GLASSY-WINGED SHARPSHOOTER TRANSMISSION OF *XYLELLA FASTIDIOSA* TO ALMOND

Project Leader:
Alexander H. Purcell
Division of Insect Biology
University of California
Berkeley, CA 94720-3112

Researcher:
Rodrigo P.P. Almeida
Department of Plant and Environmental Protection Sciences
University of Hawaii at Manoa
Honolulu, HI 96822

Reporting Period: The results reported here are from work conducted from October 2002 to October 2003.

ABSTRACT

The acquisition of *Xylella fastidiosa* (*Xf*) from dormant almond by the glassy-winged sharpshooter (GWSS) was attempted in January 2003 near Bakersfield with 16 groups of 4 adult GWSS per group caged on branches of almond trees with ALS symptoms and confirmed by culturing as having *Xf* infections. None of the surviving GWSS transmitted *Xf* to 16 Non-pareil almond test plants. Culture assays for *Xf* in naturally-infected field almonds and greenhouse plants documented densities of about 1,000,000 to 10, 000,000 live *Xf* per gram of leaf vein. These are 10 to 100 times lower than in grapes with PD symptoms and may explain in part why vector acquisition of *Xf* from almond is lower than from grape. In other experiments, we found that previously reported, genetically-determined groupings of *Xf* strains from almond (Groups I and II) and grape or almond (typed as "Grape" strains) had distinctive biological characteristics that corresponded to the genetic groupings. All strains mechanically inoculated into grape and almond caused almond leaf scorch (ALS), but only grape strains caused Pierce's disease. Almond Group I strains did not grow on PD3 medium, but Almond Group II strains and Grape strains did. Almond strains of *Xf* survived winter dormancy in almond at Berkeley better than did grape strains, but the reverse was true in grape.

INTRODUCTION

One of the unknown but feared impacts of the glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*) in the San Joaquin Valley of California is its role in the spread of almond leaf scorch disease (ALS). GWSS includes numerous species of trees among its favored plant hosts (Turner and Pollard 1959a) and is considered to be one of the most important vectors for the phony peach disease (Turner and Pollard 1959b), so it should be expected to feed on almond. We previously reported that GWSS transmits *Xf* to almond less efficiently than to grape, with insect numbers per plant and days of exposure approximately equal in influence on transmission probability (Purcell 2002; Purcell and Almeida 2002). We also found that GWSS transmitted *Xf* to dormant almond trees in greenhouse experiments. Our objectives for the past year were to determine if GWSS could acquire *Xf* from dormant almond in the field, what populations of *Xf* occurred in ALS-symptomatic plants in the field, and to determine if both grape and almond strains colonized and caused disease in both almond and grape. Some of the results re-summarized here were recently published (Almeida and Purcell 2003b).

OBJECTIVES

1. Determine the efficiencies of acquisition and inoculation of *Xf* by the GWSS to almonds.
2. Quantify populations of *Xf* in infected almonds in the field throughout a season.
3. Determine the ability of the GWSS to inoculate *Xf* to mature (> 1 year) and dormant woody tissues of almond.

RESULTS

GWSS transmission of Xf to almond

Objectives 1 and 3 were completed and the results reported last year (Purcell 2002) and in a publication (Almeida and Purcell 2003b). To briefly summarize: GWSS acquired and inoculated *Xf* from/to almonds with an acquisition efficiency of ~7% per insect per day, and inoculation of 4% per insect per day. This is less than half of GWSS transmission rates to grape (Almeida and Purcell 2003a). GWSS transmitted *Xf* to 1-year old woody tissue of almonds with equal efficiency as to green tissue, and survived about the same on both types of plant tissues. Because of concerns regarding transmission of *Xf* from grape to almonds, we did a transmission experiment using two grape strains (one from Napa Valley, another from Bakersfield), and found that both were transmitted to both grape and almonds. Napa Valley strains were slightly more virulent (more severe symptoms) in grape than were Bakersfield strains.

We tested the ability of GWSS to acquire *Xf* from dormant almonds in the field. In experiments in a commercial 'Non-pareil' almond orchard north of Bakersfield, we caged lab-reared, non-infective GWSS adults. The insects first were tested for four days on grape seedlings to insure that they were *Xf*-free and then transferred in the field to almond branches that had previously tested as positive for ALS symptoms and *Xf* populations by culturing. After one week on dormant almonds we transferred the 16 surviving GWSS (of the initial 64 used) to almond test plants (Non-pareil on Lovell rootstock) in the greenhouse. Almost all of these GWSS survived in the lab, but there was no transmission of *Xf* to almond, as determined by culturing assays (Hill and Purcell 1995).

Populations of Xf in infected almonds in the field and greenhouse

Xf bacterial populations within plants have been correlated with vector transmission efficiency (Hill and Purcell 1997). Following that assumption, we quantified *Xf* from infected almonds in the field growing in 3 different areas of California and in the laboratory. Details about our experiments have been published (Almeida and Purcell 2003b). We found that bacterial populations were 10- to 100-fold lower in almond than in grapes. We also surveyed bacterial populations in an infected orchard at UC Davis over 4 years (1997-99, and 2001-02). Bacterial populations were low in April, reached 10^6 CFU/g of tissue in June and 10^7 CFU/g in September (Almeida and Purcell 2003b, Almeida and Purcell in press). These low populations suggest that GWSS transmission of *Xf* to almonds during spring and early summer is most likely to occur with infective insects migrating from grape or other plants. During late summer, when bacterial populations increase, transmission from infected to healthy almonds might become important within orchards, but the overwinter survival of *Xf* infections established in August is unknown.

A significant finding from our field inoculations of almond at Davis and Parlier that is important for growers is that first year infections (needle inoculation) are almost certain not to be noticed in visual surveys for ALS symptoms. Further, about half of infections established in early spring die out during the subsequent winter, with even further substantial die-out of infections during the second winter (Almeida and Purcell 2003 in press). Moreover, ALS symptoms from two-year-old infections were not pronounced and would likely be overlooked the second year as well. This means that growers cannot reliably assess the effects of vector control for ALS control on the same or following year's ALS incidence based on ALS symptoms. Extensive ALS symptoms that are noticed for the first time probably result from infections established at least 3 or more years before. In general, ALS symptoms (and presumably *Xf*) spread within trees more rapidly in the more susceptible varieties (e.g., Peerless, Non-pareil) than in less susceptible varieties (e. g., Mission, Carmel), as well as occurring at a higher incidence in the more susceptible varieties (unpublished data).

Biological characteristics of grape and almond Xf strains

PD and ALS have long been considered to be caused by the same strain of *Xf*, but recent genetic studies revealed differences among *Xf* isolated from these host plants (Hendson et al. 2001). We tested the hypothesis that ALS is caused by PD and ALS strains in the field, and found that both groups of *Xf* caused ALS and over wintered within almonds after mechanical inoculation. Under greenhouse conditions, all isolates caused ALS, and all grape isolates caused PD. However, isolates belonging to almond genetic groupings did not cause PD in inoculated grape but systemically infected grape with lower frequency and much lower populations than grape strains. Isolates able to cause both PD and ALS developed 10-fold higher concentrations of *Xf* in grape than in almond. In the laboratory, grape isolates over wintered with higher efficiency in grapes than in almonds; almond isolates over wintered significantly better in almonds than in grapes (results reported in Almeida and Purcell in press).

Based on our *Xf* collection, we never recovered Almond strain isolates from PD grapevines, but have recovered Grape strain isolates from infected almonds. We assigned almond strains into groups I and II based on their genetic characteristics, growth on PD3 solid medium (Davis et al. 1981), and bacterial populations within inoculated grapevines. Our results show that genetically distinct grape and almond strains differ in population behavior and pathogenicity in grape and ability to grow on two different media. Details about the genetic grouping of the various *Xf* strains in California are reported in Hendson et al. (2001).

CONCLUSIONS

In summary, we found that GWSS is a less efficient vector of *Xf* to or from almond in field and lab tests than vectors such as the blue-green sharpshooter and less efficient than in transmitting to grape. GWSS did not acquire *Xf* from or survive well on dormant almond in the field, but transmitted *Xf* to dormant almond in lab tests. This does not mean that GWSS is not a threat for spreading ALS where populations of *Xf*-infective GWSS enter almond orchards. Other factors such as GWSS population levels, infectivity rates, and residence times in orchards, and plant-to-plant movement rates can be as important as transmission efficiency.

The populations of *Xf* in almond were generally 10 to 100-fold less than in comparable weights of leaf veins of PD-grape. This may explain in part why vector transmission of *Xf* from almond is lower than from grape. Growers should be aware that ALS symptoms progress slowly from a single point of infection the same year of infection and most are not noticeable even the second year of infection. Thus most ALS foliar symptoms will not be noticed until the third year or later after infection.

Our finding that grape and almond strain groupings based on genetic characteristics are biologically distinct explains at least in part why PD does not occur near hotspots of ALS. However, our findings cannot explain the reverse situation: "Why doesn't ALS occur near PD hot spots?" The lower rates of overwinter survival of grape strains in almond can only partly explain this anomaly.

REFERENCES

Almeida, R.P.P., and A.H. Purcell. 2003a. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). Journal of Economic Entomology 96(2): 264-271.

Almeida, R.P.P., and A.H. Purcell. 2003b. *Homalodisca coagulata* (Hemiptera: Cicadellidae) transmission of *Xylella fastidiosa* to almond. Plant Disease 87: 1255-1259.

Almeida, R.P.P., and A.H. Purcell. 2003 (in press). Biological traits of grape and almond strains of *Xylella fastidiosa*. Applied & Environmental Microbiology.

Davis, M.J., R.F. Whitcomb, and A.G. Gillaspie, Jr. 1981. Fastidious bacteria of plant vascular tissue and invertebrates (including so called rickettsia-like bacteria), p. 2172-2188. In M.P. Starr, H. Stolp, H.G. Truper, A. Balows, and H.G. Schlegel (eds), The Prokaryotes: A Handbook on Habits, Isolation, and Identification of Bacteria Springer-Verlag, Heidelberg.

Hendson, M., et al. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Applied & Environmental Microbiology 67(2): 895-903.

Hill, B.L., and A.H. Purcell. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology 85(2): 209-212.

Hill, B.L., and A.H. Purcell. 1997. Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. Phytopathology 87: 1197-1201.

Purcell, A.H., and R.P.P. Almeida. 2002. Transmission of *Xylella fastidiosa* to almonds by the glassy-winged sharpshooter. pp. 8-11 in Proc. 30th Annual Almond Industry Conference. Modesto, Calif. Dec. 4-5, 2002.

Purcell, A.H. 2002. Characterization and studies on the fundamental mechanisms of *Xylella fastidiosa* transmission to grapevines by the glassy-winged sharpshooter. Pages 57-58 in Proceedings of Pierce's Disease Research Symposium, Coronado, CA, Dec. 15-18, 2002.

Turner, W.F., and H.N. Pollard. 1959a. Life histories and behavior of five insect vectors of phony peach disease, U.S.D.A. Technical Bulletin 1188.

Turner, W.F., and H.N. Pollard. 1959b. Insect transmission of phony peach disease. United States Department of Agriculture Technical Bulletin 1193: 1-27.

FUNDING AGENCIES

Funding for this project was provided by the Almond Board of California, and the College of Natural Resources'AES Institute at UC Berkeley.