

How to be an ideal vector: four crucial steps in the transmission mechanism of *Xylella fastidiosa* by sharpshooters

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Pierce's Disease of grape, caused by the bacterium *Xylella fastidiosa* (*Xf*), has become a serious threat to California vineyards since the introduction of a new vector, glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*. Research in my lab seeks to understand how the feeding of GWSS controls transmission (acquisition and inoculation) of *Xf*, and to apply that knowledge to development of resistant grape varieties, microbial biological control, and epidemiological modeling of disease progression. Our research tools include electrical penetration graph (EPG) monitoring of feeding, confocal and scanning electron microscopy of insect and grape tissues, and green fluorescent protein (GFP)-transformed *Xf*.

Studies to date support a model of **four crucial steps** that must all occur simultaneously during GWSS feeding, for successful *Xf* inoculation. These are: **1)** the titer of acquired bacteria (bacterial load) present in the vector foregut must be high, **2)** bacteria must be present in a precise location in the foregut, probably the anterior precibarium, **3)** the vector must perform a specific inoculation behavior, comprising both egestion and salivation, and **4)** the vector's stylet tips must be in a functional xylem cell at the time of inoculation.

Evidence for these steps, in brief, is: **1)** A related paper in this Proceedings (Backus 2007) describes microscopic comparisons of microbial colonization of foreguts of 'clean', lab-reared GWSS vs. 'dirty' field-collected GWSS. Clean GWSS acquired many more GFP-*Xf* than did dirty GWSS (see figure in Backus 2007). Preliminary, post-acquisition inoculation assays with these insects suggests that originally clean, 'maximally loaded' GWSS inoculate far more *Xf* than do originally dirty, 'topped off' GWSS. **2)** Close examination of pre-inoculation vs. post-inoculation GWSS suggests that the anterior precibarium within the foregut is the location from which bacteria are dislodged during inoculation (matching and expanding on the findings of Almeida and Purcell 2006). **3 and 4)** We have recently identified an EPG waveform for GWSS, called the X-wave, which uniquely identifies intracellular puncture of a xylem cell (Holmes 2007) prior to ingestion. Components of the X-wave have been correlated with egestion and salivation behaviors (Backus et al. 2005, Joost et al. 2006) that may sweep bacteria out of the anterior precibarium; thus, the X-wave may represent the inoculation behavior.

Evidence that these four steps must occur for optimum inoculation came in a recent experiment. A single, individual GWSS can be an "ideal vector" (i.e. 100% inoculation efficiency within one probe on susceptible grape), when all four of these steps are maximized.

Clean GWSS were allowed 7 – 10 days acquisition access period on symptomatic, 'Cabernet Sauvignon' grape that had been mechanically inoculated with GFP-*Xf*. Each maximally loaded, *Xf*-inoculative insect was then EPG-recorded for a single, standardized probe (composed of sheath salivation plus 3-6 min of ingestion, including at least one X-wave) on a healthy 'Caber-net Sauvignon' grapevine petiole (Fig. 1A). Next, each plant was held in an insect-exclusion cage in the greenhouse for one of 4 time periods: 0, 10, 20 or 40 days (the 0 d tissue actually was excised 2 – 4 min after the end of the probe). After the holding period, the petiole tissue in the immediate vicinity of the marked probe site was excised (Fig. 1B) and prepared for later histology (in process). In addition, when plants became obviously symptomatic for PD at 3 mo. (Fig. 1C), 8 – 10 leaves above the probed petiole were assayed for *Xf* using PCR (Fig. 1D).

All of the 36 plants (9 reps per treatment) became symptomatic for PD and died within 5 months after the inoculation probe. Control, healthy plants subjected to all the same treatments except inoculation thrived,

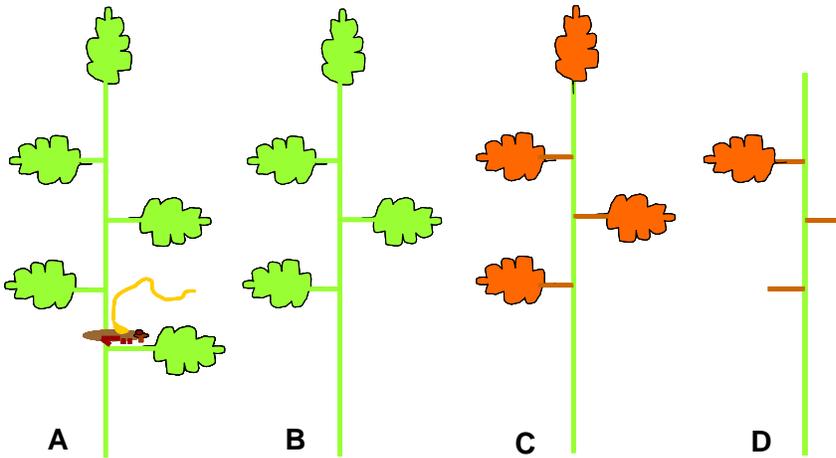


Figure 1. **A.** Wired, EPG-recorded GWSS is allowed one probe on healthy grapevine petiole; plant is held in greenhouse. **B.** After holding time, probed petiole is excised for histology; plant is returned to greenhouse. **C.** After 3 months, leaves above probed petiole show PD symptoms. **D.** Ten leaves above probed petiole are excised and tested for *Xf* via PCR.

and none died. A majority of plants in each treatment were PCR-positive for *Xf*, i.e. 100% of the 0 d plants, and 56% each for the 10, 20 and 40 d plants. The titers of bacteria in the negative 44% of leaves were probably below the detection threshold for our non-optimized PCR tests.

The strong symptom results plus PCR results, combined, suggest that 100% of these grape plants were inoculated with *Xf* by a single GWSS probe. We postulate that inoculated, 'pioneer' bacteria infected the initial xylem cells, colonized (reproduced), and eventually spread systemically to the rest of the

plant, leading to a lethal PD infection. We are repeating this experiment, with an improved histological component, for increased sample size prior to publication. If results are similar, this will be the first time that GWSS has been experimentally shown to exhibit 100% vector efficiency per individual insect, *let alone from a single probe*. Other researchers have documented much lower individual vector efficiencies. We hypothesize that their results were caused, primarily, by use of dirty GWSS for acquisition, and secondarily, by the inability to exactly control inoculation probing, as can be done via EPG.

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