Serological Estimates of the Seasonal Dynamics of *Erwinia tracheiphila* in *Acalymma vittata* (Coleoptera: Chrysomelidae)

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ABSTRACT Serological assays were used to estimate the proportion of *Acalymma vittata* (F.) that harbored *Erwinia tracheiphila* (E. F. Smith) Holland, the causal agent of bacterial wilt in cucurbits. These proportions were related to the proportion of *A. vittata* that transmitted disease in single beetle caged bioassays. The serological assays classified beetles as harboring the bacteria when the titer was above ≈10^5 cells per beetle. From 7.1 to 10.7% of the *A. vittata* captured as they emerged from soil that had been in cucurbits the previous year tested positive for the presence of *E. tracheiphila.* Also, from 0 to 8.3% of beetles captured on squash traps during this time of beetle emergence tested positive. This provides strong serological evidence for *A. vittata* serving as the primary overwintering reservoir for *E. tracheiphila.* During the growing season, the proportion of beetles testing positive with serological assays varied and ranged up to ≈53, 78, and 39% in 1995, 1996, and 1997, respectively. These serological proportions were 3.6–5.1 times higher than the proportion of beetles that transmitted disease in single beetle caged bioassays, and explained 44–49% of the variation in the proportion of beetles that were able to transmit disease in the caged bioassays. We suggest the proportion of *A. vittata* that harbored at least some *E. tracheiphila* cells may be >5 times higher than the proportion of beetles that can, alone, transmit disease in a short time. We discuss these data as supporting the hypothesis of beetle aggregation behavior as an important component of bacterial wilt epidemiology.

KEY WORDS *Acalymma vittatum, Erwinia tracheiphila,* bacterial wilt, cucurbits, epidemiology

Diabroticite beetles, including the striped cucumber beetle, *Acalymma vittata* (F.), and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Barber), are among the most serious insect pests of fresh-market cucumber, *Cucumis sativa* L., and cantaloupe, *Cucumis melo* L., in the eastern, midwestern, and southern United States. These beetles cause damage by feeding on young plants (Brewer et al. 1987), by feeding on rinds of fruit, or by larval feeding on roots (Ellers-Kirk 1996). However, the most significant damage is caused by their ability to transmit plant pathogens. Diabroticites are able to harbor or transmit viruses or virus-like particles (Coudriet et al. 1979, Kim 1980), and larval feeding has been correlated with fusarium wilt (Latin and Reed 1985). The most significant pathogen transmitted by diabroticites in much of the United States is the bacterium *Erwinia tracheiphila* (E. F. Sm) Holland (Rand and Enlows 1920), which causes bacterial wilt. Recent studies have considered a range of management tools, including rhizobacteria (Zehnder et al. 1997), plastic mulch (Necibi et al. 1992, Ellers-Kirk 1996), microbial metabolites (Johnson et al. 1993, Reed et al. 1986), trap crops (Pair 1997), host plant resistance (Brust and Rane 1995), entomophagous nematodes (Reed et al. 1986, Ellers-Kirk 1996), kairomonal baits (Fleischer and Kirk 1994, Brust and Foster 1995), and imidacloprid (Fleischer et al. 1998). However, current programs rely primarily on insecticides and allow little tolerance for Diabroticite beetles because of their ability to transmit *E. tracheiphila.*

Erwinia tracheiphila is presumed to overwinter in diabroticite beetles. De Mackiewicz et al. (1998) showed that earlier serological evidence for herbaceous weeds serving as overwintering reservoirs (see Bassi 1982, Blua et al. 1994) was explained by a serological response to bacterial cells that died after introduction into nonhost plants. However, little is known about the proportion of beetles that harbor the pathogen. Caged bioassays conducted in the early 1900s resulted in very low proportions of diseased indicator plants. Doolittle (1921) captured ≈1,300 beetles before the time of planting cucurbits, and caged groups of ≈50 beetles on seedlings. He obtained symptoms from only 2 groups of 50 beetles. Rand and Cash (1920) wound-inoculated cucumber seedlings with guts from 1,643 beetles from collections spanning May through August, and obtained symptoms in only 12 plants. More recently, however, Brust (1997b) obtained higher transmission rates from caged bioassays. Transmission rate increased from ≈1% from caging overwintering beetles to 8–12% from beetles collected at the end of the season. Direct inoculation of cucurbits with *E. tracheiphila* have revealed important biological factors that would influence caged bioassays. Disease transmission frequency was increased by us-

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ing larger wounds, wounding before inoculation, and by placing inoculum on wounds within 3 h of wounding (Brust 1997a). Also, disease progression was directly influenced by inoculum dose (Lukezic et al. 1996). Brust (1997b) also showed that caging beetles for 72 h, as opposed to shorter intervals, increased the frequency of transmission.

Although caged bioassays have not shown high transmission rates, relationships exist between beetle density, behavior, and bacterial wilt incidence. Rand and Enlows (1920) concluded that the maximum numbers of "wilt-carrying" beetles together with succulent, rapidly growing vines were the principle factors influencing disease incidence. Yao et al. (1996) developed regressions of the area under the disease progress curve against beetle density soon after immigration, and showed that even low immigration densities resulted in significant disease. Brust (1997b) noted a higher probability of transmission associated with concentrated feeding, and suggested that congregation of beetles on individual plants, especially soon after immigration, is important for disease progression. However, these studies had no measure of the proportion of infective beetles and based conclusions on beetle density. Caged bioassays are also not direct measurements of whether or not a beetle is harboring the pathogen. Understanding the seasonal dynamics of the proportion of *A. vittatum* harboring *E. tracheiphila* both early in the season (from overwintering beetles, before crop planting) and throughout the growing season is an essential step in understanding the epidemiology of bacterial wilt in cucurbits. Serological assays are 1 method for estimating the proportion of biological samples harboring a microbe, and de Mackiewicz et al. (1998) describe enzyme-linked immunosorbant assays (ELISA) for detection of *E. tracheiphila.* The objectives of this study were to estimate the seasonal dynamics of the proportion of *A. vittata* harboring *E. tracheiphila.* We used ELISA to estimate the proportion of field-collected *A. vittata* harboring *E. tracheiphila*, and related the serological estimates to proportions of plants that developed wilt symptoms in single-beetle caged bioassays.

Materials and Methods

Serological Assays. Polyclonal antisera were produced against the *E. tracheiphila* type strain (ATCC 33245) in female New Zealand white rabbits, as described in de Mackiewicz et al. 1998. The final concentration of the IgG component of the collected serum was ≈12 mg/ml for each treatment. Double antibody sandwich ELISA (DAS-ELISA) were conducted according to Clark and Bar-Joseph (1984) and McLaughlin and Chen (1990) with the trapping and conjugated antibodies at a dilution of 1:400. The antisera was validated against field-collected *E. tracheiphila* (see de Mackiewicz et al. 1998). The antisera was not cross-reactive against similar bacteria (*E. amylovora, E. carotovora* pv. *carotovora, E. herbicola* [=*Pantoea agglomerans*] and *E. stewartii* [=*Pantoea stewartii* subsp. *stewartii*] with DAS-ELISA at $\approx 10^6$ colony forming units (cfu) (de Mackiewicz et al. 1998). Results of the ELISAs were determined by measuring absorbance of each well at 405 nm with a plate reader (Dynatec, Chantilly, VA) 30 min after adding the substrate to the wells. To estimate the number of bacterial cells associated with the ELISA absorbance values, we made serial dilutions from *E. tracheiphila* cultured in broth media (10 g nutrient broth and 5 g Bacto-peptone per liter of water, Difco, Detroit, MI) for 72 h at 28 ± 1°C. Each dilution was streaked on nutrient agar plates supplemented with extra agar and peptone (NAP plates) (23 g Difco nutrient agar, 5 g Difco agar, and 5 g Difco Bacto-peptone per liter water). After an additional 72 h at 28 ± 1°C, the number of cells per dilution was estimated by counting the number of single colonies on the agar plates, and the absorbance value from the broth culture determined with ELISA. The relationship between ELISA absorbance values and number of cfu was estimated with nonlinear regression using JMP software (SAS Institute 1995).

Assays with *A. vittata.* Before the time of planting of cucurbits in central Pennsylvania, adult *A. vittata* were collected using both emergence traps and squash traps. Emergence traps were used to collect *A. vittata* in 1995 and 1997 as they emerged from the soil in fields planted to muskmelon the previous year at the Russell E. Larson Research Farm, Rock Springs, PA. All vegetation was removed from under the emergence traps, and these beetles had no access to any plant food source after emergence. In 1995, 27 emergence cages (0.9 by 0.9 m) and 3 walk-in screen cages (1.8 by 3.6 m) were used; and in 1997, 50 emergence cages (0.9 by 0.9 m) were used. Cages were placed in mid-April, and monitored every other day or daily until curcurbits were growing in nearby fields. Squash traps were also used as a means of increasing the sample size of beetles collected before the growing season. A continuous supply of squash ('Hubbard') was grown in 3.8-liter pots in the greenhouse until it was producing flowers, or started in 64-cell flats until the cotyledon stage. Pots of flowering squash or flats of cotyledon-stage squash were placed in fields in Center County, PA, monitored daily, and replaced frequently because of nightly frosts. Beetles collected from the emergence cages or aspirated from the squash traps were frozen. ELISA analyses were conducted on subsamples of these early-season beetles.

During the growing season, adult *A. vittata* were collected from cucurbits at the Russell E. Larson Research Farm, Rock Springs, PA, for both caged bioassays and for ELISA assays. Eighty-six *A. vittata* beetles were collected randomly from a muskmelon ('Cordell') field every other week during the summers of 1995 and 1996, and from a cucumber field in 1997, because of the availability of the cucurbit crops and beetles on the farm. The caged bioassays were conducted following Brust 1997b. For these, 50 of the field-collected beetles were individually caged using plastic tubes (10 cm diameter by 11 cm tall) with screen tops placed over 3-wk-old muskmelon ('Superstar', a susceptible variety [Brust and Rane 1995])

seedlings grown in 11-cm-diameter plastic pots. After 72 h the beetles and cages were removed from the muskmelon seedlings, and the melons were monitored for bacterial wilt symptoms for 3 wk. The remaining 36 field-collected beetles were frozen at −18°C and later tested with DAS-ELISA to determine if they were serologically positive for *E. tracheiphila.* For both the overwintered and in-season beetles, 5 negative control beetles and 5 positive control beetles were included in each immunoassay. The negative control beetles were collected from a colony maintained in the Entomology Department, Pennsylvania State University, with annual introductions of eggs from field-collected beetles (see Ellers-Kirk 1996 for rearing methods). The positive control beetles were colony beetles caged with *E. tracheiphila* smeared between two 4-mm cucumber ('Flurry') cotyledon disks for 48 h. The *E. tracheiphila* was the type strain 33245 (American Type Culture Collection, Rockville, MD) maintained by biweekly subculturing on Difco nutrient agar supplemented with extra agar and peptone (NAP plates). All samples were tested in duplicate wells and the mean from the 2 tests used for analysis.

Analyses. The negative control beetles for each year were used to estimate a threshold by taking the mean ELISA absorbance value plus 3 standard deviations. The probability distribution of the ELISA absorbance values were graphed for the beetles from the laboratory colony (the negative controls) and for the beetles from the colony that had been caged with cotyledons smeared with *E. tracheiphila* (the positive controls) as a visual check on the ability of the estimated threshold to discriminate between these 2 groups. Using the threshold, field-collected beetles were classified into those harboring a sufficient titer to be serologically positive and those with a titer insufficient to distinguish from a laboratory colony. The proportion of field-collected beetles that had ELISA absorbance values greater than the threshold was graphed over time, and overlaid onto the proportion of bioassay plants that developed wilt symptoms in single-beetle caged bioassays. The functional relationship between these proportions was estimated with regression, and an analysis of heterogeneity of slope was used to determine if the relationship was consistent among years, using JMP software (SAS Institute 1995). Voucher specimens were placed in the Frost Entomology Museum, Pennsylvania State University.

Results

The distribution of ELISA absorbance values determined from laboratory colony beetles, which served as our negative control, was skewed toward low values (Fig. 1). Thresholds, above which a beetle was defined as harboring *E. tracheiphila* cells, were estimated as the mean plus 3 times the standard deviation of these values. Thresholds were 0.183, 0.171, and 0.150 for work conducted with beetles collected in 1995, 1996, and 1997, respectively. Using these thresholds, the observed probability of incorrectly classifying a laboratory beetle (a negative control) as harboring *E. tracheiphila* was 0.00, 0.03, and 0.00 for 1995, 1996, and 1997, respectively. Laboratory reared beetles that had been caged on cotyledons smeared with *E. tracheiphila* for 24 h were used as positive controls. The frequency distribution of ELISA absorbance values from these laboratory-reared positive controls were much wider, and the observed error rates higher (0.44, 0.14, and 0.40 in 1995, 1996, and 1997, respectively; Fig. 1). Both feeding behavior and insensitivity of the ELISA at low titers may have contributed to these error rates (see *Discussion*). Sensitivity of the ELISA at varying concentrations of *E. tracheiphila* cells followed an exponential increase (Fig. 2) modeled as $y = 0.000068\ (\pm 0.00001)\ e^{[1.4388\ (\pm\ 0.024)\ \log_{10}\ \mathrm{dilution}]}$, where y is the ELISA absorbance value and dilution is the $\log_{10}$ exponent of the cfu per milliliter. Using this model, the absorbance values we used as thresholds correspond to $\approx 10^{5.49}$, $10^{5.44}$, and $10^{5.35}$ cfu of *E. tracheiphila* per mil-

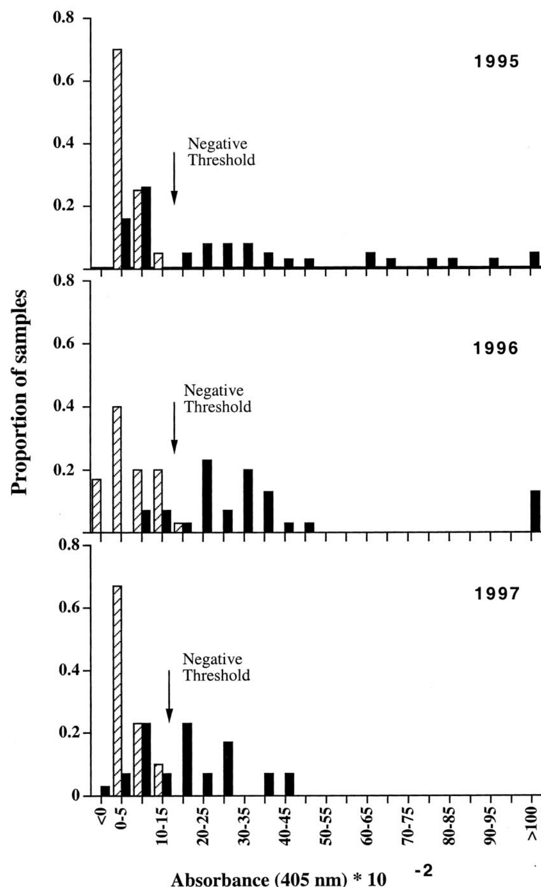

Fig. 1. Probability distributions of ELISA absorbance values from *A. vittata* reared in the laboratory (negative controls, hashed bar), and *A. vittata* reared in the laboratory and caged with *E. tracheiphila* smeared on cucurbit cotyledons (positive controls, closed bar). Data are presented for 1995 (n = 40 negative controls, 39 positive controls), 1996 (n = 30 negative controls, 30 positive controls), and 1997 (n = 30 negative controls, 30 positive controls). Arrow indicates the threshold, calculated as the mean ± 3 * SD from the negative controls, used to classify field collected beetles.

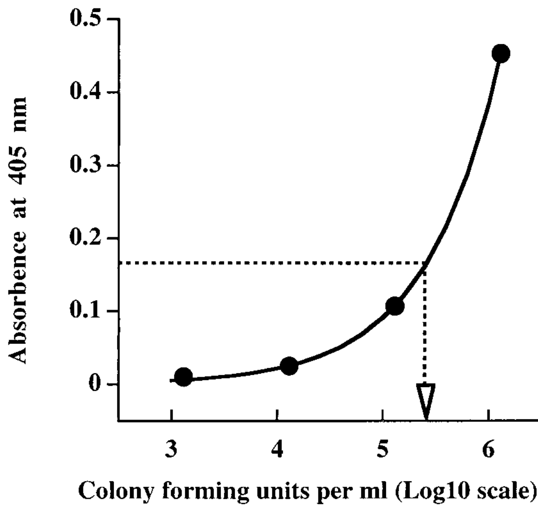

Fig. 2. Dose-response of polyclonal ELISA for *E. tracheiphila.* Solid line indicates modeled fit, $y = 0.000068\ e^{(1.4388 * \log_{10} \text{dilution})}$, where y is the ELISA absorbance value and dilution is the $\log_{10}$ exponent of the cfu/ml. Dashed line indicates threshold absorbance values described in Fig. 1.

liliter. Our methods used 1 ml per beetle, therefore our thresholds can be viewed as estimating when a single beetle was harboring $>10^5$ cfu of *E. tracheiphila.*

From 7.1–10.7% of *A. vittata* captured as they emerged from soil that had been in cucurbits the previous year tested positive for the presence of *E. tracheiphila,* even with these fairly stringent thresholds (Table 1). The area under the emergence cages was kept clear of any above-ground vegetation, and beetles collected from the emergence cages had no access to any plants as food. This provides strong serological support for *A. vittata* serving as an overwintering reservoir for *E. tracheiphila.* It was difficult to capture a large sample size using emergence traps, and squash traps were used to increase the numbers of *A. vittata* collected early in the season. From 0 to 8.3% of *A. vittata* collected on squash traps during the same approximate time as they emerged from the soil tested positive for the presence of *E. tracheiphila* (Table 1).

The seasonal dynamics of field-collected beetles that tested positive for *E. tracheiphila* with DAS-ELISA during the growing season varied among the 3 yr. In 1995, 8.3% of the beetles tested positive on the 1st sampling date (2 June), ≈1 wk after transplanting, and rose quickly to 52.8% by 13 June (Fig. 3, top). These percentages remained high into early and mid-July (52.8% on 4 July, and 33.3% on 16 July), and declined throughout August and early September. In 1996, there also was a unimodal peak in the seasonal dynamics, although the time of high percentages was shifted ≈2–3 wk later. Values were 4.4% on 2 July, the 1st sampling date, rose steadily throughout July (61.9% on 16 July and 77.7% on 30 July), and then declined through August and early September (Fig. 3, middle). The seasonal dynamics in 1997 also had relatively high values during July, but differed from the previous 2 yr because of a high estimate early in the season, resulting in a bimodal pattern (Fig. 3, bottom). In 1997, 36.1% of the field-collected beetles tested positive with ELISA on 27 May, which was during the week most transplanting of cucumbers and melons was occurring. Percentages were lower during June (13.9, 11.1, and 11.1 on 1, 11, and 24 June, respectively), rose again in early July (38.9% on 8 July), before beginning

Table 1. Percentage of *A. vittata* collected early in the field season that tested positive for *E. tracheiphila* with ELISA

Trap type	Year	Collection date	n	% Positive
Emergence cages	1995	25 April–1 June	14	7.1
	1997	27 April–13 June	28	10.7
Squash traps	1995	22 April	36	8.3
		1 June	36	5.5
	1996	26 April–8 June	30	0
		9 June–20 June	24	0
	1997	22 April	36	0
		29 April	36	0
		10 June	36	5.5

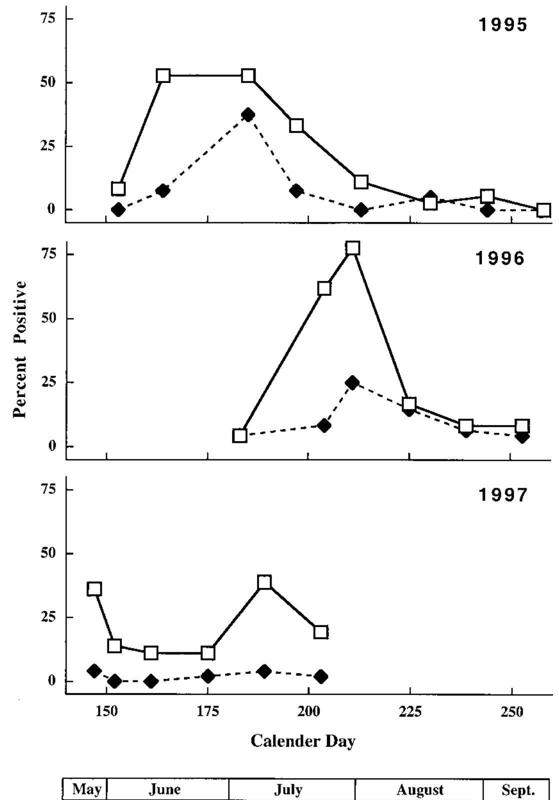

Fig. 3. Seasonal dynamics of the percentage of field-collected *A. vittata* ($n = 36$) that tested positive with ELISA for *E. tracheiphila* (open square), and the percentage of seedling muskmelons ($n = 50$) that developed wilt symptoms within 3 wk after being caged individually with field-collected beetles for 72 h (closed diamond). Data are from 1995 (top), 1996 (middle), and 1997 (bottom).

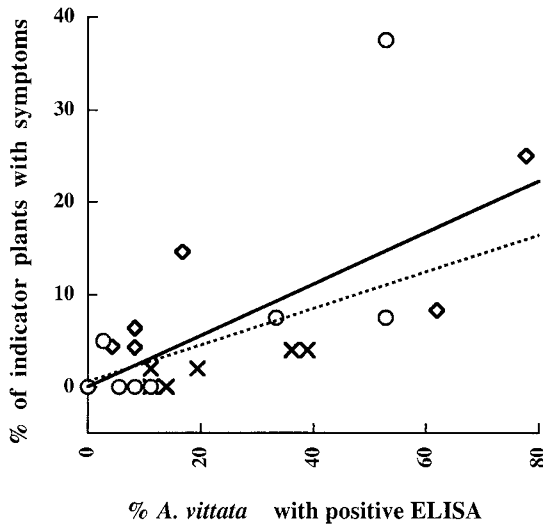

Fig. 4. Relationship estimating the percentage of seedling muskmelon that developed wilt symptoms within 3 wk after being caged individually with field-collected beetles for 72 h as a linear function of the percentage of field-collected *A. vittata* that tested positive with ELISA for *E. tracheiphila*. Data are for 1995 (circle), 1996 (diamond), and 1997 (×). Solid line ($y = 0.044 + 0.278x$) shows a fit to all data, and the dashed line ($y = 0.621 + 0.197x$) shows a fit with 1 outlier (with 37.5% wilted indicator plants) deleted.

the decline in late July (19.4% on 22 July). Samples were not taken in August and September in 1997.

The seasonal dynamics of muskmelon seedlings that developed bacterial wilt symptoms in single-beetle caged bioassays followed the same pattern as that observed with serological tests of individual beetles, but with lower peak values when the serological tests were high (Fig. 3). Values ranged from 0 to 37.5% in 1995, 4.3–25% in 1996, and 0–4% in 1997. There was no significant difference among years in the relationship expressing the percent of diseased indicator plants as a function of the percent of beetles testing positive with ELISA ($F = 0.79$; df = 2, 19; $P = 0.48$ for testing the effect of year, and $F = 1.31$; df = 2, 19; $P = 0.30$ for testing the interaction of year by percent of beetles testing positive with ELISA, in a test of heterogeneity of slope). Therefore, we pooled data among years. The percent of beetles testing positive with ELISA explained 44% of the variation in the percent of indicator plants that developed disease, and 49% of this variation when 1 outlier was deleted (Fig. 4). The regression using all the data were $y = 0.044\ (\pm 2.40) + 0.278\ (\pm 0.07)\ x$, where y is the percentage of indicator plants that developed bacterial wilt, and x is the percentage of beetles that tested positive with ELISA. When the outlier was deleted, the regression was $y = 0.621\ (\pm 1.50) + 0.197\ (\pm 0.05)\ x$. In both cases, the intercept was not significantly different than zero ($t = 0.02$; df = 1, 19; $P = 0.98$ when using all data, and $t = 0.42$; df = 1, 18; $P = 0.68$ when the outlier was deleted), and the slope was significant ($t = 3.74$; df = 1, 19; $P = 0.0015$ when using all data, and $t = 4.07$; df = 1, 18; $P = 0.0008$ when the outlier was deleted). If we assume the intercept to be negligible, then the inverse of the slope suggests that, on average, the percent of beetles that harbored sufficient *E. tracheiphila* cells to be considered positive ($\approx 10^5$ cfu/ml) was 3.6–5.1 times higher than the percent of beetles that resulted in diseased indicator plants in caged bioassays.

Discussion

Absorbance values determined from the laboratory beetles were used to estimate a threshold for determining when beetles were harboring *E. tracheiphila*, which assumes that ELISA values from beetles reared in a laboratory reflect what occurs with field-collected beetles. It was not possible to use field-collected beetles as a negative control, because it was not possible to know whether any given field-collected beetle harbored *E. tracheiphila* cells. The distribution was not Gaussian, and we did not assume a predicted error rate. Our use of the mean plus 3 standard deviations as a threshold was empirical, designed to minimize the proportion of beetles being incorrectly classified as positive (i.e., to minimize the probability of false positives). The observed error rate (the proportion of laboratory beetles classified above the threshold) was very low (<0.00–0.03 for any of the 3 yr; Fig. 1). However, our observed error rate from classifying laboratory beetles caged with cotyledons smeared with *E. tracheiphila* as below threshold (incorrectly classifying positives as negatives, or false negatives) was higher (0.14–0.44; Fig. 1). This error may have been caused by beetles not fully consuming the *E. tracheiphila* sandwiches, resulting in a low titer of cells (see below). This suggests that our error rates were not the same: we had a greater probability of incorrectly classifying a true positive as a negative (false negative) than incorrectly classifying a true negative as a positive (false positive).

Several mechanisms can lead to errors with polyclonal serological tests. As with any work with polyclonal antibodies, 1 potential error is classifying a negative as a positive because of cross-reactivity. Our ELISA did not cross-react with similar bacteria, including other *Erwinia* spp. (de Mackiewicz et al. 1998). Also, ELISA estimates of the proportion of *A. vittata* harboring *E. tracheiphila* generally tracked rates of diseased plants from caged bioassays. It is more probable that the ELISA was tracking *E. tracheiphila* as opposed to another cross-reactive bacteria that occurred with the same seasonal trend as observed in the caged bioassays. However, a wide range of bacteria is associated with the gut of diabroticites (Schalk et al. 1987). Our antibody, or the substrates and enzymes used in the ELISA, can produce titer-dependent positive responses to a few (between 1 and 3) other currently unidentified bacterial isolates from the alimentary canal of *A. vittata* (Garcia-Salazar et al., unpublished data). Our threshold minimized this source of error (the false positives caused by cross reactivity), because the ELISA absorbance values from negative control beetles also reflected cross-reactivity associ-

ated with beetles, and our threshold required an absorbance value 3 standard deviations above the negative controls. A 2nd source of error comes from the fact that the ELISA reaction is influenced by the concentration of bacterial cells, and our thresholds classified a beetle as positive only when the *E. tracheiphila* titer exceeded $\approx 10^5$ cfu per beetle (Fig. 2). Presumably, some field-collected beetles and some of the beetles caged were classified as negative when they harbored *E. tracheiphila* at lower titers, resulting in false negatives as a result of low titers. We feel our errors were more likely the result of low titers (resulting in false negatives) than to cross-reactivity (resulting in false positives). Thus, we feel our estimates of the proportion of *A. vittata* that are harboring any *E. tracheiphila* cells are low. Development of further tools with monoclonal antibodies or tests based on DNA may provide more accurate and sensitive estimates in the future.

The serological data from *A. vittata* collected in emergence cages strongly support the contention that the beetles serve as an overwintering reservoir. From 7.1 to 10.7% of the *A. vittata* collected as they emerged in the spring and with no access to plant sources of inoculum tested positive in these assays (Table 1), suggesting that these beetles harbored approximately $>10^5$ cfu of *E. tracheiphila* per beetle. This is supporting evidence that *E. tracheiphila* in *A. vittata* collected from emergence cages precludes the possibility of the beetles acquiring the pathogen from feeding on nonsymptomatic reservoirs early in the spring. Although we used large numbers of emergence cages, the sample size of beetles from emergence cages was low. Collections from squash traps increased the sample size, and may be more reflective of *A. vittata* that immigrate into young plantings. The serological evidence suggests positive but variable rates of *A. vittata* (from 0 to 8.3%) that harbor approximately $>10^5$ cfu in late May and early June in central Pennsylvania. If all collections are combined, 3.0% of the *A. vittata* immigrating into squash traps tested positive with this serological assay. Herbaceous weeds are not ecologically important overwintering reservoirs of *E. tracheiphila* in central Pennsylvania (de Mackiewicz et al. 1998), and the data presented here suggest that the beetles are the primary overwintering reservoir in central Pennsylvania.

The proportion of beetles that harbored *E. tracheiphila* estimated with ELISA showed a seasonal trend that generally followed the seasonal trend in the transmission of wilt in single beetle caged bioassays (Fig. 3), and explained 44–49% of the variation in percent of indicator plants that showed disease symptoms in caged bioassays (Fig. 4). In 2 of 3 yr, there was a unimodal rise to a proportion that was >50%, followed by a decline in the later part of the season (Fig. 3, top and middle). In 1 yr, there was a bimodal pattern over time as a result of an addition peak very early in the growing season (Fig. 3, bottom). The crop host may have been a factor, because the unimodal pattern came from beetles collected on muskmelon, and the bimodal pattern from beetles collected on cucumber. Brust (1997b) also showed dramatic seasonal variation in the proportion of diseased indicator plants in caged bioassays, but his pattern was a continuous, perhaps exponential, increase with time, with rates of ≈1% early in the season rising to 8–12% at the end of the season. Our rates of diseased indicator plants in caged bioassay (0–37.5%) bracket those of Brust (1997b), but our pattern included a decline toward the end of the growing season. Together, these results suggest that dramatic seasonal variation occur in both the proportion of *A. vittata* that harbor *E. tracheiphila*, and the probability of disease transmission by single beetles. Further work is needed to determine if there is any consistency to the pattern with time, or if there is geographic variation or variation related to crop host in this pattern.

As discussed above, polyclonal serological estimates of the proportion of *A. vittata* harboring *E. tracheiphila* are subject to error. Single beetle caged bioassays also do not completely reflect the probability of disease transmission because expression of bacterial wilt is strongly influenced by dose (Lukezic et al. 1996) and factors related to the wound that allows access to vascular tissue (Brust 1997a). Although both estimates are subject to error, both are valuable tools to infer information about epidemiology. On average, the proportion of beetles that tested positive with ELISA was 3.6–5.1 times higher than the proportion of indicator plants that developed bacterial wilt in single beetle bioassays (based on the inverse of the slope in Fig. 4 and assuming that the intercept is negligible). If the data include more false negatives than false positives, the proportion of beetles that harbor at least some *E. tracheiphila* cells may be >5 times higher than the proportion of beetles that can, alone, cause disease in a short time. This has important implications about understanding the epidemiology of bacterial wilt in cucurbits, and about disease management. Brust (1997a) observed that disease was more common on plants with concentrated feeding, which he suggests is related to adult aggregation on few leaves soon after immigration. Our data support this idea, by suggesting that a higher proportion of beetles harbor the pathogen than are able to, individually, transmit disease. Although we cannot use our data to estimate the dose needed to cause disease, or to estimate bacterial titer in frass, we suggest that beetle aggregation serves, in part, to deliver a sufficient dose by concentrating frass from multiple beetles at young feeding wounds (it may also serve to ensure wounding before delivery of inoculum, and wounds of sufficient size). Also, only low numbers of adults harbored the pathogen or caused disease in caged bioassays early in the season, suggesting that low numbers of plants would be infected early in the season. These infected plants could serve as inoculum sources. Beetle aggregation on infected plants would help ensure pathogen acquisition, and Haynes and Jones (1975) found increased feeding on infected plants with cucumber cultivars carrying the bitter gene. Thus, the serological estimates of the proportion of beetles harboring *E. tracheiphila* supports suggestions that adult aggregation behavior is

important for both pathogen acquisition and transmission, and thus for disease progression within fields. Further work should consider more sensitive and accurate methods for determining the proportion of the beetles that harbor the pathogen, and the way in which beetle density and behavior interact to influence epidemiology of bacterial wilt.

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