Transmission of *Xylella fastidiosa* to Grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae)

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ABSTRACT Pierce's disease (PD) of grapevines is caused by a xylem-limited bacterium *Xylella fastidiosa* (Wells, Raju, Hung, Weisburg, Mandelco-Paul, and Brenner) that is transmitted to plants by xylem sap-feeding insects. The introduction of the sharpshooter leafhopper *Homalodisca coagulata* (Say) into California has initiated new PD epidemics in southern California. In laboratory experiments, the major characteristics of *H. coagulata*'s transmission of *X. fastidiosa* to grapevines were the same as reported for other vectors: short or absent latent period; nymphs transmitted but lost infectivity after molting and regained infectivity after feeding on infected plants; and infectivity persisted in adults. Adult *H. coagulata* acquired and inoculated *X. fastidiosa* in <1 h of access time on a plant. Inoculation rates increased with access time, but acquisition efficiency (20% per individual) did not increase significantly beyond 6-h access. Estimated inoculation efficiency per individual per day was 19.6, 17.9, and 10.3% for experiments where plant access was 1, 2, and 4 d, respectively. Freshly molted adults and nymphs acquired and transmitted *X. fastidiosa* more efficiently than did older, field-collected insects. *H. coagulata* transmitted *X. fastidiosa* to 2-yr-old woody tissues of grapevines as efficiently as to green shoots. *H. coagulata* transmitted *X. fastidiosa* 3.5 mo after acquisition, demonstrating persistence of infectivity in adults. About half (14/29) of the *H. coagulata* from which we failed to culture *X. fastidiosa* from homogenized heads (with a detection threshold of 265 CFU/head) transmitted the pathogen to grape, and 17 of 24 from which we cultured *X. fastidiosa* transmitted.

KEY WORDS glassy-winged sharpshooter, Pierce's disease, vector, xylem

Xylella fastidiosa (Wells, Raju, Hung, Weisburg, Mandelco-Paul, and Brenner) is a gram-negative, xylem-limited bacterium that causes disease in many crops, including Pierce's disease (PD) of grapevines (*Vitis vinifera* L.) (Davis et al. 1978). Xylem sap-feeding insects such as sharpshooter leafhoppers (Cicadellidae: Cicadellinae) and spittlebugs (Cercopidae) transmit *X. fastidiosa* (Houston et al. 1947, Severin 1949, 1950). The bacterium is widespread in the American tropics and subtropics and also has been reported from Taiwan and southeastern Europe (Purcell 1997). *X. fastidiosa* has a very broad host range, colonizing members of at least 28 plant families (Hill and Purcell 1995b). There is no curative treatment for grapevines with PD. Current control strategies in coastal California are based on preventing entrance of vectors into vineyards during the spring by spraying riparian vegetation with insecticides, and management of adjacent vegetation to eliminate hosts of *X. fastidiosa* and sharpshooters (Goodwin and Purcell 1990, Purcell and Feil 2001).

The introduction of *Homalodisca coagulata* (Say) into southern California in early 1989 (Sorensen and Gill 1996) drastically increased the threat of PD and related diseases to the state's agriculture (Blua et al. 1999). *H. coagulata* appears to have been responsible for spreading oleander leaf scorch (also caused by *X. fastidiosa*) in the Los Angeles Basin (Purcell et al. 1999). In southern California, grape growers in Temecula Valley detected PD on a limited number of grapevines in 1997 for the first time in that region, and associated it with presence of *H. coagulata* in neighboring citrus groves (Blua et al. 1999). The incidence of PD increased exponentially so that within 2 yr all areas of Temecula Valley were affected (Perring et al. 2001, Purcell and Feil 2001). Because it readily feeds on almond trees, *H. coagulata* also threatens to increase the importance of almond leaf scorch disease, which also is caused by *X. fastidiosa* (Davis et al. 1980).

Virtually all sharpshooters and spittlebugs are vectors of *X. fastidiosa* (Purcell 1989). Although many studies have identified vector species, only a few have determined transmission characteristics. Detailed transmission studies with *Graphocephala atropunctata* (Say), the principal *X. fastidiosa* vector in coastal California vineyards, revealed that *X. fastidiosa* is transmitted without any latent period (delay after acquisition) (Purcell and Finlay 1979) and for an in-

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definite period of time by adults (Severin 1949), but molting stops vector transmission until the insects feed again on infected plant sources (Purcell and Finlay 1979). The lack of latent period and nymphal loss of infectivity after molting suggest that *X. fastidiosa* inoculum is limited to the foregut of vectors (Purcell and Finlay 1979). Transmission efficiency varies with vector and host plant species (Severin 1949). *X. fastidiosa* populations within grape plants directly influenced vector acquisition efficiency; higher bacterial densities in acquisition source plants resulted in higher transmission efficiencies, although the underlying mechanisms of this correlation were not identified (Hill and Purcell 1997). *G. atropunctata* and other sharpshooters for which there is information on transmission are in a different tribe (Cicadellini) than *H. coagulata* (Proconiini). The proconiine sharpshooters are larger than the Cicadellini and may differ in behavioral aspects (Young 1968), such as a tendency to often feed on woody tissues (Turner and Pollard 1959, Purcell and Saunders 1999).

In coastal California vineyards, *X. fastidiosa* infections that persist beyond the year of inoculation occur primarily during the spring months. In these areas, inoculations by vectors in summer establish infections that seldom survive the winter (Purcell 1981). *X. fastidiosa* appears only after mid-June (Smart et al. 1998) in the new vine growth where the sharpshooters prefer to feed (Hewitt et al. 1949, Purcell 1975) in bacterial populations that would enable vector acquisition (Hill and Purcell 1997). Thus, most vine-to-vine spread of *X. fastidiosa* by vectors has been postulated to occur only in summer, but because *X. fastidiosa* that infect green tissues of grape during summer survive the following winter so poorly (Purcell 1981), summer inoculations are least likely to establish chronic disease. A plausible explanation for the poor over-wintering survival of summer infections is that they occur near the growing tips of new stems of grapevines, which are severely pruned during winter dormancy. This winter pruning should eliminate infections that have not progressed below the point of pruning by the end of autumn. A major difference observed for *H. coagulata*, but not for sharpshooters in the tribe Cicadellini, is that *H. coagulata* will often feed on mature woody tissues of grapevine, even feeding on dormant vines during winter (Purcell and Saunders 1999). With this feeding behavior, *H. coagulata* may inoculate vines at the bases of canes during summer, avoiding the possibility that pruning will eliminate summer infections and thus could increase the likelihood that vine-to-vine spread of *X. fastidiosa* (summer spread) will result in chronic PD. In addition, the feeding of *H. coagulata* on vines during winter may contribute to PD spread if these inoculations establish chronic infection. The logistic increase of current PD epidemics because of *H. coagulata*, as observed in a preliminary epidemiological study of PD in Temecula Valley (Perring et al. 2001), suggests that dissemination is polycyclic, having more than one cycle of pathogen spread per year (Van der Plank 1963).

Proconiine sharpshooters in Brazil transmit the citrus disease-causing strain of *X. fastidiosa* very inefficiently when compared with cicadelline vector species (Krugner et al. 1998). Turner and Pollard (1959) found that *H. coagulata* was a vector of phony peach disease in the southern United States but that it had the lowest transmission rates of all the vectors tested. Purcell and Saunders (1999) found that *H. coagulata* transmits *X. fastidiosa* to grapevines. However their estimates of transmission efficiency were based on few individuals, and they did not determine the effects of molting, nor determine whether *H. coagulata* could transmit the bacterium persistently. Our objectives were to describe and quantify the characteristics of transmission of *X. fastidiosa* to grape by *H. coagulata*, including its ability to transmit *X. fastidiosa* to woody stems of grapevines. These characteristics are fundamental for future epidemiological analyses that incorporate transmission by *H. coagulata.*

Materials and Methods

Insects, Plants and Experimental Conditions. The use of field-collected *H. coagulata* for some experiments was necessary because of difficulties in rearing insects in the laboratory, and maintaining colonies for more than one generation. We collected *H. coagulata* adults from citrus groves in southeastern Kern Co. and maintained them in a secure greenhouse insectary at Berkeley, CA. During our first year of collection, no PD had been reported from surrounding vineyards since the 1950s, so we reasoned that this region was the best area to collect *H. coagulata* that were free of *X. fastidiosa.* Insects were kept in Plexiglas cages with nylon screening and containing various combinations of sweet basil [*Ocimum basilicum* (L.)], European grape (*V. vinifera* L.), California mugwort [*Artemisia douglasiana* (Besser)], and okra [*Abelmoschus esculentus* (L.)]. We changed plants frequently to maximize succulent plant growth on which these insects preferred to feed. For nymphal transmission experiments and some experiments to determine the effect of acquisition access period (AAP) of *X. fastidiosa*, we allowed field collected insects to lay eggs and then transferred nymphs to separate cages as soon as they hatched to ensure that they were free of *X. fastidiosa*, because no transovarial transmission of the pathogen occurs (Freitag 1951). This method relied on loss of nymphal infectivity with molting, which was one of our objectives to confirm for *H. coagulata*, so we also exposed the molted insects to 'pretest' grape plants before using them in tests requiring noninfectious insects. We used seedlings of susceptible grapevine varieties 'Cabernet sauvignon' and 'Pinot noir' for all experiments. Rooted cuttings of the two varieties also were occasionally used for assays on persistence of infectivity in adults. For experiments with woody plants, we used 2-yr-old, rooted cuttings of 'Cabernet sauvignon'. All experiments had greenhouse negative controls, grapevines from the same lots of tested plants that were not exposed to vectors. None of the controls was positive for *X. fastidiosa.* We reared, kept, and

tested all insects in a greenhouse insectary heated and ventilated to maintain an average temperature of 25°C ± 5°C, but episodically reaching 30–37°C.

Detection of *X. fastidiosa*. We tested grapevine samples for the presence of the bacterium by culturing, following the dilution plating method of Hill and Purcell (1995a). Briefly, leaf petioles were surface sterilized, cut in small pieces, ground in 2 ml of phosphate saline buffer with a tissue homogenizer, and two 20 μl drops of the suspension were spread on modified periwinkle wilt (PWG) solid medium. We identified *X. fastidiosa* based on growth rate and colony characteristics. Periodic samples of our cultures tested by polymerase chain reaction (PCR) (Minsavage et al. 1994) always proved positive for *X. fastidiosa.* We quantified populations of *X. fastidiosa* from insect heads with the same culturing protocol, but used 1 ml of buffer for sample homogenization to increase detection sensitivity. Homogenization was done with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) with a PT-10 generator probe. We calculated the number of colony forming units (CFU) per head by plating two 100-fold dilutions in addition to the original homogenate. Our threshold for detecting at least one CFU in an undiluted 20-μl homogenate of an insect head in 95% of the cultures was 265 CFU. We calculated this value with the Poisson distribution of zero for 5% of the cases, assuming a random distribution of *X. fastidiosa* within the homogenized 1 ml. The detection threshold for 90% confidence was 115 CFU per sample. *H. coagulata* heads were obtained from individuals tested in experiments to study the effects of inoculation access period (IAP) and AAP on transmission. Because we found positive cultures from all test plants with definitive PD symptoms on leaves and stems (Goodwin and Purcell 1990), a few plants in the experiment to analyze the effect of molting on transmission were only diagnosed by characteristic PD symptoms, independently assessed by both authors and at least one other member of our lab.

Transmission Experiments. The STL grape strain of *X. fastidiosa* (American Type Culture Collection ATCC 700963) was used for all experiments. To minimize source plant variability, we pooled three or four PD-symptomatic grapevines into a single 45 by 75 by 40-cm (width, height, depth) cage, and randomly collected *H. coagulata* after various access times, depending on the transmission experiment. This limited the number of insects that could be used per single repetition to ≈50–100 individuals per source plant cage if various access times were to be evaluated during the same experimental repetition. Insects had access to entire plants in the tests, except as noted below. We used voile ('bridal veil') sleeve cages (30 by 10 cm) or cylindrical cellulose acetate-butyrate plastic cages ventilated with dacron organdy windows to confine the insects on test plants. We used 4 d as a standard period to attempt to maximize acquisition and inoculation efficiencies by *H. coagulata* in our experiments; this period maximized efficiency when transmission of *X. fastidiosa* to grapevines was studied with another vector, *G. atropunctata* (Purcell and Finlay 1979).

Nymphal Transmission and the Effect of Molting on Infectivity. To determine the effect of molting on vector transmission of *X. fastidiosa,* we allowed last instar nymphs a 4 d AAP on grapevine with PD. We transferred the insects individually to healthy seedlings for an IAP until adult emergence (1–21 d) and checked individuals daily. After molting, the adults were transferred to another seedling for an IAP of 7–9 d. Because of concerns that *X. fastidiosa* might multiply in and be acquired from the plants on which nymphs molted, we did a second experiment, in which we successively transferred individual nymphs of various instars to healthy seedlings every other day, checking for exuviae during each transfer to determine if the nymph had molted. After one or two transfers (2 or 4 d) after a molt, we transferred the insects to a PD-symptomatic grapevine to allow an AAP of 2 d on a source of *X. fastidiosa,* so that insects had access to one more test plant (in comparison to the experiment described above) before another molt occurred. These insects were confined in clamshell-type cages (6-cm-long with an i.d. of 2.5 cm) made of foam (water pipe insulation) with open ends covered with a nylon mesh (Hill and Purcell 1995a), on the petiole of a symptomatic leaf and the adjacent stem.

Effect of Time on Transmission Efficiency. We determined *X. fastidiosa* acquisition and inoculation efficiencies by testing *H. coagulata* adults under variable AAP and IAP, respectively. A total of seven and four repetitions through time were required for experiments with variable AAP and IAP, respectively, to obtain a minimum of 30 (AAP) or 46 (IAP) insects per access period. We tested approximately the same number of insects per access period for each repetition of each experiment. We first confined *H. coagulata* to be used in acquisition efficiency experiments for 4 d on healthy pretest grapevines to determine if any individual were naturally infected before transferring them to a source plant with PD symptoms. We did not detect any insects as naturally infected with *X. fastidiosa* and transmitting to any pretest plant (tested for *X. fastidiosa* eight or more weeks later). One, 6, 12, 24, 48, and 96 h after the AAP began, we transferred individual insects to grape for 4 d of IAP (n = 30–38 per AAP). We used a similar design to determine the effect of IAP on transmission, this time varying IAP and using a constant 4-d AAP. The same *H. coagulata* individual inoculated more than one plant. To minimize possible circadian (time of day) effects on transmission, we tried to minimize short and long access periods by different insects by transferring insects that had a 12 h IAP to another healthy grapevine for a subsequent 96 h; the other insects had 1, 6, 24, and 48 h as an IAP sequence. We tested a total of 46–56 insects for each period. The one and 12 h IAP started at the same time (0800 hours), thus the 6 h IAP also ran during the same time period as the 12 h IAP. We also began experiments with variable AAP at this time of day.

Persistence of Infectivity. We used young adults (<1 wk after emergence) to assess the persistence of infectivity. Seventeen insects had a 4-d AAP on PD-

symptomatic grape, and then were transferred individually to healthy grape seedlings for a 4-d IAP to check for infectivity. They were then transferred to mugwort for 2 wk. Mugwort is a nonsystemic, low titer host of *X. fastidiosa* that should not support vector acquisition of the bacterium for at least 2 wk (Hill and Purcell 1995b). They were subsequently transferred again to two successive test plants for 4-d IAP each. After these transfers, we kept the insects on healthy mugwort for 2 wk and then transferred them to grapevines for 2-d IAP, to avoid reacquisition of *X. fastidiosa* from the plant on which the IAP was occurring. This sequence was repeated for 7 mo to observe vector transmission and survival over time, as long as a few insects remained alive.

2-yr Old Wood Inoculation. We tested the transmission of *X. fastidiosa* to woody tissues of grapevines by *H. coagulata* by caging groups of four insects either on 2-yr-old woody stems with mature bark or new green shoots of 'Cabernet sauvignon' cuttings (40 and 44 groups, respectively). These groups had a 4-d AAP on a PD source plant and a 4-d IAP on a healthy cutting. For each repetition through time of the experiment, we divided the cohort, so that approximately the same number of groups inoculating wood also inoculated shoots.

Data Analysis. Mean IAP of insects that did or did not transmit *X. fastidiosa* were compared with Student's *t*-test (MS Excel 2002). The linear regressions between transmission as the dependent variable and plant access period as the independent variable were calculated with MS Excel 2002; transmission efficiency percentages were transformed into probits to linearize the relationship between access time (logarithmic scale) and transmission rates. Transmission frequencies to shoots compared with woody tissue and determination of the effect of molting on infectivity were compared by calculating Chi-squared using the Yates correction for continuity (Agresti and Finlay 1997), as was the relationship between transmission and detection of *X. fastidiosa* in insect heads.

Results

Nymphal Transmission and the Effect of Molting on Infectivity. Nymphal transmission rates and loss of infectivity were determined in two experiments. In the first one, in which IAP was governed by the days to adult emergence (1–21 d), the fifth instars that transmitted *X. fastidiosa* had a mean IAP of 11.2 ± 1.2 d (mean ± SE, range of 1–18 d, n = 18). This was not different (Student's t-test = 0.356; df = 23; P = 0.362) from the IAP for those that did not transmit (12.1 ± 2.8 d, range of 1–21 d, n = 7). After adult emergence, each of the insects was transferred for 7–9 d to a fresh test plant, of which three became infected. The insects that transmitted after molting had IAPs before molting of 4, 9, and 14 d.

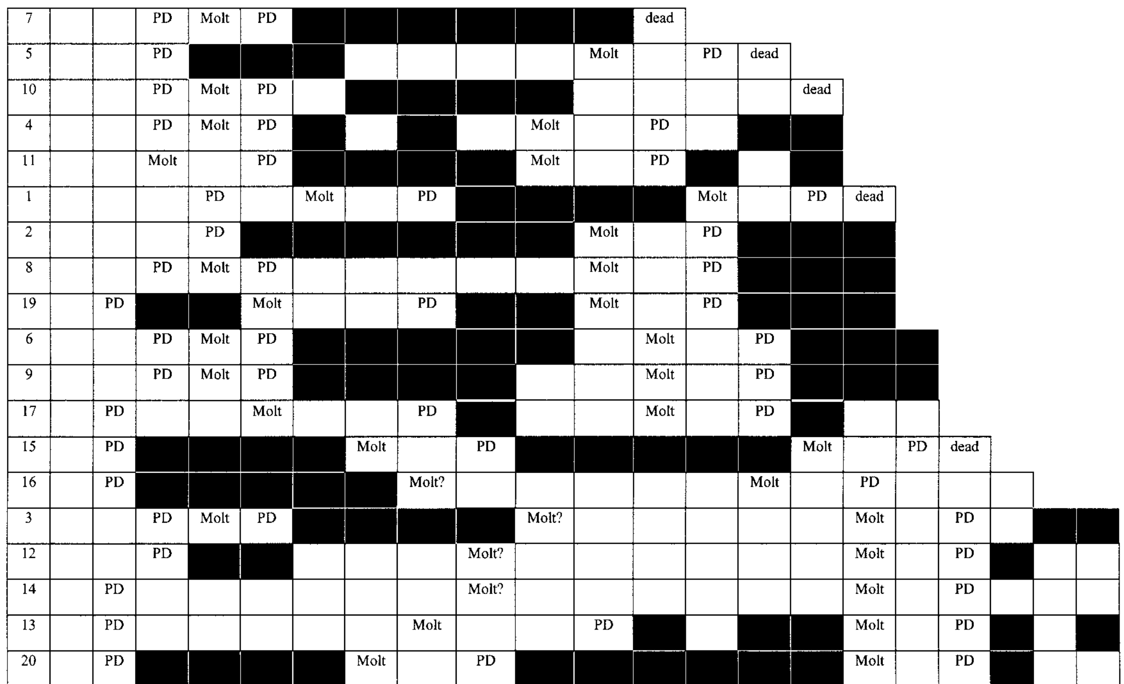

Fig. 1. Transmission of *X. fastidiosa* to grape by *H. coagulata* nymphs sequentially transferred from healthy grapevines every other day, with occasional 2 d acquisition access period on plants with Pierce's disease. Each row represents a different insect; each block represents 2 d of plant access. White blocks, no transmission; black blocks, successful transmission; PD, acquisition access period on source plant; Molt, molting that occurred on the previous plant (block); Molt?, probable molt but no exuvia observed.

Because we obtained transmission after molting with insects having variable and often lengthy IAPs, in a second experiment we limited nymphs to only 2 d of IAP (Fig. 1). Only one *H. coagulata* out of 19 nymphs tested did not transmit *X. fastidiosa* to a test plant at any time during the experiment. After having AAP on source plants, insects fed on 179 seedlings (before molting or after having another AAP after a molt), of which 102 plants became infected (not eliminating plants for insects that never transmitted). Nineteen plants were fed on by infective insects (assumed infective by transmitting to at least one plant after AAP) after a molt was determined. None of these plants became infected with *X. fastidiosa,* demonstrating loss of infectivity after molting ($P < 0.0001$; 1 df; χ^2 test). Four cases of probable but unobserved molts (labeled 'Molt?' in Fig. 1), were identified based on length of time between two molts (usually not longer than 10–14 d). In the last molt during the experiment, all *H. coagulata* emerged as adults, with the exception of nymphs #6 and #8 (fifth instars). Nymphs acquired *X. fastidiosa* in 22 of 27 (81%) opportunities and adults in 10 out of 12 (83%), as determined by at least one positive plant after acquisition and before a molt. By eliminating the insects where a probable molts occurred ('Molt?' in Fig. 1) from the analysis, nymphs transmitted *X. fastidiosa* (considering only those that transmitted at least once after acquisition) in 83% of opportunities (72/87). The same rate calculated for adults in this experiment was 67% (20/30).

Effect of Time on Transmission Efficiency. Increase in AAP was not associated with increases in transmission efficiency (Fig. 2: y (probits) $= 4.018 + 0.300x$ (log time), $r^2 = 0.289$, $P = 0.271$). The regression equation for IAP with transmission rate was significant (y (probits) $= 2.991 + 0.831x$ (log time), $r^2 = 0.667$, $P = 0.047$). *H. coagulata* acquired and inoculated *X. fastidiosa* with 1 h of source and test plant access time, respectively. We observed that transmission rates were variable among experiments and replicates. For example, using the 48 h IAP percentages of transmission as a reference (from experiments with variable IAP), efficiencies obtained for three repetitions were 4.8, 45.5, and 60%. Inoculation efficiency did not increase significantly from 48 to 96 h (32.6 and 35.3, respectively). Because the same individual was used for successive IAPs, we tried to determine if transmission rates increased for the same insects with longer IAP. Insects that transmitted with only a few hours of access did not necessarily transmit with a longer IAP. Although the 12 h IAP had a lower efficiency than the six and 24 h, we know that more of those individuals were infective, because they also performed the 96 h IAP (which was the IAP with highest transmission rate of all tested). For one IAP experiment repetition (with 21–27 adults per IAP), we used *H. coagulata* adults that had been collected ≈2 mo earlier. Transmission rates for this test were 5–10 times lower than those obtained in earlier tests that used the same cohort of insects (summaries of all IAP experiments). Although we pooled the results from various experiments to obtain enough insects for the regression equation for both

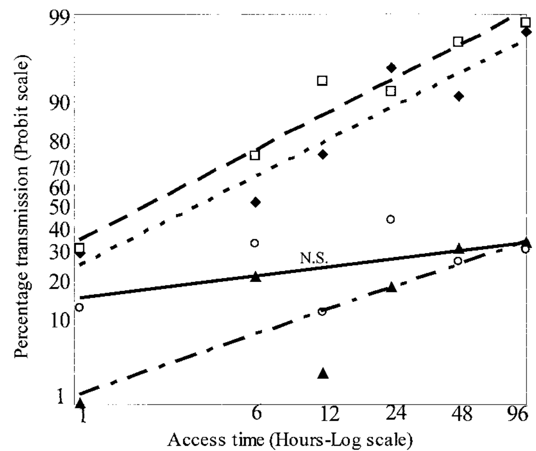

Fig. 2. *H. coagulata* transmission of *X. fastidiosa* to grapevines for various acquisition and inoculation access periods. Open circles indicate the percentage of *H. coagulata* that transmitted after various acquisition access periods and 4 d of inoculation access. Filled triangles indicate the percentage that transmitted after 4 d of acquisition access period and variable inoculation access periods. Solid line (transmission probability y (probits) $= 4.018 + 0.300$ access time x (log time), $r^2 = 0.289$, $P = 0.271$) is the regression (N.S. = not significant) for *H. coagulata* acquisition; semidashed line (transmission probability y (probits) $= 2.991 + 0.831$ access time x (log time), $r^2 = 0.667$, $P = 0.047$) is the regression for inoculation. Open squares and filled diamonds represent the data points for *G. atropunctata*'s acquisition and inoculation, respectively; long and short dashed lines show, respectively, regressions of acquisition and inoculation rates by *G. atropunctata* (Purcell and Finlay 1979).

AAP and IAP effects on transmission, we found that the repetitions for the IAP experiments should not be pooled (hypothesis of no difference in effect across repetitions on different days was rejected using chi-square test of independence). The driving factor was the low transmission rate obtained during one experiment with a large number of individuals. Interestingly, by pooling the experiments, our regression equation was significant for IAP, whereas by eliminating the repetition with large numbers of nontransmitting insects, the regression was not significant ($P >$ 0.05).

Persistence of Infectivity. *H. coagulata* transmitted *X. fastidiosa* to 5 of 34 plants during the first two bi-weekly transfers and to 3 of 51 during subsequent transfers made 1.5–2.5 mo after acquisition. Only one of 17 *H. coagulata* transmitted 3.5 mo after the 4 d AAP, the longest persistence period observed. A total of six different insects transmitted *X. fastidiosa* at least once. Eight *H. coagulata* individuals survived the first 6 mo, and four for the entire experiment (7 mo), demonstrating the potential longevity of this species. At the end of the 7-mo experiment, we observed the insects excreting honeydew, but usually in lower amounts compared with 6 mo earlier. These older adults preferred feeding on leaves of grapevines and mugwort rather than stems, in contrast to when they were

Table 1. Transmission of *X. fastidiosa* to 2-yr-old wood and shoots of grapevine, with groups of 4 *H. coagulata* adults per plant

	Tissue inoculated	
	New Shoot	2-yr old wood
No. uninfected plants	15	21
No. infected plants	29a	19a
Avg no. of *H. coagulata* live after IAP	3.68	2.85

Values within a row followed by the same letter are not significantly different by Chi-square test using the Yates correction for continuity ($\alpha = 0.05$).

young adults, when they preferred stems (data not shown).

2-yr Old Wood Inoculation. *H. coagulata* successfully inoculated *X. fastidiosa* into 2-yr-old woody tissues of grapevine. Transmission efficiency into woody stems was similar to that in young shoots (Table 1). *H. coagulata* survived well on 2-yr-old wood despite the lack of access to green tissues during 4 d IAP (Table 1). We routinely observed in the field *H. coagulata* adults feeding on grape stems with scaly bark that were even older and thicker than the 2-yr old stems used for these experiments.

Detection of *X. fastidiosa* in *H. coagulata*. Detection of *X. fastidiosa* in *H. coagulata* heads by culturing was not associated with transmission of *X. fastidiosa* to grape (Table 2). Contamination of cultures with other bacteria that obscured culturing results for detecting *X. fastidiosa* varied among experiments. We also cultured *X. fastidiosa* from the heads of *H. coagulata* that were used as groups or individuals in other transmission experiments. Pooling all the culture attempts that were positive for *X. fastidiosa* ($n = 71$), the mean population of *X. fastidiosa* detected in a *H. coagulata* head was 4.4×10^3 CFU/head (range, 2.5×10^1–2.5×10^5 CFU/head) estimated by dilution-plating.

Discussion

We found that *H. coagulata* transmitted *X. fastidiosa* to grapevines in a persistent manner, that nymphs lost infectivity with molting, and found no evidence of a latent period. These basic characteristics are the same as observed for another vector of *X. fastidiosa* to grapevines, *G. atropunctata* (Severin 1949, Purcell and Finlay 1979). We also found that *H. coagulata* can inoculate 2-yr-old woody tissues of grapevines. Although

Table 2. Relationship between *X. fastidiosa* transmission by *H. coagulata* and its detection in insect heads by culturing

H. coagulata head	Transmission to grape[a]	
	Negative	Positive
Negative culture	15a	14a
Positive culture	7a	17a
Contaminated culture	24	26

Values within a column followed by the same letter are not significantly different by Chi-square test using the Yates correction for continuity ($\alpha = 0.05$). Data of 'contaminated culture' were not used for analysis.

[a] Number of insects tested.

the numbers tested were low (19 positive out of 40 plants tested), our results clearly established that infection of woody tissues was possible, suggesting that the vine-to-vine spread of *X. fastidiosa* by this vector when inoculating woody tissues may be more likely to persist as chronic infections in subsequent seasons. Field experiments are needed to confirm that this can occur in vineyards during winter. *X. fastidiosa* transmission efficiency by *H. coagulata* was lower and more variable among experiments compared with the highly efficient vector (to grape), *G. atropunctata* (Purcell and Finlay 1979).

The three exceptions in the first molting experiment where nymphs transmitted *X. fastidiosa* after molting probably resulted from the insects acquiring the bacterium from the same plant they had inoculated 4–14 d earlier. Purcell and Finlay (1979) also had three cases of nymphal transmission after molting under similar circumstances. Hill and Purcell (1997) demonstrated that *G. atropunctata* can acquire *X. fastidiosa* from plants that were insect-inoculated just 4 d earlier. Because the insects were confined to small seedlings, it is plausible that they reacquired the bacteria they had previously inoculated into the plant. Even so, transmission was very low (3/18) from grapevines inoculated only a few days before, consistent with the conclusions (Hill and Purcell 1997) that plants with low populations of *X. fastidiosa* were poor sources. When we used only 2 d of IAP (Fig. 1) to reduce the chances of *X. fastidiosa* reacquisition from test plants, no case of transmission after a molt was observed. The cases of probable molt ('Molt?') were likely true molts, because of the loss of infectivity and length of time between instars.

H. coagulata acquired and inoculated *X. fastidiosa* with 1 h of plant access period, further suggesting the absence of a latent period for transmission. Absent or short latent period can also be inferred from the tests with sequential transfers of *H. coagulata* nymphs. The same was previously observed with *G. atropunctata* (Purcell and Finlay 1979). The lack of evidence for a latent period also supports the hypothesis that *X. fastidiosa* does not have to circulate within its vector. For both vector species, acquisition occurred more efficiently than inoculation in short periods of time. Acquisition may occur very efficiently after insects feed on infected vessels, whereas inoculation that leads to successful infection may require a specific feeding-associated behavior by the insect or a required response by the plant that occurs less frequently. In the experiments conducted with *H. coagulata*, acquisition of *X. fastidiosa* did not increase with plant access periods of >6 h.

In general, nymphs and recently emerged adults from the molting experiments transmitted more efficiently than older adults or adults of unknown age (IAP and AAP experiments) that had been maintained in the greenhouse insectary longer than 3 wk as adults. We also observed that transmission by adults decreased over time in the experiment to assess the persistence of infectivity. These results suggested that newly molted insects were better vectors of *X. fastid-*

iosa than older insects. Because insects collected from the field used in most experiments had reasonable transmission rates, insect age alone may not have been the only limiting factor for transmission efficiency. The reduction in transmission efficiency may be a result of niche competition between *X. fastidiosa* and other microbes for the attachment site(s) in vectors' mouthparts, sites needed for successful transmission. We cultured various microbes (mostly bacteria) from about half of *H. coagulata* heads ('Contamination' in Table 2), but have not identified them. Comparable assay methods failed to detect other bacteria as frequently in *G. atropunctata,* which was a more efficient and consistent vector of *X. fastidiosa* to grapevines (Hill and Purcell 1995a, Severin 1950) than *H. coagulata.* Future transmission experiments with *H. coagulata* should further test the effect of age and other related factors (such as other microbes) on vector efficiency. We do not know if the different plant tissues for bacterial acquisition affect efficiency (discussed below), but the hypotheses are not mutually exclusive, and both plant tissue type and microbes, and even age-related changes in sharpshooter behavior, may have contributed to our results.

Transmission rates were much lower for *H. coagulata* than those determined for *G. atropunctata* by various authors (Severin 1949, Purcell and Finlay 1979). Extensive transmission experiments of *X. fastidiosa* to citrus with 12 vector species in Brazil (Krugner et al. 1998, J.R.S. Lopes, Universidade de São Paulo, Piracicaba, SP, Brazil, personal communication), demonstrated that members of the Cicadellini transmit with higher efficiency than Proconiini. A major morphological difference between members of the two tribes is size. Proconiines are much larger, with larger mouthpart structures. The location of *X. fastidiosa* inoculum in vectors' foregut and the inoculation mechanism(s) are still unknown. There is little published information available about sharpshooter probing behavior except for histological studies (Houston et al. 1947) and analysis of excrement (Brodbeck et al. 1995). Therefore, we do not know if any specific probing behavior may be directly related to pathogen inoculation. In our second experiment to study nymphal transmission, nymphs had access to leaf petioles instead of entire plants as in other experiments. These results suggest that plant tissue where acquisition occurs may influence transmission, but to our knowledge there are no data comparing acquisition rates of *X. fastidiosa* vectors feeding on different tissues.

Detection of cultured *X. fastidiosa* in the vector's head was not associated with transmission to plants. Hill and Purcell (1995a) had a similar result with *G. atropunctata.* The culturing method is theoretically less sensitive than others based on DNA amplification (PCR) (Pooler et al. 1997), but our culturing protocol should be capable of detecting at least one CFU in 95% of the cases where there are 265 or more CFU/head and in 63% of the cases where there are 50 CFU/head distributed at random in the homogenate (Poisson distribution). Apparently small numbers of bacteria were adequate for efficient vector transmission, with no indication that higher populations of *X. fastidiosa* in the vector's head increased transmission. This suggests that the area(s) of the foregut necessary for transmission of *X. fastidiosa* is very small. Logically some bacteria must be present in the foregut to be transmitted to plants. PCR has been used to detect *X. fastidiosa* in sharpshooters (Pooler et al. 1997), but not correlated with transmission of *X. fastidiosa* by the same specimens. The PCR detection method may be more sensitive than culturing but has the disadvantage that it does not reveal whether the bacteria detected are viable. Nonetheless, comparisons of PCR or other highly sensitive diagnostic methods for bacterial detection with transmission efficiency of *H. coagulata* and other vector species might be useful in developing an index to estimate levels of natural infectivity without the extended time commitment and greenhouse facilities required for transmission tests. Natural infectivity is an important component in relating vector abundance and activity to PD spread (Purcell and Feil 2001).

PD in California has historically been a monocyclic disease that occurs along the edges of vineyards. Chronic disease occurs when sharpshooters (Cicadellini) inoculate shoots of grapevines during the spring months, when *X. fastidiosa* has time to move basally in stems to reach woody portions of the vine where it can over winter. Current epidemics of PD in California that are associated with *H. coagulata* appear to be polycyclic, with exponential increase early in the epidemic, suggesting that the vine-to-vine phase of *X. fastidiosa* spread by *H. coagulata* creates a large number of chronic infections. *H. coagulata* feeds on woody tissues of grapevines throughout the year, and transmits *X. fastidiosa* into stems 2-yr-old or older, most of which will not be pruned off; thus many inoculations made in summer and fall may become chronic infections. Vector-mediated infections of dormant vines by *H. coagulata* have not been documented, but our preliminary results indicate that dormant vines can be infected under laboratory and field conditions (unpublished). This implies that control of *H. coagulata* populations in vineyards may need to be maintained year-round to control PD. Thresholds for control of *H. coagulata* for satisfactory control of PD have not yet been determined.

The introduction of *H. coagulata* into California dramatically changed the epidemiology of PD wherever this insect occurred. Our results suggest that although *H. coagulata* is a less efficient vector of *X. fastidiosa* to grape than *G. atropunctata*, it has other characteristics that may make it a more effective vector in the field. Its propensity to feed on and ability to inoculate woody tissues may lead to establishing a higher incidence of chronic infections during summer and fall (Purcell and Saunders 1999, Purcell and Feil 2001). In addition, *H. coagulata*'s dispersal abilities (Ball 1979, Blua et al. 1999) and its propensity to aggregate in high numbers along crop borders (Blua et al. 1999) may mandate that strategies for control of PD (Goodwin and Purcell 1990) must be reconsidered in the light of this new threat to the grape industry.

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