Patterns of *Xylella fastidiosa* Colonization on the Precibarium of Sharpshooter Vectors Relative to Transmission to Plants

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ABSTRACT *Xylella fastidiosa* is a bacterial plant pathogen that causes many plant diseases, including Pierce's disease of grapevines. Sharpshooter leafhopper (Hemiptera: Cicadellidae: Cicadellinae) vectors transmit this bacterium to plants. Although the basic mechanism of pathogen transmission is not completely understood, previous studies implicated the foregut of infected insects as the source of bacterial inoculum because infective nymphs lose transmissibility after molting and no latent period is required for transmission. Scanning electron microscopy documented the spatial distribution of *X. fastidiosa* in the foreguts of the sharpshooter *Graphocephala atropunctata* (Signoret) after short (1-d) and long (4-d) acquisition access periods on infected plants and various inoculation access periods on grape test plants. After 1-d acquisition and 1-d inoculation periods, cells had only colonized portions of the precibarium between the stylets and the cibarial pump, often embedded in a matrix-like material. After long (15-d) periods after acquisition, we found bacterial cells attached polarly to the insect's cuticle in a regular pattern throughout the precibarium but absent from specific locations on both pharynges. Insects sampled for microscopy 2 wk after long acquisition access periods and that contained bacteria in their mouthparts transmitted *X. fastidiosa* to healthy grapevines independently of the length of the acquisition access period. In contrast, individuals free of bacteria on the precibarium did not transmit the pathogen. After successive short acquisition and inoculation periods, 5 of 12 *X. fastidiosa*-positive insects did not transmit to plants. We also did transmission experiments with the sharpshooter *Homalodisca coagulata*; however, only one of 30 individuals had *X. fastidiosa* attached to its precibarium, and none transmitted it to plants. Our results suggest that sharpshooters introduce into plants *X. fastidiosa* cells that detach from the precibarial canal during feeding.

KEY WORDS attachment, *Graphocephala*, *Homalodisca*, Pierce's disease

Xylella fastidiosa Wells et al. is a xylem-limited bacterial plant pathogen that causes various plant diseases, including Pierce's disease (PD) of grapevines (Hopkins and Purcell 2002). This bacterium is transmitted to plants by sharpshooter leafhoppers (Hemiptera: Cicadellidae), spittlebugs (Hemiptera: Cercopidae) (Severin 1949, 1950), and cicadas (Hemiptera: Cicadidae) (Paião et al. 2002). Vector specificity for *X. fastidiosa* transmission is very broad and seems to be limited to those specialized hemipteran insects that preferentially ingest xylem sap (Frazier 1966). Sharpshooter nymphs and adults can vector *X. fastidiosa*, but nymphs lose infectivity after molting. This characteristic suggests that the foregut, from which the cuticular lining is shed with each molt, is the source from which vectors introduce bacterial cells to accomplish transmission (Purcell and Finlay 1979, Almeida and Purcell 2003). *Xylella fastidiosa* is transmitted by vectors without a latent period (time between pathogen acquisition and its inoculation into a new plant) (Purcell and Finlay 1979), is persistent, and multiplies in its vectors (Severin 1949, Hill and Purcell 1995). These attributes are also consistent with the hypothesis that *X. fastidiosa* is transmitted from the foregut.

Sharpshooters have a simple but extremely efficient sap-sucking mechanism for feeding on a very dilute diet that is usually under negative tension. Sap ingestion is possible because of a diaphragm pump where flow direction is controlled by two valves. The anterior valve, i.e., precibarial valve, occurs in the precibarium of the foregut; the precibarium directs fluid from the food canal into the cibarium (Backus and McLean 1982, 1983). The cibarium is a pump chamber with a rigid, ovoid, cuticular bottom and an elastic, membranous diaphragm (Purcell et al. 1979) (Fig. 1). Cibarial dilator muscles attached to a longitudinal ridge on the dorsal side of the membrane contract to elevate the diaphragm, thereby increasing pump volume and lowering the pressure within the chamber relative to the sap on which the insect is feeding. This pulls fluid into the cibarium. When the muscles relax, the bellows-like diaphragm collapses to the bottom of the cibarium, pushing fluid along a groove in the floor of the cibarium into the esophagus (Purcell et al. 1979). A second valve, the cardiac (esophageal) valve is located between the esophagus and midgut and is assumed to passively open because of positive pressure, allowing fluid to pass to the mid-

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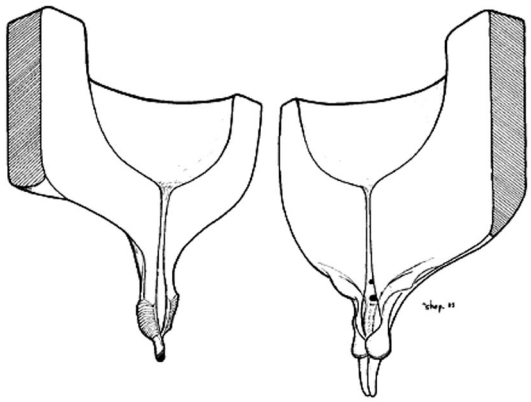

Fig. 1. Schematic drawing of the epipharynx (right) and hypopharynx (left) of *G. atropunctata.* Precibarium is shown in the center of each pharynx, opening into the cibarium chamber (top section of figure).

gut (Backus and McLean 1983; McLean and Kinsey 1984). The cardiac valve collapses shut when the pump muscles contract to prevent the backflow of midgut fluids into the foregut. This general mechanism is based on morphological studies and is assumed to be similar for leafhoppers (Backus and McLean 1982, 1983), aphids (McLean and Kinsey 1984), psyllids (Ullman and McLean 1986), and thrips (Hunter and Ullman 1994).

Purcell et al. (1979) showed that *X. fastidiosa* occurs on the cuticle of the cibarium and proximal precibarium of *Graphocephala atropunctata* (Signoret). However, it is not clear whether cells from this area of the foregut serve as an inoculum source. Brlansky et al. (1983) demonstrated that *X. fastidiosa* also occurs on the precibarium of other sharpshooters, both anterior and posterior to the precibarial valve, but they did not correlate bacterial presence or location with transmission to plants. In both studies authors found that *X. fastidiosa* occurred in dense populations of cells that formed a solid mat or "carpet" of rod-shaped bacteria. However, the numbers of cultivable cells of *X. fastidiosa* that can be enumerated by dilution plating from vectors' heads were not correlated with transmission ability of the sharpshooters *G. atropunctata* (Hill and Purcell 1995) or *Homalodisca coagulata* (Say) (Almeida and Purcell 2003). Sharpshooters that had numbers of bacteria below the detection threshold (estimated to be <100 cells at a confidence level of 95% of culturing at least one cell per head) transmitted about as well as sharpshooters estimated to have thousands of cells in their heads. One implication of the finding that small numbers of bacteria can enable efficient vector transmission is that the location from which bacteria must occur to enable transmission is very small (Hill and Purcell 1995). *X. fastidiosa* cells present on the precibarium are closer to the stylets than those on the cibarium and thus may more readily be inoculated into xylem vessels during feeding (Hill and Purcell 1995). The exact location or locations that *X. fastidiosa* must colonize to enable vector transmission are unproven.

The sharpshooter *G. atropunctata* is the most important vector of *X. fastidiosa* in coastal California (Redak et al. 2004). It is an efficient vector when compared with other sharpshooters (Severin 1949, Purcell and Finlay 1979, Almeida and Purcell 2003) and is the most-studied vector of *X. fastidiosa* in relation to its transmission biology. For these reasons, we used *G. atropunctata* to study the spatial distribution of *X. fastidiosa* on the precibarium of sharpshooter vectors and its transmission to plants after short and long incubation periods (i.e., the total time from initial access on infected plants to time of fixation of the insect mouthparts) by using scanning electron microscopy (SEM). Our objective was to determine the location of *X. fastidiosa* in relation to transmitting or nontransmitting sharpshooters.

Materials and Methods

Bacterium and Leafhoppers. We used the *X. fastidiosa* grape isolate STL (ATCC700963) from Napa Valley, CA, for experiments (Hendson et al. 2001). Bacterial suspensions were mechanically inoculated into healthy grapevines following previously described methods to produce source plants of the pathogen (Hill and Purcell 1995). We collected adults and nymphs of the sharpshooter *G. atropunctata* from urban areas of Berkeley, CA, and reared their progeny from eggs in the greenhouse. We reared *H. coagulata* from insects originally collected from citrus in Kern County, CA. *X. fastidiosa* is not transmitted transovarially (Freitag 1951). We kept both species of insects in a temperature-controlled greenhouse, in cages on grape, *Vitis vinifera* L.; California mugwort, *Artemisia douglasiana* (Besser); or basil, *Ocimum basilicum* L. The plants were changed weekly during the insects' development to produce adults free of *X. fastidiosa.* We further screened sharpshooters assumed to be free of *X. fastidiosa* by confining them in groups (typically 10) on grape seedlings (*Vitis vinifera* L. 'Cabernet Sauvignon') for at least 4 d and holding the plants for 3–6 mo for symptoms of PD and evaluation by culturing for the presence of *X. fastidiosa.* None of the pretest screening plants were positive for *X. fastidiosa* for any of the insects used in these experiments.

Transmission and Distribution of *X. fastidiosa*. We conducted three experiments, labeled A, B, and C (Table 1), with *G. atropunctata* given the opportunity to transmit *X. fastidiosa* to grape, followed by examination of the insects for the distribution of *X. fastidiosa* in the foreguts of individuals from each group of vectors. Insects acquired the pathogen during the acquisition access period (AAP), and then they were transferred to nongrape plants (California mugwort) to allow bacterial multiplication in the foregut, and finally they were transferred to healthy grape for the inoculation access period (IAP). Incubation period (in place of latent period) is defined here as the total period from beginning of AAP to the end of the IAP.

Table 1. Summary of transmission experiments and their AAPs, bacterial multiplication, and IAPs

Exp	Insect transfer sequence			No. insects[a]	No. positive heads	No. PD plants
	AAP	Multiplication period	IAP			
A	4 d	7 d	4 d	10	7	7
B	8 h	13 d	1 d	9	3	4
C	1 d	0 d	1 d	22	12	7

The total period for *X. fastidiosa* colonization of the precibarium before fixation is the sum of the three periods in the transfer sequence (incubation period).

[a] Includes only the number of insect heads that were not damaged during dissection for SEM analysis.

In experiment A, we used 4 d for both AAP and IAP to maximize *X. fastidiosa* transmission efficiency. Experiment A and B had similar incubation periods; however, in B the AAP was reduced to 8 h to determine its effect on *X. fastidiosa* distribution and transmission patterns. In experiment C, we used a short incubation period of 1 d AAP followed directly by a 1-d IAP (total incubation of 2 d). The objective of experiment C was to determine regions of initial bacterial attachment in the precibarium before thorough colonization of the canal occurred. After all of the IAPs, we fixed the insect heads (see below) and kept the grape test plants in a greenhouse for later diagnosis. We evaluated test plants for *X. fastidiosa* infection by visual symptoms of PD and by culturing (Hill and Purcell 1995). In preliminary studies, we also analyzed five *G. atropunctata* adults before an AAP and 16 individuals that had a 4-d AAP followed by 2 wk on basil (only to allow bacterial multiplication, no IAP). We also analyzed the precibarium of *H. coagulata* adults by using the same protocols described above. Insects were reared in the laboratory as described previously (Almeida and Purcell 2003). Sixteen adults from our colony were fixed without access to *X. fastidiosa*-infected plants. Two *H. coagulata* groups of 15 individuals had a 4-d or a 1-wk AAP, respectively, followed by a 2-wk IAP on grape (individually).

Scanning Electron Microscopy. We fixed insect heads in 2% (vol:vol) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h at room temperature. Samples that could not be further processed immediately were stored at 4°C for up to 2 mo. We rinsed the fixed heads three times with 0.1 M sodium cacodylate buffer; postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer; rinsed once again in buffer, and dehydrated them in an ethanol series. After dehydration, samples were critical point dried and stored within a desiccator until dissected. The pharynges were dissected, mounted on sticky carbon dots on aluminum stubs, and sputter-coated with gold-palladium (similarly to Backus and McLean 1982). Specimens damaged during dissections were not examined or included in our study. We examined samples with an Electroscan E3 ESEM or a Hitachi field emission SEM and saved the images as digital files.

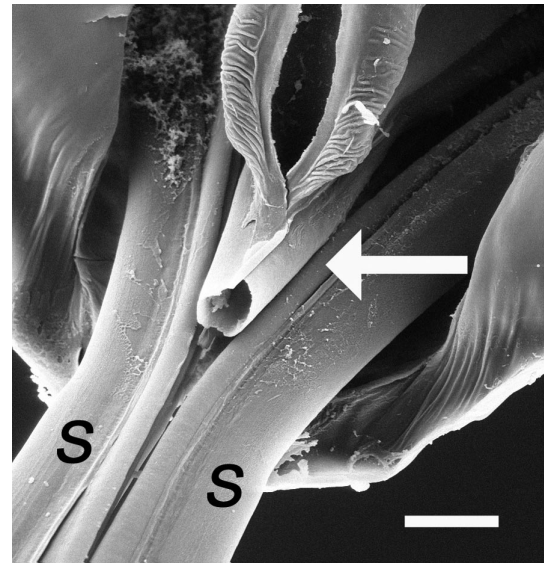

Fig. 2. Hypopharynx of *G. atropunctata*; the HEF (arrow) is located above the stylets (S). Bar = 20 μm.

Results

Sharpshooter Precibarium. The precibarial and cibarial morphology of *G. atropunctata* was similar to previously published reports (Purcell et al. 1979, Backus and McLean 1983; Fig. 1). The channel was divided into proximal and distal regions by the flap-like precibarial valve located on the epipharynx. We observed the 10 D-sensilla, eight P-sensilla, and the two H-sensilla identified by Backus and McLean (1983). At the proximal end of the stylet, where the stylets diverge into grooves exterior to the hypopharynx, we found a conically shaped extension of the hypopharynx, formed by overlapping flanges, which connects the food canal to the precibarium (Fig. 2). This hypopharyngeal extension that inserts into the food canal (HEF) has not been described for leafhoppers, but Ullman and McLean (1986) reported it in psyllids.

In side view, the precibarium is "step-like," elevating dorsally proximal to the valve on the epipharynx, in the region of the P-sensilla (i.e., epipharyngeal basin; Backus and McLean 1983). Its surface shifts ventral-proximally into a tubular channel with parallel ridges, distal to the cibarium (Fig. 3). We use the term epipharyngeal trough for this section of the precibarium, following suggestion of E. A. Backus. Distal to the valve, we found the precibarial valve's pit, which is the interior of an apodeme on which the valve muscles insert (Backus and McLean 1982). The structure of the epipharynx and how it interlocks with the hypopharynx suggest that some fluid may flow into the pit. However, we were not able to determine with SEM if there is an exit structure for that fluid beyond the precibarial valve (Figs. 3 and 4).

***X. fastidiosa* Distribution on Precibarium.** For experiments A and B, we observed *X. fastidiosa* cells on

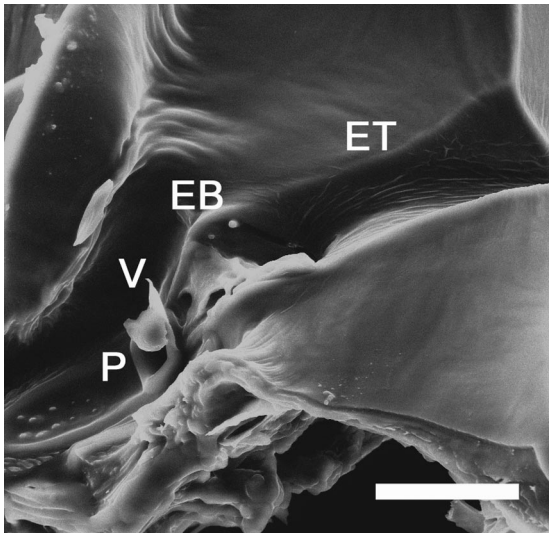

Fig. 3. Epipharynx of *H. coagulata* showing the step-like characteristic of the precibarium and precibarial valve's pit. P, precibarial valve's pit; V, precibarial valve; EP, epipharyngeal basin; and ET, epipharyngeal trough. Bar = 50 μm.

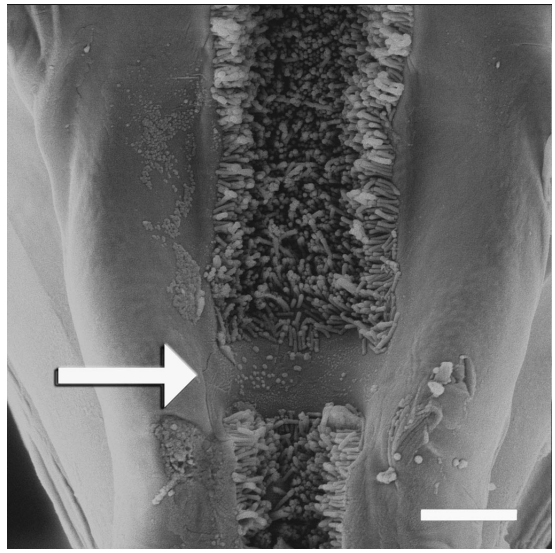

Fig. 5. Hypopharynx of *G. atropunctata* showing region always free of *X. fastidiosa* (arrow). This region matches the location of the precibarial valve on the epipharynx. Absence of cells may be associated with movement of the valve or contact to structures associated with the valve present in the epipharynx. Bar, 10 μm.

both pharynges, always polarly attached to the insect's cuticle. Probably because of the long incubation period given the insects, we found mats of bacteria in both cohorts. The hypopharyngeal surface was covered with homogeneous mats (Fig. 5). However, an area on the hypopharynx directly opposite the valve (located on the epipharynx), was always *X. fastidiosa*-free (Fig. 5). Based on measurements of both pharynges, this is probably the area where the precibarial valve interlocks with the hypopharynx. We often found bacteria located only proximally to the precibarial valve; if present distally, bacteria also were always observed proximally (Fig. 6). We did not find bacteria on the precibarial valve (Fig. 4) or at the center of the epipharyngeal trough (view not shown). Cells often occurred within the pit distal to the precibarial valve (Fig. 4). *X. fastidiosa* also attached to the proximal area of the precibarium (Fig. 7A) and in the groove at the distal portion of the cibarium (view not shown).

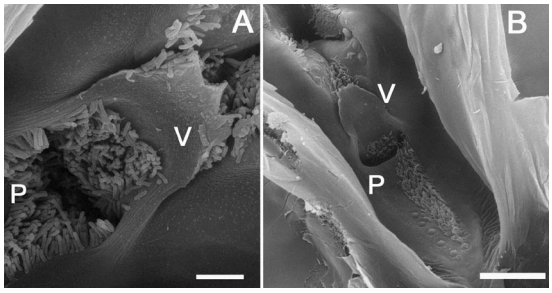

Fig. 4. Epipharynx of *G. atropunctata* with *X. fastidiosa* occurring on the precibarium. A and B (A, bar = 5 μm; B, bar = 20 μm) show cells within the precibarial valve's pit (P) and their absence on the precibarial valve (V). Inset in B shows the epipharyngeal basin lies above (top left) the valve; the D-sensilla lie below (bottom right).

Transmission of *X. fastidiosa*. The occurrence of *X. fastidiosa* cells in the precibarium of *G. atropunctata* was highly correlated with its transmission to grape (Table 1). Only one insect in which we failed to find bacteria, in experiments A and B, transmitted *X. fastidiosa* to plants. All other bacteria-free insects did not transmit *X. fastidiosa* to plants. In contrast, after a 1-d AAP and 1-d IAP (experiment C), five of 12 positive insects did not transmit *X. fastidiosa* to plants. Experiment C provided information on the sites of initial bacterial attachment after acquisition (Fig. 6). In all cases, *X. fastidiosa* had not fully colonized the precibarium. Most of the heads (11/12) were colonized by clusters of cells proximal to the precibarial valve area (Fig. 6). These colonies were assumed to be located at sites of initial attachment on the precibarium by *X. fastidiosa.* All insects that transmitted *X. fastidiosa* to plants had small colonies in the precibarium. Cells were also found attached sideways when short incubation periods were used, contrary to the more usual polar attachment seen after long incubation periods. Many insects had cells attached both ways (i.e., polarly and sideways) to the precibarial cuticle. Sideways attachment was observed in small microcolonies and at the fringe of bigger colonies of attached cells. In one case, cells were only observed distally to the valve, entering the valve's pit (Fig. 7B).

***H. coagulata* Observations.** None of the groups of tested *H. coagulata* transmitted *X. fastidiosa* to grape. Morphological characteristics of the precibarium of *H. coagulata* were similar to *G. atropunctata* and as

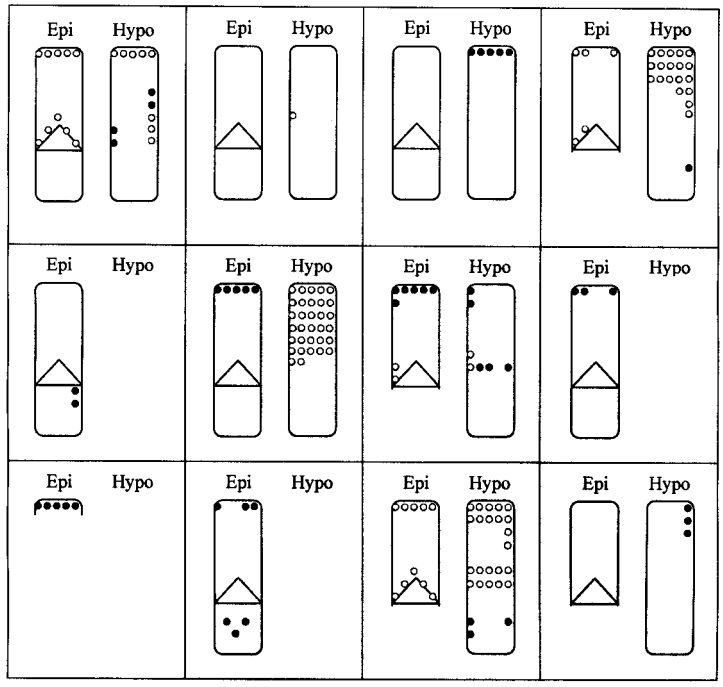

Fig. 6. Diagrammatic illustration of areas with *X. fastidiosa* attached after a 2-d incubation period (experiment C) in the precibarium of 12 *G. atropunctata*. Epipharynx (Epi) and hypopharynx (Hypo) are represented; the stylets would be below (distal) and the cibarium above (proximal) in each figure. Precibarial valve is shown as a triangle; filled and empty circles indicate regions colonized by *X. fastidiosa* where cells were found to be attached sideways or polarly, respectively. Figures not drawn to scale. Sections of cuticle that could not be visualized are not included in the diagrams.

described previously by Backus and McLean (1983). Only one of the 30 dissected and analyzed *H. coagulata* was infected with *X. fastidiosa.* The distribution of the bacteria on the precibarium was similar to that described for *G. atropunctata*, occurring proximally to the valve.

Discussion

We analyzed the spatial distribution of *X. fastidiosa* in the precibarium of *G. atropunctata* after short and long incubation periods and correlated bacterial presence and location to its transmission to plants. We identified specific areas of the precibarium not colonized by the bacterium. This may be due to how the pharynges interlock, to differences in fluid dynamics within the precibarial canal, or to abrasion by the closing of the precibarial valve. Insects that transmitted *X. fastidiosa* to plants had bacterial cells in the precibarium. When short incubation periods were used, most (but not all) bacterial clusters were present proximally to the precibarial valve. The small numbers of bacteria present in transmitting insects after a short (2 d) latent period is consistent with the findings that small numbers of live (cultivable) *X. fastidiosa* are sufficient for efficient transmission (Hill and Purcell 1995).

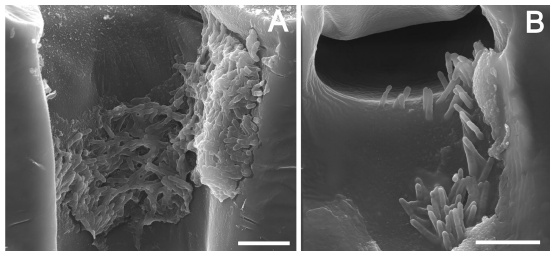

Fig. 7. *X. fastidiosa* colonies on the epipharynx of the precibarium after a 2-d incubation period (experiment C). (A) Early colonization of the proximal end of the precibarium; cells are embedded in matrix and not polarly attached. (B) Few cells were found distally to the precibarial valve. Bars = 5 μm.

In addition to the morphology of the precibarium of sharpshooter leafhoppers previously published (Backus and McLean 1983), we report the presence of an HEF, also described in psyllids and present in aphids and leafhoppers (Ullman and McLean 1986, and unpublished observations cited therein). The HEF probably seals the connection between the stylet's food canal and the precibarium, as suggested by Ullman and McLean (1986). The anterior section of the precibarium on the epipharynx has a blind end that closes against the hypopharynx. The maxillary stylets are constantly moving in and out while the insect is probing (Backus 1988), and a tight seal can be maintained by the HEF, which presumably acts to prevent leakage during fluid ingestion. In one *G. atropunctata* and seven *H. coagulata* specimens, we found a mem-

brane-like structure of unknown composition (view not shown) within the lumen of the precibarium. This structure may be an artifact of fixation or tissue that is present in only some insects.

X. fastidiosa occurred throughout the precibarium, with the exception of two locations on the epipharynx (the precibarial valve and the epipharyngeal trough), and one location on the hypopharynx (the site against which the flap of the valve closes or part of the apodeme is located). Brlansky et al. (1983) noted that bacteria formed mats distally and proximally to the valve, but they did not determine any areas where *X. fastidiosa* was not present, nor did they relate bacterial location to transmission. Because the precibarial valve is a flap-like structure on a hinge, Backus and McLean (1982) suggest that it acts as a pressure-sensitive check-valve, closing against the hypopharynx each time the cibarium dilator muscles relax, to function with little or no backwash (Backus and McLean 1982). Therefore, cells cannot survive on the portion of the cuticle where the valve closes. This mechanical friction may also be one way through which bacterial cells can be dislodged and subsequently inoculated into plants. Any cells that have already detached would not be present for our examination. Thus, we suggest the location of areas within the precibarium where *X. fastidiosa* does not occur are more likely to be the site from which detached bacteria are inoculated into plants. The closing action of the precibarial valve, abrading away any attached bacteria on the hypopharynx where the valve contacts the hypopharynx, is the most reasonable explanation of why we found no bacteria at that location. Bacterial biofilms occurred at the proximal portion of the precibarium, but these cells would have to move farther than those at the distal (anterior) portion of the precibarium to be expelled out of the stylets. Nevertheless, our results do not exclude the possibility that cells attached to any regions of the precibarium may be inoculated into plants.

Purcell et al. (1979) correlated *X. fastidiosa* transmission to plants with culturing of the pathogen from the precibarium (or "food meatus"; Snodgrass 1935). That report emphasized the importance of the foregut in *X. fastidiosa* transmission to plants. Those authors also cultured *X. fastidiosa* from the stylet bundle of infective insects, but these results were not further investigated. We have now shown that presence of bacteria on the precibarium, as detected microscopically, correlates well with transmission to plants. We did not find *X. fastidiosa* attached to maxillary stylets of *G. atropunctata*, although we examined the stylets of only a few individuals (data not shown). The extensive colonization of the precibarium and cibarium by *X. fastidiosa* is further evidence that this bacterium multiplies in the foregut of vectors (Purcell et al. 1979, Brlansky et al. 1983, Hill and Purcell 1995). Our results show that only small numbers of *X. fastidiosa* attached to the precibarium after 2 d, yet this was sufficient for pathogen transmission. Thus, the importance of bacterial multiplication in the transmission mechanism per se is probably low, because it has been shown that *X. fastidiosa* is transmitted efficiently without a latent period and bacterial populations within insects have not been correlated with transmission efficiency because insects with fewer than 100 cultivable bacteria per head transmitted efficiently (Hill and Purcell 1995).

Our experiments corroborate previous studies showing that *X. fastidiosa* inoculum for transmission consistently colonizes the foregut of vectors (Purcell and Finlay 1979, Almeida and Purcell 2003) and also support the hypothesis that bacterial attachment and detachment from the precibarium is important for vector transmission. A recent study using an isolate of *X. fastidiosa* expressing a green fluorescent protein reporter gene showed the presence of the bacterium in the precibarium of vectors that transmitted it to plants (Newman et al. 2003). Bacterial colonization of the precibarium was also shown to be essential for vectoring capability when a *X. fastidiosa* mutant that did not attach to or colonize the precibarium was shown not to be transmissible by insects (Newman et al. 2004). Although *X. fastidiosa* transmission to plants has been associated with its presence in the precibarium, questions about differences in vector efficiency remain unanswered. *H. coagulata* is a less efficient vector than *G. atropunctata* in transmitting *X. fastidiosa* from grape to grape (Almeida and Purcell 2003). We found only one of 30 *H. coagulata* with cells on the precibarium in this study, suggesting that its lower transmission efficiency may be associated with lack of bacterial attachment to the precibarium. However, because *X. fastidiosa* has low vector specificity, the ability of cells to attach to the foregut of different vector species should be similar (Frazier 1965). Differences in feeding behavior may better explain variability among species in transmission efficiency.

The use of short AAP and IAP allowed us to determine in more detail the location of *X. fastidiosa*'s initial colonization of the precibarium of vectors. Usually, cell clusters were attached to the proximal area of the precibarium, suggesting that 1) *X. fastidiosa* cells first colonize the area above the precibarial valve and 2) bacteria can be inoculated from the proximal area of the precibarium. Assuming that there is no fluid flowing into the cibarium through the precibarial valve pit and that the current hypothesis for the functional mechanism of the precibarial valve is correct (Backus and McLean 1982, 1983; McLean and Kinsey 1984), there are different possible interpretations of our results relative to vector transmission. One hypothesis is that when the cibarial dilator muscles relax, cells may be detached from the precibarium and pushed distally past the precibarial valve before it closes. However, sharpshooters may have unknown probing behaviors, similar to the complex behaviors of aphids that transmit nonpersistent viruses (Martin et al. 1997). Electrical penetration graph (EPG) monitoring would be useful in addressing such questions; and such studies on the probing behaviors of sharpshooters have been initiated (Almeida and Backus 2004, Backus et al. 2005). Coupling of EPG with transmission experiments will provide valuable informa-

tion on behavior associated with successful inoculation. Vector transmission of *X. fastidiosa* to dormant plants with positive root pressure (Almeida et al. 2005) provides indirect evidence suggesting that an active leafhopper probing behavior is required for transmission rather than the simple suction of cells from the foregut into the negative pressure of the xylem stream of actively transpiring plants. Further understanding of how the cell–cell signaling system of *X. fastidiosa* affects transmission (Newman et al. 2004) also should provide additional details as to how vectors transmit this bacterium to plants. It would also be relevant to study the importance of fluid flowing into the pit (as evidenced by presence of *X. fastidiosa*) to sharpshooter feeding mechanics and *X. fastidiosa* transmission. It seems to us that fluid in the pit, apparently not regulated by the precibarial valve, could be easily moved back into plants through the stylets.

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