The Role of Common Weeds and Insects in the Movement of *Xylella fastidiosa* and Severity of Almond Leaf Scorch

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Interpretive Summary:

Xylella fastidiosa is a xylem-limited bacterium that causes almond leaf scorch (ALS), Pierce's disease (PD) of grapevines, and other plant diseases. To better understand the epidemiology of *X. fastidiosa* and biology of its xylem-feeding insect vectors, vegetation and Cicadomorph insects were surveyed for abundance and *X. fastidiosa* presence in ALS-infected almond orchards in the northern, central, and southern San Joaquin Valley over two years.

Vegetation samples were tested for *X. fastidiosa* by immunocapture PCR. Eleven of 38 species of common ground vegetation tested were positive for *X. fastidiosa*, including shepherd's purse (*Capsella bursa-pastoris*), filaree (*Erodium* spp.), cheeseweed (*Malva*

parvifolia), burclover (*Medicago polymorpha*), annual bluegrass (*Poa annua*) London rocket (*Sisymbrium irio*), and chickweed (*Stellaria media*). Sixty-three of 1369 samples tested positive for *X. fastidiosa*, with positive samples found between November and March.

Four point nine percent of more than 42,000 Cicadomorph insects collected were xylem feeders, overwhelmingly green sharpshooters, *D. minerva.* Insect populations were highly seasonal, with few vectors collected from December to mid-April, increasing in late April, and peaking from mid-June through mid-July. Green sharpshooters were eliminated inside the orchard when vegetation was removed for almond harvest in mid to late July, although they were still found in adjacent intact vegetation into September. Sites in Butte and Glenn counties had much higher green sharpshooter populations (2.49 and 8.77 *D. minerva*/100 sweeps) than sites in Stanislaus (0.66 *D. minerva*/100 sweeps) or Kern counties (0.08 *D. minerva*/100 sweeps). *D. minerva* were collected more frequently at the margins (8.4/100 sweeps), compared to >10m inside (1.5/100 sweeps) and > 10m outside the almond orchards (3.3/100 sweeps). There were no differences in green sharpshooter populations on riparian habitat, on orchard floor vegetation, and on weeds near roadsides. One point one percent of green sharpshooters tested were positive for *X. Fastidiosa*, which were collected between May and July inside the almond orchard. *X. fastidiosa* strains in sharpshooters matched strains isolated from ALS-infected trees and weeds.

Both ground vegetation and almond trees were most commonly infected with the almond strain of *X. fastidiosa*. ALS-infected almond samples had a *X. fastidiosa* concentration within previously reported ranges (1.84×10^6 - 2.15×10^7 CFU/g), and between 1.9 and 0.17% of almond trees at the study sites had ALS in 2004.

An insecticide spray applied to almond orchard floor vegetation in January at one of the study sites did not suppress green sharpshooter populations or populations of Cicadomorphs in general for the following growing season.

Our results show that the biology of the green sharpshooter, the main ALS vector, is much different from the biology of the blue-green sharpshooter or the glassy-winged sharpshooter, the main PD vectors. Green sharpshooters feed and breed on common ground vegetation species that can harbor *X. fastidiosa* on the almond floor. While the proportion of almond trees, insects, and alternate weedy hosts with *X. fastidiosa* is low, green sharpshooter populations can be high, and ground cover extensive in orchards with flood irrigation or in areas with abundant winter rainfall. In orchards with ALS-susceptible varieties, sampling to determine ALS prevlence and green sharpshooter occurrence, and a year-round weed control program may assist in management of *X. fastidiosa*.

Objectives:

The study objectives were:

1) to characterize the *X. fastidiosa* presence and strains found in almond leaf scorch samples and compare them with strains from nearby alternate host plants, especially almond orchard floor vegetation;

2) to collect insects in ALS-infected orchards and nearby vegetation, and the conduct laboratory analyses determine whether or not they carry *X. fastidiosa*;

3) to test *D. minerva* for *X. fastidiosa* transmission efficiency to and from almonds and weeds commonly found in almond orchards;

4) Collect regional data on ALS epidemiology with respect to orchard management (e.g., irrigation) and the surrounding environmental conditions (e.g., nearby crop plantings);

5) to describe native sharpshooter biology and development;

6) to conduct experiments to reduce native sharpshooter density during winter and spring periods.

Materials and Methods:

Sample sites were selected based on grower reports of ALS incidence, and located in the northern (ca.122°09'W, 39°39'N and 121°50'W, 39°45'N, Glenn County; ca. 121°56'W, 39°49'N and 121°55'W, 39°36'N Butte County), central (ca. 121°03'W, 37°31'N and ca. 120°44'W, 37°39'N, Stanislaus County) and southern Central Valley (ca. 119°17'W, 35°22'N and ca. 119°15'W, 35°20'N, Kern County). Vegetation and insects were collected every 2 to 6 weeks, depending on seasonal abundance.

Vegetation sampling: On each visit, researchers walked through the orchard and conducted a visual survey for the four most abundant weed species. Vegetation was identified according to the Statewide IPM Program produced by the Agriculture and Natural Resources Department at the University of California, Davis (available online). Four evenly spaced rows were then selected (300 – 400 m per row) and 3 to 5 leaves of each of the common weed species were collected from each transect. Samples were stored separately for each species and transect in a 3.8-liter plastic bag. Ten to 30 individual plants were sampled for each weed species in each transect. The collected material was stored in a cooler (ca. 7°C) and processed for *X. fastidiosa* presence within 2 days of collection.

Insect sampling: Each orchard was divided into transects based on the surrounding environment (i.e. the presence of a creek, alfalfa field, or other potential breeding site of *D. minerva*). Orchard floor vegetation in each transect was swept 100 times with a 35 cm diameter sweep net, repeated three times in each transect, approximately 1200 sweeps per site per sampling date. Five to ten yellow sticky traps (7.5cm by 13cm) were set in lower branches of almond trees, 1 to 2m above the ground, in the center and around the perimeter of each site. Insects collected by sweep net and sticky trap were

stored at -20 °C until sorting. Xylem-feeding Cicadellid and Cercopid Homopterans were identified and counted at each site and sampling date, along with Delphacid and Deltacephalinid leafhoppers, and Membracids. A subset of collected *D. minerva*, *X. fulgida*, and *S. festinus* were preserved in acetone (Fukatsu 1999) for later detection of *X. fastidiosa* in mouthparts. Specimens were identified according to Bland (1978) and Dietrich (2005), and representative vouchers submitted to the Essig Museum of Entomology at U.C. Berkeley.

Bacterial detection in vegetation: Each sample (plant species and transect) was processed separately for the presence of *X. fastidiosa* using immunocapture DNA separation, which is a sensitive antibody-linked system that selectively removes bacteria (Shapland 2006). We selected plant parts where sharpshooters were known to feed, such as leaf petioles, leaf blade bases of grasses, and plant stems. Briefly, 0.70g of plant material was cut into 1-mm sections, placed into 3 ml of modified SCP buffer, and thoroughly homogenized (Brinkmann Instruments, Westbury NY). The homogenate was strained through cheesecloth into a sterile microcentrifuge tube and centrifuged at 14000x g for 5 min. The pellet was resuspended in 500 µl PBS/BSA buffer and 100 µl of rabbit antibody to *X. fastidiosa* (diluted 1/100 in PBS/BSA) was added to each sample. Samples were incubated at room temperature for 30 min with gentle shaking, followed by centrifugation at 14000x g for 2.5 min. The pellet then was resuspended in 1.0 ml of PBS/BSA, centrifuged a second time for 2.5 min, and resuspended in 1.0 ml of PBS/BSA. Five µl of Dynabeads M-280 sheep anti-rabbit IgG (Dynal Biotech, Lake Success NY) was added to each sample and incubated at room temperature for 30 min with gentle shaking. The beads were removed with a Magnetic Particle Concentrator (Dynal MPC-S, Oslo), and washed three times with 1.0 ml, 1.0 ml, and 500 µl of PBS/BSA. Finally, the bead-antibody-bacteria complex was resuspended in 10 µl of sterile distilled water.

For PCR amplification, all samples were prepared in sterile microcentrifuge tubes with 12.5 µl Taq Master Mix (Qiagen, Valencia, CA), 6.5 µl PCR water, 1 µl of each primer RST-31 and RST-33, and 4 µl of DNA extract. PCR reactions were carried out in a thermal cycler according to the conditions described by Minsavage, et al. (1994). PCR products were separated by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and viewed under ultraviolet light. The presence of *X. fastidiosa* in the original sample was determined by a band at 733 kb.

After PCR amplification, strain differences were detected with *RsaI* restriction enzyme digestion (Hendson et al. 2001). *RsaI* cuts PCR products from oak, oleander, peach, plum, and all but three ALS strains into two fragments, while leaving PD strains intact.

Bacterial detection in insects: *X. fastidiosa* was detected in insects utilizing vacuum-extraction and PCR (Bextine 2005). Insect specimens were removed from the acetone; the heads were removed, and pinned with a #2 insect pin, avoiding the eyes. The pinned head was placed into a 96-well plate filled with 180µL of cell lysis buffer (ATL) from the DNeasy tissue kit for animal tissues (Qiagen). The plate was placed into a vacuum chamber which was pressurized and depressurized five times to flush the lytic buffer through the insect head. The ATL buffer was then processed according to the

manufacturer's directions. The extracted DNA was amplified by PCR with RST 31-33 primers as described above. The *X. fastidiosa* strain present in insects was identified with multiplex PCR (Hernandez-Martinez 2006) or restriction enzyme digestion (Hendson 2001), and compared with *X. fastidiosa* strains prevalent in ALS-infected trees in the orchard (Shapland 2006).

Mapping and detection of almond leaf scorch: All field sites were surveyed for the presence and distribution of ALS. Disease symptoms were rated visually at Butte, Glenn and Stanislaus county sites in September and October 2004 and October 2005, and in November 2004 in Kern County. The locations of diseased trees were recorded with a GPSMAP 76 handheld device (Garmin Inc., Olathee KS). All trees at Grainland Rd., Zeering Rd., Tim Bell Rd. and Kern sites 2, 6 and 7 were surveyed, while subsets of trees were surveyed at Rd. P, Rd. VV, and Anita Rd.

Bacterial titer and strain: *X. fastidiosa* was cultured from symptomatic almond trees at each site and from fresh samples of alternate host plants at sites where previously collected samples tested positive for *X. fastidiosa*, using immunocapture DNA separation and PCR amplification. In late summer and early fall, when the symptoms of ALS were most obvious we surveyed each orchard and collected symptomatic almond leaf and petiole samples. Both ground vegetation and almond samples were processed for bacterial culture on selective media within 24h. We plated samples on both PWG and PD3 media to provide a rudimentary indication of X. fastidiosa strain. Grape strains of *X. fastidiosa* grow on both PWG (Davis et al. 1983) and PD3 (Davis et al. 1981), but some almond strains grow only on PWG (Almeida and Purcell 2003). Samples were cultured (Hill and Purcell 1995) to determine both the strain and concentration of bacteria in almond and ground vegetation samples.

D. minerva *biology and development:* *D. minerva* colonies were established with insects collected from Stanislaus (near Modesto), Sonoma (near Guerneville), and Solano (near Fairfield) counties, and maintained in the greenhouse at UC Berkeley. Insects were raised on 'Sweet' basil (*Oncimum basilicum*) and bermudagrass (*Cynodon dactylon*) plants in 3.75 L pots enclosed by wood and mesh cages. The greenhouse was maintained at 25°C (+10 °C /-5°C) with natural lighting, supplemented from October to April with Plant and Aquarium lights (General Electric, Fairfield, CT) for a 14-hour day length. All plants were grown in Supersoil (Rod McLellan Co., Marysville, OH) and fertilized daily with diluted 20-20-20 fertilizer.

Seeds of burclover (*Medicago polymorpha)*, cheeseweed (*Malva parvifolia*), johnsongrass (*Sorghum halepense*), and filaree (*Erodium* sp.) were collected in the field and germinated in the greenhouse. Basil was grown from purchased seeds, and bermudagrass was vegetatively propagated by stolons. Plants were used in experiments when they were six to nine weeks old. Scientific and common names of the weeds were listed according to the Jepson Manual (Hickman 1993) and the Growers Weed Identification Handbook (Anonymous 1999), and voucher specimens deposited at the Jepson Herbarium at U.C. Berkeley. In all experiments, equal numbers of each plant species were set up in each replication to control for the effects of greenhouse

temperature, plant nutritional status, light exposure on the maturation and behavior of *D. minerva,* and the effects of plant age, phenology and nutrition.

Nymph development. Ten *D. minerva* adults, five males and five females, were transferred to bermudagrass, burclover, cheeseweed, and filaree for a 48-hour egg laying period, contained in a cylindrical plastic cage with mesh screen. Adults were removed after two days; the plant was recaged, and monitored for nymph emergence thereafter. Approximately 10 insects were gently shaken off each plant weekly, and the numbers of each instar were counted. Insects were returned to the plant after counting, and adult sharpshooters were removed from the plant as they matured. All insects were moved to a new plant of the same species as plants became senescent or infested with other insects. Experiments were conducted from June to November 2006, with eleven replications of equal numbers of plants from each species per replication.

Adult survival. To determine the suitability of the various weed species for long-term *D. minerva* survival, eight newly-moulted adults (<14 days old), four males and four females, were placed on 'Peerless' almond seedlings, bermudagrass, burclover, cheeseweed, seedling 'Cabernet Sauvignon' grapes, johnsongrass, filaree and basil. Eight replications with equal numbers of plants from each species per replication were completed from February to July 2007. Every week, all adults were gently shaken off the plant and counted, then returned to the plant. All insects were moved to a new plant of the same species when a plant was senescent or infested with other insects.

Egglaying. Five adult male, and five adult female *D. minerva* were transferred to bermudagrass, burclover, cheeseweed, johnsongrass, filaree and basil in seven replications with equal numbers of plants from each species per replication, from February through May 2007. Adults were removed after a four-day egglaying period, and the eggs present in the plant visualized plant with a Nikon SMZ800 dissecting microscope. Eggs and plants were photographed with a Nikon Coolpix digital camera. The plant was returned to the insectary, and recaged. The numbers of nymphs emerging from the plant were counted 7, 14 and 21 days after egglaying. Nymphs were removed form the plants after counting. Once all nymphs were removed, plant dry weights were determined by removal of plant material at the soil line, which was placed in a paper bag, dried for at least seven days in a heated cabinet, and weighed.

X. fastidiosa *survival in, and* D. minerva *transmission between almonds and alternate hosts* was tested between almonds and nine weed species (Shapland et al 2006): shepherd's purse (*Capsella bursa-pastoris*), filaree, cheeseweed, burclover, common groundsel (*Senecio vulgaris*), London rocket (*Sysmbrium irio*), sowthistle (*Sonchus* sp.), chickweed (*Stellaria media*), and burning nettle (*Urtica urens*). Plants were grown in the greenhouse from seed as previously described.

Mechanical inoculation of alternate hosts: *X. fastidiosa* isolates (ALS6 and Fresno-ALS) were started 10 to 14 days prior to inoculations. *X. fastidiosa* colonies from one plate were suspended in 2 ml SCP buffer by pipetting repeatedly to break up clumps of bacteria. Inoculum concentrations were determined by dilution plating on PWG media. Plants were inoculated immediately after preparing the bacterial suspension, always on

a sunny day to ensure the drop was drawn into the plant via transpiration. A 5 µl drop of *X. fastidiosa* suspension was placed on the inoculation location and probed 3 to 6 times with a #2 insect pin. Sites were marked site with tape.

Plants were assessed for *X. fastidiosa* presence and titer four weeks after inoculation. Two samples were cut immediately adjacent to the inoculation site: one for culturing and the other was frozen at -20C for immunocapture PCR (Shapland 2006) if needed due to contamination from bacteria other than *X. fastidiosa.*

Also, twenty plants of each species were inoculated as above and planted outside in screenhouses in the field, half in Bakersfield CA, and half in Parlier, CA. Plants were assessed for *X. fastidiosa* presence four, six and eight weeks after mechanical inoculation via culture and immunocapture PCR as previously described.

Insect inoculation of alternate hosts: Almond plants infected with *X. fastidiosa* were generated by mechanically inoculating almonds with ALS6 or Fresno-ALS strains two to three months prior to transmission test, as previously described. Almonds and weeds were kept in unsprayed greenhouse until symptoms developed, then tested for *X. fastidiosa* presence and titer via culture two weeks prior to use in transmission experiments.
Between 80 and 100 green sharpshooters were placed on *X. fastidiosa*-infected almonds for a 4-day acquisition access period (AAP). *D. minerva* were then moved in groups for four to alternate hosts for a four-day inoculation access period (IAP), confined to plants in small foam or mesh cages. Following inoculation, inoculation site was marked with lab tape and plants were placed in the greenhouse to develop infections.

Insect acquisition of* X. fastidiosa *from alternate hosts: Alternate hosts belonging to the nine weed species were infected with *X. fastidiosa* by mechanical inoculation with ALS6 and Fresno-ALS as previously described. Plants were four to six weeks old when inoculated. *D. minerva* free of *X. fastidiosa* were reared through three weekly changes as nymphs on basil.

D. minerva were placed in groups of ten on basil plants for a four-day pretest period, then divided into two for transfer to alternate hosts for a four-day AAP, then transferred in those groups to seedling 'Peerless' almonds for a four-day IAP. At the end of the IAP, insect heads were preserved in acetone and vacuum-extraction PCR performed to detect the presence of *X. fastidiosa* in *D. minerva*. Ten to 12 weeks after inoculation, the almond seedlings were tested for *X. fastidiosa* infection by culture.

Dormant spray to suppress vector populations: The treated orchard was located southwest of Chico (ca.121°55'W, 39°36'N, Butte County), 20.2 hectares of mixed Peerless, Nonpareil, and Price varieties, between 19 and 26 years old. In surveys in fall 2004 and 2005, ALS-infected trees were present, predominantly in the center of the orchard, but also in individual trees in the eastern and northern edges. Except for the insecticide spray, standard cultural practices were followed. Vegetation was removed

from the orchard in preparation for nut harvest on 15 July 2005, ending insect sampling on the orchard floor vegetation.

The orchard was divided into 8 blocks of 460 trees per block. The groundcover on the almond orchard floor in four of the blocks was treated with Asana XL (esfenvalerate, DuPont Corp. Wilmington, DL) insecticide at 10 oz. per acre (0.725 L per hectare) on 27 January 2005. Sites were sampled for Cicadomorph insects, particularly *D. minerva*, once a month from February through July 2005 as previously described. One hundred sweeps per block on the orchard floor vegetation were taken at each sampling date.

Data analysis: Data were entered in Excel (Microsoft Corp., Redmond WA), with averages compared using one-way and repeated measures ANOVA, and means separated using Tukey's HSD comparison (JMP, SAS Institute, Cary NC and Systat, San Jose CA).

Results and Discussion:

Vegetation sampling: From June 2003 to April 2005, 38 species of ground vegetation were commonly found **(Table 1, as presented in the 2005 report)**, with most material collected in winter and spring when ground vegetation was abundant. Between August and October, it was difficult to find live ground vegetation within the almond orchards, as the orchard floor was completely free of vegetation prior to harvest. Irrigation was also discontinued, so that the nuts can be shaken from the trees and dried on the bare ground. All orchards in this study followed these practices. At that time samples were across the orchard rows (transects) to produce a single sample for each plant species and orchard (**Figure 1, as presented in the 2005 report**).

Insect sampling: From June 2003 to September 2005, more than 42,000 Cicadomorph insects were collected in Stanislaus, Butte, Kern and Glenn counties, including 1,920 *D. minerva,* five *X. fulgida*, one *P. spumarius*, and 80 *S. festinus*. Four point nine percent were xylem feeders (sharpshooters or spittlebugs), known vectors of *X. fastidiosa* **(Figure 2)**. *X. fulgida* was only collected at the Anita Rd. site, and only in sweeps of riparian vegetation along a creek adjacent to the almond orchard. Other Cicadellid species collected in Stanislaus and Butte counties included *Exitanus exitosus* Uhler, and *Ambleysellus grex* Oman. *Thamnotettix zelleri* Kirshbaum was collected from grasses at Anita Rd. in May. *Actinopteris* sp., *Empoasca* sp., and *Exitanus exitosus* Uhler were collected in Kern County. *Nothodelphax consimilis* Van Duzee (Delphacidae) also was collected at all field sites in Butte, Glenn and Stanislaus counties, but not at the Kern County sites

Insect populations were highly seasonal, with few vectors collected from December to mid April, increasing populations in late April, and peak populations from mid-June through mid-July. Cicadellid populations were eliminated inside the orchard when vegetation was killed with herbicides in preparation for almond harvest in mid to late July, although *D. minerva* were still found in adjacent riparian areas or crops into September.

More *D. minerva*, and Cicadomorphs in general were found at northerly collection sites **(figure 2)**. The highest Cicadomorph populations were at Anita Road in Butte county (146 per 100 sweeps), and lowest were populations at Zeering Road in Stanislaus county (7 per 100 sweeps). Sites in Butte and Glenn counties had higher *D. minerva* populations (17.0, 2.9, 2.3, to 1.3 *D. minerva* per 100 sweeps) than the Stanislaus county sites (1.1 and 0.4 *D. minerva* per 100 sweeps). In Kern County, *D. minerva* were only found at Site 6 in June 2004, 1.3 per 100 sweeps). The exception to this trend was *S. festinus* populations, as 55 *S. festinus* were collected in Kern County, 25 were collected at Butte and Glenn county sites, and none were collected from sites in Stanislaus County.

Four thousand eighty-four insects, including nine *D. minerva*, were collected by sticky traps hung in the lower branches of the trees for 13,051 trap-days, compared to 42,392 collected by sweeps (including 1,912 *D. minerva*). No insects were collected in limited sampling with beat sheets at four sites (0 in 1660 beats), compared to 1,318 insects collected in 430 sweeps at the same sites and dates.

More sharpshooters were collected in sweeps at the margins of orchards (an average of 8.4 per 100 sweeps), compared to collections more than 10m inside (1.5 per 100 sweeps) and more than 10m outside the almond orchards (3.3 per 100 sweeps) at the Chico and Modesto-area study sites (Sharpshooters: one-way ANOVA, $P = 0.0001$, $n = 983$). There were no significant differences between total Cicadomorph populations at the three collection locations (Total Cicadomorphs: one-way ANOVA, $P = 0.17$, $n = 983$; **figure 3**).

There were no differences in sharpshooter populations between collections made on riparian habitat, almond orchard floor vegetation, and roadside vegetation, for collections made on the same days and at the same study sites (one-way ANOVA, $P > 0.27$, $n = 217$). Total numbers of Cicadomorphs were lower in riparian habitats compared to roadside or almond orchard floor vegetation. (One-way ANOVA, $P = 0.07$, $n = 217$; **figure 4**).

Bacterial detection in vegetation: Sixty-three of 1369 samples from the six orchards were positive for *X. fastidiosa* (4.6%). *X. fastidiosa* was recovered from 11 of the 38 ground vegetation plant species tested, including *Capsella bursa-pastoris* (shepherd's purse), *Malva parvifolia* (cheeseweed), *Senecio vulgaris* (common groundsel), *Sisymbrium irio* (London rocket), *Sonchus* sp. (sowthistle), *Stellaria media* (chickweed), *Urtica urens* (burning nettle), and *Veronica persica* (speedwell), eight species from which it had not previously been recovered in the field **(Table 1)**. There was a strong seasonal component to bacterial presence in ground vegetation, with no *X. fastidiosa* positive samples found between April and mid-October during the two years of the study **(Table 2, as presented in the 2005 report)**. Linear regression shows that there was a positive, significant relationship between the number of samples taken per plant species and the percentage of samples positive for *X. fastidiosa* ($y = 0.0553x - 0.2074$, $R2 = 0.8935$).

Restriction enzyme digestion of PCR products and bacterial culture on selective media showed that almond trees at six of seven studied orchards were infected with the almond strain of *X. fastidiosa* **(Table 3, as presented in the 2005 report)**. At Zeering Rd. in Stanislaus County, a grape strain of *X. fastidiosa* was isolated from all weeds and almond trees sampled. At each site, tissue samples from both almond trees and surrounding weeds gave the same result: each contained either the grape or almond strain of *X. fastidiosa*, but never both.

Bacterial detection in insects: *X. fastidiosa* was recovered from 17 of 1532 (1.1%) of sharpshooters tested. Three *D. minerva* tested positive at Anita Road, 13 at Road VV, and one at Tim Bell Road. Only almond strain *X. fastidiosa* was recovered from sharpshooters, matching strains isolated from ALS-infected trees and weeds at the Anita Road, Road VV, and Tim Bell Road sites. *X. fastidiosa*-positive sharpshooters were collected between 19 May and 24 July in the Chico area and on 4 April in Stanislaus County. All sharpshooters testing positive for *X. fastidiosa* were collected inside the almond orchard.

Mapping and detection of* X. fastidiosa *infection in almond trees: Overall infection percentages were low, between 1.9 and 0.17% at the study sites in 2004 **(Table 3)**. More ALS-symptomatic trees were observed in 2004 than 2005, when trees had lost many leaves due to senescence and *Tranzchelia discolor* almond rust. All sites included highly susceptible varieties of trees: Sonora, Solano, or Peerless. At Rd. VV in Glenn County, no *X. fastidiosa*-infected trees were detected; however, *X. fastidiosa* was detected in weeds and insects at that site.

Bacterial titer and strain: Petioles from ALS-symptomatic almond trees at Zeering Rd. containing the grape strain of *X. fastidiosa* had an average concentration of 2.15×10^6 CFU/g, which was significantly greater than the concentrations at other sites sampled ($P = 0.014$). Our results agreed with previous findings that the average *X. fastidiosa* titer in ALS-symptomatic almond leaves was lower than the average *X. fastidiosa* titer in PD symptomatic grapes (Almeida and Purcell 2003).

Nymph development: *D. minerva* nymphs developed from egg to adulthood on all species tested: burclover, bermudagrass, cheeseweed, and filaree **(Figure 5)**. The longest maturation time recorded was 70 days on bermudagrass, and 49 days for the other three species. The shortest maturation time was 28 days, on all plants. Although a few nymphs emerged 7 days after egglaying, most emerged after 14 days. *D. minerva* appeared to mature at similar rates on all weed species tested. Seventy-nine percent (348 of 441) of sharpshooters survived the 2-day egglaying period, similar proportions on all plants (Chi-square with pairwise comparisons, $P>0.10$).

Egglaying. Numbers of emerging nymphs were similar on all five plant species tested (one-way ANOVA, $P = 0.52$), with lots of variability in numbers of nymphs between replications **(Figure 6)**. Overall, the most nymphs emerged from johnsongrass, (19 average per plant), followed by bermudagrass.

D. minerva laid eggs in masses in lower stems and petioles of weed species tested, often quite close to the soil line. Eggs were deposited under the plant epidermis, completely covered by plant tissue **(Figure 7, A and B)**. Tests are planned for fall 2007 to determine the number of eggs per mass, and egglaying habit on the various plant species.

D. minerva survived the four-day egglaying period (8.88 adults per plant, SE = 0.20), in similar proportions on all five plant species (one-way ANOVA, $P = 0.93$). There were no differences in dry weight between species of plants used for egglaying, with average dry weights of 3.0, 2.9, 2.6, 2.1 and 1.9g per plant, for cheeseweed, filaree, johnsongrass, burclover, and bermudagrass, respectively (one-way ANOVA, $P = 0.37$). There was no correlation between plant dry weight and numbers of nymphs from that plant (simple linear regression, $R^2 = 0.009$, $P = 0.57$).

The average temperature in the insectary during the egglaying interval for replications six through ten was between 22.9 and 25.0°C. High temperatures measured from 38.7 to 36.9°C, and low temperatures from 18.4 to 20.6°C. There was no apparent relationship between total nymph emergence and average, maximum high or maximum low temperatures.

Mechanical inoculation of alternate hosts: *X. fastidiosa* infection rates and titers were lower in inoculated alternate hosts grown in the field compared to the same plants grown in the greenhouse **(Table 4)**. While experiments are ongoing, with anticipated completion in fall 2007, *X. fastidiosa* populations were roughly 10 times lower in field-grown plants. The plant species from which *X. fastidiosa* was detected regularly in surveys of almond orchard vegetation **(Table 2)** appear to be frequently-infected, high-titer hosts when mechanically inoculated, except for shepherd's purse. Experiments to test insect inoculation of *X. fastidiosa* into, and acquisition from, these nine alternate host species are ongoing in summer 2007.

Dormant spray to suppress vector populations: There were no statistically significant differences in total Cicadomorph or *D. minerva* populations between insecticide-treated and untreated blocks **(Figure 8)**. Overall, mean Cicadomorph populations were greater in treated blocks (77.6 per 100 sweeps), compared to treated blocks (58.4 per 100 sweeps), while *D. minerva* populations were lower in sprayed blocks (1.2 per 100 sweeps) than in unsprayed blocks (0.7 per 100 sweeps).

Previous field surveys for *X. fastidiosa* in alternate host plants focused on Pierce's disease management in surveys of common weed and riparian vegetation species inside and adjacent to vineyards. With the recent increase of ALS in California, there was need to identify *X. fastidiosa* hosts in almond orchard floor vegetation as well. Different vegetation types are present near grapes and almonds, as well as different vector species. In grapes, the blue-green sharpshooter (*Graphacephala atropunctata*) or glassy-winged sharpshooters (*Homalodisca vitripennis*) are the primary vectors. In almonds, the green sharpshooter, *Draeculacephala atropunctata*, is the main vector. The three species have very different life cycles and patterns of host plant use. There are also different vegetation patterns in vineyards and almond orchards. In vineyards, a

clear edge effect was found with PD incidence closely tied to adjacent riparian habitat (Purcell and Frazier 1985), whereas most previous work did not show any clear spatial patterns with ALS in almond orchards (Purcell 1980).

Sharpshooters on grapes, especially the blue-green, generally enter vineyards from adjacent vegetation, feed briefly on grapevines, and do not breed or persist inside vineyards. In contrast, almond orchards, especially in areas with abundant winter rainfall or flood irrigation, often contain *D. minerva* breeding and feeding habitat through mid-summer. Adult longevity, egg laying, and nymph development studies conducted in the greenhouse indicated that *D. minerva* feed, reproduce and mature on weed species that are *X. fastidiosa* hosts and commonly found inside almond orchards. Thus the vectors of ALS multiply inside the orchard, in contrast to PD vectors which mostly originate outside the vineyard. The distribution of *D. minerva* could may explain differences ALS and PD distribution. We are currently conducting transmission tests in the greenhouse, to determine the rates of *X. fastidiosa* transmission by *D. minerva* between the most frequently-infected alternate hosts and almonds.

D. minerva were eliminated inside the orchard when vegetation was killed with herbicides in preparation for almond harvest in mid to late July, although they were still found in adjacent riparian areas or crops into September. Sites in northerly counties had much higher *D. minerva* populations than sites in central or southern San Joaquin valley, indicating that *D. minerva* are highly dependant on vegetation present in the orchard, as opposed to the almond trees themselves, or migrating into the orchard from outside.

Four point six percent of field-collected, naturally-infected vegetation samples contained *X. fastidiosa*. The *X. fastidiosa*-positive plant species were the most-frequently found species in the orchards on most of the sampling dates. There was a positive and significant relationship between the number of samples taken per plant species and the percentage of samples positive for *X. fastidiosa*. We detected *X. fastidiosa* in weeds between October and April, whereas insect populations were highly seasonal, with few vectors collected from December to mid-April, increasing in late April, and peaking from mid-June through mid-July.

One point one percent of *D. minerva* tested were positive for *X. fastidiosa*. *X. fastidiosa*-positive sharpshooters were collected between May and July. The seasonal difference between when *X. fastidiosa* was detected in weeds (November through April) and when *X. fastidiosa* was detected in insects may be explained two ways: 1. since the overall proportion of *D. minerva* with *X. fastidiosa* was so low, infected sharpshooters were only detectable in the months when populations were highest, or 2. the orchard floor vegetation was in better condition, and temperatures more favorable to *X. fastidiosa* growth and survival in plants, in the winter and early spring. Since adult *D. minerva* survived at least 10 weeks on weed species present in the orchard in greenhouse longevity tests, it is possible that a sharpshooter could acquire *X. fastidiosa* in early spring and be detected when collected in the summer months.

Both ground vegetation and almond trees were most commonly infected with the almond strain of *X. fastidiosa*. *X. fastidiosa* strains in sharpshooters matched strains isolated from ALS-infected trees and weeds. While a low proportion of almond trees at the study sites had ALS, those trees were highly symptomatic, and had *X. fastidiosa* populations between 10^6 and 10^7 colony-forming-units per gram of petiole – thus infected trees were infrequent but had lots of inoculum sufficient for sharpshooter acquisition.

Pyrethroid insecticide treatment, applied to orchard floor vegetation in late January, was not effective in reducing ALS vector populations or total populations of leafhoppers and Delphacids. Since the label of Asana insecticide lists five Cicadomorph species that it effectively controls (Anonymous 2002), and sharpshooters are susceptible to a wide range of insecticides from organophosphates to neonicitinoids, it should effectively control D. *minerva* at label rates.

One reason for the lack of effectiveness may be the timing of the application, as the insecticide was applied four months before peak *D. minerva* populations. The very low populations at the first sampling date in February (and none in March and April) show that the few adults that survived the winter multiply to yield a much larger second generation in May and June. This population pattern was found in other almond orchards in Butte, Glenn, and Stanislaus counties, and in previously-published studies in pastures and alfalfa fields in Fresno County.

With an estimated half-life of 7.5 days in lab tests, Asana insecticide may also not persist long enough to effectively target emerging *D. minerva* in late and mid-February. The application of insecticide in late January may have been too early to kill over wintering *D. minerva* adults as they came out in February. Finally, *D. minerva* may have been physically protected from the spray, since they overwinter hidden in perennial vegetation. Future experiments should determine the most effective timing, the best cultural or chemical techniques to control *D. minerva* populations, and to determine an economic threshold for *D. minerva* infestation.

Our results show that the biology of ALS vectors is different from PD vectors, and that they feed and breed on common ground vegetation species that can harbor *X. fastidiosa* on the almond floor. While the proportion of almond trees, insects, and alternate weedy hosts with *X. fastidiosa* is low, *D. minerva* populations can be high, and ground cover extensive in orchards with flood irrigation or in areas with abundant winter rainfall. In orchards with ALS-susceptible varieties, sampling to determine ALS and *D. minerva* occurrence, and a year-round weed control program may assist in management of *X. fastidiosa*.

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Recent Publications:

Shapland, E.B., Daane, K.M., Yokota, G.Y., Wistrom, C., Connell, J.H., Duncan, R.A., and Vivieros, M.A. 2006. Ground vegetation survey for *Xylella fastidiosa* in California almond orchards. Plant Disease 90: 905-909.

A second manuscript, *Seasonal Abundance and Natural Infectivity of* Xylella fastidiosa *Vectors in California Almond Orchards*, is in preparation for submission to the Journal of Economic Entomology.

Additional presentations of research results were at the CDFA GWSS-PD Research Symposium in 2005 and 2006, and at the 2006 Almond Board Conference in Modesto, CA and the 2007 ESA Conference in Portland, OR.

Table 1: Ground vegetation collected from almond orchards and tested for *Xylella fastidiosa*.

Weed species		*X. fastidiosa*		
Scientific Name	Common Name	This study	Previous studies	Reference
Amaranthus spp.	Pigweed	-	-	6
			+	36*
Amsinckia spp.	Fiddleneck	-	-	6
Anagallis arvensis	Scarlet pimpernel	-	-	6
Avena fatua	Wild oat	-	+	11
Brassicaceae spp.	Mustards	-	+	6
Capsella bursa-pastoris	Shepherd's purse	+	N/A	
Chamaesyce maculate	Spotted spurge	-	N/A	
Chamomilla suaveolens	Pineapple weed	-	N/A	
Chenopodium album	Lambsquarter	-	N/A	
Claytonia perfoliata	Miner's lettuce	-	+	30,31
Conyza bonariensis	Fleabane	-	N/A	
Conyza canadiensus	Horseweed	-	+	36*
Coronopus didymus	Lesser swinecress	-	N/A	
Cyperus esculentus	Yellow nutsedge	-	+	11
Echinochloa crus-galli	Barnyard grass	-	+	17*, 36*
Erodium spp.	Filaree	+	+	6, 11*, 36*
Escallonia montevidensis	Escallonia	-	+	11
Festuca spp.	Fescue grass	-	N/A	
Helianthus spp.	Sunflower	-	+	6*
Hordeum murinium	Hare barley	-	+	11
Lactuca serriola	Prickly lettuce	-	+/-	30,31, 36*
Malva parvifolia	Cheeseweed	+	-	6, 11*
Medicago polymorpha	Burclover	+	+	11
Poa annua	Annual bluegrass	+	+	11
Portulaca oleracea	Common purslane	-	-	11*, 36*
Ranunculus spp.	Buttercup	-	N/A	
Rumex crispus	Curly dock	-	+	11
Salsola tragus	Russian thistle	-	N/A	
Senecio vulgaris	Common groundsel	+	N/A	
Sisymbrium irio	London rocket	+	N/A	
Sonchus oleraceus	Annual sowthistle	-	+	36*
Sonchus spp.	Sowthistle	+	-	6
Sorghum halepense	Johnsongrass	-	+/-	11*, 30, 31
Stellaria media	Chickweed	+	N/A	
Typha spp	Cat tail	-	N/A	
Urtica urens	Burning nettle	+	N/A	
Veronica persica	Speedwell	+	N/A	
Xanthium strumarium	Cocklebur	-	+	36*

Results are compared to previous field surveys near vineyards and riparian areas, except for those marked * which refer to greenhouse studies.

Table 2: *X. fastidiosa* detection in almond orchard vegetation. Data combined from six almond orchards, from June 2003 to April 2005.

Plant species	J	F	M	A	M	J	J	A	S	O	N	D
Burning nettle		■										
Common Groundsel											■	
Speedwell		●										
Sowthistle			■									
London Rocket		■	■									
Annual bluegrass			■									●
Cheeseweed	♦		■ ●							♦	■	♦ ●
Chickweed		●	●		■						●	●
Burclover			♦									
Shepherd's purse		●	♦		■						●	
Filaree			♦ ♠ ●							♦	■ ●	●

■ = Kern, ● = Stanislaus, ♦ = Butte, ♠ = Glenn

Table 3: Almond orchards surveyed for Cicadomorph insects, vegetation, and *Xylella fastidiosa* strains and titers.

Site (County)	GPS Coordinates	No. trees surveyed	No. trees XF +	Strain in almonds	Strain in weeds	*X. fastidiosa* titer[2]
Grainland Rd. (Butte)	121°55'W, 39°36'N	3700	7	Almond	Almond	1.84×10^6 ab
Anita Rd. (Butte)	121°56'W, 39°49'N	~6000	38	Almond	Almond	2.30×10^6 b
Rd. P (Glenn)	122°09'W, 39°39'N	~3600	6	Almond	Almond	1.19×10^7 ab
Rd. VV (Glenn)	121°50'W, 39°45'N	~2500	0	--	Almond	--
Zeering Rd. (Stanislaus)	121°03'W, 37°31'N	2560	49	Grape	Grape	2.15×10^7 a
Tim Bell Rd. (Stanislaus)	120°44'W, 37°39'N	900	2	Almond	Almond	6.27×10^6 b
Site 2 (Kern)	-	-	4	Grape	--	3.90×10^6
Site 6 (Kern)	-	-	3	Almond	--	1.84×10^7
Site 7 (Kern)	-	-	14	Almond	Almond	1.08×10^7 b

[1] Results based on PCR and restriction enzyme digestion with *Rsa1*.
[2] Ave. CFU/g cultured from ALS symptomatic petioles: 27 July, 30 Sept. and 14 October 2004.
[3] Different letters after each mean indicate a significance difference ($P < .05$), Tukey's pairwise comparison. Kern sites 2 and 6 not included due to small number of sampled trees.

Table 4: *X. fastidiosa* infection in greenhouse (GH) and field-grown alternate hosts following needle inoculation with ALS6 and Fresno-ALS strains. Infections were assessed after four to eight weeks in field-grown plants, and four weeks in greenhouse-grown plants. Results are preliminary for greenhouse-grown plants, with anticipated completion in fall 2007.

Species	Growing location	No. tested	No. infected (%)	CFU/g
Burclover - *Medicago polymorpha*	GH	36	21 (58)	1.77×10^7
	field	54	13 (24)	3.00×10^6
Cheeseweed - *Malva parviflora*	GH	-	-	-
	field	38	6 (16)	1.35×10^7
Chickweed - *Stellaria media*	GH	35	30 (86)	5.93×10^7
	field	5	3 (60)	-
Filaree - *Erodium* sp.	GH	-	-	-
	field	6	2 (33)	6.44×10^7
Common groundsel - *Senecio vulgaris*	GH	-	-	-
	field	28	3 (11)	3.34×10^3
London rocket - *Sysimbrium irio*	GH	17	8 (47)	1.61×10^6
	field	17	0 (0)	-
Burning nettle - *Urtica urens*	GH	27	9 (33)	1.09×10^6
	field	14	1 (7)	-
Shepherd's purse - *Capsella bursa-pastoris*	GH	-	-	-
	field	2	0 (0)	-
Sowthistle - *Sonchus oleraceus*	GH	50	15 (30)	3.87×10^7
	field	38	3 (8)	8.33×10^4

Figure 1: *Xylella fastidiosa* presence in vegetation in almond orchards. Combined results from 6 almond orchards in Butte, Glenn, Stanislaus, and Kern Counties from June 2003 to April 2005.

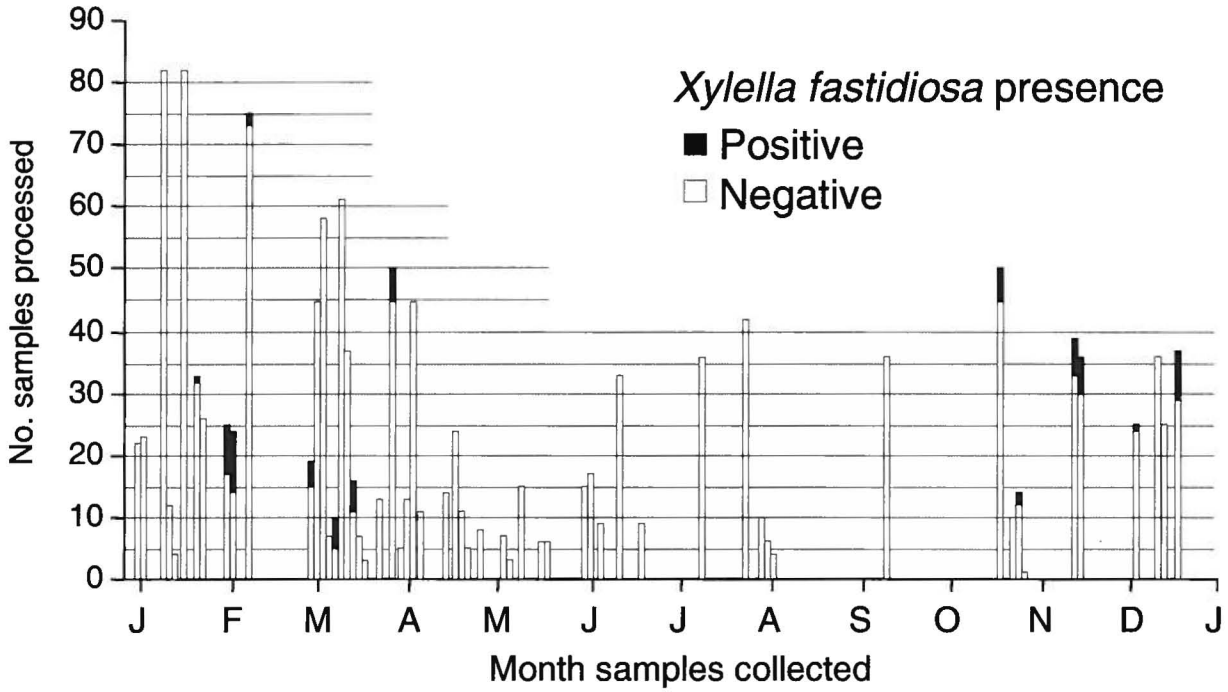

Figure 2: Average Cicadomorph and sharpshooter populations from June 2003 to September 2005 inside and adjacent to **A**) four almond orchards near Chico, CA (Butte and Glenn Counties) and **B**) two almond orchards near Modesto, CA (Stanislaus County).

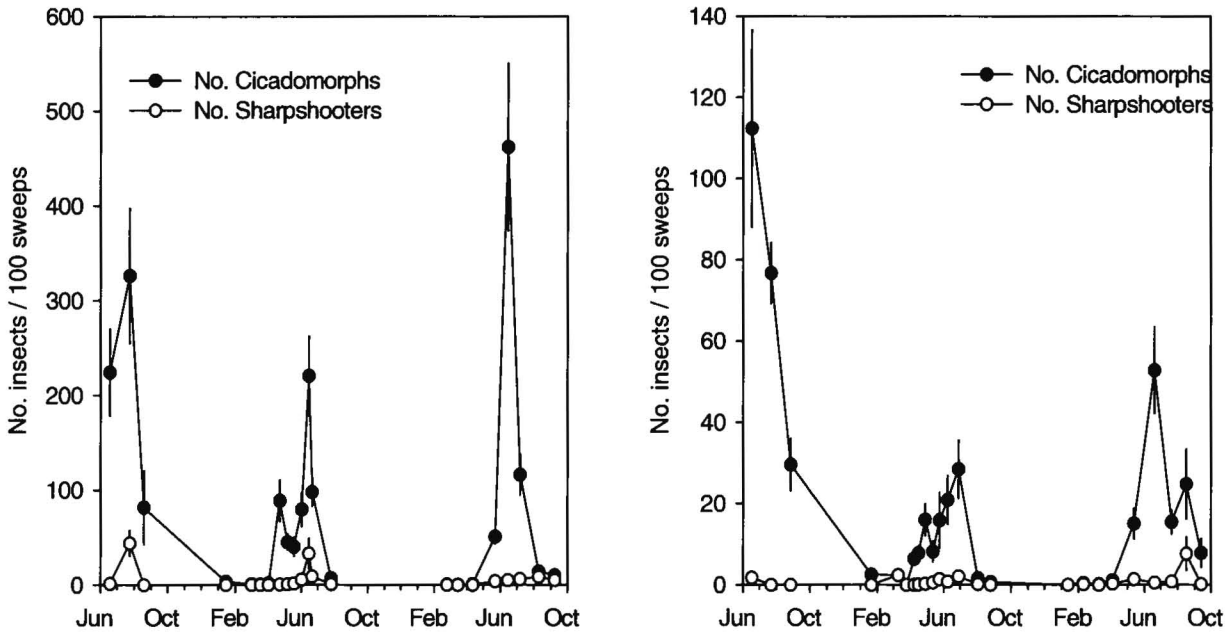

Figure 3: Average insect populations collected from sweeps of orchard floor and adjacent vegetation >10m outside, within 10m of the margins, and >10m on the interior of study sites.

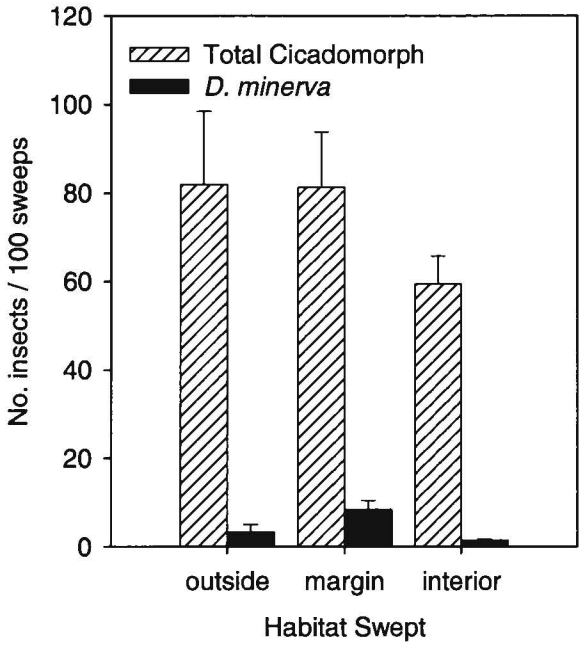

Figure 4: Average insect populations collected from sweeps of vegetation on the almond orchard floor (almond), in adjacent riparian habitat (riparian), and on roadsides adjacent to study sites. Insects were collected on the same days at the same study sites.

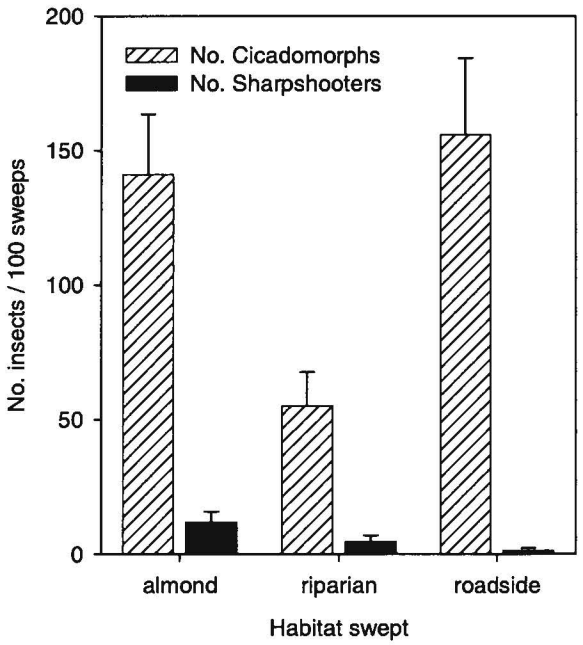

Figure 5: *D. minerva* maturation on four species, in average number of insects per plant, on alternate hosts in seventy days in the greenhouse: **A)** filaree, **B)** cheeseweed, **C)** burclover, and **D)** bermudagrass.

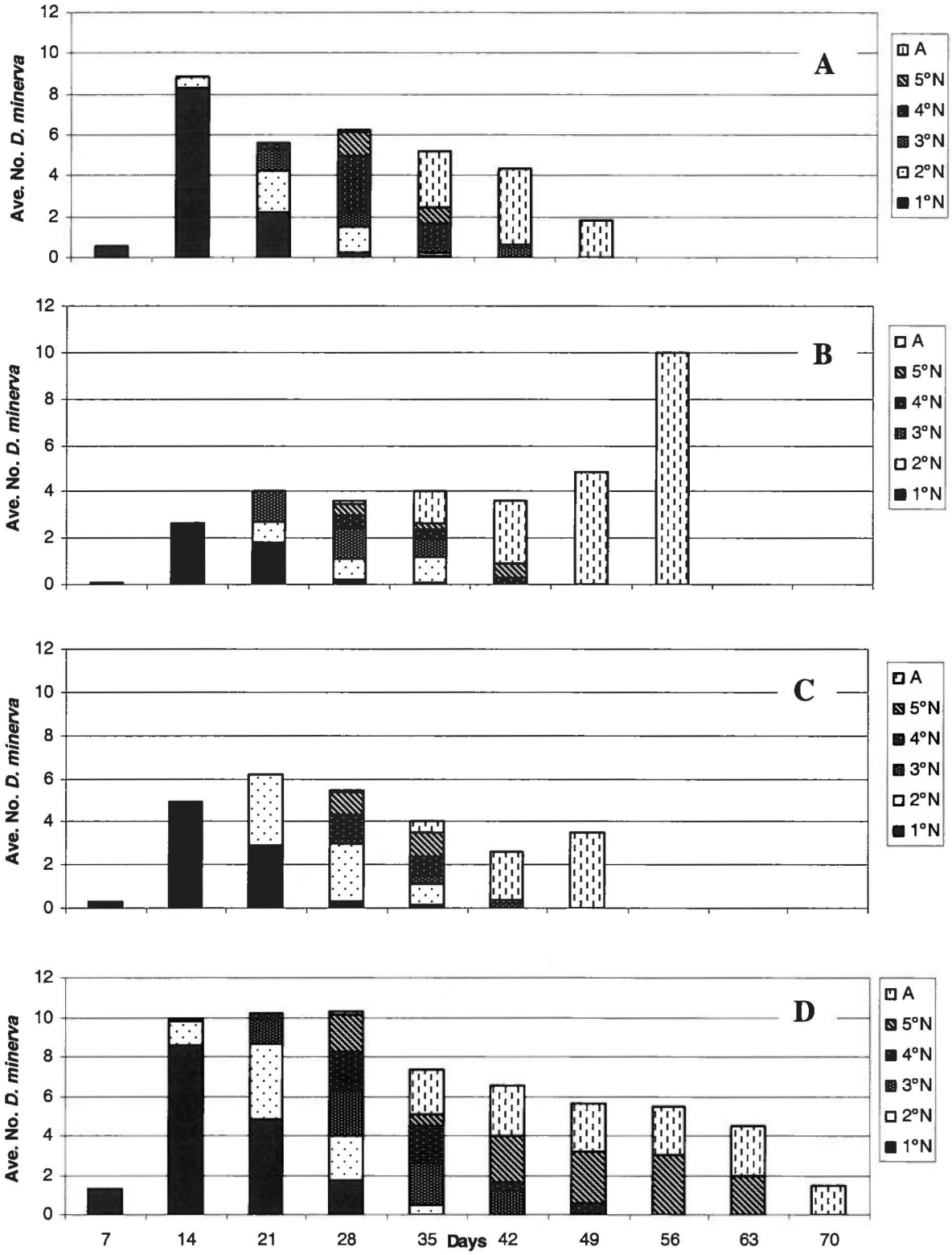

Figure 6: *D. minerva* nymph emergence from five weed species in greenhouse tests. Eight adult *D. minerva* were given a 4-day egglaying interval on alternate host plants of similar age and size; adults were removed, and emerged nymphs counted weekly thereafter.

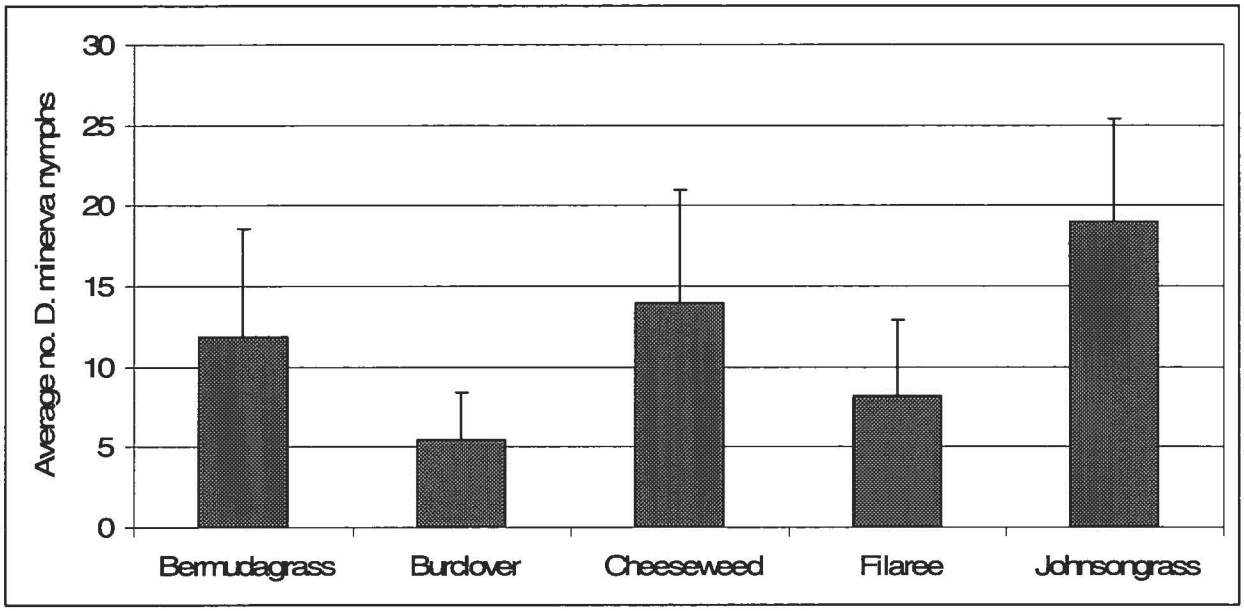

Figure 7: *D. minerva* egg masses in johnsongrass approximately 10 days after laying. **A.** dissected to remove flap of plant epidermis and show developing eggs; **B.** intact egg mass.

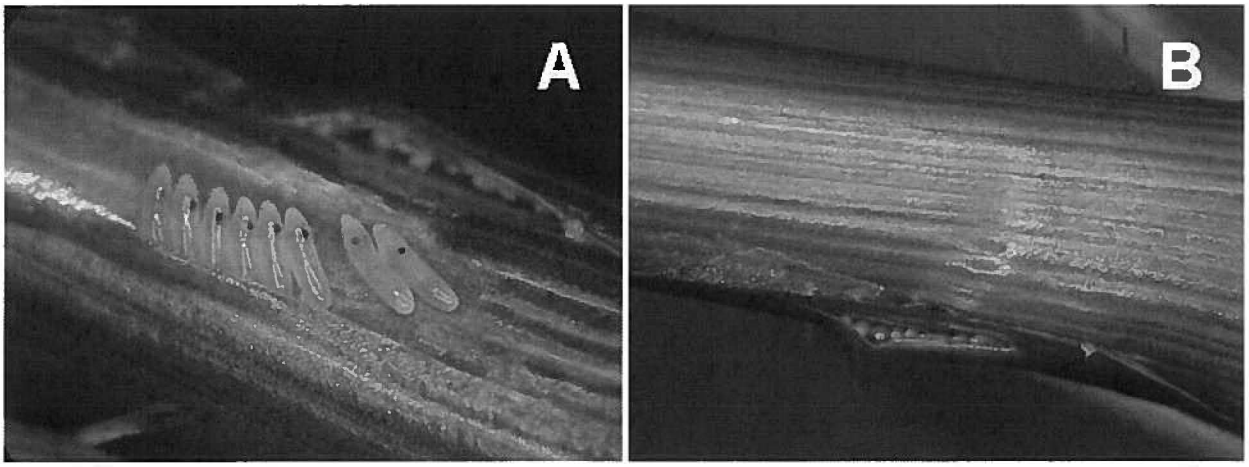

Figure 8: Total Cicadomorph **(A: top)** and *D. minerva* **(B: bottom)** populations swept from vegetation in a Butte County almond orchard in 2005. Treatment was with Asana insecticide on 27 January 2005. Means of the two treatments are not significantly different in analysis with repeated measures ANOVA (Cicadomorphs: $P = 0.25$, n = 47, *D. minerva*: P = 0.68, n = 47).