Quantifying the Feeding Periods Required by Corn Flea Beetles to Acquire and Transmit *Pantoea stewartii*

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ABSTRACT

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The feeding periods required by corn flea beetles to acquire and transmit *Pantoea stewartii* were investigated in the Stewart's disease of corn pathosystem. To quantify the effect of acquisition feeding period on percentage of acquisition, field-collected corn beetles were allowed to feed for 6, 12, 24, 36, 48, and 72 h on corn seedlings previously inoculated with a rifampicin- and nalidixic acid-restraint strain of *P. stewartii*. Acquisition of *P. stewartii* by corn flea beetles was considered positive if the rifampicin- and nalidixic acid-marked strain was recovered on selective media. To quantify the effect of transmission feeding period on percent transmission of *P. stewartii* by corn flea beetles, *P. stewartii*-infested corn flea beetles were allowed to feed on healthy corn seedlings for periods of 3, 6, 12, 24, 36, 48, and 72 h. After the appropriate transmission feeding period, leaf tissues surrounding the sites of feeding scars were cultured for the presence of the *P. stewartii*-marked strain. Transmission of *P. stewartii* was considered positive if the marked strain was recovered on selective media. Acquisition of *P. stewartii* occurred within 6 h and the percentage of corn flea beetles that had acquired *P. stewartii* after 72 h ranged from 68 to 94%. The change in *P. stewartii* acquisition by corn flea beetles (Y) with respect to acquisition feeding period (X) was best described by the Gompertz model, with R^2 values ranging from 91 to 99%. The mean time for acquisition by 50% of the corn flea beetles was 36.5 ± 11.6 h. The minimum transmission feeding time required for corn flea beetles to transmit *P. stewartii* following a 48-h acquisition feeding period was less than 3 h. The percent transmission of *P. stewartii* by corn flea beetles was nearly 100% after a 48-h transmission feeding period and was 100% by 72 h. Among population growth models evaluated, the monomolecular model best described the relationship between percent transmission (Y) and transmission feeding periods (X), with R^2 values of up to 84%. However, a nonlinear form of the monomolecular model better quantified the relationship between percent transmission and transmission feeding period, because pseudo- R^2 values ranged between 98.1 and 99.5%. The predicted transmission feeding time required for 50% of *P. stewartii*-infested corn flea beetles to transmit the pathogen was 7.6 ± 0.87 h. These results suggest that the corn flea beetle is a highly efficient vector that can quickly acquire and transmit *P. stewartii*, thereby requiring insecticide seed treatments and foliar insecticides that act quickly to prevent corn flea beetles from acquiring and transmitting *P. stewartii* to corn plants.

Additional keywords: *Chaetocnema pulicaria*

Stewart's disease of corn, caused by *Pantoea (Erwinia) stewartii* subsp. *stewartii* (Smith) Dye (11,17,29,31), has significant economic implications for sweet corn and seed corn producers in the U.S. Corn Belt (21). Phytosanitary regulations are in place to prevent the introduction of *P. stewartii* into countries where this pathogen has not been reported to occur (1). The bacterium is a nonmotile, non-spore-forming, capsule-forming gram-negative rod that is approximately 0.4 to 0.8 μ m by

0.9 to 2.2 μ m in size (22,23,29). *P. stewartii* is an insect-borne pathogen. The corn flea beetle *Chaetocnema pulicaria* Mel-sheimer (order: Coleoptera, family: Chrysomelidae) is the primary vector of *P. stewartii*. Corn flea beetles are approximately 1.8 mm in size and typically are shiny and black with enlarged hind femurs (7,22).

In addition to being an insect-borne pathogen, another potential source of inoculum is seed transmission. Early reports suggested that seed transmission played an important role in the occurrence of Stewart's disease (24,30). However, recent studies by Michener et al. (18), Block et al. (2–4), and Khan et al. (12) all have shown that the risk of seed-to-seedling transmission of *P. stewartii* is extremely low. In their study, Khan et al. (12) grew 75,000 plants from seed that was harvested from *P. stewartii*-

infected plants and did not find any seed-to-seedling transmission of *P. stewartii* in the test plants; however, they did not determine the actual percentage of *P. stewartii*-infected (or infested) seed. Both Michener et al. (18) and Block et al. (2–4), however, adjusted their estimates of seed-to-seedling transmission to account for the percentage of infected seed in a seedlot. Michener et al. (18) reported that seed-to-seedling transmission was 0.038% after testing 58,300 kernels obtained from infected plants. Studies by Block et al. (2–4) revealed only 0.14% seed-to-seedling transmission of *P. stewartii* from infected kernels harvested from artificially inoculated plants (29 of 19,735 seed), and 0.022% (1 of 4,563) seed-to-seedling transmission from *P. stewartii*-infected kernels harvested from naturally infected plants (3).

After conducting several field experiments during the 1920s in Maryland and Maine using multiple *P. stewartii*-infected seed lots, Rand and Cash (25,26) were convinced that the onset and secondary spread of *P. stewartii* in the field suggested the involvement of an insect vector. Later, the corn flea beetle (*C. pulicaria*) was shown to be the primary vector of *P. stewartii* in the Stewart's disease of corn pathosystem (8). In spite of the fact that the corn flea beetle was shown to be the principal vector of *P. stewartii* as early as 1934, little is actually known about the feeding times required for successful acquisition and transmission of the pathogen. This knowledge not only would help to improve our understanding of the biology of this pathosystem, but also may help in developing more resistant genotypes or insecticide programs to better manage corn flea beetle populations by preventing the acquisition and transmission of *P. stewartii*. The objectives of this study were to quantify the feeding periods required by the corn flea beetles for both acquisition and transmission of *P. stewartii*.

MATERIALS AND METHODS

The susceptible sweet corn cv. Jubilee (Syngenta Seeds Inc., Boise, ID) was planted in the greenhouse in 15-cm-diameter plastic pots containing a steam-pasteurized 1:2:1 mixture of peat, perlite, and soil. Two seed were planted per pot and thinned to one plant per pot after

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emergence. The plants were fertilized weekly with a solution of 21-5-20 (N-P-K) fertilizer (200 ppm N; Miracle-Gro, Marysville, OH).

Inoculum preparation and plant inoculation. *P. stewartii* isolate ES Rif-9A was obtained from Charles B. Block, United States Department of Agriculture (USDA) Plant Introduction Station, Iowa State University, Ames. This isolate is a rifampicin- and nalidixic acid-resistant strain derived from a wild-type strain of *P. stewartii* (SS104) (14). Stock cultures of this isolate were stored at -75°C in 15% glycerol and, before experiments began, this strain was twice inoculated and recovered from sweet corn cv, Stowell's Evergreen, which is moderately resistant to *P. stewartii*. Studies by Lincoln (15,16) have shown that *P. stewartii* strains that are inoculated repeatedly and recovered from resistant plants maintain their aggressiveness better than strains passed through susceptible plants. Isolate stability was confirmed by serial transfers onto nonselective media and by plant inoculation and re-isolation. No evidence of back mutation was observed (2). Prior to inoculation, cultures were incubated for 48 h at 25°C on nutrient broth yeast extract agar (NBY) amended with cycloheximide (100 $\mu\text{g/ml}$), rifampicin (50 $\mu\text{g/ml}$), and nalidixic acid (40 $\mu\text{g/ml}$ (NBY-CRN). Inoculum concentrations were adjusted to approximately 1×10^8 CFU/ml in normal saline solution (0.85% NaCl). At corn growth stage V5 (fifth-leaf stage), the second youngest leaf of each corn seedling was inoculated with *P. stewartii* (ES Rif-9A) using the pinprick method (2,32) and plants were kept at a temperature between 18 and 21°C . All plants were reinoculated 5 days later using the same procedure.

Collection of corn flea beetles. For both the acquisition and transmission feeding period experiments, bulk samples of corn flea beetles were collected using a 38.1-cm-diameter sweep net (Gempler's, Belleville, WI). Each bulk sample consisted of 30 to 60 sweeps within a corn plant canopy. Samples of field-collected corn flea beetles were separated from debris and other insects that were collected during field sampling by emptying the contents of a sample bag into a 5.6-liter plastic pan that contained approximately 3 liters of tap water. Individual corn flea beetles then were collected using a moistened, small-bristle paintbrush and transferred to insect cages as described below.

Acquisition experiments. For the acquisition experiments, there were six treatments (duration of the acquisition feeding period) and 45 replications or beetles per treatment. The acquisition treatment periods were 3, 6, 12, 24, 36, 48, and 72 h. After Stewart's disease symptoms were present on the inoculated leaves (7 to 9 days after inoculation), one field-collected corn flea beetle was placed in a

cage consisting of a 2.5-cm-diameter acrylic hollow rod that was approximately 2.5 cm long. The top end of the hollow transparent rod was sealed with a polyester mesh (32 by 32 per 2.5 cm) and a circular sheet of armaflex insulation (3.55 cm in diameter) was placed on the opposite end. This piece was affixed with two 1.25-cm nails.

To quantify the relationship between acquisition feeding time and percent acquisition of *P. stewartii* by corn flea beetles, one corn flea beetle per cage was placed on a diseased corn leaf at the edge of *P. stewartii* lesions. A diseased leaf was one in which lesions typical of *P. stewartii* were observed along the leaf veins. Corn flea beetles were allowed to feed for the appropriate amount of time (treatments), then removed from the diseased seedlings. Upon removal, corn flea beetles were individually ground in 300 μl of phosphate-buffered saline Tween buffer (PBST; 8.0 g of NaCl, 1.15 g of Na_2HPO_4 , 0.2 g of KH_2PO_4 , 0.2 g of KCL, 0.5 g of Tween 20, and 1,000 ml of dH_2O , pH 7.4) in an autoclaved microcentrifuge tube. Each beetle suspension (100 μl) was spread onto duplicate plates of NBY-CRN agar to isolate the rifampicin-nalidixic acid-resistant isolate of *P. stewartii*. The bacterial cultures then were placed in an incubator at 25°C . After 48 h, the plates were examined for colonies typical of *P. stewartii*. Acquisition of *P. stewartii* by individual corn flea beetles was considered positive if the ES Rif9A strain was recovered from the NBY-CRN media. Pathogenicity tests were conducted to confirm that the colonies were *P. stewartii*. This was done by inoculating corn seedlings with individual isolates at corn growth stage V2 (second-leaf stage) using 1×10^8 CFU/ml in normal saline solution (0.85% NaCl). Plants were observed for symptoms of Stewart's disease after 8 days. The acquisition experiments were performed three times.

Transmission experiments. Based on the results obtained from the acquisition study, the transmission study was conducted using a single acquisition feeding period of 48 h. The 48-h acquisition feeding period was chosen because more than 50% of the corn flea beetles (63.3%) were predicted to have acquired *P. stewartii* after a 48-h acquisition feeding period (based on the acquisition experiments). In all, 270 corn flea beetles were placed individually in cages and allowed to feed for 48 h on diseased plants. Caged beetles then were transferred to healthy plants at corn growth stage V3 (third-leaf stage) and allowed transmission feeding periods of 3, 6, 12, 24, 36, 48, or 72 h. Thirty beetles were tested individually for each feeding period. During the course of each experiment, a small number of corn flea beetles did not survive until the end of their respective transmission feeding periods; therefore, the corn seedlings receiving

these beetles were omitted from the analysis. After the appropriate transmission feeding period, surviving beetles were removed and placed individually in sterile microcentrifuge tubes and ground in PBST buffer. Of the ground suspension, 100 μl was placed onto NBY-CRN to determine if the corn flea beetles had acquired the rifampicin- and nalidixic acid-resistant strain of *P. stewartii*. The proportion of successful transmissions was operationally defined on the number of plants testing positive for *P. stewartii* divided by the number of corn flea beetles that had successfully acquired the bacterium within 48 h. Thus, plants receiving corn flea beetles that had not acquired *P. stewartii* after the 48-h acquisition feeding period were deleted from the analysis. This standardized value for pathogen transmission was used for modeling purposes.

After removal of the cages, corn leaves were examined carefully for corn flea beetle feeding scars. The leaves that had been exposed to feeding were sampled 7 days later by taking 0.3 g of leaf tissue in the area surrounding and including each feeding scar and grinding it individually in 800 μl of $1 \times$ PBST buffer (based on the sample weight). Leaf sap (200 μl) obtained from the ground leaf samples was plated onto the NBY-CRN selective medium. Percent transmission of *P. stewartii* was operationally defined as the number of corn leaves that tested positive for *P. stewartii* based on recovery of the Rif9A strain from the selective media, divided by the total number of corn seedlings that had a corresponding live corn flea beetle that tested positive for the NBY-CRN-marked strain. Pathogenicity tests were conducted with these isolates as described previously. The transmission experiments were performed a total of three times.

Data analysis. Data from both the acquisition and transmission experiments for specific variables (e.g., percent acquisition at 6 h, time to 50% acquisition, time to 50% transmission, percentage acquisition or transmission at 72 h, and so on) were analyzed using analysis of variance and mean separations were performed using the Waller-Duncan K-ratio test ($P \leq 0.05$; Statistical Analysis System, SAS Institute, Gary, NC).

To select the most appropriate modeling approach, percent acquisition of *P. stewartii* by corn flea beetles was plotted with respect to acquisition feeding period for each acquisition experiment, and percent transmission of *P. stewartii* by corn flea beetles was plotted with respect to transmission feeding period for each transmission experiment (Sigma Plot; SPSS INC, Chicago, IL). Next, the change in percent acquisition (or transmission) (dy) with respect to the change in time (dt) was each plotted with respect to time (t) for each experiment. Based upon the shapes of the percentage of acquisition or transmission

curves and their respective rate curves (dy/dt versus t), one or more population growth models were selected and evaluated for goodness of fit (6,33). To accomplish this, pathogen acquisition and transmission data first were converted to proportions and then transformed using linearized models (logistic, linear, Gompertz, monomolecular, or logarithmic) that appeared to best fit these data (6,13,20). The criteria used to select the best linearized model were (i) the F test for each overall model, (ii) the coefficient of determination (R^2), (iii) the standard error of the estimate for y (SE E_y), and (iv) the T statistic for the slope (33). After selecting the most appropriate population growth model and transforming pathogen acquisition and transmission data, linear regression was performed for each experiment to estimate the intercepts and rates (slopes) for pathogen acquisition (or transmission) with respect to time, as well as to calculate regression statistics (13,20,33). The resulting linear models from both the acquisition and transmission experiments were used to calculate the time to 50% acquisition or transmission by rearranging each equation to solve for the time (in hours) that 50% of corn flea beetles had acquired or transmitted the pathogen.

RESULTS

Acquisition experiments. The earliest measured acquisition of *P. stewartii* from diseased corn plants by corn flea beetles occurred the first within 3 to 6 h of feeding. The percentage of corn flea beetles that acquired *P. stewartii* after a 6-h acquisition access period ranged from 0 to 26.7%. (Fig. 1A). Acquisition increased at the fastest rate from 12 to 36 h, and the rate of acquisition then decreased between 36 and 72 h (Fig. 1A). After 72 h, the percentage of corn flea beetles that had acquired *P. stewartii* ranged from 68 to 94% (Fig. 1A).

Based upon the shape of the acquisition (Fig. 1A) and rate curves (Fig. 1B), the Gompertz model and the logistic population growth models were selected for further consideration to develop a linearized population growth model that best fit the acquisition experiments (6,20). Based upon model selection criteria (F statistic, R^2 , and so on), the linearized form of the Gompertz model was found to best fit the three acquisition experiments, with acquisition feeding period (X) explaining 91 to 99% (R^2) of the variation in percentage of acquisition (Y). The slopes (rates of acquisition) relating percent acquisition of *P. stewartii* by corn flea beetles to acquisition feeding period for the three experiments were 0.043, 0.058, and 0.022 gompits/h, respectively, which equates to an approximate rate of 1 to 2% acquisition per hour over the 72-h acquisition feeding period (with rates increasing fastest up to 37% acquisition and subsequently slowing after

37% of the corn flea beetles had acquired the pathogen; Fig. 1C). The three experiments could not be combined into a single model because there were significant differences ($P \leq 0.05$) among the slopes for the three individual experiments. The SE E_y values for the three linearized acquisition curves were 0.38, 0.15, and 0.19 gompits, respectively. Using linear regression (Fig. 1C), the duration of the acquisition feeding period required for 50% of the

corn flea beetles to acquire the rifampicin- and nalidixic acid-resistant strain of *P. stewartii* from diseased corn plants was 49.8, 29.2, and 30.4 h for the three experiments, respectively. The average acquisition feeding period for 50% of the corn flea beetles to acquire *P. stewartii* was 36.5 ± 11.6 h.

Transmission experiments. The earliest measured transmission (11%) from *P. stewartii*-infested corn flea beetles to corn

Fig. 1. A, Pathogen acquisition progress curves for corn flea beetles feeding on corn plants inoculated with a rifampicin- and nalidixic acid-resistant strain of *Pantoea stewartii* (isolate ES-Rif 9A). B, Rate of change in pathogen acquisition between consecutive acquisition feeding period time points. C, Linear regression lines and equations using the Gompertz model ($-\ln(-\ln(y))$) to transform the proportion of *P. stewartii* corn flea beetles that had acquired *P. stewartii* in response to increasing acquisition feeding period.

seedlings occurred after a 3-h transmission feeding period in one experiment, and between 3 and 6 h in the other two experiments (38 and 50% transmission, respectively; Fig. 2A). After a 48-h transmission feeding period, percent transmission of *P. stewartii* by *P. stewartii*-infested corn flea

beetles to corn seedlings was close to 100% for all three transmission experiments (Fig. 2A). The rate (dy/dt versus t) of transmission of *P. stewartii* to corn seedlings by *P. stewartii*-infested corn flea beetles was fastest at the beginning of the transmission feeding period, then slowed

as transmission feeding periods increased, which is also characteristic of the monomolecular growth model (Fig. 2B) (6,20).

An initial examination of the linearized pathogen transmission data (using an assumption that the maximum transmission was 99.5%) indicated that the linearized form of the monomolecular model performed well for two of the three transmission experiments. We found, however, that the nonlinear form of the monomolecular model better fit the percent transmission data for all three experiments (5). The nonlinear form of the monomolecular model is:

$$y = 1 - [(1 - y_0) * \exp(-r * \text{time})]$$

where y_0 represents a constant of integration and r represents the rate in units of time^{-1} (5,6). A pseudo- R^2 value ($1 - \text{SSE/SSTO}$) and examination of residuals was used to verify the model fit for each experiment (28). Therefore, we used the nonlinear form of the model to quantify pathogen transmission with respect to time (Fig. 2C) (6). Pseudo- R^2 values ranged from 98.1 to 99.5%, indicating a strong model fit. The rates of *P. stewartii* transmission using the nonlinear monomolecular model for each experiment were 0.07, 0.06, and 0.12 units/h, respectively (i.e., 7, 6, and 12% transmission per hour with the rate decreasing as time increases; Fig. 1A). Using the nonlinear monomolecular model, the predicted transmission feeding times required for 50% of *P. stewartii*-infested corn flea beetles to transmit the pathogen to corn seedlings were 9.2, 6.2, and 7.5 h for the three experiments, respectively (mean: 7.6 ± 0.87 h; Fig. 2C). After approximately 20 to 35 h in each experiment, greater than 90% transmission was observed, after which there was only a slight change in percentage of transmission through 72 h.

DISCUSSION

Vector efficiency is a complex process involving several subprocesses: (i) acquisition efficiency, (ii) transmission efficiency, and (iii) the period of time that the vector remains infectious. Each of these subprocesses may be influenced by vector biology, host resistance, and environmental conditions. This study is the first to quantify two of these subprocesses in the corn flea beetle-*P. stewartii* pathosystem: acquisition and transmission efficiency. Both of these subprocesses were affected by the length of the feeding periods for each subprocesses. Acquisition of *P. stewartii* by corn flea beetles first was detected after a 6-h acquisition feeding period with up to 24% acquisition. By 24 h, 24 to 48% of the corn flea beetles in this study had acquired the marked strain (isolate ES Rif-9A), and the average time for 50% of the corn flea beetles to acquire the marked strain was 36.5 ± 11.6 h. The rapid acquisition of *P. stewartii* by corn flea beetles is epidemiologi-

Fig. 2. **A**, Pathogen transmission progress curves for corn flea beetles feeding on corn plants inoculated with a rifampicin- and nalidixic acid-marked strain of *Pantoea stewartii* (isolate ES-Rif 9A) after feeding on healthy corn plants. **B**, Plot of the change in pathogen transmission with the change in transmission feeding time (transmission rate, dy/dt versus t) with respect to time. The negative decrease in the rate is typical of data that is best fit with the monomolecular growth model. **C**, Plot of pathogen transmission curves with the fitted equations with the nonlinear form of the monomolecular growth model are presented. The value * represents the case for false negatives in corn flea beetles that tested negative for *P. stewartii* acquisition by corn flea beetles, even though a small number of corn seedlings tested positive for *P. stewartii*.

cally important in this pathosystem because it has been reported that *P. stewartii* is not passed from adult corn flea beetles to eggs (7). Therefore, the first and second field generations of *C. pulicaria* that occur after the overwintering generation has completed their life cycles must acquire *P. stewartii* from diseased plants in the field (9).

To study the effects of insecticide seed treatments on corn flea beetle leaf feeding and transmission of *P. stewartii* to corn plants, Munkvold et al. (19) arbitrarily used an acquisition feeding period of 9 to 10 days for corn flea beetles to acquire *P. stewartii* from diseased corn plants. Based upon our models using the Gompertz transformation and extrapolating beyond 72 h (3 days) in our study to 9 or 10 days, as was used in Munkvold et al.'s study, we estimate that percent acquisition of *P. stewartii* by corn flea beetles was close to 100% and that an acquisition period of 6 to 7 days would actually be sufficient to obtain close to 99% acquisition. Using a different approach, Dill (7) also allowed corn flea beetles to feed on infected corn plants to determine the minimum amount of leaf tissue that corn flea beetles needed to consume before they could acquire the bacterium. The duration of the acquisition feeding period and the efficiency of acquisition with respect to acquisition feeding time, however, were not reported.

This study provides new quantitative information to explain how rapidly corn flea beetles can acquire and transmit *P. stewartii* from diseased corn plants in the field. Epidemiologically, the ability of 50% of corn flea beetles to acquire the pathogen within 36.5 h and for 50% of *P. stewartii*-infested beetles to transmit *P. stewartii* within 6 to 8 h facilitates the rapid increase in pathogen inoculum in corn fields, as well as the rapid dissemination of that inoculum. The subprocesses of acquisition and transmission may contribute directly to the rapid increase in the incidence of *P. stewartii*-infested corn flea beetles (10) and the rapid temporal spread of Stewart's disease in the field. Under normal field conditions, the actual percentage of diseased plants resulting from the transmission of a fixed population size of *P. stewartii*-infested corn flea beetles may be higher than reported in our study, because corn flea beetles in the field can move readily from plant to plant, and a single infectious corn flea beetle may lead to multiple plant infections, although this hypothesis needs to be evaluated experimentally. Also, we did not test the possibility that percent transmission may increase in response to a longer acquisition access period. Our study was based on using a single acquisition access period (48 h) to ensure that more than 50% of the corn flea beetles would be infested by *P. stewartii*, and the actual percentage of corn flea beetles that had successfully acquired *P. stewartii* for use in the three transmission feed-

ing period studies was 72, 71, and 72%, respectively.

Esler and Nutter (10) observed that the incidence of *P. stewartii*-infested corn flea beetles in the first summer generation was as high as 20 to 40% in newly emerged beetles. In that study, corn flea beetles were sampled and tested by enzyme-linked immunosorbent assay just after the beetle-free period that occurs in early-to-mid June. This is the period after which the overwintering corn flea beetles have completed their life cycle (and died), but before the first summer generation of corn flea beetles has emerged from eggs laid by the overwintering generation (7,9). During the first 20 days that the first summer generation had begun to emerge (day of year 180 to 200), the proportion of *P. stewartii*-infested corn flea beetles increased at a rate of 1.2 to 3.3% per day (10). Although the present study found the rate of acquisition by corn flea beetles to be 2 to 3% per hour (not days), our study does not include information concerning the probability that corn flea beetles will come into contact with and feed on *P. stewartii*-infected leaf tissue. In the present study, corn flea beetles were placed directly into contact with *P. stewartii*-infected leaf tissue. Esler and Nutter (10) further reported that, in spite of beetle death at the end of the first summer generation and the emergence of the second corn flea beetle summer generation, the incidence of *P. stewartii*-infested corn flea beetles in the second summer generation was as high as 85.6% by late August. Thus, our findings that corn flea beetles can rapidly acquire *P. stewartii* supports field observations by Esler and Nutter (10) regarding the rapid increase in *P. stewartii*-infested corn flea beetle populations during the corn growing season. Moreover, our study is the first to report that the Gompertz population growth model best describes *P. stewartii* acquisition by corn flea beetles over time and explains how *P. stewartii*-infested corn flea beetle populations can reach incidence levels as high as 85.6% in the field.

Whether or not there is a true latent period required within the corn flea beetle vector before transmission can occur remains unknown. However, the fact that we did not detect the presence of *P. stewartii* after a 3-h acquisition feeding period, but did observe up to 24% acquisition of *P. stewartii* by corn flea beetles after a 6-h acquisition feeding period, suggests that either corn flea beetles did not acquire a sufficient number of bacterial cells to be detected after a 3-h acquisition feeding period (using our operational definition for successful acquisition that requires the recovery of the marked strain from corn flea beetles using a selective medium), or that the bacterium must multiply within the corn flea beetle to reach a population level that could be detected using our detection method. Dill (7) believed that re-

gurgitation or defecation on the leaf surface was needed before successful transmission of *P. stewartii* could occur near corn flea beetle feeding scars. In our study, the fact that up to 24% (0.24 probability) of the corn flea beetles could acquire *P. stewartii* after a 6-h acquisition feeding period and that up to 11% (0.11 probability) of *P. stewartii*-infested corn flea beetles could successfully transmit the pathogen after a 3-h transmission feeding period indicates that it is possible for 2.64% ($0.11 \times 0.24 \times 100$) of corn flea beetles to acquire and transmit *P. stewartii* in 6 h or less. Thus, if a latent period is required within the feeding periods of acquisition and transmission, it is less than 6 h. Moreover, the efficiency of both subprocesses increases with increasing feeding time.

We did not examine the length of time that *C. pulicaria* remains infectious (retention time); however, previous studies by Robert et al. (27) and Dill (7) have postulated that corn flea beetles remain infectious throughout a single generation once they have acquired the bacterium. Although it is likely that *C. pulicaria* can acquire *P. stewartii* and transmit it to more than one plant, additional experiments are needed to determine how long *P. stewartii*-infested corn flea beetles remain infectious and how long they remain feeding on a single corn plant before moving to other corn plants.

In the transmission study, there was a positive relationship between the duration of the transmission feeding period and the increase in percent transmission of *P. stewartii*. It is important to note that, although percentage transmission increased as the transmission feeding period increased, the rate of transmission (dy/dt versus t) actually decreased as the transmission feeding period (X) increased in all three experiments. This study is the first to report that percent transmission of *P. stewartii* by corn flea beetles is best described by the monomolecular model. According to this model, percent transmission of *P. stewartii* increases the fastest at the beginning of the transmission feeding period and the rate of transmission decreases with respect to time without an inflection point. Thus, biologically, corn flea beetles (as a population) tend to transmit *P. stewartii* more efficiently early in the post-acquisition phase than later in the post-acquisition phase.

Following an acquisition access period of 48 h on diseased plants, the minimum transmission feeding period required for successful transmission of *P. stewartii* by corn flea beetles was 3 h, and 50% transmission occurred by 7.6 ± 0.87 h. Thus, corn flea beetles are highly efficient in both acquiring and transmitting *P. stewartii* and, combined with the supposition that corn flea beetles remain infectious for the remainder of their adult lives, this makes

the corn flea beetle a highly efficient vector. Our results suggest that insecticide seed treatments and foliar insecticides must act quickly to prevent corn flea beetles from acquiring and transmitting *P. stewartii*. Furthermore, our results suggest that the development of resistant corn hybrids that effectively reduce or delay pathogen acquisition and transmission may offer an extremely effective measure to better manage Stewart's disease in the future.

We observed nontransmission of *P. stewartii* by corn flea beetles that were known to be carrying *P. stewartii* (based on positive tests for *P. stewartii* by plating beetle contents on NBY-CRN media). Nontransmission by infested corn flea beetles could be due to factors such as insufficient feeding times (in cages) to transmit the bacterium, the need for a period of latency in the corn flea beetle before it becomes infectious, the possibility that injury to beetles occurred when transferring them from diseased to healthy plants, or a required prolonged settling time for some caged beetles to initiate feeding.

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LITERATURE CITED

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