

The effect of buckwheat flowers and cahaba vetch extrafloral nectaries on fitness of the vine mealybug parasitoid *Anagyrus pseudococci* (Hymenoptera: Encyrtidae)

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Abstract

The effect of access to buckwheat flowers (*Fagopyrum esculentum* Moench; Polygonales: Polygonaceae) or vetch extrafloral nectaries (*Vicia sativa* L. cv. 'cahaba white'; Fabales: Fabaceae) on the fitness of *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) (a parasitoid of the vine mealybug, *Planococcus ficus* [Signoret]) (Hemiptera: Pseudococcidae) was determined in the laboratory. Female *A. pseudococci* provided with vetch survived 4 days longer compared with water only. Buckwheat had no effect on the average longevity of *A. pseudococci*. *Anagyrus pseudococci* offspring production increased by 132% and 152% when females were provided either vetch or buckwheat, respectively, when compared with water. There was no significant difference in parasitoid longevity or fecundity between plant species. Buckwheat increased the percentage of female *A. pseudococci* offspring by 15% and 19% when compared with water and vetch, respectively.

Key Words: cover crops; extrafloral nectar; fecundity; longevity; sex ratio

Resumen

Se determinó el efecto al acceso de flores de alforfón (*Fagopyrum esculentum* Moench; Polygonales: Polygonaceae) o nectarios extraflorales de veza blanca (*Vicia sativa* L. cv 'cahaba blanco'; Fabales: Fabaceae) sobre el estado físico de *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) (un parasitoide de la cochinilla harinosa de la vid, *Planococcus ficus* [Signoret]; Hemiptera: Pseudococcidae) en el laboratorio. Hembras de *A. pseudococci* proveídas con veza sobrevivió 4 días más que las hembras proveídas con sólo agua. El alforfón no tuvo ningún efecto sobre el promedio de la longevidad de *A. pseudococci*. La producción de progenie de *Anagyrus pseudococci* incrementó en un 132% o un 152% cuando las hembras recibieron ya sea veza o alforfón, respectivamente, en comparación con las hembras proveídas con agua. No hubo una diferencia significativa en la longevidad o la fecundidad del parasitoide entre las especies de plantas. El alforfón incrementó el porcentaje de la progenie de las hembras de *A. pseudococci* en un 15% y un 19% en comparación con agua y veza, respectivamente.

Palabras Clave: cultivos de cobertura; néctar extrafloral; fecundidad; longevidad; proporción de sexos

Conservation biological control aims to enhance the efficacy of beneficial insects, such as parasitoids and predators. One approach is to deliberately provide resources to natural enemies, such as nectar, which may be absent from agricultural habitats where they are foraging for pests. Floral and extrafloral nectar can maximize the longevity, fecundity, searching activity of most beneficial insects and increase parasitism/predation rates and enhance female sex ratios of offspring (Berndt & Wratten 2005; Kost & Heil 2005; Irvin et al. 2006; Hogg et al. 2011). Incorporating nectar producing cover crops in orchards and vineyards is potentially one-way to enhance populations of beneficial insects in agricultural systems with the intention of improving pest control (Gurr et al. 2004). Cover crops can enhance beneficial insects that attack vineyard pests and with corresponding reductions in damaging spider mite and leafhopper populations in grapes (Hanna et al. 1996; Nicholls et al. 2000; English-Loeb et al. 2003). Additionally, cover crops

have additional benefits such as maintaining soil quality and reducing erosion (Dlott et al. 2002).

The vine mealybug (*Planococcus ficus* [Signoret] [Hemiptera: Pseudococcidae]) was found in the Coachella Valley, Imperial County California USA on table grape (*Vitis vinifera* L.; Vitales: Vitaceae) in 1994 (Daane et al. 2008), and has since become the most serious mealybug pest in California vineyards (Daane et al. 2006). Females undergo three instars before developing into a gravid mature adult (Daane et al. 2011). Males have a different life cycle than that of females; developing from third instar, to a prepupa stage, to a pupa, and then to a winged adult. *Planococcus ficus* has 4 to 7 generations per year in much of California's grape growing regions. Economic damage occurs when this pest infests fruit or excretes honeydew that covers fruit and leaves, often resulting in sooty mold growth, and associated leaf drop can result in sunburned fruit (Daane et al. 2008). Furthermore, *P. ficus* vec-

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tors grape leaf roll causing viruses (Engelbrecht & Kasdorf 1990), and is therefore considered economically important even at low densities.

Insecticide applications in the Coachella Valley are often ineffective against *P. ficus*, because they are protected underneath bark of trunks and cordons and can overwinter underground on roots (Daane et al. 2011). The thick layers of protective wax secreted by *P. ficus* may also contribute to them being difficult to control with pesticides (Güleç et al. 2007). Biological control may offer a possible alternative method to suppress *P. ficus* populations. One of the most important biological control agents of *P. ficus* is the polyphagous, cosmopolitan, solitary koinobiont parasitoid, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) (Noyes & Hayat 1994; Daane et al. 2004 a, b). Currently, *A. pseudococci* is the dominant biological control agent of *P. ficus* throughout California (Daane et al. 2008). Due to its wide host and geographic range, *A. pseudococci* is also one of the most commonly commercially reared mealybug parasitoids and has often been used for biological control of pseudococcids in several countries (Güleç et al. 2007). However, natural levels of control of *P. ficus* by *A. pseudococci* in California may be reduced because overwintered parasitoids remain in an immature stage inside *P. ficus* until April to early May, thereby delaying their window of activity until after the mealybug is active (Daane et al. 2004b). It is unknown whether supplying *A. pseudococci* with pollen and nectar via a nectar producing cover crop during this critical time may enhance fitness of emerging overwintered parasitoids and subsequent biological control of *P. ficus*.

Buckwheat (*Fagopyrum esculentum* Moench) can promote female-biased parasitoid sex ratios when it is planted as a cover crop in vineyards (Berndt et al. 2002) and lower abundance and increased parasitism of leafhoppers in vineyards has been documented as result (Nicholls et al. 2000; English-Loeb et al. 2003). This plant shows promising potential as a cover crop in vineyards as seed is inexpensive and readily available, it germinates easily, is adaptable to poor growing conditions, and has a short sowing-flowering time (Angus et al. 1982; Bowie et al. 1995). Alternatively, cahaba vetch (*Vicia sativa* L. cv. 'cahaba white'; Fabales: Fabaceae) is suggested in the California Code of Sustainable Winegrowing Workbook as a cover crop in vineyards because it can improve soil nutrition, fertility and structure, and reduce erosion and dust (Dlott et al. 2002). This cover crop also suppresses populations of damaging nematode species in Californian vineyards (McKendry 1992). Cahaba vetch produces extrafloral nectaries at the base of each stipule. Extrafloral nectar, which is easily accessible to beneficial insects, is often produced in larger volumes and for longer periods of time when compared with floral nectar (Koptur 2005). However, not all nectar sources are of benefit to mealybug parasitoids (Davies et al. 2004). A parasitoid's access to nectar is influenced by flower morphology (Wäckers 2004; Patt et al. 1999), nectar quality (Baker & Baker 1983), and structure of its mouthparts (Baggen et al. 1999; Jarvis 1998). At the time this work was conducted, it was unknown whether buckwheat flowers or extrafloral nectaries or cahaba vetch could improve the fitness of *A. pseudococci*. Consequently, the following study sought to investigate whether buckwheat flowers and cahaba vetch extrafloral nectaries increase longevity and fecundity of *A. pseudococci* in the laboratory. This study is a first step in determining whether these cover crops have potential for conservation biological control in California vineyards.

Materials and Methods

Parasitoid Survival and Fecundity in the Laboratory on Nectar Resources

Maintenance of Insect Colonies. *Planococcus ficus* mummies parasitized by *A. pseudococci* were provided by cooperators at University of

California, Berkeley, California (UCB), USA. Maintenance of *A. pseudococci* and host colonies at UCB is described in Daane et al. (2004b). On arrival, mummies were placed in Petri dishes (10 × 1.5 cm) and held at 26 ± 2 °C and 30-40% RH under a 14:10 h L:D photoperiod. Petri dishes were checked daily for parasitoid emergence.

Planococcus ficus colonies were held at 26 ± 2 °C and 30-40% RH under a 14:10 h L:D photoperiod with fluorescent lighting and maintained at University of California, Riverside, California (UCR). One organically grown butternut squash fruit heavily infested with *P. ficus* (provided by Kearney Agricultural Research and Extension Center, Parlier, California) was placed in a wooden cage (32 × 34 × 37 cm) painted white, with a glass top, mesh back for ventilation, and hinged front door containing a cloth sleeve for access. Squash were rested on a wooden stand. One additional *P. ficus* colony was set up each week from this initial colony by gently brushing ovisacs laid by *P. ficus* in the initial colony onto a new squash.

Maintenance of Nectar Plants. Plants of buckwheat (*F. esculentum*; obtained from Outsidepride, Salem, Oregon) and vetch (*Vicia sativa* L. cv. 'Cahaba White'; obtained from Bailey Seed Company, Salem, Oregon) were grown from seed in a greenhouse at 26 ± 3 °C under 14: 10 h L:D natural light. Seeds were sown in 1-gal (3.8-L) pots, containing 4 seeds per pot. Synchronous nectar production was ensured by performing staggered sowings at 7-10 day intervals. Plants were fertilized every 3 weeks with Miracle-Gro (20 mL/3.5 L of water, Scotts Miracle-Gro Products Inc., Marysville, Ohio). Prophylactic applications of pyrethrin + canola oil (Garden Safe Brand Fruit & Vegetable Insect Spray, Schultz Company, Bridgeton, Missouri) were applied to vetch plants every 7-10 days to control greenhouse insect pests. Plants used for experiments were free of pyrethroid applications for at least 14 days and were hand sprayed thoroughly with water and dried before use in experiments.

Experimental Set Up. Three treatments (water, buckwheat, and vetch) were evaluated in the laboratory at 26 ± 2 °C and 30-40% RH under a 14: 10 h L:D photoperiod. Each treatment was placed in a wooden cage (as previously described), and 16 cages of each treatment were arranged in a completely randomized design. A white piece of cardboard was placed on the bottom of each cage to allow ease of finding dead parasitoids. Water was provided via a 7.4 mL glass vial (2 dram Fisherbrand Glass Vial, Fisher Scientific, Pittsburgh, Pennsylvania) with a 5 cm cotton wick, which was placed on the bottom of each cage; and the water was topped up daily. Plant treatments consisted of one 1 gal potted buckwheat or vetch plant with the bottom and top of the pot wrapped in Parafilm (Parafilm 'M' Laboratory Film, Pechiney Plastic Packaging, Chicago, Illinois) to prevent parasitoid access to moisture. Plants in cages were watered as needed with an 8 oz (237 mL) wash bottle inserted through a hole in the Parafilm. Tape was placed over the hole after each watering to prevent parasitoid access. 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 *A. pseudococci* were released inside each cage. Longevity of female and male parasitoids was recorded daily until death.

Hosts were provided in treatment cages to individual mated *A. pseudococci* females by placing one butternut squash fruit infested with a range of host ages, including at least 50 third instar and adult *P. ficus* (preferred for oviposition by *A. pseudococci*) on a wooden stand inside the cage. Host densities were estimated and relatively equal host densities were provided in each treatment. The squash was removed and replaced after 6 days to ensure that *A. pseudococci* females were provided with life stages suitable for parasitism during their entire lifetime. Host numbers and age were selected based on previous studies of *A. pseudococci* (Daane et al. 2004b). Small pieces of tissue paper were placed over large areas of honeydew excreta daily to prevent parasitoids from getting trapped and dying prematurely. Parasitoids could potentially feed from honeydew absorbed into tissue paper.

Exposed squash bearing *P. ficus* and plants removed from cages were placed into labeled wooden cages for 3 weeks to allow offspring to emerge. The number of male and female *A. pseudococci* offspring was recorded for each cage replicate.

Statistical Analyses

Only parasitoids that died of natural causes were included in statistical analyses. Those females that did not mate (producing only male progeny) were excluded from the male progeny totals and sex comparison analyses. This resulted in 10-16 replicates depending on the treatment. The effect of treatment on the total number of offspring and female longevity was determined using ANOVA in SAS (1990). Total offspring and female longevity data were square-root transformed prior to analyses. 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 & Lemeshow 2000). Pair-wise contrast tests at the 0.05 level of significance were used to separate means. Means (\pm SEM) presented here were calculated from untransformed data.

Results

Female *A. pseudococci* provided with 'cahaba' vetch plants survived, on average, 4 days longer when compared with females provided water only ($t = 3.81$; $df = 1$; $P < 0.01$) (Fig. 1). Buckwheat had no significant effect on the longevity of *A. pseudococci* when compared with water treatments ($t = 2.29$; $df = 1$; $P = 0.07$) (Fig. 1). There was no significant difference in longevity of *A. pseudococci* between plant species ($t = -1.33$; $df = 1$; $P = 0.39$) (Fig. 1). Total *A. pseudococci* offspring production increased by 132% and 152% when females were provided vetch or buckwheat, respectively, compared with water (vetch: $t = 5.05$; $df = 1$; $P < 0.0001$, buckwheat: $t = 2.83$; $df = 1$; $P = 0.05$) (Fig. 2). There was no significant difference in fecundity of *A. pseudococci* between plant species ($t = -1.98$; $df = 1$; $P = 0.13$) (Fig. 2). The presence of buckwheat enhanced *A. pseudococci* female offspring sex ratio by 15% and 19% compared with water and vetch, respectively (water: odds ratio point estimate = 1.46; $P < 0.01$; vetch: odds ratio point estimate = 1.76; $P < 0.0001$) (Fig. 3). Vetch had no significant effect on the sex ratio of offspring produced by *A. pseudococci* (odds ratio point estimate = 0.83; $P = 0.17$) (Fig. 3).

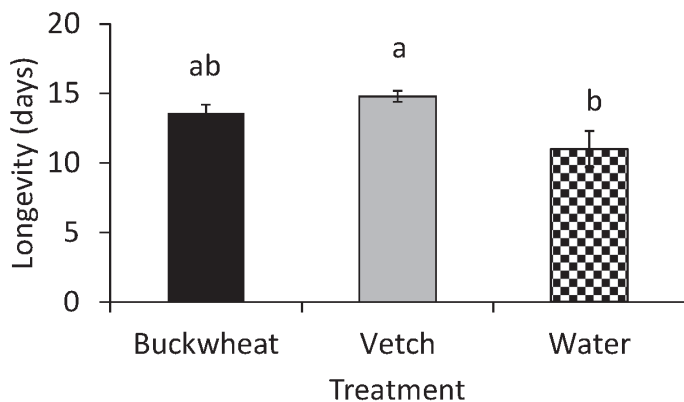


Fig. 1. Mean longevity when female *Anagyrus pseudococci* were provided with 1 potted vetch (*Vicia sativa*) plant, 1 potted buckwheat (*Fagopyrum esculentum*) plant, or only water in the laboratory. Different letters indicate significant differences (Tukey's studentized range test: $P < 0.05$) between treatment foods (ANOVA: $F = 5.56$, $df = 2$, $P < 0.001$).

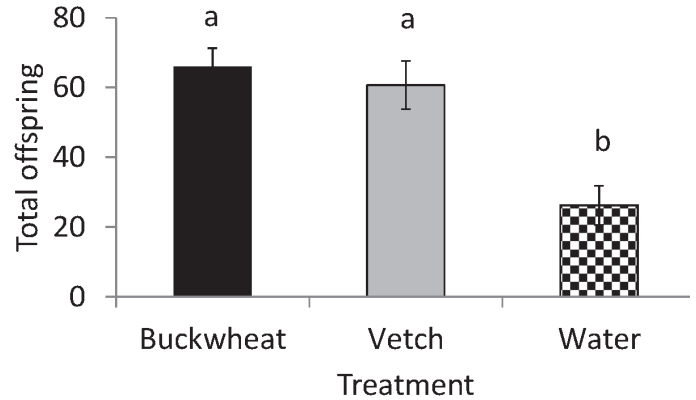


Fig. 2. Total offspring produced when female *Anagyrus pseudococci* were provided with 1 potted vetch (*Vicia sativa*) plant, 1 potted buckwheat (*Fagopyrum esculentum*) plant, or only water in the laboratory. Different letters indicate significant differences (Tukey's studentized range test: $P < 0.05$) between treatment foods (ANOVA: $F = 14.93$, $df = 2$, $P < 0.0001$).

Discussion

Our results suggest that *A. pseudococci* can use the extrafloral nectar of vetch as a food source. Access to extrafloral nectar from vetch significantly increased longevity and fecundity of *A. pseudococci* by 36% and 132%, respectively, when compared with 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 beneficial insects because it is usually more concentrated than floral nectar (Koptur 2005). Additionally, extrafloral nectar is often produced in larger volumes and for a longer period of time when compared with floral nectar (Koptur 2005). It is more exposed and easily accessible because it is not confined within a corolla where petals and stamen filaments can impede small parasitoids (Patt et al. 1997).

Life-table studies conducted by Güleç et al. (2007) demonstrated that the average number of offspring produced by female *A. pseudococci* over its lifespan ranged between 22 and 35, depending on host (*P. ficus*) age. In the current study, average offspring production of parasitoids in the water treatment was 26, but ranged from 61-66 in the

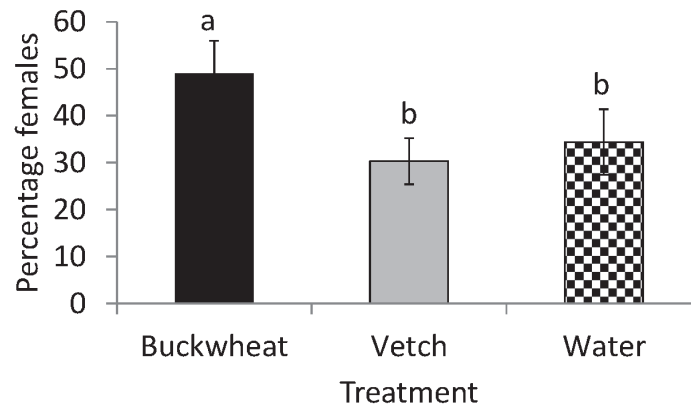


Fig. 3. Mean offspring sex ratio when female *Anagyrus pseudococci* were provided with 1 potted vetch (*Vicia sativa*) plant, 1 potted buckwheat (*Fagopyrum esculentum*) plant, or only water in the laboratory. Different letters indicate significant differences (Pair-wise contrast tests: $P < 0.05$) between treatment foods (logistic regression: $\chi^2 = 32.83$, $df = 2$, $P < 0.0001$).

buckwheat and vetch treatments. Progeny production of parasitoids fed plant nectar in the currently study was also higher than other fitness studies previously conducted with *A. pseudococci* (Anidov et al. 1967; Chandler et al. 1980; Tanga et al. 2013). However, differences in offspring production between studies may be attributable to differences in temperature (Tingle & Copland 1989; Daane et al. 2004b), photoperiod (Sagarra et al. 2000), host and female parasitoid density (Sagarra et al. 2000; Chong & Oetting 2006), host age (Daane et al. 2004b; Güleç et al. 2007), host species (Bugila et al. 2014), host plant (Cloyd & Sadof 2000; Tanga et al. 2013), host exposure time and food source between studies. The current study exposed a range of host ages on infested squash to parasitoids for 6 day periods until parasitoid death, whereas, Güleç et al. (2007) used a fixed number of a specific host age on infested potatoes exposed to parasitoids for 24 h periods until parasitoid death. The methodology reported by Güleç et al. (2007) did not detail whether test parasitoids were provided with a food source other than hosts/honeydew.

Buckwheat failed to significantly enhance longevity of *A. pseudococci*. This may be attributed to parasitoids having access to honeydew excreted by *P. ficus*. Mealybug and aphid honeydew can be a significant source of food for some parasitoids (Sandanayaka et al. 2009; Hopkinson et al. 2013), but not always (Davies et al. 2004). Suma et al. (2012) demonstrated that exposure to hosts and honeydew enhanced longevity of *A. sp. nr. pseudococci* 291% compared with water without hosts (mean longevity = 5 days). However, providing parasitoids the opportunity to feed from hosts and/or honeydew was not as beneficial as providing parasitoids with sugar syrup in the absence of hosts (Suma et al. 2012). In the current study, host and honeydew exposure was equal for all 3 food treatments suggesting that buckwheat maybe less beneficial for enhancing *A. pseudococci* survival compared with vetch, which enhanced survival compared with water by 36%. However, differences in survival between plant species were not significant. Although buckwheat had no significant effect on longevity of *A. pseudococci*, fecundity of *A. pseudococci* was significantly enhanced (152% increase) when females were provided buckwheat compared with water. This indicates that access to nectar is beneficial for maximizing fecundity of *A. pseudococci* even in the presence of host feeding and honeydew.

The *A. pseudococci* offspring sex ratios produced in all 3 treatments were male-biased (30-49% female). This may be attributable to *A. pseudococci* parasitizing mostly 3rd instar nymphs which were more prevalent on infested squash than adult *P. ficus*. Previous laboratory studies indicated that sex ratio of *A. pseudococci* was male-biased when 3rd instar *P. ficus* were available (Güleç et al. 2007; Danne et al. 2004b) and equal or female-biased in young adult female hosts (Güleç et al. 2007; Daane et al. 2004b). Islam & Copland (1997) reported that *A. pseudococci* displayed maternal adjustment of sex ratio as a function of host size with an increased proportion of females with increasing host size.

The success of a conservation biological control program may largely depend on the number of female offspring produced by a female natural enemy in the presence of unlimited prey/hosts (Kean et al. 2003). Results from the current study demonstrated that providing *A. pseudococci* with buckwheat in the laboratory significantly increased percentage female offspring compared with water and vetch, but the ratio was still male biased possibly because of the age of the hosts available for parasitism. A similar result was found for *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae), in which a higher proportion of female offspring were reared from sentinel hosts in buckwheat field plots compared with control plots (Berndt et al. 2002). This illustrates the possibility of enhancing efficiency of beneficial insects through resource subsidies that increase female sex ratios (Kean et al. 2003).

Although buckwheat and vetch increased fecundity of *A. pseudococci* in the laboratory, it is unknown how this finding translates to the field environment. Results from laboratory studies can differ to those conducted in the field due to differences in the relative humidity between lab and field studies effecting 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 egg maturation and oviposition rates (Rosenheim & Rosen 1991), higher energy requirements of parasitoids in the field that are not caged and allowed to move freely, and predation of beneficial insects in the field (Heimpel et al. 1997). Baggen et al. (2000) demonstrated the use of buckwheat flowers by the encyrtid, *Copidosoma koehleri* Blanchard, in the field since catches of this parasitoid were high for traps close to buckwheat flowers. Olson & Wäckers (2007) demonstrated that *Meteorus autographae* Meusebeck (Hymenoptera: Braconidae) fed from nectaries of 'cahaba' vetch in the field, which resulted in a significantly higher sugar gut content when compared with unfed control parasitoids. In addition, Koptur & Lawton (1988) observed *Scambus plantatus* (Hartig) (Hymenoptera: Ichneumonidae) feeding from extrafloral nectar of common vetch in the field. However, it may be important to consider that some parasitoids may only respond innately to olfactory floral cues, and not extrafloral nectar of the same plant species, even though floral nectar may not be accessible to parasitoids due to flower structure (Géneau et al. 2013).

In southern California, arid conditions during spring and frequent weed control in vineyards removes potential floral resources (e.g., Dent 1995; Gurr et al. 2003) for beneficial insects during critical times when pest control is needed. Although rainfall in other parts of California may allow growth of flowering weed species, a single suitable nectar source can have more impact on sugar gut content of beneficial insects than a diverse range of plant species (Olson & Wäckers 2007). However, this may not always be the case and a variety of plant species with different flowering phenologies may be better (Gurr et al. 2004).

'Cahaba' vetch and buckwheat may be suitable food sources for enhancing fecundity of beneficial insects of grape pests in the field when sown as a spring nectar cover crop. Enhanced fitness of beneficial insects as a result of access to floral resources could lead to increased parasitism and predation rates, as demonstrated by Géneau et al. (2012) and Hogg et al. (2011), respectively, which may consequently enhance biological control of grape pests.

Further research is required to determine whether parasitoids commonly visit vetch extrafloral nectaries and buckwheat flowers in the field, and whether these plants are hosts for *P. ficus* or increase pest status of any other grape infesting species (e.g., the glassy-winged sharpshooter, *Homalodisca vitripennis* [Germer] [Hemiptera: Cicadellidae], the vector of *Xylella fastidiosa* Wells et al., the causative agent of Pierce's disease in grapes). In addition, it is important to consider cover crop strategies within the context of the full integrated pest and disease management program. Cover cropping may not be compatible with chemical control practices currently used in conventionally grown grapes in California since research has demonstrated that soil-applied systemic imidacloprid is translocated to nectar of treated buckwheat plants, consequently killing nectar feeding *A. pseudococci* (Krischik et al. 2007). Instead, this technique may be a useful tool when used in conjunction with mating disruption (Millar et al. 2002) or chemical control using newer selective insecticides such as Prev-Am® or spiro-tetramat, which have demonstrated to be compatible with biological control of *P. ficus* with *A. sp. near pseudococci* (Mansour et al. 2011). It is also important to consider that vetch may harbor other pest species (Olson & Wäckers 2007), and both vetch and buckwheat may be hosts of *X. fastidiosa* (Irvin et al. 2014). Any benefit from cover crops

comprised of vetch or buckwheat 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* (Irvin et al. 2014).

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