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Evaluating the potential of flowering plants for enhancing predatory hoverflies (Syrphidae) for biological control of *Diaphorina citri* (Liviidae) in California

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HIGHLIGHTS

- Plants were evaluated for conservation biocontrol of Asian citrus psyllid (ACP).
- Alyssum and buckwheat attracted 10–20 times more hoverflies than California poppy.
- Alyssum increased hoverfly oviposition and predator abundance on ACP colonies.
- 10% more ACP nymphs survived to adulthood in the controls compared to alyssum plots.
- Individual hoverfly larvae consumed, on average, 421 ACP nymphs before pupation.

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ABSTRACT

Diaphorina citri (Hemiptera: Liviidae) is a notorious citrus pest that vectors a bacterium that causes huanglongbing, a lethal citrus disease. Studies evaluating the potential of buckwheat (*Fagopyrum esculentum*), alyssum (*Lobularia maritima*), phacelia (*Phacelia tanacetifolia*), and California poppy (*Eschscholzia californica*) as insectary plants for conservation biological control of *D. citri* were conducted in California citrus orchards. Field studies assessed flowering phenology and attractiveness to hoverflies and other natural enemies, hoverfly oviposition on *D. citri* colonies, and the effect of potted alyssum and predation from hoverflies on the mortality of *D. citri* nymphs. Laboratory studies quantified lifetime consumption of *D. citri* nymphs by individual *Allograpta obliqua* (Diptera: Syrphidae) larvae, the only hoverfly species attacking *D. citri* in the field. Results indicated that alyssum and buckwheat possessed a short sowing to flowering time (30–33 days when sown in March) and attracted 10–20 times more syrphids than California poppy. No hoverflies were observed feeding from phacelia. California poppy was marginally attractive to hoverflies, and failed to attract ladybugs, parasitoids, or predatory wasps. When alyssum was present in an unsprayed citrus orchard, the number of hoverfly eggs laid on *D. citri* colonies and abundance of predators was 3.5 times and 2.5 times higher, respectively, when compared to control plots lacking alyssum. Consequently, 10% more *D. citri* nymphs survived to adulthood in the control plots compared to alyssum plots. In the laboratory, individual *A. obliqua* larvae consumed, on average, 421 *D. citri* nymphs before pupation. Results demonstrate that *A. obliqua* is an important and voracious predator of *D. citri* nymphs, and that alyssum and buckwheat show potential as insectary plants for conservation biological control of *D. citri*.

1. Introduction

Conservation biological control attempts to improve habitat in agroecosystems for natural enemies through the planting and maintenance of floral resources which provide shelter, nectar, alternative hosts/prey, and pollen (Gurr et al., 2004; Heimpel and Jervis, 2005; González-Chang et al., 2019). Floral and extrafloral nectar can maximize the longevity, fecundity, searching activity and parasitism/predation rates of natural enemies, and may increase sex ratios in favor of female offspring (Berndt and Wratten, 2005; Kost and Heil, 2005; Irvin et al., 2006; Hogg et al., 2011b). Incorporating nectar producing non-crop resources (i.e., insectary plants) in citrus orchards, for example, can

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enhance populations of natural enemies and reduce citrus pest densities (Liang and Huang, 1994; Silva et al., 2010; Aguilar-Fenollosa et al., 2011). However, not all flowering resources can function as insectary plants that benefit natural enemies as they may fail to provide resources that predators and parasitoids can exploit (Davies et al., 2004; Karp et al., 2018). Poorly selected insectary plants may act as 'sinks' for some species of natural enemies or sources for pests, which negates pest suppression (MacLeod, 1999; Landis et al., 2000). For a particular cropping system of interest, research to determine what species of insectary plants, where, how, and when to deploy them is needed for developing effective conservation biological control programs (Gillespie et al., 2011).

In California, the $3.4 billion citrus industry is threatened by *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), a notorious citrus pest first found in California in 2008. *D. citri* vectors the phloem-dwelling bacterium, *Candidatus* Liberibacter asiaticus (*C*Las). *C*Las causes the deadly citrus disease huanglongbing (HLB) and has infected more than 1400 citrus trees in California since first detection in 2012 (Bové, 2006; Halbert and Manjunath, 2004; Hall et al., 2012; Bayles et al., 2017; Center for Environmental and Research Information Systems [CERIS], 2019; Graham et al., 2020). In Florida, *C*Las was detected in 2005 and more than US$4 billion in economic losses have resulted (Farnsworth et al., 2014). To control *D. citri* and reduce spread of *C*Las, insecticidal and biological control, and removal of *C*Las-infected trees are employed (Grafton-Cardwell et al., 2015; Hornbaker and Kumagai, 2016; Kistner et al., 2016b). Classical biological control of *D. citri* in California has utilized two species of parasitoid imported from Pakistan, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Argarwal) (Hymenoptera: Encyrtidae) (Hoddle et al. 2014). *D. aligarhensis* failed to establish in California, but *T. radiata* established and spread rapidly and joined a resident *D. citri* natural enemy guild comprised exclusively of predators (Kistner et al., 2016a, 2016b, 2017). However, the efficacy of some natural enemy species attacking *D. citri* (e.g., *T. radiata*) is significantly compromised by Argentine ant, *Linepithema humile* Mayr (Hymenoptera: Formicidae), which has developed a food-for-protection mutualism with *D. citri* nymphs (Schall and Hoddle, 2017).

Kistner et al. (2016a) demonstrated that larvae of two species of *Allograpta* (Diptera: Syrphidae), were the dominant predators of *D. citri* in southern California, accounting for ~60% of all recorded attacks on *D. citri* nymphs (Kistner et al., 2017). Predatory hoverflies (i.e., syrphids) are important natural enemies because of their high reproductive rates, high rates of prey search and consumption, and strong dispersal capabilities (Chambers and Adams, 1986; Poehling, 1988; Gilbert, 1993; Tenhumberg and Poehling, 1995; Jervis and Kidd, 1996; Murdoch and Briggs, 1996). Hoverfly larvae may exhibit defensive behaviors towards aggressive ants that protect colonies of honeydew producing pests (Detrain et al., 2017). While hoverfly larvae are predacious, adults feed on nectar and pollen for energy and egg maturation, respectively, and females lay their eggs on or near patches of prey (Bugg, 1992). The enhancement of *D. citri* predators, especially syrphids, through habitat manipulation may increase biological control and subsequent population suppression of *D. citri*.

Insectary plants that are especially attractive to adult syrphids include buckwheat (*Fagopyrum esculentum* Moench), tansy phacelia (*Phacelia tanacetifolia* Benth), and sweet alyssum (*Lobularia maritima* [L.] Desv.) (Ozols, 1964; Bugg, 1992; Lövei et al., 1993; Burgio et al., 2016). Flowering phacelia has been used to enhance adult syrphid abundance and reduce aphid populations in sugarbeet and cereals (Sengonça and Frings, 1988; Hickman and Wratten, 1996). Strips of alyssum have been intercropped with lettuce in California to increase aggregation and oviposition of hoverflies which improved biological control of aphids (Smith and Chaney, 2007; Bugg et al., 2008; Smith et al., 2008). Plots of alyssum grown in apple orchards enhanced numbers of syrphids and reduced woolly apple aphid on apple trees (Gontijo et al., 2013). Native plants may have additional advantages for use in habitat manipulation because they are adapted for growing under local conditions, may be more resistant to insects and diseases, are less likely to be invasive, and could have good tolerance to drought (Fiedler and Landis, 2007; Isaacs et al., 2009). Phacelia and California poppy (*Eschscholzia californica* Cham.) are California natives that are attractive to predators and parasitoids (Lundin et al., 2018).

To develop a conservation biological control program for *D. citri* in California citrus orchards, studies reported here were conducted to determine whether buckwheat, alyssum, phacelia or California poppy attract key guilds of natural enemies. These studies sought to investigate: (1) flowering phenology and attractiveness to hoverflies and other natural enemy guilds (e.g., parasitoids); (2) hoverfly oviposition on *D. citri* colonies and hoverfly species identification in the field; (3) quantification of life time consumption of *D. citri* nymphs by individual *A. obliqua* (Say) larvae in the laboratory; and (4) the effect of potted alyssum and predation from hoverflies on the mortality of *D. citri* nymphs in the field.

2. Materials and methods

2.1. Flowering phenology and attractiveness to hoverflies and other species of natural enemies

A total of 51 test plots (three rows of seventeen plots) were set up at Agricultural Operations, University of California Riverside (GPS coordinates: N 33.96503°; W 117.34058°) which were subjected to staggered sowings to accommodate the number of experimental plots (see below). Individual plots were 1.5 m^2 and plots were separated by a 30 cm furrow on two opposite sides and by 1 m on the remaining two sides. A relatively small plot size was used to evaluate the relative attractiveness of flowering plants to hoverflies in a free-choice feeding environment (Colley and Luna, 2000). On February 19th and March 29th, 2018, five replicates of buckwheat, alyssum, phacelia and California poppy were sown in 20 randomly selected plots following recommended sowing rates (buckwheat: 20.4 kg/0.4 ha = 5.04 g/m^2 = 7.57 g seed per plot; alyssum: 0.9 kg/0.4 ha = 0.22 g/m^2 = 0.34 g seed per plot; phacelia: 4 kg/0.4 ha = 1 g/m^2 = 1.51 g seed per plot; poppy: 2.7 kg.0.4 ha = 0.67 g/m^2 = 1.01 g seed per plot). Seed was sourced from Outsidepride (Salem, OR). Sequential sowings were conducted to ensure all species of plants flowered simultaneously for an extended period in order to conduct predator visitation counts and assess their relative attractiveness (see below). Since buckwheat has a short flowering period (Irvin et al., 2014), staggered sowings of buckwheat seed in buckwheat plots were made every 3 weeks. 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 90 min time periods every 2–4 days from February 2018 until January 2019, depending on time of year. Water was delivered by two Maxijet Stake sprinklers (Maxijet Inc., Dundee, FL) per plot coupled on 6.35 mm line (Landscape Products, Tolleson, AZ). Sprinklers had 180 or 90 degree spray coverage depending on position within the experimental plot. An adjustable pressure regulator was installed to deliver 30 PSI under which sprinklers emitted 5 GPH. Weeds were removed by hand as necessary. Monthly mean temperature, precipitation, relative humidity and day length during this experiment are presented in Supplementary Material 1.

2.1.1. Germination counts and flowering phenology

At 7 weeks post-sowing, the number of plants per plot were counted to assess germination rates. At 8 weeks post-sowing, the height of ten randomly selected plants per plot was measured and average 8 week height estimates were calculated for each plot. Plots were checked weekly and then every 2–3 days when plants were nearing nectar production. The number of days until at least 5% of plants were flowering was recorded per plot. Plants were monitored until flowering ceased (i.e., when less than 5% of plants were flowering). The length of the flowering period in days was recorded per plot.

A two-way ANOVA was used to determine whether sowing date, plant species and their interaction had a significant effect on germination, plant height, days until flowering, and length of flowering (Proc. GLM in SAS [2011]). Germination and plant height data were transformed using a natural logarithm scale prior to analyses to normalize data distribution. Length of flowering data were square root transformed prior to analyses. No transformation was required for days until flowering data. Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. Means reported here are back-transformed.

2.1.2. Predator visitation to flowers

Relative attractiveness of alyssum, buckwheat, phacelia and California poppy was assessed 2–3 times a week, between April 6th and August 22nd 2018, by observing the frequency of hoverfly feeding visits to flowers. To assess the relative attractiveness of insectary plants, each flowering plot was visually assessed for 2 min and the number of hoverflies, ladybugs (Coleoptera: Coccinellidae), large predatory wasps and minute parasitic wasps (both Hymenoptera) feeding from flowers were recorded. Hoverflies entering the plot and feeding from one or more flowers were counted as one feeding visit, unless they left the plot and returned for additional feeding (Colley and Luna, 2000). The number of non-feeding hoverflies and ladybugs were also recorded. Visual assessments were conducted between 9.00 am and 12.00 pm.

A zero inflated Poisson regression model was used to determine whether plant species and sampling month had a significant effect on the number of non-feeding adult ladybugs, feeding adult ladybugs, total ladybugs (non-feeding + feeding), parasitic wasps, predatory wasps, non-feeding adult hoverflies, feeding adult hoverflies and total hoverflies (non-feeding + feeding) (Proc. GENMOD in SAS [2011]) (Lambert, 1992). Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. Wilcoxon rank sum test at the 0.05 level of significance was used to compare means of insect counts between two plant species when zeroes were counted for one plant species (Proc. NPAR1 in SAS [2011]) (Mann and Whitney, 1947). Similarly, Wilcoxon rank sum test was used to compare means of insect counts between two months when zero feeding observations occurred for one of the months. Means were calculated using the zero inflated Poisson regression model for each plant species and month and then back-transformed from the logarithm scale to the original scale. Results from determining the effect of sampling month on insect counts are not reported here.

2.1.3. Species of hoverflies

Approximately once a week, between May 7th and August 22nd 2018, hoverflies visiting flowers were collected with a sweep net (1134 cm^2, Heavy Duty Sweep Net, BioQuip Products, Inc., Rancho Dominquez, CA) for 2 min for each of the five plots for each species of flowering plant. Hoverflies visually observed visiting flowers were captured. Both the number of hoverflies observed and those captured were recorded by plant species. Captured hoverflies were immediately placed in ventilated clear plastic cages (15.24 × 15.24 × 30.48 cm, with 2 mesh sides and a cloth sleeve for easy access) labelled by plant species. Cages were transported to the laboratory and placed into a refrigerator for 4–24 h before transferring hoverflies into labelled 7.4 ml glass vials. Representative specimens were identified to species by Martin Hauser, California Department of Food and Agriculture, Sacramento, and subsequent identifications were identified from reference specimens, a key supplied by M. Hauser outlining main distinguishing features between *Allograpta exotica* Wiedemann and *A. obliqua*, and identification guides in Bugg et al. (2008).

A *t*-test was used at the 0.05 level of significance to compare the difference in sweep net counts between observed and captured hoverflies for all species combined ("total hoverflies") (Proc. TTEST in SAS [2011]).

2.2. Field deployment of *D. citri* infested citrus and foraging activity by hoverflies

2.2.1. Preparation of experimental plants for field deployment.

Diaphorina citri used for these experiments were sourced from *C*Las-free colonies maintained in the Insectary and Quarantine Facility (IQF) at University of California, Riverside, CA (UCR). *D. citri* were reared on potted *Citrus volkameriana* V. Ten. & Pasq plants (25 cm tall). Citrus plants were obtained as rooted seedlings ~2 years of age (Willits and Newcomb Inc., Arvin, CA) and reared in greenhouses at UCR Agricultural Operations (see Bistline-East et al. [2015] for management practices). Experimental plants were pruned to produce heavy flush growth which is necessary to stimulate *D. citri* oviposition. Approximately 15–20 colony-sourced *D. citri* adults were introduced into individual ventilated clear plastic cages containing one citrus plant and allowed to oviposit for 7 days. Cages containing ovipositing adults were maintained in IQF at 29 ± 2 °C and 40–60% RH, under a L14:10D photoperiod. After 7 days, adults were removed and plants were maintained in cages until eggs hatched. The number of 1st–3rd instar nymphs on the stem of each experimental plant deployed in the field was counted with the assistance of a 3.5× magnifying OptiVISOR (Donegan Optical Company, Lenexa, KS). If required, excess *D. citri* nymphs or unhatched eggs were removed using a fine paintbrush or by removing infested flush growth until plants or stems contained desired densities of *D. citri* for experiments. For example, the "Hoverfly oviposition on *D. citri* colonies" experiment and "Effect of alyssum and predation from hoverflies on field survival of *D. citri* nymphs" experiment required a *D. citri* density of 100–400 and ~100 per plant, respectively.

2.2.2. Hoverfly oviposition on *D. citri* colonies and hoverfly species identification

Once a month, between June 22nd and December 11th 2018, 3–19 potted citrus plants (depending on availability) infested with 100–400 1st–3rd instar *D. citri* nymphs were placed in an unsprayed citrus orchard (GPS co-ordinates: N 33.97268°; W 117.31819°) on top of inverted plastic 19l buckets (37 cm high) that were anchored to the ground with steel tent pegs. Plants were secured to buckets using 25 cm long bungee cords. Bucket sides were coated around the entire circumference with a sticky barrier (Tanglefoot insect barrier, Contech Enterprises Inc., Victoria, Canada) to prevent Argentine ants (*Linepithema humile* [Mayr]) from accessing plants and protecting *D. citri* nymphs from natural enemies (Kistner and Hoddle, 2015). After 2 days, *D. citri* infested plants were removed and placed in a temperature controlled room in IQF and held at 26 ± 2 °C and 60–80% RH under a L16:8D photoperiod. The number of hoverfly eggs laid on each citrus plant was counted with the assistance of an OptiVISOR. To determine the species of hoverfly ovipositing on *D. citri* colonies in the field, a cohort of 4–20 hoverfly eggs were removed from experimental plants each month and reared inside a ventilated clear plastic cage on potted citrus plants containing 400–800 1st–3rd instar *D. citri* nymphs. One hoverfly egg was placed on a single leaf of a caged plant. Eggs were adhered to leaves using a small amount of 50% honey-water solution. A total of 41 eggs were set up in 41 cages. Rearing cages were held at 26 ± 2 °C and 60–80% RH under a L16:8D photoperiod. Citrus plants containing *D. citri* were replaced after 7 days and hoverfly larvae were transferred to the new plants to ensure an adequate food supply for developing larvae. Hoverfly pupae were removed from cages using a fine paint brush, placed into labeled microcentrifuge tubes and held at 26 ± 2 °C and 60–80% RH under a L16:8D photoperiod for emergence. Thirty larvae were successfully reared to adulthood and identified to species.

A zero inflated Poisson regression model at the 0.05 level of significance was used to determine the effect of month on the number of hoverfly eggs laid on citrus plants (Proc. GENMOD in SAS [2011]). Means were calculated using the zero inflated Poisson regression model for each month, and back-transformed from the logarithm scale to the original scale.

2.2.3. *Consumption of D. citri nymphs by individual hoverfly larva in the laboratory*

Hoverfly larvae (*A. obliqua* only, see results) that hatched from eggs laid on citrus plants described in the previous experiment were used to determine lifetime consumption of *D. citri* nymphs by individual hoverfly larvae. Small 130 ml plastic vials with ventilation (three 2 cm holes [one on the bottom, and one on each of two sides] covered with mesh netting [80 μm mesh width JelliV Corporation, Southport, CT]) and plastic lids were used as rearing units. A hole was drilled in the bottom of the lid and cut from the hole to the side of the lid. A stem attached to a potted citrus plant containing a known number of 1st-3rd instar *D. citri* nymphs (ranging from 100 to 400 nymphs) was slid through the hole and sealed with cotton balls and putty (Aleene's Instant Tacky Reusable Craft Putty, Duncan Enterprises, Fresno, CA). Using a fine paint brush, one 1st instar hoverfly larva was placed on the stem containing *D. citri* nymphs, and the vial was placed over the stem and sealed to the lid. The vial enclosing the stem was supported with wooden skewers. The whole plant containing vials on skewers (up to three vials were attached to a single plant) was placed inside a clear plastic ventilated rearing cage. After 48 h, larvae were removed from plants using a paintbrush and surviving nymphs were counted. Larvae were placed on new stems containing *D. citri* and this 48 h cycle continued until larvae pupated. Controls to determine naturally occurring mortality of *D. citri* nymphs consisted of enclosing a stem with a known number of *D. citri* nymphs in a vial without a hoverfly larva as previously described. Fourteen replicates of hoverfly larva vials and control vials were set up. The cumulative mortality of *D. citri* nymphs in each hoverfly larva replicate was calculated across all 48 h feedings until pupation. Natural mortality of *D. citri* nymphs over 48 h was calculated for each control vial. Since controls were not paired with larvae treatment replicates, average natural mortality was calculated across the 14 control replicates, and this average was used to correct mortality in larval vials for naturally occurring nymph mortality using the Schneider-Orelli formula for even sample sizes (Püntener, 1981). The adjusted mortality for each hoverfly larva replicate was used to estimate the number of 1st-3rd instar *D. citri* nymphs consumed until pupation using the following equation: Total number of *D. citri* exposed to hoverfly larva until pupation * (adjusted percentage mortality/100). The lifetime consumption of *D. citri* by individual hoverfly larvae was calculated across all 14 replicates.

Wilcoxon rank sum test at the 0.05 level of significance was used to compare the mean difference in consumption of *D. citri* nymphs between female and male *A. obliqua* (Proc. NPAR1WAY in SAS [2011]).

2.2.4. *Effect of alyssum and predation from hoverflies on D. citri survival in the field*

Four blocks of citrus at the Biocontrol Grove at UCR (GPS coordinates: N 33.97268°; W 117.31819°) were used for this experiment: 11 rows of Washington Navel oranges, 16 rows of Oro Blanco grapefruit, 10 rows of Valencia oranges and 14 rows comprised of a combination of Eureka lemons, Valencia oranges, Washington Navel oranges and Oro Blanco grapefruit. Machine cultivation between rows was conducted prior to the experiment to control weeds. Removal of weeds within the row was conducted every 3 weeks using an electric weed whacker (Ego Power + 15″ String Trimmer with Rapid Reload, ST1502SA, Naperville, IL). Orange and grapefruit trees used in this study were not in flower. The three rows of lemon trees in the combination citrus block were flowering, and it is unknown whether these are beneficial to natural enemies. During the four weeks this experiment was conducted (June 20th through July 22nd, 2018), average temperature, average solar radiation, total precipitation, average relative humidity and average day length was 23.6 ± 0.3 °C, 678.7 ± 27.4 Ly/day, 1.0 mm, 58.1 ± 0.7%, and 14. h/day, respectively (CIMIS weather database, Station 44 UC Riverside [https://cimis.water.ca.gov/Default.aspx]; "Timeanddate.com", Riverside, California [https://www.timeanddate.com/astronomy/usa/riverside]). The four blocks of citrus were divided into two plots comprised either of oranges or grapefruit. The two plots were subdivided and separated by ~ 100 m to accommodate two treatments: twenty 19 l pots of flowering alyssum were placed in two sections and the two control sections received 20 19 l pots containing soil only. All pots were fitted with one 11.3 l/h dripper (Spot-Spitter Mini Flow, Primerus Products LLC, Carlsbad, CA) and were irrigated for 6 h, two times a week, when surrounding citrus trees were irrigated.

Over the period June 20th-July 23rd, 2018 four potted *C. volkameriana* plants infested with ~100 1st–3rd instar *D. citri* nymphs were secured onto four buckets stands (as previously described) situated beneath the lower canopy of two citrus trees in each of the four experimental plots for a total of 16 experimental trees deployed. Nearby tree foliage was pruned to ensure that no citrus foliage touched the potted plants or exclosure cages. Four treatments were used to assess natural enemy impact on *D. citri* survival. Four exclusion treatments similar to those utilized by Kistner et al. (2016a) were employed. First, an exclosure (15 by 30 cm) made of fine mesh (95 μm^2 holes) organdy was placed over potted plants, which was supported using two 28 cm long sticks inserted into potting soil. This control treatment excluded all natural enemies thereby providing *D. citri* survivorship rates in the absence of natural enemies (i.e., baseline mortality rates from natural causes and abiotic factors). Second, exclosures (15 by 30 cm) made of coarse mesh organdy (3 mm^2 holes) were placed over potted plants to exclude large predators while allowing entry of small natural enemies like *T. radiata*. Third, potted plants with no mesh exclosures were checked daily for hoverfly eggs and larvae which were removed using a fine needle and paint brush and placed into microcentrifuge tubes (Fisherbrand 1.5 ml polypropylene microcentrifuge tubes, Fisher ScientiWc, Pittsburgh, PA). This third treatment directly investigated the impact hoverflies have on *D. citri* survivorship. In the laboratory, a cohort of 6 hoverfly eggs or larvae removed daily from experimental plants were reared in cages on potted citrus plants containing 400–800 1st −3rd instar *D. citri* nymphs to determine species of hoverfly associated with *D. citri* colonies in the field. Eighteen larvae were reared to adulthood and identified to species.

The fourth treatment, non-exclusion, consisted of potted plants which were fully exposed to allow all natural enemies free access to *D. citri* life stages. All four treatments were protected from *L. humile* by applying a sticky barrier around the entire circumference of bucket stands as previously described. Plants were inspected between 8:00am and 12:00 pm every other day and numbers of *D. citri* eggs, first-third instars, fourth-fifth instars, and adults were recorded per treatment. Adult *D. citri* emerging into exclosures as well as fifth-instar nymphal exuviae left on twigs/leaves were counted and removed at each inspection. Predators and parasitoids observed on *D. citri* colonies during inspections were recorded. Unknown arthropods on *D. citri* colonies were collected and preserved in 95% ethanol in labeled vials and identified. Counts continued until all immature *D. citri* had died from unknown causes, disappeared, been eaten, or emerged as adults. This experiment was repeated starting August 8, 2018 and plot assignments were reversed. Additionally, from June 15th through August 29th, 2018, hoverflies visiting flowers were collected on 11 dates with a sweep net for 5 min for each of the two plots of potted alyssum. Sweep net protocol and subsequent hoverfly identification were conducted as previously described.

A negative binomial regression model was used to determine whether flower treatment, *D. citri* treatment and their interaction had significant effect on the number of predators observed and hoverfly eggs laid on citrus plants (Proc. GENMOD in SAS [2011]) (Hilbe, 2011). There was no significant effect of the interaction between flower treatment and *D. ctiri* treatment, therefore, this term was removed and the model rerun. Significant means were separated using Tukey's Studentized range test at the 0.05 level of significance. Wilcoxon rank sum test at the 0.05 level of significance was used to compare means of hoverfly eggs between two *D. citri* treatments when zero was counted in one of the *D. citri* treatments (Proc. NPAR1WAY in SAS [2011]). A logistic regression model was conducted to determine whether flower treatment, *D. citri* treatment and their interaction had a significant effect on the proportion of *D. citri* nymphs surviving to adulthood (Proc. LOGISTIC in SAS [2011]) (Walker and Duncan, 1967). Tukey's Studentized range test at the 0.05 level of significance was used to separate significant means. Fisher's Exact test was conducted at the 0.05 level of significance to compare mean proportion of *D. citri* survival between two *D. citri* treatments when zero nymphs survived to adulthood in one of the treatments (Proc. FREQ in SAS [2011]) (McDonald, 2009). Means and SEMs were calculated for each variable using the appropriate model described and back-transformed. A Wilcoxon Signed Rank Test was used at the 0.05 level of significance to compare the difference in sweep net counts between observed and captured hoverflies for all species combined ("total hoverflies") (Proc. TTEST in SAS [2011]).

3. Results

3.1. Flowering phenology and attractiveness to hoverflies and other natural enemy species

3.1.1. Germination counts and flowering phenology

Table 1 lists the date that each species flowered (at least 5% of plants flowering) for each sowing date. Buckwheat flowers were available for all predator visitation assessments (April 6th and August 22nd, 2018) due to staggered sowings of this species which was used as a baseline. Flowering plots of alyssum were available between April 6th and July 3rd, 2018. All four plant species were flowering simultaneously between April 24th and June 5th, 2018. Plant species had a significant effect on 7 week plant counts ($F = 33.36$, $df = 3, 32$, $p < 0.0001$). There was no significant effect of sowing date ($F = 1.56$, $df = 1, 32$, $p = 0.22$) or sowing date by species interaction ($F = 0.38$, $df = 3, 32$, $p < 0.77$). Alyssum plots contained a significantly higher (51%-194% higher) number of germinated plants after 7 weeks compared with buckwheat, phacelia and poppy (Table 1). Buckwheat plots contained the lowest plant density at 122 plants per 1.5 m^2 plot. Plant species ($F = 35.95$, $df = 3, 32$, $p < 0.0001$), sowing date ($F = 10.47$, $df = 1, 32$, $p < 0.005$) and their interaction ($F = 9.01$, $df = 3, 32$, $p < 0.005$) had a significant effect on plant height. Sowing date had a significant effect on height of phacelia at week eight with shorter plants occurring later in the spring (Table 1; Supplementary Material 2). There was no significant effect of sowing date for the remaining plant species. For sowing dates, average height of alyssum plants was significantly shorter (up to 49% shorter) than buckwheat, phacelia and poppy (Table 1; Supplementary Material 2). Plant species ($F = 467.24$, $df = 3, 31$, $p < 0.0001$), sowing date ($F = 421.53$, $df = 1, 31$, $p < 0.0001$) and their interaction ($F = 6.92$, $df = 3, 31$, $p < 0.005$) had a significant effect on the number of days until flowering was observed. The number of days required from sowing to flowering for poppy and phacelia were up to 99% longer compared with buckwheat and alyssum (Table 1; Supplementary Material 2). For the March sowing date, buckwheat and alyssum required 30 and 33 days, respectively, from sowing to flowering while California poppy and phacelia flowered after 55 and 60 days, respectively. For all four plant species, the number of days required from sowing to flowering was significantly shorter when seeds were sown in March compared with February (Table 1; Supplementary Material 2). Plant species ($F = 166.06$, $df = 3, 31$, $p < 0.0001$), sowing date ($F = 11.44$, $df = 1, 31$, $p < 0.005$) and their interaction ($F = 5.47$, $df = 3, 31$, $p < 0.01$) had a significant effect on the length of the flowering period. California poppy seed sown in February and March produced flowers for 155–164 days which was significantly longer than all remaining plant species tested. Alyssum flowered 41 days longer than buckwheat when seeds were sown in February, whereas, the length of the flowering period was statistically equivalent between these plant species for the March sowing date (Table 1; Supplementary Material 2).

Table 1

The date that each plant species started flowering, mean seven week plant counts, plant height, the number of days until plants produced flowers, and the length of flowering (days) for alyssum (*Lobularia maritima*), buckwheat (*Fagopyrum esculentum*), California poppy (*Eschscholzia californica*) and phacelia (*Phacelia tanacetifolia*) plants sown on February 19th, 2018 and March 29th (means ± SEMs; different letters indicate significant [$p < 0.05$] differences between plant species; asterisks indicates significant [$p < 0.05$] differences between sowing dates).

	Alyssum	Buckwheat	Phacelia	Poppy
Date flowering started (February sowing)	March 31st	April 4th	April 26th	April 26th
Date flowering started (March sowing)	May 1st	April 28th	May 25th	May 22nd
7 week plant counts (both sowing dates)	359.9 ± 23.5 a	122.4 ± 5.8b	238.3 ± 29.3c	180.2 ± 12.2c
Height (cm) (February sowing)	22.7 ± 1.0 a	35.7 ± 1.0b	53.8 ± 3.4 * c	30.6 ± 1.5b
Height (cm) (March sowing)	19.6 ± 1.0 a	38.7 ± 3.0b	30.5 ± 3.3c	30.7 ± 3.2c
Days to flowering (February sowing)	44.8 ± 0.8 * a	48.0 ± 0.0 * a	70.3 ± 0.0 * b	67.2 ± 0.8 * b
Days to flowering (March sowing)	33.2 ± 0.0 a	30.0 ± 0.0 a	59.7 ± 2.8b	54.6 ± 0.6b
Length of flowering (February sowing)	98.1 ± 5.8 * a	57.5 ± 2.8b	36.2 ± 2.4 * c	163.7 ± 18.3 d
Length of flowering (March sowing)	74.1 ± 2.0 a	65.3 ± 5.8 a	14.8 ± 3.2b	154.9 ± 4.3c

3.1.2. Predator visitation to flowers

Plant species had a significant effect on the number of all insect groups counted (Supplementary Material 3). Counts of non-feeding ladybugs were 2–7 times higher in phacelia plots compared with alyssum, buckwheat and poppy (Fig. 1). Counts of feeding ladybugs were 12 times higher in buckwheat plots compared with alyssum. Total ladybugs (i.e., non-feeding and feeding combined) was 8–14 times higher in buckwheat and phacelia plots compared with alyssum and poppy (Fig. 1). Twelve times more parasitoids were observed feeding from alyssum flowers compared with buckwheat, whereas, feeding predatory wasps were 4 times higher in buckwheat plots compared with alyssum (Fig. 1). Ladybugs, parasitoids and predatory wasps were not recorded feeding from phacelia and poppy flowers. There was a significantly higher (2–21 times higher) number of hoverflies feeding from alyssum flowers compared with buckwheat, phacelia and poppy. Counts of feeding hoverflies were 12 times higher in buckwheat plots compared with phacelia and poppy. No hoverflies were recorded feeding from phacelia flowers (Fig. 1).

3.1.3. Species of hoverflies

A total of 72 feeding hoverflies were observed and 54 were captured in flowering trial plots during May and August 2018 (Table 2). Captured hoverflies were comprised of five species; *Paragus tibialis* (Fallen), *A. obliqua*, *Sphaerophoria sulphuripes* (Thomson), *Toxomerus marginatus* (Say), and *Syritta pipens* L. (Table 2). The number of hoverflies captured during sweep netting (mean = 1.3 ± 0.3 hoverflies captured in 10 mins) was half the number of total hoverflies observed (mean = 3.0 ± 0.7 hoverflies observed in 10 mins) ($t = 3.65$, $df = 32$, $p < 0.001$).

3.2. Interactions between D. citri and hoverflies

3.2.1. Hoverfly oviposition on D. citri colonies and hoverfly species identification

Allograpta obliqua was the only species of hoverfly to oviposit on citrus plants infested with *D. citri* nymphs. There was a significant effect of month on the number of hoverfly eggs laid on experimental citrus plants ($F = 5.07$, $df = 6, 51$, $p < 0.001$). The number of hoverfly eggs per 25 cm citrus plant was, on average, 20 eggs in July and 14 in November (Fig. 2). Zero eggs were laid on plants deployed in December. Hoverfly oviposition on deployed citrus plants were significantly higher (up to 18

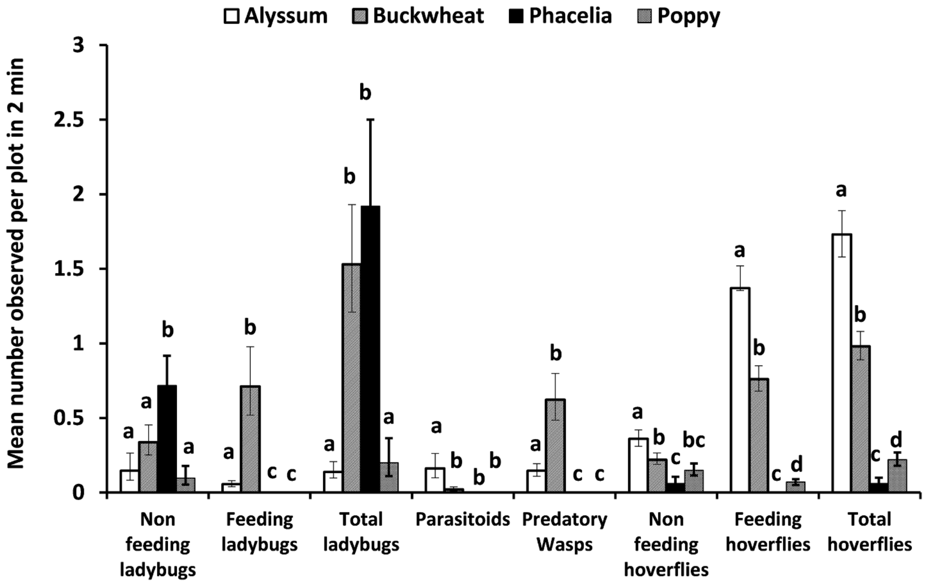

Fig. 1. Mean number of several groups of insects observed within 2 min observations in plots of alyssum (*Lobularia maritima*), buckwheat (*Fagopyrum esculentum*), phacelia (*Phacelia tanacetifolia*) and California poppy (*Eschscholzia californica*) set up at Agricultural Operations, University of California, Riverside, CA from April to August 2018 (error bars indicate ± SEMs; different letters indicate significant [$p < 0.05$] differences in insect counts between plant species).

Table 2
Percentage of each hoverfly species observed and captured at the flowering trial and the citrus orchard study during 10 min observations.

	Observed in flowering trial May – Aug n = 72	Captured in flowering trial May – Aug n = 54	Observed in citrus orchard Jun – Aug n = 58	Captured in citrus orchard Jun – Aug n = 25
Paragus tibialis (predacious)	49	50	36	40
Allograpta obliqua (predacious)	1	2	12	24
Syritta pipens (detritivorous)	14	13	36	32
Sphaerophoria sulphuripes (predacious)	1	15	2	4
Toxomerus marginatus (predacious)	8	20	0	0
Scaeva pyrastri (predacious)	0	0	5	0
Unknown	26	0	9	0

times higher) in July and November compared with August, September, October and December (Fig. 2).

3.2.2. Consumption of *D. citri* nymphs by individual hoverfly larvae in the laboratory

In the absence of hoverfly larvae, the mean rate of natural morality of *D. citri* in vials was 27 ± 6%. There was a significant effect of sex on the estimated number of *D. citri* consumed by individual *A. obliqua* larvae (Wilcoxon rank sum of scores = 21, $p < 0.01$). Male *A. obliqua* consumed 39% (mean male consumption = 523 ± 34 nymphs) more *D. citri* nymphs when compared with female *A. obliqua* (mean female consumption = 319 ± 26).

3.2.3. Effect of alyssum and predation from hoverflies on *D. citri* survival in the field

After two weeks of observations, no hoverfly eggs were laid on plants during the August 8th, 2018 deployment. Therefore, only results from the June 22nd–July 28, 2018 deployment are presented here. There were 80 observations of predators on potted citrus plants containing colonies of *D. citri*. Of these, 88% were hoverfly larvae, 11% were predatory mites, and 1% were lacewing larvae. Three lacewing eggs and 133 hoverfly eggs were laid on citrus plants infested with *D. citri*. One lacewing larva was observed consuming a *D. citri* nymph. Zero *T. radiata* were observed interacting with *D. citri* nymphs and no parasitism of *D. citri* nymphs was recorded. *Allograpta obliqua* was the only species of hoverfly that oviposited on citrus plants and larvae were observed consuming *D. citri* nymphs. Over all *D. citri* treatments, the number of hoverfly eggs laid on potted citrus plants ($F = 8.78$, $df = 1, 8$, $p < 0.01$) and abundance of predators ($F = 5.69$, $df = 1, 10$, $p < 0.05$) was 3.5 times and 2.5 times higher, respectively, on potted citrus plants deployed in alyssum plots compared with control plots lacking alyssum (Fig. 3). Over all flowering treatments, *D. citri* treatment had a significant effect on predator abundance ($F = 4.08$, $df = 3, 10$, $p < 0.05$) and number of hoverfly eggs on citrus plants ($F = 4.08$, $df = 3, 10$, $p < 0.05$). Predator abundance was 5 times higher in the no-exclusion treatment compared with full exclusion (Fig. 4). Three predatory mites were counted in the "full exclusion" treatment across all flowering treatments. Zero hoverflies eggs were counted on potted citrus plants placed under fine netting that fully excluded predators and parasitoids (Fig. 4). Hoverflies oviposited statistically equivalent numbers of eggs on the no exclusion and hoverfly removed treatments. However, occasionally *A. obliqua* were able to oviposit through the coarse mesh netting onto plants in partial exclusion treatments. The number of hoverfly eggs laid on potted citrus plants was 5–8 times higher in the no-exclusion and hoverfly eggs removed treatments compared with the partial exclusion and full exclusion treatments (Fig. 4).

In flowering alyssum plots, *D. citri* survival was 2–3 times higher in the full exclusion treatment compared with the partial and hoverfly removed treatments (Fig. 5, Supplementary 4). *D. citri* nymph survival in control plots was statistically equivalent in the full exclusion, partial exclusion and hoverfly removed treatments. Comparing nymph survival between plant treatments exposed to natural enemies (no exclusion) indicated that 10% more nymphs survived to adulthood in the control plots compared with plots containing potted flowering alyssum (Fig. 5, Supplementary 4). Nymph survival on citrus plants under the partial exclusion and hoverfly removed treatments was 2.5 times higher in control plots compared with alyssum. There was no significant difference in *D. citri* survivorship rates in the absence of natural enemies (full

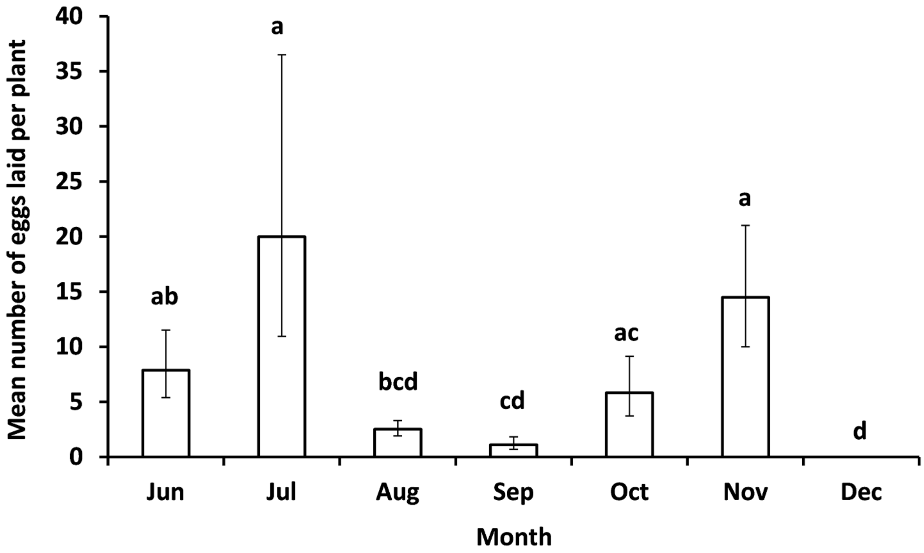

Fig. 2. The mean number of eggs laid on sentinel citrus plants deployed in an organic citrus orchard at Biocontrol Grove, University of California, Riverside, CA every month from June 22nd through Dec 11th, 2018 (error bars indicate ± SEMs; different letters indicate significant [$p < 0.05$] differences in egg number between months).

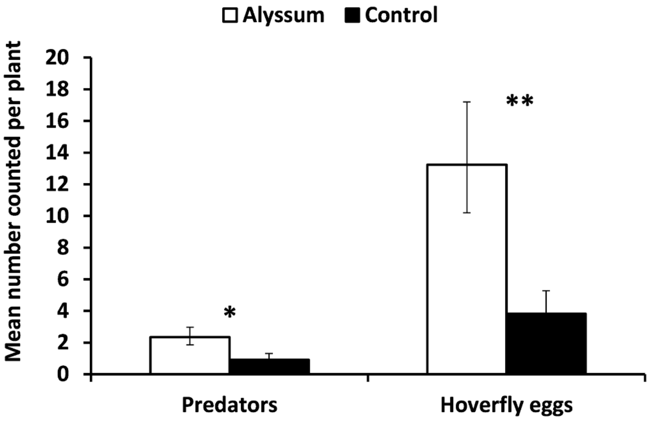

Fig. 3. The mean number of predators observed and hoverfly eggs laid on potted citrus plants deployed in alyssum and control plots set up in an organic citrus orchard at Biocontrol Grove, University of California, Riverside, CA in June 2018 (error bars indicate ± SEMs; asterisks indicate a significant [* = $p < 0.05$; ** = $p < 0.01$] difference between flower treatments).

exclusion treatment) between alyssum and control plots indicating that the baseline survival of *D. citri* was consistent between treatments (Fig. 5, Supplementary 4).

In the alyssum plots during June and August 2018, a total of 58 feeding hoverflies were observed and 25 were captured (Table 2). Captured hoverflies were comprised of four species; *P. tibialis*, *A. obliqua*, *S. sulphuripes, and S. pipens* (Table 2). Zero *T. marginatus* were observed or captured. Sweep netting hoverflies feeding on alyssum flowers resulted in 47% less total hoverflies captured (mean = 3.6 ± 1.0 captured in 10 min) than those observed (5.3 ± 2.3) (Wilcoxon Signed Rank Test = 14.0, $p < 0.05$).

4. Discussion

4.1. Flowering phenology and attractiveness to hoverflies and other natural enemies

Hoverflies exhibited a high degree of selectivity among the four insectary plants tested most likely due to their suitability in terms of nectar accessibility. Alyssum and buckwheat were identified as food resources for four species of predatory hoverflies when compared with California poppy and phacelia. Alyssum and buckwheat have flowers with short corollas which increases the availability of nectar to natural enemies (van Rijn and Wäckers, 2016). California poppy flowers have been reported to provide pollen to natural enemies, but little or no nectar (Percival, 1955). However, the dry climate in southern California may cause hoverflies to select plants that provide nectar because nectar may be the only source of water in the environment (Petanidou, 2007), even though poppies provide pollen, a resource needed for egg maturation (Bugg, 1992). Alyssum was more attractive to hoverflies compared with buckwheat, attracting twice as many syrphids. This may be due to the higher floral area of this plant species attracting greater numbers of predators (Lundin et al., 2018). No hoverflies were observed feeding from phacelia throughout the course of this study. The deep corolla of

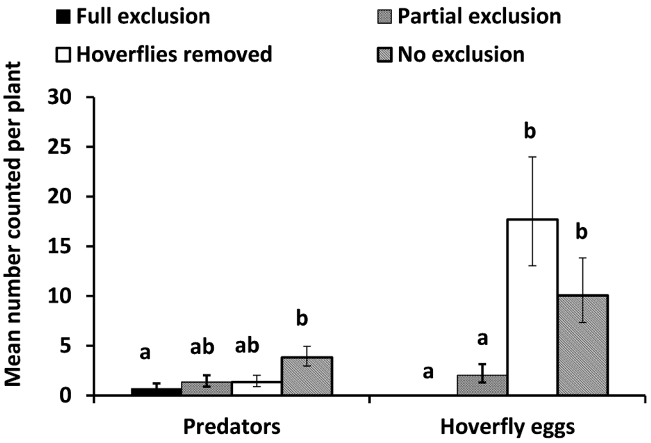

Fig. 4. The mean number of predators observed and hoverfly eggs laid on potted citrus plants under four *D. citri* treatments (Full Exclusion: fine mesh netting fully excluding all predators and parasitoids; Partial Exclusion: coarse mesh netting excluding large predators; No Exclusion: no netting; Hoverflies Removed: no netting, but with removal of hoverfly eggs and larvae) set up in an organic citrus orchard at Biocontrol Grove, University of California, Riverside, CA in June 2018 (error bars indicate ± SEMs; different letters indicate significant [$p < 0.05$] differences in the abundance of predators and hoverfly eggs between *D. citri* treatments).

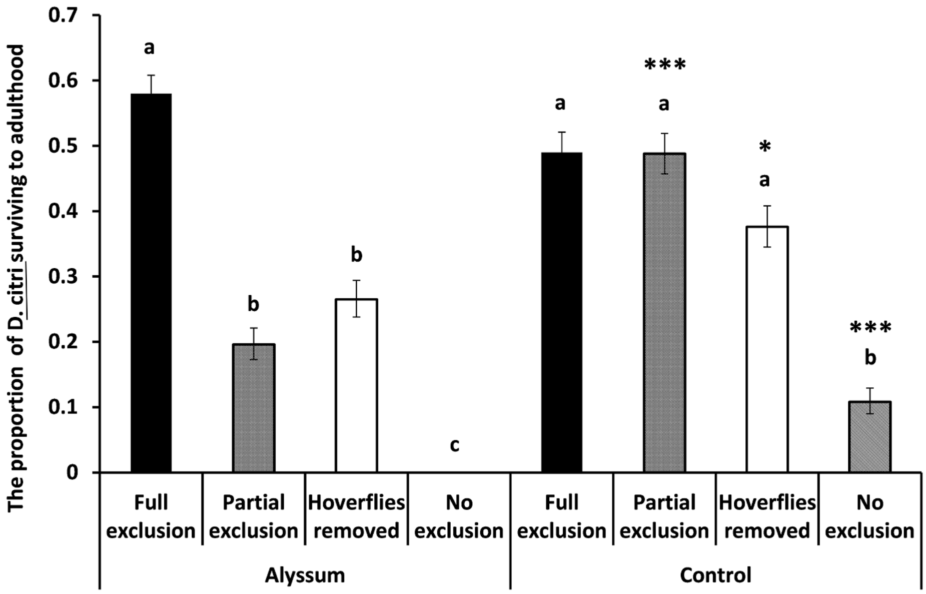

Fig. 5. The proportion of *D. citri* surviving to adulthood in four *D. citri* treatments (Full Exclusion: fine mesh netting fully excluding all predators and parasitoids; Partial Exclusion: coarse mesh netting excluding large predators; No Exclusion: no netting; Hoverflies Removed: no netting, but with removal of hoverfly eggs and larvae) and two floral treatments (irrigated potted alyssum, and irrigated pots containing soil as a control) set up at an organic citrus orchard at Biocontrol Grove, University of California, Riverside, CA in June 2018 (error bars indicate ± SEMs; different letters indicate significant [$p < 0.05$] differences between *D. citri* treatments; asterisks indicate a significant [* = $p < 0.05$; *** = $p < 0.0001$] difference between floral treatments).

phacelia flowers limits nectar use by hoverflies, and the blue/purple flowers are not a preferred color for stimulating feeding by hoverflies (Cowgill, 1989; Lunau and Wacht, 1994; van Rijn and Wäckers, 2016).

In contrast to results here, previous studies have demonstrated syrphids are attracted to phacelia and feed on phacelia pollen (Sengonça and Frings, 1988; Hickman and Wratten, 1996; Colley and Luna, 2000). Species of hoverfly in southern California may have different floral preferences than hoverfly species occurring in other regions. Alternatively, the presence of alyssum and buckwheat in our experimental plots may have diverted hoverflies from nearby phacelia and California poppy that they otherwise may have visited. The attractiveness of phacelia and California poppy to syrphids may have been different if these plants were evaluated in the absence of more preferred plants. Colley and Luna (2000) noted the relative attractiveness of different species of insectary plants changed over time when highly attractive species ceased flowering and less preferred species were still in flower.

Buckwheat attracted up to 13 times more ladybugs and predatory wasps when compared with alyssum. However, eight times as many parasitoids were observed feeding on alyssum compared with buckwheat. This result illustrates the potential importance of using mixed species of insectary plants since each species may support different communities of natural enemies (Chaney, 1998; Hogg et al., 2011a). Although ladybugs and hoverflies were observed in phacelia plots they were not observed feeding from phacelia flowers. Attraction to phacelia may have been attributable to the presence of prey (e.g., aphids). This result illustrates the importance of using direct measures of flower use by natural enemies rather than abundance of natural enemies in experiments assessing the value of insectary plants as food resources for conservation biological control.

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. In this study, buckwheat and alyssum started flowering 19–30 days earlier than phacelia and California poppy. Insectary plants with a short sowing to flowering time is a desirable trait because they rapidly provide nutrition for natural enemies. Alyssum sown in February started flowering at the same time as buckwheat and then continued blooming for 42 days longer than buckwheat. Mixed species sowings may be useful to simultaneously take advantage of quick flowering species and those that have long nectar production periods. California poppy flowered for 160 days which was longer than all other insectary plants tested. However, California poppy was only marginally attractive to feeding hoverflies and zero ladybugs, parasitoids, or predatory wasps were observed feeding from flowers.

Alyssum and buckwheat exhibit potential as insectary plants in citrus orchards because they germinated readily, exhibited a short sowing to flowering time, and were attractive to a range of natural enemies. Alyssum may have two advantages over buckwheat. Firstly, the perennial life cycle of alyssum and its ability to flower uninterrupted for extended periods (Pico and Retana, 2003). Secondly, alyssum is significantly shorter than buckwheat. The low-growing habit of alyssum may be advantageous because it would interfere minimally with standard orchard-management practices (Gontijo et al., 2013). When selecting an insectary plant for natural enemy enhancement, it is important to consider ease and cost of attaining seed, the costs of establishing and maintaining the floral resource (especially water costs in southern California), potential to become a weed, whether the insectary plant is of value and could be harvested and sold, potential to attract pests or host pathogens, and the phenology of flowering relating to synchronizing nectar production with critical times in pest control (Irvin et al., 2014, 2016). Currently, alyssum and buckwheat cost (in $US) $47/kg and $3/kg, respectively (Outsidepride.com), and at recommended sowing rates (2.5 kg/ha and 56 kg/ha, respectively), seed costs for alyssum and buckwheat would total $118/ha and $168/ha, respectively. Placing insectary plants around field margins or in small patches dispersed throughout the orchard, may be a cost effective way to plant. Our results indicated that insectary plant species with overlapping flowering periods are needed to extend the window of nectar as syrphids were present in from April – August and peak abundance was observed in June, indicating an extensive period of nectar requirement.

This study measured syrphid flower visitation rate which is a relative measure and is dependent on the number of flowering species present, competition with other flower visitors, and previous experience (Wäckers and van Rijn, 2012). Furthermore, flower attractiveness does not necessarily indicate which flowers enhance fitness and research investigating the effect of alyssum and buckwheat on survival and fecundity of *A. obliqua* is warranted. Amorós-Jiménez et al. (2014) demonstrated that although syrphids visited alyssum more frequently than coriander (*Coriandrum sativum* L.), female *Sphaerophoria rueppellii* (Wiedeman) that fed on coriander oviposited more eggs than those that fed on alyssum. Interestingly, *S. rueppellii* larvae whose parents fed on alyssum flowers had shorter developmental times, increased survival to adulthood rates, and produced larger adults compared with larvae whose parents fed on coriander flowers. This outcome supports the proposed use of mixed species of insectary plants in citrus since each flowering species may enhance different components of syrphid fitness. However, mixed species resources may increase the risk of non-target effects, such as inadvertently promoting populations of pests (Baggen et al., 1999), syrphid parasitoids, higher-order predators, or hyper-parasitoids (Stephens et al., 1998).

4.2. Interactions between D. citri and hoverflies

Only 2% of hoverflies captured in the open plot flowering trial were *A. obliqua*, whereas, in the citrus orchard study, 24% of hoverflies captured were *A. obliqua*. Citrus orchards may contain higher numbers of *A. obliqua* compared with the flowering trial site because *A. obliqua* is predatory and likely feeds on a variety of citrus pests, like *D. citri*, as psyllids are a common prey for some species of syrphid (Rojo et al., 2003; MacDonald et al., 2016). In California citrus orchards, typically one or more hoverfly larvae in high density *D. citri* patches can consume more than 100 *D. citri* nymphs within 48 h (Kistner and Hoddle, 2015). The current study demonstrated that individual *A. obliqua* larva consumed on average 421 *D. citri* nymphs before pupation. This highlights the potential of *A. obliqua* as a voracious biological control agent of this pest. Consumption rates of *D. citri* in the field may differ to those reported here since laboratory larvae do not need to search for prey, instar encountered and consumed may vary, and temperature can effect consumption rates (Tenhumberg and Poehling, 1995; Soleyman-Nezhadiyan and Laughlin, 1998). It may be unlikely that naturally occurring colonies of *D. citri* would contain sufficient nymphs to sustain and complete hoverfly larval development. In addition to psyllid nymphs, hoverfly larvae may consume thrips, whiteflies, aphids, and springtails, and can survive for several days without food while searching for new pest colonies (Rojo et al., 1996, 2003; Gomez-Polo et al., 2015). In the absence of prey, *A. obliqua* larvae can subsist on plant materials, such as pollen (Schneider, 1969). In the laboratory, *A. obliqua* larvae ate *D. citri* honeydew which may sustain hoverfly larvae in the field when prey are absent or scarce.

Results from the field trial investigating potted alyssum as an insectary plant showed that alyssum enhanced hoverfly oviposition and abundance compared with control plots lacking alyssum and this translated to 10% fewer *D. citri* nymphs surviving to adulthood in plots containing potted flowering alyssum. When hoverfly eggs and larvae were removed from citrus plants, *D. citri* survival was still 1.5 times lower in alyssum plots compared with control plots. A diverse range of predators are associated with growing alyssum agricultural crops (Chaney, 1998; Hogg et al., 2011a, 2011b; Gontijo et al., 2013) which may have further suppressed *D. citri*.

Although results from the current study are encouraging, there are caveats that need consideration. The experiment investigating the effect of alyssum and predation from hoverflies on *D. citri* survival in the field was conducted over 7 weeks using small plots of alyssum separated by buffer zones $\geq$ 30 m. Pots of alyssum were only established for 2 weeks before *D. citri*-infested sentinel plants were deployed for evaluation. The beneficial effects of flowering plants on hoverfly abundance and suppression of *D. citri* may be greater if evaluated with large-scale field trials over an entire growing season. For example, establishment of insectary plants early in spring would provide resources for egg maturation and foraging (Bugg, 1992) and hoverfly impacts on *D. citri* populations should be monitored from April through November to encompass the times of the year that *D. citri* (found on flush growth in spring and fall [Kistner et al., 2016b]) and *A. obliqua* are most prevalent.

Argentine ants can reduce parasitism of *D. citri* by *T. radiata* by up to 80% (Tena et al., 2013) and may inhibit predators from accessing *D. citri* colonies (Kistner and Hoddle, 2015). A sticky insect barrier was used in this study to prevent ants from accessing experimental plants. In the absence of these measures, ants may have accessed some experimental plants and protected *D. citri* nymphs from natural enemies, therefore increasing *D. citri* survival or creating inconsistency between treatments and replicates.

The current study used sentinel plants containing high densities of colony reared *D. citri* (approximately 100 1st-3rd instar nymphs per potted plant) to investigate hoverfly oviposition and predation of nymphs. Such high densities of *D. citri* nymphs may not reflect the reality of naturally occurring lower densities (approximately 5 1st-3rd instar nymphs per branch [Kistner et al., 2016b]) that currently prevail on citrus in southern California. Hoverflies may not oviposit until prey reach a critical threshold density (e.g., greater than 50 aphids on broccoli stimulates egg laying in four species of syrphid [Ambrosino et al., 2006]). Results from the current study, Kistner et al. (2016b) and Kistner et al. (2017) demonstrated that adult *A. obliqua* locate and oviposit on large artificially-established *D. citri* colonies. However, it is unknown whether female *A. obliqua* would locate smaller sized naturally occurring colonies of *D. citri* and select these for oviposition. Host patch selection by *A. obliqua* in citrus, especially with respect to *D. citri*, warrants investigation.

Results presented here suggest that alyssum and buckwheat show promising potential as insectary plants in citrus orchards for conservation biological control of *D. citri* in California. Insectary plants could be grown in small plots as a cover crop between rows, or as a field margin resource, to enhanced hoverfly oviposition and abundance, and reduce survival of *D. citri* nymphs. Further research using naturally occurring populations of *D. citri* and investigating potential effects of irrigated insectary plants on citrus tree growth, yield, or fruit quality is required (Irvin et al., 2016). Additional work could identify species of efficacious insectary plants that can be grown as “cash crops” to provide complementary sources of income for farmers. These revenue streams could include use in folk medicine, cuisine (e.g., harvestable herbs like coriander), as cut flowers, or for seed (Pinheiro et al., 2013; Hogg et al., 2011a).

CRediT authorship contribution statement

Nicola A. Irvin: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization. **Carly Pierce:** Investigation, Writing - original draft. **Mark S. Hoddle:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2021.104574.

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