and in uninoculated Valois silt loam.](image-url)
in unoinoculated soil remained reasonably constant, with only minor fluctuations at a cell density level of about $5 \times 10^4$/g of soil. By contrast, the abundance of these organisms in inoculated soil increased from an initial value of $9.2 \times 10^3$ to $5.4 \times 10^4$ cells/g of soil in a period of 4 days. The corresponding decrease in the *X. campestris* population was from $1.3 \times 10^8$ to $8.4 \times 10^4$ cells/g of soil. The soil protozoa, which included ciliates and flagellates but only rarely included amebae, did not respond to a further diminution in the *X. campestris* population. These findings argue for the direct involvement of soil protozoa in the decline of *X. campestris* in soil.

It was observed in a preliminary experiment that protozoa were suppressed in Valois silt loam receiving 2.0 mg of actidione per g. The ability of *X. campestris* to persist in such a treated soil would show whether protozoa were indeed regulating pathogen density. Therefore, one sample of soil was amended with the antibiotic at the stated concentration, and a second sample received no actidione. The bacteria was added in large numbers to the two samples, and counts were made at regular intervals. The data in Table 1 confirm the previously noted increase in protozoa and decrease in xanthomonads in the actidione-free sample, and they also are in agreement with the preliminary test that suggested that the antibiotic held the animals in check. Furthermore, only a slight fall, rather than the usual abrupt decline, in the *X. campestris* level was evident in the soil supplemented with the chemical.

**DISCUSSION**

The pattern of population changes of *X. campestris* in natural soil—a rapid initial decrease followed by a stabilization in the cell density—is not unique to this organism. Thus, a similar trend was observed by Van Donsel et al. (18), who reported that 90% of the *Escherichia coli* and *Streptococcus faecalis* cells added to soil died in from 3 days to 3 weeks, depending on the prevailing conditions, whereas low counts of the organisms were evident for as long as 142 days. No experimental evidence has yet been provided to explain why low but not high cell densities can be maintained, but, if predation by protozoa is the means by which the bacteria are eliminated, then it might be hypothesized that the prey may persist in the presence of its predators when the energy that could be gained by predation is equal to or less than the energy expended in seeking the few surviving prey individuals. Such an explanation also may account for the persistence of *Rhizobium* in the presence of bdellovibrios (S. O. Keya and M. Alexander, unpublished data). In addition to such a prey density-dependent feeding by the protozoa, the physical obstruction to predation imposed by soil particles and the possible diminished edibility of prey sorbed to soil colloids may allow for survival.

The ability of an organism, like *X. campestris*, to withstand starvation conditions and persist in the absence of carbon and nitrogen sources could be a beneficial characteristic in soil where competition for nutrients, particularly for carbon, probably often takes place. The mechanisms by which bacteria maintain themselves under starvation conditions, which would prevail for a poor competitor, are not well defined. In the absence of exogenous substrates and if the organisms do not enter a metabolically inactive stage, bacteria probably derive energy from intracellular constituents such as poly-$\beta$-hydroxybutyrate, polyphosphates, and polysaccharides, and several studies (3, 17) have demonstrated that internal poly-$\beta$-hydroxybutyrate reserves may be important in the resistance of microorganisms to starvation. On the other hand, bacteria surviving in nutrient-deficient solutions may persist because nutrients released from dying cells allow the growth of survivors (2), but the significance of cryptic growth in an environment with many species is probably small inasmuch as neighboring species will compete for the nutrients so released and probably are often better adapted to make use of them.

Because the number of antibiotic-producing microorganisms remained remarkably constant and the level of tartrate-extractable toxins did not increase, it seems plausible to believe that toxin production is not a significant factor in

**Table 1. Effect of adding actidione to Valois silt loam inoculated with X. campestris on microbial numbers**

| Days | Counts in actidione-treated soil (no./g) | Counts in untreated soil (no./g) |
|------|-----------------------------------------|---------------------------------|
|      | *X. campestris* | Protozoa | *X. campestris* | Protozoa |
| 0    | $2.0 \times 10^4$ | 170 | $1.5 \times 10^4$ | 5.4 $\times 10^4$ |
| 2    | $1.9 \times 10^4$ | 60  | $2.3 \times 10^4$ | 2.8 $\times 10^4$ |
| 4    | $1.0 \times 10^4$ | ND  | $9.7 \times 10^4$ | 2.1 $\times 10^4$ |
| 6    | $8.0 \times 10^4$ | 170 | $4.7 \times 10^4$ | 2.7 $\times 10^4$ |
| 8    | $6.0 \times 10^4$ | 70  | $3.2 \times 10^4$ | 1.7 $\times 10^4$ |
| 10   | $9.0 \times 10^4$ | 68  | $4.0 \times 10^4$ | 7.0 $\times 10^4$ |
| 12   | $2.7 \times 10^4$ | ND  | $8.2 \times 10^4$ | 1.6 $\times 10^4$ |

*None detected.*
the decline of the pathogen. Similarly, although the number of lytic bacteria and actinomycetes was high, their abundance was unaffected by the inoculation and subsequent decline of the pathogen. Furthermore, no microorganisms were found in soil which lysed live X. campestris cells, at least by the methods and with the sensitivity of the procedures employed. Nevertheless, the soil may contain a steady-state level of lytic enzymes or nonextractable toxins that do indeed contribute to the demise of an introduced alien species, and no relationship may exist between organisms producing antibiotics in a particular agar medium and those generating antibacterial compounds in soil.

The reason for the resistance of live cells to lysis as compared to autoclaved X. campestris cells is not clear. Similar findings have been made in studies of the lysis of other microorganisms by actinomycetes (20) and myxobacteria (19). Webley et al. (19) proposed that the resistance of living cells to lysis probably results from the excretion by these cells of compounds inhibitory to the lytic enzyme or from the synthesis of cell walls at a rate faster than the enzymatic destruction of the walls.

The appreciable increase in protozoan abundance associated with the decline of X. campestris and the failure of the pathogen population to be decimated in soil treated so that protozoa were inactive argue for a key role of predators in regulating the bacterial numbers. This hypothesis is also supported by the finding that the pathogen density reached a reasonably constant value when the protozoan number reached its maximum level. Further investigation is required to ascertain whether the protozoa are the sole agents responsible for elimination of the aliens and why the prey is able to maintain a reasonably large number of propagules despite the presence of its predators.

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LITERATURE CITED

1. Alexander, M. 1971. Microbial ecology. Wiley, New York.
2. Boylen, C. W., and J. C. Ensign. 1970. Long-term starvation survival of rod and spherical cells of Arthrobacter crysalllopisites. J. Bacteriol. 103:569–577.
3. Chen, M., and M. Alexander. 1972. Resistance of soil microorganisms to starvation. Soil Biol. Biochem. 4:283–288.
4. Cochrane, W. G. 1960. Estimation of bacterial densities by means of the "most probable number." Biometrics 6:105–116.
5. Cook, F. D., and C. Quadling. 1959. A modified technique for isolation of bacteriophage from contaminated materials. Can. J. Microbiol. 5:311–312.
6. Danso, S. K. A., M. Habte, and M. Alexander. 1973. Estimating the density of individual bacterial populations introduced into natural ecosystems. Can. J. Microbiol. 19:1450–1451.
7. Goto, M. 1972. The significance of the vegetation for the survival of plant pathogenic bacteria. p. 39–54. In H. P. Maas (ed.), Proceedings of the third international conference on plant pathogenic bacteria. North Holland Publishing Co., Wageningen.
8. Johnson, L. F., and E. A. Curl. 1972. Methods for research on the ecology of soil-borne plant pathogens. Burgess Publishing Co., Minneapolis.
9. Maloy, O. C., and M. Alexander. 1968. The most probable number method for estimating populations of plant pathogenic organisms in the soil. Phytopathology 48:126–128.
10. Menzies, J. D. 1963. Survival of microbial plant pathogens in soil. Bot. Rev. 29:79–122.
11. Okabe, N., and M. Goto. 1963. Bacteriophages of plant pathogens. Annu. Rev. Phytopathol. 1:397–418.
12. Patrick, Z. A. 1964. The antibiotic activity of soil microorganisms as related to bacterial plant pathogens. Can. J. Bot. 33:705–735.
13. Scherff, R. H. 1973. Control of bacterial blight of soybean by Bdellovibrio bacteriovorus. Phytopathology 63:400–402.
14. Singh, B. N. 1985. Culturing soil protozoa and estimating their numbers in soil. p. 403–411. O. M. Kevan (ed.), Soil zoology. Butterworths Scientific Publishers, London.
15. Starr, M. P., and N. L. Baigent. 1966. Parasitic interaction of Bdellovibrio bacteriovorus with other bacteria. J. Bacteriol. 91:2006–2017.
16. Stolp, M., and M. P. Starr. 1963. Bdellovibrio bacteriovorus gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:217–248.
17. Stokes, J. L., and W. L. Paras. 1968. The role of poly-β-hydroxybutyrate in survival of Sphaerotilus discophorus during starvation. Can. J. Microbiol. 14:785–789.
18. Van Donsel, D. J., E. E. Geldreich, and N. A. Clarke. 1967. Seasonal variation in survival of indicator bacteria in soil and their contribution to storm-water pollution. Appl. Microbiol. 15:1362–1370.
19. Webley, D. M., E. A. C. Follett, and I. F. Taylor. 1967. A comparison of the lytic action of Cytaphage jhonsonii on a eubacterium and a yeast. Antonie van Leeuwenhoek. J. Microbiol. Serol. 33:159–165.
20. Welsch, M. 1942. Bacteriostatic and bacteriolytic properties of actinomycetes. J. Bacteriol. 44:571–580.
21. Zeitoun, F. M., and E. E. Wilson. 1969. The relation of bacteriophage to the walnut tree pathogens, Erwinia nigrifluens and Erwinia rubrifaciens. Phytopathology 59:756–761.