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Evaluating the potential of buckwheat and cahaba vetch as nectar producing cover crops for enhancing biological control of *Homalodisca vitripennis* in California vineyards

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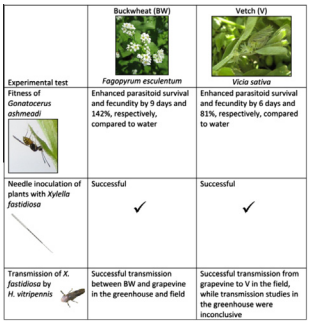
HIGHLIGHTS

- Buckwheat (BW) and vetch (V) enhanced parasitoid survival and fecundity.
- BW usually required 23–32 days from sowing to nectar-producing flowers.
- *X. fastidiosa* (*Xf*) infected and replicated in BW, V, sweet alyssum and coriander.
- *H. vitripennis* transmitted *Xf* between BW and grapevine in greenhouse and field.
- *H. vitripennis* successfully transmitted *Xf* from grapevine to V in the field.

GRAPHICAL ABSTRACT



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ABSTRACT

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) is a significant pest of grapevines in California due to vectoring *Xylella fastidiosa* Wells et al., Buckwheat (*Fagopyrum esculentum* Moench) and vetch (*Vicia sativa* L. cv. 'cahaba white') are potential nectar-producing cover crop plants in California vineyards that could enhance *Gonatocerus ashmeadi* Girault, a parasitoid of *H. vitripennis*. Three aspects of these 2 plant species were evaluated. (1) Effect on parasitoid fitness: In the laboratory, buckwheat and vetch enhanced survival of *G. ashmeadi* by 9 and 6 days, respectively, compared to water. *G. ashmeadi* offspring production was 81% and 142% greater when females were provided vetch or buckwheat, respectively, compared to water. Buckwheat decreased *G. ashmeadi* female offspring by 19% compared to water and vetch. (2) Nectar production phenology in southern California: From April through September, buckwheat required only 23–32 days from sowing to nectar-producing flowers. Vetch required an additional 14–33 days to produce extrafloral nectar during June–August. The range in length of the nectar producing period between August 2007 and November 2007 was 41–52 days and 163–164 days for buckwheat and vetch, respectively. (3) *X. fastidiosa* Wells et al. host testing and *H. vitripennis* transmission studies: Needle inoculation of cover crop plants showed that *X. fastidiosa* successfully infected and replicated in buckwheat, vetch, sweet alyssum [*Lobularia maritima* (L.) *Desv.*] and coriander (*Coriandrum sativum* L.), plants that were assessed here or elsewhere as nectar producing cover crops for use in vineyards. *H. vitripennis* successfully transmitted *X. fastidiosa* between buckwheat and grapevine in the greenhouse and field. *H. vitripennis* successfully transmitted *X. fastidiosa* from grapevine to vetch in the field, while transmission studies investigating movement from vetch to grapevine in the greenhouse were inconclusive.

1. Introduction

Conservation biological control aims to reduce pest populations by attracting, retaining, and promoting populations of natural

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enemies (Gurr et al., 2004). One aspect of this approach is habitat manipulation where plant diversity is increased, improving conditions for natural enemies that include floral resources (nectar and pollen), alternative hosts/prey, shelter, and mating sites (Heimpel and Jervis, 2005). Floral and extrafloral nectar can maximize the longevity, fecundity, searching activity and parasitism/predation rates of most natural enemies, and increase female sex ratios (Berndt and Wratten, 2005; Kost and Heil, 2005; Irvin et al., 2006; Hogg et al., 2011). Incorporating nectar producing cover crops in orchards and vineyards is one-way to enhance natural enemy populations in agricultural systems with the intention of improving pest control by providing natural enemy food and shelter (Gurr et al., 2004). Cover crops have been shown to enhance natural enemies of vineyard pests and reduce spider mite and leafhopper populations (Hanna et al., 1996; Nicholls et al., 2000; English-Loeb et al., 2003). Additionally, cover crops help maintain soil quality and prevent erosion (Dlott et al., 2002).

In Temecula, Riverside County California (CA), sustainability of the grapevine industry is severely threatened by *Xylella fastidiosa* Wells et al., a xylem dwelling plant pathogenic bacterium that causes Pierce's Disease (PD). Multiplication of *X. fastidiosa* in vines blocks xylem vessels, which causes leaf scorch symptoms and typically kills susceptible vines within a few years (Purcell, 1997). In August of 1999, 300 acres of grapevines in Temecula were destroyed by PD and over 1100 acres of grapevines were devastated statewide by 2002 (CDFA, 2010). This epidemic was related to large numbers of glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) (formally *Homalodisca coagulata* [Takiya et al., 2006]), which vectors *X. fastidiosa*. To control *H. vitripennis* and help prevent the spread of PD, approximately 75% of Temecula grape growers currently apply prophylactic imidacloprid applications which cost $150–$200 per acre (Daugherty and Pinckard, 2010). The enhancement of biological control for *H. vitripennis* natural enemies offers an additional, sustainable method for sharpshooter management in the Californian grape industry. *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) is a small solitary egg parasitoid [1.28–1.76 mm in length (Triapitsyn, 2006)], and is the key natural enemy attacking egg masses of the *H. vitripennis* in California (USA). Laboratory studies have shown that the average fecundity for this parasitoid species is 94–130 *H. vitripennis* eggs and the ovigeny index is 0.22 (Irvin and Hoddle, 2007, 2009). Cover crops can contribute to enhancement of biological control, potentially helping growers reduce pesticide use and possibly producing grapes for organic markets. Cover crops are promoted by the Californian wine industry which promotes sustainable practices through the Code of Sustainable Winegrowing Workbook (CSWW) (Dlott et al., 2002).

Buckwheat (*Fagopyrum esculentum* Moench) has been shown to improve parasitoid sex ratios in vineyards (Berndt et al., 2002) thereby increasing parasitism and lowering abundance of leafhoppers (Nicholls et al., 2000; English-Loeb et al., 2003). This plant shows promising potential as a cover crop in vineyards because its seed is inexpensive and readily available, it germinates easily, it is adaptable to poor growing conditions, and it has a short sowing to flowering time (Angus et al., 1982; Bowie et al., 1995). However, despite this potential, it is unknown whether buckwheat, if planted in CA vineyards where PD is present, could act as a host for *X. fastidiosa* and whether *X. fastidiosa* can be transmitted between buckwheat and grapevines by adult *H. vitripennis*.

Another cover crop, cahaba vetch (*Vicia sativa* L. cv. 'cahaba white') is suggested in CSWW since it improves soil nutrition, fertility and structure, and it reduces soil erosion and dust (Dlott et al., 2002). This cover crop also has been shown to suppress populations of damaging nematode species in Californian vineyards (McKendry, 1992). Cahaba vetch produces extrafloral nectaries at the base of each stipule. Extrafloral nectar is easily accessible to natural enemies, and it often is produced in larger volumes and for longer periods of time than floral nectar (Koptur, 2005). It is unknown whether extrafloral nectaries of cahaba vetch benefit natural enemies of grape pests, especially egg parasitoids of *H. vitripennis*.

The studies reported here are the first steps in determining whether buckwheat and cahaba vetch have potential in conservation biological control as nectar producing cover crops in CA vineyards. These studies sought to investigate: (1) whether buckwheat flowers and cahaba vetch extrafloral nectaries increase longevity and fecundity of *G. ashmeadi* in the laboratory; (2) the nectar production phenology and agronomy of buckwheat and cahaba vetch growing in southern California; and (3) whether buckwheat and cahaba vetch are hosts of *X. fastidiosa* and whether this bacterium can be reciprocally transmitted between these plants and grapevines by adult *H. vitripennis*. In addition, sweet alyssum [*Lobularia maritima* (L.) *Desv.*] and coriander (*Coriandrum sativum* L.) were included in the mechanical inoculation studies to determine if these two plants are hosts for *X. fastidiosa*. Alyssum and coriander have previously shown promise as nectar producing floral resources for natural enemy enhancement in crops (Baggen and Gurr, 1998; Irvin et al., 2006).

2. Materials and methods

2.1. Parasitoid survival and fecundity in the laboratory on nectar resources

2.1.1. Maintenance of insect colonies

Colonies of *G. ashmeadi* and *H. vitripennis* were maintained at the University of California, Riverside, CA (UCR). *G. ashmeadi* colonies were reared on *H. vitripennis* eggs laid on *Citrus limon* cv 'Eureka' lemon leaves. Maintenance of trees used for *H. vitripennis* colonies and *H. vitripennis* and *G. ashmeadi* colony maintenance is described in Irvin et al. (2012). Petri dishes containing leaves with *H. vitripennis* eggs previously exposed to *G. ashmeadi* were held at 26 ± 2 °C and 30–40% RH under a L16:8D photoperiod and checked daily for parasitoid emergence. Adult parasitoids which emerged were used in experiments.

2.1.2. Maintenance of nectar plants

Plants of *F. esculentum* (obtained from Outsidepride, Salem, OR) and *V. sativa* cv. 'Cahaba White' (obtained from Bailey Seed Company, Salem, OR) were grown from seed in a greenhouse at 26 ± 3 °C under natural L14:10D light. Seeds were sown in 1 gal pots, at 4 seeds per pot. Synchronous nectar production was ensured by performing staggered sowings at 7–10 day intervals. Plants were fertilized every three weeks with Miracle-Gro (20 ml/3.5 L of water, Scotts Miracle-Gro Products Inc., Marysville, OH). Prophylactic applications of pyrethrin + canola oil (Garden Safe Brand Fruit & Vegetable Insect Spray, Schultz Company, Bridgeton, MO) were applied to vetch plants every 7–10 days to control greenhouse insect pests. Plants used for experiments were not treated for at least 14 days before being used, and they were washed thoroughly with water and allowed to dry before use in experiments.

2.1.3. Experimental set up

Three treatments (water, buckwheat, and vetch) were evaluated in the laboratory at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod. Each treatment was placed in a cage, and 16 cages of each treatment were arranged in a completely randomized design. Wooden cages (32 × 34 × 37 cm) painted white, with a glass top, mesh back for ventilation and hinged front door containing a material sleeve for access were used for treatment replicates. A white

piece of cardboard was placed on the bottom of each cage to allow ease of finding dead parasitoids. Water was provided to parasitoids via a 7.4 ml glass vial (2 dram Fisherbrand Glass Vial, Fisher Scientific, Pittsburgh, PA) with a 5 cm cotton wick. The vial was placed on the bottom of each cage and filled daily. Plant treatments consisted of 1 gal pot with buckwheat or vetch plants with the bottom and top of the pot wrapped in Parafilm (Parafilm'M' Laboratory Film, Pechiney Plastic Packaging, Chicago IL) to prevent parasitoid access to moisture inside pots. Plants in cages were watered as needed with an 8 oz wash bottle inserted through a hole in the Parafilm. Tape was placed over the hole after each watering to exclude parasitoids. Plants were removed and replaced every 4–5 days to ensure a constant supply of nectar. One newly emerged (⩽12 h old) naive male and female *G. ashmeadi* was released inside each cage. Female parasitoid longevity was recorded daily until death of each individual. Survival was assigned to the last day the parasitoid was found alive.

Hosts were provided to *G. ashmeadi* by placing the petioles of 'Eureka' lemon leaves containing *H. vitripennis* eggs through holes drilled in the lid of a 130 ml plastic vial (40 dram plastic vial, Thornton Plastics, Salt Lake City, UT) filled with water. Leaves bearing hosts exposed to parasitoids were removed and replaced every three days until death of the female parasitoid. On the 1st, 4th, and 7th replacement day, 80, 80 and 60 host eggs (<24 h old) were provided to *G. ashmeadi,* respectively. On subsequent host changing days 40 *H. vitripennis* eggs were provided. Host numbers and age were selected based on previous fecundity studies for *G. ashmeadi* (Irvin and Hoddle, 2007). After exposure to parasitoids, leaves bearing the *H. vitripennis* egg masses were placed into Petri dishes (9 × 1 cm, Becton Dickinson Labware, Becton Dickinson and Co., Franklin Lakes, NJ) lined with moist filter paper (9 cm Whatman Ltd. International, Maidstone, England) and left at 26 °C for three weeks to allow parasitoids to emerge. Leaves sometimes decayed which prevented successful insect emergence. Therefore, unemerged eggs were dissected and numbers of unemerged parasitoids were recorded and included in progeny calculations.

2.1.4. Statistical analyses

Only parasitoids that died of natural causes (i.e., not from drowning, squashing from handling errors, or escaping) were included in statistical analyses of longevity. Those females that did not mate (i.e., produced only male progeny) were excluded from the male progeny totals and sex ratio comparison analyses. This resulted in 10–16 experimental units depending on treatment. The effect of treatment on the total number of parasitoid offspring and longevity was determined using ANOVA with unbalanced replicates in SAS (1990). Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. Logistic regression was used to determine the effect of treatment on logit offspring sex ratio (percentage female) (Hosmer and Lemeshow, 2000) and the effect of time on percentage of male offspring. Pair-wise contrast tests at the 0.05 level of significance were used to separate means. Means (±SEM) presented here were calculated from untransformed data.

2.2. Cover crop phenology and agronomy

2.2.1. Experimental set up

A tractor and cultivator were used to create a furrow surrounding each of 156 plots (twelve rows of thirteen plots) at Agricultural Operations, UCR (GPS co-ordinates: N 33°57′56.0; W 117°20′25.1). Monthly temperature, precipitation, relative humidity and day length during this experiment are presented in Table 1. Plots were 1 m^2 and they were separated by 1.8 m. In the middle of each month from August 2007 until August 2008, five replicates of buckwheat and vetch were sown in 10 randomly selected plots following recommended agricultural sowing rates (buckwheat: 22.6 kg/0.4 ha = 5.62 g seed/m^2; vetch: 27.2 kg/0.4 ha = 6.71 g seed/m^2). Seed was sourced as described previously. Seed was sown in each plot, covered with approximately 2.5 cm of soil using a rake, and watered with 9.5 L of water from a watering can.

Plots were irrigated for 2 h time periods via a 0.89 mm orange O-Jet 6000 Series Micro-Spray sprinkler head (Olson Irrigation Systems, Santee, CA) installed in the middle of each plot. An adjustable pressure regulator was installed to deliver 15 PSI under which sprinklers emitted 7.2 GPH. Plots were irrigated every 2–4 days from August 2007 until July 2009 depending on time of year. Irrigation was turned off during periods of rain.

Vetch plots were highly susceptible to mites (*Tetranychus urticae* Koch), aphids, thrips (*Frankiniella* spp.), the three-cornered alfalfa hopper (*Spissistilus festinus* [Say]) and the false chinch bug (*Nysius* sp.) which significantly reduced plant vigor. Therefore, prophylactic applications of Ortho Systemic Insect Spray (8% acephate and 0.5% fenbutatin-oxide; The Ortho Group, Marysville, OH) were applied once a month in March 2008 and April 2008 following label directions, and then every 7–10 days from May 2008 until September 2008. From October 2008 until July 2009, vetch plots were sprayed once a month to control pests.

Weeds were removed by hand as necessary. Plots were checked weekly and then every 2 days when plants were nearing nectar production. The number of days until at least one plant produced nectar (i.e., floral nectar from flowers of buckwheat or extrafloral nectar from the stipples of vetch) was recorded per plot. At six weeks, the height of ten randomly selected plants per plot was measured and average six weeks height estimates were calculated for each plot. Plants were monitored until nectar production ceased (i.e., when all flowers died for buckwheat or when vetch plants stopped producing extrafloral nectar). The length of the nectar producing period was recorded per plot.

2.2.2. Statistical analyses

Multiple regression was used to determine the effect of sowing date, plant species and sowing date* plant species interaction on average six weeks height (data logged transformed), days until nectar production (raw data) and length of nectar production (raw data) (Hosmer and Lemeshow, 2000). Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. Means (±SEM) are presented for untransformed data.

2.3. X. fastidiosa host testing and H. vitripennis transmission studies

Cover crop plants may be a host for *X. fastidiosa* and act as potential reservoirs of PD that may be reciprocally transmitted by *H. vitripennis* from cover crop to grapevines. Mechanical inoculations of cover crop plants were conducted to investigate whether buckwheat and vetch are hosts for *Xylella fastidious* subspecies *fastidiosa* (the Temecula strain of bacteria that causes PD in this area). Further testing was conducted on buckwheat and vetch to determine whether *H. vitripennis* could acquire *X. fastidiosa* from buckwheat or vetch and successfully transmit the pathogen to grapevines, and from grapevines to cover crops. In addition, sweet alyssum and coriander were included in the mechanical inoculation studies to determine if these two plants are hosts for *X. fastidiosa*.

2.3.1. Plant maintenance

Buckwheat, cahaba vetch (see previous source), coriander (*C. sativum*; obtained from Burpee & Co. Warminster, PA) and sweet alyssum (*L. maritima cv.* '*Carpet of Snow*'; obtained from Burpee & Co.) were grown from seed in a greenhouse at 26 ± 3 °C under natural L14:10D light. Seeds were sown in 1 gal pots, with

Table 1
Monthly weather statistics during phenology experiment conducted during August 2007 and August 2008 at Agricultural Operations, University of California, Riverside, CA.

Date	Average air temperature[a] (°C)	Average solar radiation[a] (Ly/day)	Precipitation[a] (mm)	Average relative humidity[a] (%)	Daylength[b] (h/day)
August 2007	25.6	537	0	46	13.42
September 2007	21.8	458	0	48	12.38
October 2007	18.9	349	0	43	11.35
November 2007	15.8	254	0	48	10.40
December 2007	10.8	241	0	47	9.90
January 2008	10.9	241	70.3	52	10.13
February 2008	12.4	323	17.0	52	10.90
March 2008	15.1	464	0.78	41	11.90
April 2008	16.9	535	0	44	13.05
May 2008	18.0	535	0.3	50	13.93
June 2008	23.2	632	0.5	45	14.40
July 2008	24.4	597	0	50	14.18
August 2008	25.1	566	2.5	48	13.40

[a] Data was downloaded from the CIMIS weather database (i.e., Station 44) (http://www.cimis.water.ca.gov/cimis/dataInfoType.jsp).
[b] Data downloaded from "Timeanddate.com" for Riverside, California; http://www.timeanddate.com/worldclock/astronomy.html?n=880&month=8&year=2007&obj=sun&afl=-11&day=1.

4–5 seeds per pot. After emergence of the first true leaves, plants were thinned to one plant per pot. Plants were fertilized weekly with Miracle-Gro All Purpose Plant Food at a rate of 1.25 ml per 3.78 liters of water. Grapevines (cv. Redglobe; Sunridge Nurseries, Bakersfield, CA) used for transmission studies were grown in 1 gal pots and fertilized with Miracle Gro as described previously. Grapevines were placed in a separate greenhouse at 23–27 °C under natural L12:12D light with yellow sticky traps (Olsen Sticky Traps, Gempler's, Madison, WI) deployed to attract and capture any potential *H. vitripennis* present and prevent accidental *X. fastidiosa* contamination of test plants.

2.3.2. Needle inoculation of cover crop plants

The PD strain of *X. fastidiosa* was cultured on PWG media (Hill and Purcell, 1995) for 7 days, and resuspended in cold, sterile SCP buffer (Hopkins and Thompson, 1984) for needle inoculation. Approximately twenty-five buckwheat, vetch, alyssum and coriander plants were needle inoculated in the laboratory with *Xylella fastidious* subspecies *fastidiosa* (Temecula strain of PD) by pipetting a 10 μL drop of bacterial solution onto a stem and probing the drop with a sterilized #1 insect pin (Indigo Instruments, Tonawanda, NY) until up take of the drop was observed. Cover crop plant stems were inoculated once near the base of the plant usually between the 2nd and 3rd nodes, or the first available internode above the 2nd node. Grapevines were inoculated at the first internode of new canes growing from the crown. After needle inoculation, plants were tested with (1) enzyme-linked immunosorbent assay (ELISA) and (2) a plate culturing technique for the presence of *X. fastidiosa*.

Plants were tested with ELISA according to manufacturer's instructions (PathoScreen Kit, Agdia, Elkhart, IN) after four weeks to determine the presence or absence of *X. fastidiosa*. The first available petiole above the inoculation site was sampled for plants that had substantial petioles. For plants with very small petioles, a small shoot was sampled instead. Petioles or shoots were cut to approximately 2 cm, weighed, and ground in an Agdia Mesh Sample bag (Agdia, Elkhart, IN) in 10× w/v General Extraction Buffer. 100 μl of each sample was loaded into 96 well microtiter plates coated with *X. fastidiosa*-specific antibodies and processed. The results of the analysis were evaluated using a Benchmark microtiter plate reader (BioRad, Hercules, CA) at a wavelength of 490 nm. Negative controls were used for each plant species. Positive and negative grapevine controls, plus the positive kit control, and buffer only negatives were used for each test on every plate. Samples were considered positive for *X. fastidiosa* if the absorbance reading was greater than two times the average of the negative controls. Test results were valid only if positive control wells tested positive and negative control buffer wells remained clear.

Plate culturing technique was used to culture live *X. fastidiosa* cells from cover crop test plants and grapevines. For culturing, 3 cm samples of petiole, leaf blade, and/or stem (depending on plant morphology) were cut from plant samples collected. Samples were surface-sterilized in a series of 5 × 30 s sterile baths as follows: 20% bleach (1:5 dilution of 5.25% hypochlorite solution), 95% ethyl alcohol, then 3 sterile deionized water rinses. Surface sterilized samples then were finely chopped (roughly 1–3 mm thick disks or pieces) in 600 μl sterile SCP buffer using sterile forceps and scalpels and allowed to sit for 10–15 min. Two hundred microliters of buffer were pipetted from the chopped mash onto 2 PWG media plates (Hill and Purcell, 1995). The plates were wrapped with Parafilm and, after allowing the sample to settle into the media for about 30 min, were incubated, inverted at room temperature in a drawer (dark) for 10–30 days. Plates were checked for the presence of *X. fastidiosa* colonies at 10, 20, and 30 days.

2.3.3. Greenhouse transmission of X. fastidiosa by H. vitripennis

Further testing was conducted on buckwheat and vetch to determine whether *H. vitripennis* could acquire *X. fastidiosa* from buckwheat or vetch and successfully transmit the pathogen to grapevines, and then from grapevines to the cover crop of interest. *H. vitripennis* used for this work were sourced from previously established colonies. Original colonies were started with eggs laid and hatched in captivity from field collected females. Eggs in colonies were checked daily for nymphs, which were immediately transferred to large cages (91 cm × 61 cm × 122 cm) containing clean corn, basil, and sunflower plants. Cages containing nymphs were located in a greenhouse at 23–27 °C under natural L12:12D light. Randomly collected individuals were tested periodically for *X. fastidiosa* with PCR. All PCR tests of colony insects were negative for *X. fastidiosa*.

Transmission tests were performed on 5 vetch and 5 buckwheat plants, and 2 control grapevines for each plant species. Sometimes cover crop plants died before completion of adequate testing (see Table 4 for final replicate numbers). Forty adult *H. vitripennis* were released into cages (BugDorm-2120, [60 cm × 60 cm × 60 cm], MegaView Science Education Services, Taiwan) containing cover crop plants previously needle inoculated and successfully infected with *Xylella fastidious* subspecies *fastidiosa*. The insects were allowed 48 h feeding and acquisition access period (AAP) (Almeida and Purcell, 2003). Insects were collected and five *H. vitripennis* were placed into a nylon organdy sleeve cage on each of five Redglobe grape plants that had been tested with ELISA and demonstrated to be free of *X. fastidiosa*. Sleeve cages were 61 cm long and 20 cm in diameter with cable wire sewn into tubes around the outside to maintain their shape and nylon drawstring cords on each end. Sleeve cages were doubly secured with rubber

bands around the pots and twist ties at the top. All *H. vitripennis* were given a 96 h inoculation access period (IAP) (Almeida and Purcell, 2003). The grape test plants were placed in a greenhouse as described previously and tested for *X. fastidiosa* infection 8, 12 and 16 weeks post-feeding using ELISA and the plate culturing technique as described previously. Using the same protocols, *H. vitripennis* transmission from cover crop to cover crop, and grapevine to grapevine (controls) were tested. Following implementation of the transmission tests, the soil in each potted grapevine was treated with a systemic insecticide (Admire) at a rate of 0.75 mL/cu ft soil to prevent inadvertent transmission *of X. fastidiosa* by insect vectors.

2.3.4. Transmission of X. fastidiosa by H. vitripennis in the field

Trials that investigated natural inoculation of buckwheat and vetch under field conditions were conducted at Agricultural Operations, UCR where *H. vitripennis* and *X. fastidiosa* co-occur. Buckwheat and vetch were sown in the field in August 2008 and after three weeks, 10 buckwheat and 10 vetch plants were randomly selected and individually covered with acetate cages. Acetate cages were 30.5 cm tall and 10.2 cm diameter, with 5.1 × 10.2 cm 'windows' on opposite sides, covered with nylon mesh organdy. The top of the cylinder also was covered with nylon mesh organdy. The seam of the acetate tube and the fabric were glued using a hot glue gun. Cages were secured in place with a 91.4 cm length of 2.5 cm diameter PVC pipe positioned in the ground directly east of the plant. The cage was placed over the plant and fastened to the PVC pipe using a 114 g rubberband (Office Max brand #32 rubberbands; Office Max, Naperville, IL) to prevent the cage from being blown over by wind.

Adult *H. vitripennis* (125) were collected from the field and placed in a Bug Dorm with a potted grapevine (cv. Redglobe). The grapevine had been needle-inoculated and infected (confirmed by ELISA) with *Xylella fastidiosa* subspecies *fastidiosa*. Insects were left to feed for a 48 h AAP. Live adults were aspirated into plastic 40 dram vials (five adults per vial). One vial was placed into each cage for each plant. Four potted grapevine plants that were confirmed free of *X. fastidiosa* using ELISA were used as controls. These grapevines were placed beside the buckwheat and vetch plants and fitted with nylon organdy sleeve cages. One vial of adult *H. vitripennis* was opened onto each potted grapevine. All *H. vitripennis* were given a 96 h IAP. Grapevine controls were treated once with Admire as described previously and returned to the greenhouse.

Buckwheat plants started dying at 2–3 weeks post-IAP, so all plants were collected at three weeks post-IAP and tested for *X. fastidiosa*. Four buckwheat plants were too dry for culture testing (which tests live cells), so these were tested with ELISA only in case dead cells could be detected. The remaining 6 buckwheat plants were tested with ELISA and the plate culturing technique as described previously. The vetch plants were sampled at 4-weeks post-IAP by collecting a small branch from the base of the plant. Lowest leaves from each branch of the grapevine controls were collected and the lowest 2 cm of petiole tissue from each leaf was used. Field planted cover crop plants died before cover crop to grapevine tests could be conducted for field transmission tests.

3. Results

3.1. Parasitoid survival and fecundity in the laboratory on nectar resources

Providing *G. ashmeadi* with buckwheat or cahaba vetch plants enhanced parasitoid survival by an average of 9 and 6 days, respectively, compared to water (Fig. 1a). Parasitoid survival was statistically equivalent between plant species (Fig. 1a). *G. ashmeadi* offspring production was increased 81% and 142%, respectively, when females were provided vetch or buckwheat compared to water (Fig. 1b). There was no significant difference in fecundity of *G. ashmeadi* between plant species (Fig. 1b). Buckwheat decreased *G. ashmeadi* female offspring sex ratio by approximately 19% compared to water and vetch (Fig. 1c). Female offspring sex ratio was statistically equivalent between water and vetch (Fig. 1c). There was a significant effect of time on the percentage of male progeny for buckwheat ($\chi^2 = 92.78$, df = 4, $p < 0.0001$) and vetch ($\chi^2 = 31.50$, df = 3, $p < 0.0001$) (Fig. 2). The percentage of male progeny in the buckwheat treatments significantly increased 42% as female parasitoids aged from 0 to 12 days (Fig. 2). The percentage of male progeny in the vetch treatments significantly increased 17% as female parasitoids aged from 0 to 6 days. There was no effect of time on percentage of male progeny for older parasitoids (7–12 days) provided with vetch (Fig. 2).

3.2. Cover crop phenology and agronomy

There was a highly significant effect of sowing date ($F = 93.92$, df = 12, $p < 0.0001$), plant species ($F = 2089.87$, df = 1, $p < 0.0001$) and sowing date* plant species interaction ($F = 16.29$, df = 12,

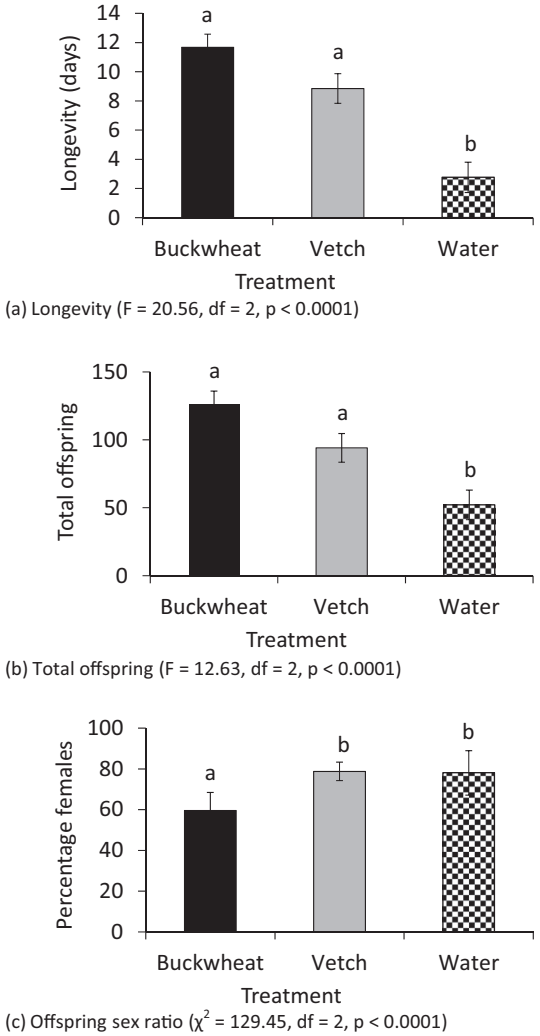

Fig. 1. Mean (a) longevity, (b) total offspring and (c) offspring sex ratio produced when female *G. ashmeadi* were provided with one potted vetch (*Vicia sativa*) plant, buckwheat (*Fagopyrum esculentum*) plant or water only in the laboratory (different letters indicate significant differences [$p < 0.05$] between treatments).

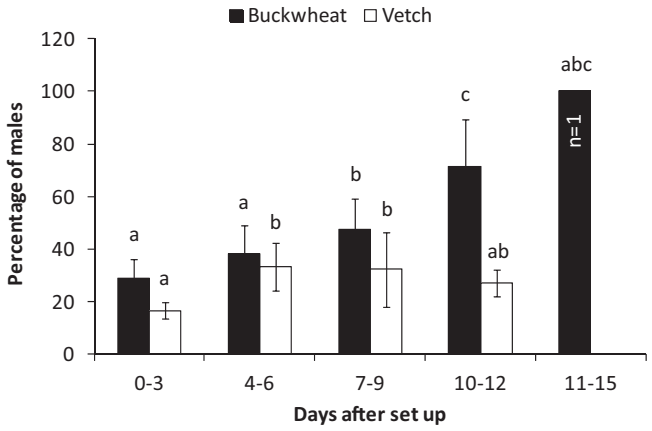

Fig. 2. The effect of time on the mean percentage of male offspring produced when female *G. ashmeadi* were provided with one potted vetch (*Vicia sativa*) plant or buckwheat (*Fagopyrum esculentum*) plant in the laboratory (different letters indicate significant differences [$p < 0.05$] between time periods within each plant treatment).

$p < 0.0001$) on mean six weeks plant height. Sowing date had a significant effect on six weeks height of buckwheat ($F = 83.29$, df = 12, $p < 0.0001$) and cahaba vetch ($F = 32.67$, df = 12, $p < 0.0001$) with shorter plants occurring during the winter months (Table 2). For each sowing date, vetch was significantly shorter than buckwheat ($p < 0.001$) (Table 2).

There was a highly significant effect of sowing date ($F = 33.73$, df = 12, $p < 0.0001$), plant species ($F = 77.15$, df = 1, $p < 0.0001$) and sowing date* plant species interaction ($F = 7.17$, df = 12, $p < 0.0001$) on the number of days from sowing to flowering. Sowing date had a significant effect on days until flowering of buckwheat ($F = 22.31$, df = 12, $p < 0.0001$) and vetch ($F = 18.65$, df = 12, $p < 0.0001$). The number of days required from sowing to flowering for buckwheat was significantly shorter during the warmer summer months, compared to cooler months during November through January (Table 2). From April through September, buckwheat required only 23–32 days from sowing to nectar-producing flowers (Table 2). For vetch, the number of days required from sowing to nectar production was significantly shorter during the spring months of March through May compared to the winter months of November through January (Table 2). In August 2007, November 2007, June 2008, July 2008 and August 2008 vetch took significantly longer (14–32 days longer) to start producing nectar compared to buckwheat (Table 2).

There was a highly significant effect of sowing date ($F = 7.16$, df = 12, $p < 0.0001$), plant species ($F = 98.86$, df = 1, $p < 0.0001$) and sowing date* plant species interaction ($F = 12.39$, df = 12, $p < 0.0001$) on the length of nectar production. Sowing date had no significant effect on the length of nectar production of buckwheat flowers ($F = 1.72$, df = 12, $p = 0.07$) and a highly significant effect on length of nectar production of vetch extrafloral nectaries ($F = 20.67$ df = 12, $p < 0.0001$). Length of nectar production in vetch was significantly longer (98–206 days longer) when seeds were sown in July and August compared to the rest of the year (Table 2). In contrast to results for the number of days until flowering, once extrafloral nectaries were present on vetch plants, vetch produced nectar for on average, 129 days longer than buckwheat in August 2007, September 2007, October 2007, November 2007, July 2008 and August 2008 (Table 2).

3.3. *X. fastidiosa* host testing and *H. vitripennis* transmission studies

3.3.1. Mechanical inoculation of cover crop plants

Four weeks after mechanical inoculation, 63% and 53% of buckwheat plants inoculated with *X. fastidiosa* tested positive by ELISA and plate culture tests, respectively (Table 3). Results from mechanical inoculation of vetch showed that 45% and 15% of plants became infected with *X. fastidiosa* as detected by ELISA and culture tests, respectively (Table 3). For alyssum and coriander plants inoculated with *X. fastidiosa*, 50% and 35% of plants tested positive by ELISA, respectively. Culture testing demonstrated that 35% and 70% of alyssum and coriander plants inoculated with *X. fastidiosa*, respectively, tested positive (Table 3). These results demonstrated that *X. fastidiosa* can successfully infect and replicate in buckwheat, vetch, alyssum and coriander.

3.3.2. Greenhouse transmission of *X. fastidiosa* by *H. vitripennis*

Greenhouse studies testing the ability of *H. vitripennis* to transmit *X. fastidiosa* from cover crop plants indicated transmission of *X. fastidiosa* from buckwheat plants to buckwheat plants, and from buckwheat plants to grapevines was possible (Table 4). The grapevine to grapevine controls also were positive. Results for greenhouse transmission of *X. fastidiosa* from vetch to grapevines were inconclusive. Vetch plants grown in the greenhouse were highly susceptible to pest problems and were extremely difficult to keep alive long enough to allow for adequate testing. Four cohorts of vetch plants were evaluated during 2 years of testing and only plants from the last cohort survived long enough for testing. Results from the ELISA testing showed that 40–60% of the vetch to vetch and vetch to grapevine tests were positive for *X. fastidiosa*

Table 2
The mean six weeks plant height, the number of days until plants produced nectar, and the length of nectar production in buckwheat (*Fagopyrum esculentum*) and vetch (*Vicia sativa*) plants sown in the middle of each month from August 2007 until August 2008 (error bars indicate SEMs; different letters indicate significant [$p < 0.05$] differences between months; asterisks indicates significant [$p < 0.05$] differences between vetch and buckwheat plants).

Date	6 weeks height		Days until nectar production		Length of nectar production	
	Buckwheat	Vetch	Buckwheat	Vetch	Buckwheat	Vetch
August 07	48.2 ± 2.8 ab*	8.6 ± 0.7 a	23.8 ± 0.5 a*	52.2 ± 3.4 ab	41.2 ± 0.5 *	162.8 ± 3.4 a
September 07	54.6 ± 0.8 b*	7.4 ± 0.5 a	28.6 ± 0.2 ab	37.2 ± 1.9 ac	34.8 ± 0.4 *	144.8 ± 1.9 abc
October 07	35.4 ± 2.1 ab*	3.2 ± 0.4 b	38.2 ± 0.4 ab	41.2 ± 0.8 abc	44.0 ± 0.3 *	154.4 ± 0.9 ac
November 07	10.5 ± 0.3 c*	2.1 ± 0.5 c	56.0 ± 1.1 c*	70.6 ± 1.6 d	51.6 ± 1.7	96.4 ± 1.9 abcd
December 07	4.5 ± 0.2 d*	3.0 ± 0.4 bc	66.8 ± 0.2 c	68.6 ± 0.6 d	109.0 ± 3.6	83.2 ± 1.0 bcd
January 08	6.5 ± 0.5 d*	3.0 ± 0.3 bc	57.4 ± 1.7 c	56.0 ± 0.8 bd	73.8 ± 5.8	67.6 ± 1.7 bd
February 08	31.5 ± 0.7 a*	4.1 ± 0.2 bd	39.6 ± 0.2 b	41.4 ± 1.8 abc	75.0 ± 3.9	84.0 ± 1.9 abcd
March 08	36.0 ± 1.6 ab*	5.4 ± 0.4 ad	35.8 ± 0.4 ab	36.4 ± 1.7 c	77.2 ± 0.4	63.8 ± 3.4 d
April 08	50.8 ± 2.5 ab*	6.9 ± 0.2 a	29.2 ± 0.5 ab	34.6 ± 2.0 c	75.8 ± 5.7	78.8 ± 9.6 bcd
May 08	43.4 ± 3.7 ab*	8.6 ± 0.5 a	31.4 ± 0.4 ab	35.6 ± 0.4 c	60.4 ± 5.8	47.0 ± 7.5 d
June 08	48.7 ± 5.6 ab*	7.4 ± 0.3 a	32.3 ± 0.3 ab*	56.0 ± 0.0 abd	67.3 ± 6.9	110.8 ± 67.9 abcd
July 08	47.8 ± 4.6 ab*	8.2 ± 1.3 a	24.0 ± 0.8 a*	56.6 ± 12.4 bd	55.6 ± 7.1*	252.0 ± 17.3 e
August 08	52.5 ± 2.5 ab*	9.5 ± 0.2 a	25.0 ± 1.0 ab*	39.0 ± 3.4 ac	61.5 ± 10.5*	253.6 ± 15.1 e

Table 3
Evaluation of pathogenicity for four test plant species that were mechanically inoculated with *Xylella fastidiosa* subspecies *fastidiosa* in the laboratory.

Test plant	ELISA[a]	Culture[b]	Successful inoculation?
Fagopyrum esculentum (buckwheat)	19/30 (63%)	16/30 (53%)	Yes
Vicia sativa (cahaba vetch)	9/20 (45%)	3/20 (15%)	Yes
Lobularia maritima (alyssum)	10/20 (50%)	7/20 (35%)	Yes
Coriandrum sativum (coriander)	7/20 (35%)	7/10 (70%)	Yes

[a] Number of plants testing positive for the presence of *X. fastidiosa* based on the number of plants inoculated using enzyme-linked immunosorbent assay (ELISA).
[b] Number of plants testing positive for the presence of *X. fastidiosa* based on the number of plants inoculated using direct culturing on PWG.

Table 4
Greenhouse studies using *H. vitripennis* to transmit *Xylella fastidiosa* from buckwheat (*Fagopyrum esculentum*) and vetch (*Vicia sativa*) cover crop plants previously needle inoculated with *X. fastidiosa* subspecies *fastidiosa*.

Transmission test	ELISA[a]	Culture[b]	Successful transmission?
Buckwheat-to-Buckwheat	1/3 (33%)	2/3 (67%)	Yes
Buckwheat-to-Grapevine	4/4 (100%)	4/4 (100%)	Yes
Grapevine-to-Grapevine Controls for buckwheat	2/2 (100%)	2/2 (100%)	Yes
Vetch-to-Vetch	3/5 (60%)	0/5 (0%)	Inconclusive
Vetch-to-Grapevine	2/5 (40%)	0/5 (0%)	Inconclusive
Grapevine-to-Grapevine Controls for vetch	0/2 (0%)	0/2 (0%)	No

[a,b] See Table 3.

transmission (Table 4). However, the culture technique resulted in 0% transmission from vetch to vetch and vetch to grape (Table 4). Additionally, the grape to grape controls resulted in no transmission (Table 4) which may indicate there was a problem with *H. vitripennis* used for this cohort.

3.3.3. Transmission of *X. fastidiosa* by *H. vitripennis* in the field

Studies testing the ability of *H. vitripennis* to transmit *X. fastidiosa* from grapevine to cover crop in the field showed that 40% and 33% of the grapevine to buckwheat tests were positive for *X. fastidiosa* transmission, as detected by ELISA and culture testing, respectively. Results from the ELISA and culture testing showed that 60% and 30% of the grapevine to vetch tests, respectively, were positive for *X. fastidiosa* transmission (Table 5). These results demonstrate that transmission from grapevine to buckwheat and grapevine to vetch were successful in the field.

4. Discussion

4.1. Parasitoid survival and fecundity in the laboratory on nectar resources

Our results suggest that *G. ashmeadi* can use the extrafloral nectar of vetch as a food source. Access to extrafloral nectar from vetch significantly increased longevity and fecundity of *G. ashmeadi* by 221% and 81%, respectively, compared to water. Similar results were found by Géneau et al. (2012) with *Microplitis mediator* Haliday (Hymenoptera: Braconidae) feeding on extrafloral nectar of common vetch (*V. sativa*). Extrafloral nectar can be a highly valuable food source for natural enemies because it is usually more concentrated than floral nectar (Koptur, 2005). Results from our phenology experiment demonstrated that vetch plants produced nectar for, on average, 194 days longer than buckwheat plants during July and August 2008, therefore supporting previous research comparing length of nectar production between extrafloral nectar and floral nectar (Koptur, 2005).

This study also demonstrates the importance of floral nectar for *G. ashmeadi,* since females with access to nectar from buckwheat flowers significantly increased fecundity when compared to water only treatments. Previous laboratory studies have demonstrated that realized fecundity of *G. ashmeadi* was statistically equivalent between buckwheat and honey-water treatments (Irvin and Hoddle, 2007). In studies by Irvin and Hoddle (2007), realized fecundity of *G. ashmeadi* provided excised buckwheat flowers was 52 lower than average fecundity reported for buckwheat in the current study. This may be due to intact flowers, rather than excised flowers, being used in the current study, or differences in host density and exposure time between studies.

Vetch and buckwheat may be suitable food sources for *G. ashmeadi* for enhancing longevity and fecundity in the field when sown as a cover crop. Enhanced parasitoid fitness as a result of access to floral resources could lead to an increase in parasitism rates as demonstrated by Géneau et al. (2012), and improved biological control of *H. vitripennis* in field situations. The success of a conservation biological control program largely may depend on the number of female offspring produced by a female natural enemy in the presence of unlimited prey/hosts (Kean et al., 2003). Percentage of female offspring decreased by ~19% when *G. ashmeadi* were provided flowering buckwheat compared to water and vetch. A higher proportion of male *G. ashmeadi* offspring occurred in buckwheat treatments and this was attributable to this treatment resulting in longer-lived females which lay proportionately more male progeny to female progeny because longer lived

Table 5
ELISA and culture test results for field GWSS transmission experiment involving placing GWSS on needle inoculated host for acquisition.

Transmission test	ELISA[a]	Culture[b]	Successful transmission?
Grapevine-to-Buckwheat	4/10 (40%)	2/6 (33%)	Yes
Buckwheat Grapevine-to-Grapevine Controls	2/2 (100%)	2/2 (100%)	Yes
Grapevine-to-Vetch	6/10 (60%)	3/10 (30%)	Yes
Vetch Grapevine-to-Grapevine Controls	1/2 (50%)	2/2 (100%)	Yes

[a,b] See Table 3.

females lay fewer eggs even though the total number of male eggs laid does not change significantly (Irvin and Hoddle, 2007). In contrast, Irvin and Hoddle (2007) demonstrated that percentage of female *G. ashmeadi* progeny was statistically equivalent between buckwheat and water treatments.

Although buckwheat and vetch increased longevity and fecundity of *G. ashmeadi*, it is unknown how this finding translates to the field environment. Results from laboratory studies can differ from those conducted in the field due to differences in the relative humidity between lab and field studies which affect nectar viscosity (Winkler et al., 2009a), depletion of nectar in the field by more competitive nectarivores e.g., bees and bumble bees (Winkler et al., 2009b), differences in temperature affecting parasitoid egg maturation and oviposition rates (Rosenheim and Rosen, 1991), higher energy requirements of parasitoids in the field which are not caged and allowed to move freely, and predation of parasitoids in the field (Heimpel et al., 1997).

4.2. Cover crop phenology and agronomy

Information on growth time required to flowering and duration of nectar production is important for synchronizing nectar production to the phenology of natural enemies of key pests. Results indicated that buckwheat may be a better cover crop as it is a quick growing plant that rapidly provides nutrition for natural enemies. Vetch extrafloral nectaries provided prolonged periods of nectar production which is similar to other extrafloral nectary producing plants (Koptur, 2005). However, there is a trade-off; the rapid time to floral production and subsequent short-term production versus prolonged time to production and significantly greater longevity of extrafloral nectar production. Therefore, mixed species sowings may be useful to simultaneously take advantage of quick flowering species and those that have long extra-floral nectar production periods.

For each sowing date, vetch height was significantly shorter than buckwheat. Height information may be useful when selecting cover crops with the aim of preventing moisture-loving diseases in the crop being protected. The phenology study also demonstrated high susceptibility of cahaba vetch to pests in comparison to buckwheat in southern California. Additionally, buckwheat seed was more easily obtainable than cahaba vetch during the course of our studies.

4.3. X. fastidiosa host testing and H. vitripennis transmission studies

Results showed that *X. fastidiosa* can successfully infect and replicate in buckwheat and cahaba vetch and that transmission of *X. fastidiosa* between buckwheat and grapevine and vetch and grapevine by *H. vitripennis* is possible in the greenhouse and field. It is unknown what threat these cover crops pose to vineyards and whether they would act as a reservoirs for PD which may be transmitted to grapevines by adult *H. vitripennis* feeding on the cover crop and moving into vines. Our studies conclusively demonstrated buckwheat to grape movement of PD by *H. vitripennis*. Buckwheat sown as a summer cover crop in a Temecula, CA vineyard demonstrated that *H. vitripennis* counts were 3536% higher in grape foliage compared to flowering buckwheat plants (unpublished data, Irvin and Hoddle). That is, only one *H. vitripennis* was captured during sweep netting of buckwheat flowers across all dates and replicates (16 plots), whereas, sweep netting grape foliage resulted in 50 *H. vitripennis* (22 plots). This suggests that while it is possible for *H. vitripennis* to feed on buckwheat cover crops and transmit *X. fastidiosa* from infected buckwheat plants to grapevines, it may be unlikely that a buckwheat cover crop would act as a significant reservoir of *X. fastidiosa* in vineyards since *H. vitripennis* prefer feeding on grapes and high populations would not be found in buckwheat cover crops. However, it could be argued that even the smallest risk of transmission of PD from cover crop to grapevines is not acceptable, and this potential may reasonably exist in the field since results showed successful transmission from grape to cover crop by *H. vitripennis*. It may be difficult to find a cover crop species for use in vineyards that demonstrates a benefit to natural enemies that is not a host for *X. fastidiosa*. Alyssum and coriander, two additional plants that have been shown to increase natural enemy abundance and subsequent pest control in crops (Baggen and Gurr, 1998; Irvin et al., 2006), also tested positive as hosts of *X. fastidiosa* in the current study. Screening for cover plants that are not hosts for *X. fastidiosa* should be conducted prior to testing their value as food source for parasitoids. Results for greenhouse transmission of *X. fastidiosa* to grapevines from vetch were inconclusive. Transmission from vetch to grapevine was successful using detection with ELISA, but unsuccessful using the culture technique. Additionally, results indicated that there may have been a problem with the *H. vitripennis* used for the vetch tests detected by the culture technique. ELISA tests are very sensitive, simple to complete and do not require expensive equipment. However, they can occasionally cross-react resulting in false positives (Costa et al., 2004). Plant samples testing positive with ELISA need to be confirmed positive via other methods such as culturing or polymerase chain reaction (Costa et al., 2004). The culturing technique involves culturing live *X. fastidiosa* on media and is thought to be a more reliable method of detection of *X. fastidiosa* than ELISA. However, inhibiting compounds found in some plant species can interfere with detection of *X. fastidiosa* via culturing (Purcell and Saunders, 1999). Differences between results obtained from ELISA and culturing methods also may be attributed to low level or transitory inoculations of *X. fastidiosa* which are too low to be detected by both methods used (Costa et al., 2004).

Although the results of the greenhouse transmission studies were inconclusive for vetch, results from the field studies showed that transmission of *X. fastidiosa* between grapevine and vetch was possible. Field testing may be a better indication of transmission efficiency of *X. fastidiosa* because results from greenhouse transmission studies may differ to transmission studies in the field due to differences in climatic conditions that plants are exposed to before and after infection (Costa et al., 2004). Interestingly, 30% of field grown vetch acquired *X. fastidiosa* by *H. vitripennis*, but only 13% of greenhouse grown vetch acquired *X. fastidiosa* by mechanical inoculation. Mechanical inoculation is dramatically different from inoculation via *H. vitripennis* because high concentrations of bacteria are used in the inoculum and delivery by needle destroys plant tissue which may affect uptake of bacteria (Chatterjee et al., 2008).

Transmission studies conducted in the greenhouse and field involved confining infected *H. vitripennis* in cages and forcing them to remain on plants for four days. Transmission efficiency may be lower under natural field conditions where *H. vitripennis* can freely move from plant to plant. Additionally, cover crop plants were inoculated with five infected *H. vitripennis*. Densities counted from sweep net samples conducted in flowering buckwheat grown between rows of an organic Cabernet Sauvignon vineyard in Temecula, CA never exceeded one *H. vitripennis* per plant (unpublished data, Irvin and Hoddle). Although *H. vitripennis* densities used in the current study were higher than those seen under natural conditions, Costa et al. (2000) demonstrated that the probability of infection by inoculation with one insect did not significantly differ from that with three insects. This suggests that densities of caged *H. vitripennis* on experimental cover crop plants may not have biased the outcomes of observed results.

5. Conclusions

In central and southern California, arid conditions during spring and summer typically removes potential floral resources (e.g.,

weeds; Dent, 1995; Gurr et al., 2003) that could be used by natural enemies during this critical time for pest control. Vetch and buckwheat may be suitable food sources for enhancing longevity and fecundity of grape pest natural enemies in the field when sown as an irrigated cover crop. Enhanced natural enemy fitness as a result of access to floral resources can lead to increased parasitism and predation rates, as demonstrated by Géneau et al. (2012) and Hogg et al. (2011), respectively, which consequently enhanced biological control of grape pests. However, it is important to consider cover crop strategies within the context of the full integrated disease management program. Evidence presented here suggests that growers in southern California should not plant buckwheat as a summer cover crop due to this plant supporting replication of *X. fastidiosa* and reciprocal transmission between cover crop and grapevine. Furthermore, any benefit from cover crops comprised of vetch, alyssum, and coriander may be significantly offset by development of *X. fastidiosa* that could potentially be acquired from the cover crop and spread to grapevines by *H. vitripennis*.

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