Predator demographics and dispersal in alfalfa trap-cropped strawberry

James R. Hagler1*, Diego J. Nieto2#, Scott A. Machtley1 & Sean L. Swezey2

1United States Department of Agriculture, Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138, USA, and 2Center for Agroecology and Sustainable Food Systems, University of California, 1156 High Street, Santa Cruz, CA 95064, USA

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Abstract

Alfalfa, Medicago sativa L. (Fabaceae), is a highly preferred host plant of Lygus spp. (Hemiptera: Miridae). As such, intercropping alfalfa trap-crops in strawberry production can serve as a sink for both Lygus (primarily Lygus hesperus Knight) and its natural enemies. Here we investigated the population dynamics and dispersal characteristics of the generalist predator complex in strawberry fields with alfalfa trap-crops spaced 50 rows (62 m) apart. Predator abundance was determined by counting six focal taxa collected from strawberry and alfalfa. The data revealed that Orius spp. (Hemiptera: Anthocoridae) were the numerically dominant predator taxa, comprising 84% of the focal predator population. In general, the population densities obtained for the various taxa throughout this agroecosystem were unexpectedly uniform. Predator movement from a central alfalfa trap-crop row was determined using a protein mark–capture procedure. Most protein-marked predator specimens were collected less than 2 m from the centrally marked alfalfa row, indicating that the trap-crop often produces a predator sink. Results suggest that alfalfa is a useful cultural (trap-cropping) and a biological (refuge for natural enemies) control tactic for managing Lygus spp. in strawberries.

Introduction

California growers produce over 90% of the strawberries, Fragaria ananassa Duchesne (Rosaceae) marketed in the USA (Marzolo, 2015). The Lygus species complex – primarily Lygus hesperus Knight (Hemiptera: Miridae) – is considered a key pest of this industry (Strand, 2008). Lygus feeding on flowers and immature fruit produces distorted strawberries (Handley & Pollard, 1993). Such cosmetic damage reduces fresh market yield, which constitutes most of the economic value to California strawberry growers (CDFA, 2018).

Lygus spp. in California strawberry are commonly managed with broad-spectrum insecticides and tractor-mounted vacuums. Culturally and biologically based management of this pest has been achieved through the use of alfalfa, Medicago sativa L. (Fabaceae), trap-crops. As Lygus have a reliable host plant preference for alfalfa (Stern et al., 1964; Mueller & Stern, 1974; Goodell, 2009), integrating managed trap-crops into organic strawberry production has been shown to aggregate Lygus populations (Swezey et al., 2013) and reduce associated fresh market yield losses (Swezey et al., 2007).

Trap-cropping also affects the biological control of Lygus, as spatially concentrated prey densities in alfalfa likely influence the behavior of predaceous insects and spiders. Specifically, how generalist predators respond to an aggregated distribution of Lygus can help determine whether alfalfa acts as a sink or a source for these predators relative to adjacent organic strawberries. The goals of this study were to document the population dynamics and dispersal characteristics of predaceous arthropods inhabiting an alfalfa trap-cropped strawberry agroecosystem. Here we use conventional sampling techniques, in combination with the protein mark–capture method, to investigate the
spatial and dispersal patterns of the generalist predator complex to clarify potential sink–source dynamics.

Materials and methods

Study site
This study was conducted in 2008 and 2009 at two proximate organically certified strawberry ranches (40 and 20 ha) located near Prunedale, CA, USA. At both farms, alfalfa trap-crops were established on every 50th strawberry row, 62 m apart.

Sampling and mark–capture procedures
The resident arthropod predators in a central alfalfa row were marked with a 12% (vol/vol) solution of chicken egg whites (All Whites; Papetti Foods, Elizabeth, NJ, USA) using a gas-powered backpack sprayer (Mist Duster MD155DX; Maruyama US, Denton, TX, USA) at a rate of 1 l per 6 m of row. The mark was applied to the central alfalfa row in each of four replicated plots on 19 and 25 August in 2008 and 2009, respectively. Arthropod predators were collected from the centrally marked alfalfa trap-crop, adjacent strawberry rows 1, 2, 3, and 10, and the neighboring (unmarked) alfalfa trap-crops 50 rows away, 1 and 2 days after the protein mark was applied. Strawberry rows were sampled using a hand-held vacuum suctioning modified reverse leaf blower (model BG75; Stihl, Virginia Beach, VA, USA) fitted with a 13-cm-diameter netted intake orifice. Predator collections from trap-crops were made using a standard 30-cm-diameter sweep net. In each case, a single sample consisted of either 200 suctions or 200 sweeps per respective strawberry or alfalfa row.

Protein mark detection
Each field-collected predator was placed into a 1.6-ml microcentrifuge tube (VWR, Atlanta, GA, USA) and examined for the presence of the protein mark by the anti-chicken egg albumin enzyme-linked immunosorbent assay (ELISA) described by Hagler et al. (2014). Any predator testing positive for the presence of the mark was assumed to have originated from the centrally located, protein-marked, alfalfa trap-crop. Predators collected from the mark–capture study site were scored positive for the presence of the protein mark if their ELISA values were six standard deviations above the negative control mean value (Hagler, 2011). It should be noted that the negative control predator specimens were collected from nearby alfalfa fields prior to the application of the protein marks. Additional information regarding the farm sites, protein marking protocols, collection methods, or ELISA techniques used in this study are provided by Swezey et al. (2013, 2014).

Data presentation
The predator taxa collected from each designated sampling row in each replicated plot were pooled over the two sample dates each year. The total number of individuals collected, along with the total number of predators containing the protein mark, were tallied for each predator taxon. Only the adult stage was counted for the insect predators. However, the juvenile and adult stages of spiders and harvestmen were counted. The Orius spp. and Geocoris spp. counts were also pooled and classified as predaceous bugs, due to the small counts obtained for both species. The average distance that each predator taxon traveled from the centrally marked alfalfa trap-crop was determined by dividing the total distance traveled by protein-marked specimens by the total number of protein-marked specimens collected. The distance from the marked alfalfa to the unmarked strawberry rows (rows 1, 2, 3, and 10 in each direction) and neighboring trap-crops 50 rows away in each direction were based on global positioning system (GPS) coordinates.

Results

Orius
The Orius spp. complex (Hemiptera: Anthocoridae), composed almost entirely of Orius tristicolor (white), were the most abundant predator taxa encountered. There were over 2.5× as many Orius adults collected in 2008 (n = 4,888) than in 2009 (n = 1,777). The Orius populations were uniformly distributed throughout the study site each year (Figure 1A). The marking efficiency for Orius (i.e., the percentage of marked individuals collected from the central-marked alfalfa trap-crop) was 64.5% for 2008 (458 out of 710 individuals) and 38.7% for 2009 (46 out of 119) (Figure 1A). Most (73%) of the protein-marked Orius were collected from the centrally marked row of alfalfa. All remaining protein-marked specimens were captured within three strawberry rows of the centrally marked trap-crop. As such, the average dispersal distance for protein-marked Orius was only 0.92 m in 2008 and 0.87 m in 2009.

Other predaceous bugs
Adult Nabis spp. – primarily Nabis alternatus Parshley (Hemiptera: Nabidae) – and Geocoris spp. – primarily Geocoris punctipes (Say) (Hemiptera: Geocoridae) – were the next most abundant true bug predator taxon encountered at the study sites, albeit at relatively low densities. These true bugs were about twice as dense in 2009 (n = 91) as in 2008 (n = 52). The marking efficiency for these true bugs was 83.3% in 2008 (Figure 1B). However, it should be
stipulated that this outcome was derived from a small sample size (i.e., five of the six samples were marked). There were no marking efficiency data available in 2009 due to a lack of collected true bugs from the centrally marked trap-crop. Nevertheless, the relatively high proportion of these protein-marked true bugs collected from the nearby strawberry rows suggests that we had a well-marked population of true bugs originating from the protein-marked trap-crop. The average dispersal distance, based on small sample sizes, was only 0.35 m in 2008 and 1.96 m in 2009.

Lacewings
The adult lacewings (Neuroptera) collected at the study sites included both green *Chrysoperla carnea* (Stephens) s.l., Chrysopidae) and brown lacewings (*Hemerobius* spp., Hemerobiidae). Total lacewing abundance was over 3× greater in 2009 (n = 133) than in 2008 (n = 40) (Figure 1C). In 2008, *C. carnea* comprised about 90% of collected lacewings. Conversely, in 2009 *Hemerobius* spp. made up about 86% of the lacewing community. Regarding collection location, lacewings (mostly *C. carnea*) were most prevalent in the alfalfa trap-crop rows in 2008 (Figure 1C). Yet in 2009, lacewings (mostly *Hemerobius* spp.) were more prevalent and uniformly distributed throughout the strawberry rows. The marking efficiency for lacewings, albeit based on an extremely small sample size (n = 4 for both years combined), was 100%. Only two protein-marked lacewings were captured beyond the centrally marked alfalfa in 2008. Of these, one had dispersed 62 m into an adjacent alfalfa trap-crop. As such, the calculated average movement of protein-marked lacewings (n = 6) in 2008 was 11.4 m. In 2009, there were more of both unmarked and marked lacewings that were uniformly collected in all the strawberry rows. The average dispersal distance for lacewings in 2009 was 4.2 m from the centrally marked alfalfa row.
Lady beetles
The lady beetle species complex (Coleoptera: Coccinellidae) inhabiting the trap-cropped strawberry farms consisted primarily of *Hippodamia convergens* Guérin-Méneville (83% of coccinelids). The remaining lady beetle taxa consisted of *Coccinella californica* (Mannerheim) (12.3%) and *Coccinella septempunctata* L. (4.7%). The beetle population was 6.5× higher in 2008 (n = 130 specimens collected) than in 2009 (n = 20). Almost all the specimens were collected from the alfalfa trap-crop rows (Figure 1D). The efficiency of the marking procedure was 78.6% in 2008 (n = 22 of the 28) and 100% in 2009 (n = 5). All protein-marked beetles were captured in trap-crops. In 2008, 13 of the 89 beetles (14.6%) collected from the eastern-most alfalfa trap-crop contained the protein mark. This yielded a relatively long average dispersal distance of 23.2 m. We suspect this unidirectional movement could be attributed to the prevailing wind patterns typically exhibited at the study sites. Conversely, protein-marked lady beetle specimens were only from the centrally marked alfalfa trap-crop row in 2009, and therefore had an average dispersal of 0 m.

Spiders
Collected spiders (Araneae) included various species within the families Lycosidae (60.5% of the arachnids), Salticidae (22.2%), and Thomisidae (17.3%). These hunting spiders were about 1.5× more abundant in 2008 (n = 169) than in 2009 (n = 115). Spider abundance was much higher in the strawberry rows (Figure 1E), as no spiders were collected in the centrally marked alfalfa in 2008, and only five were captured in 2009. Of these five, three (60% marking efficiency) contained the protein mark. Overall, only 10 protein-marked spiders were collected in 2008 and 2009. All 10 were captured within two rows of the centrally marked alfalfa. The average dispersal distance rate of the protein-marked spiders, albeit based on small sample sizes, was 1.4 m in 2008 (n = 1) and 0.96 m (n = 9) in 2009.

Harvestmen
Harvestmen (Opiliones: *Phalangium*) were present in relatively high numbers. There were over 3× as many collected (n = 410) in 2008 than in 2009 (n = 121). Harvestmen were more abundant in the strawberries than in the alfalfa (Figure 1F). Marking efficiency data for the 11 captured harvestmen in 2008 was 63.6% (n = 7). No harvestmen were collected in 2009 from the centrally marked alfalfa. As protein-marked harvestmen were frequently collected in the three strawberry rows nearest to the protein-marked alfalfa both years of the study, their average dispersal distance beyond the centrally marked trap-crop was 1.9 m in 2008 and 1.8 m in 2009.

Discussion
Predator census data for both the alfalfa trap-cropped strawberries reported here and by Hagler et al. (2018a) showed that *Orius* was the most numerically dominant predator taxon, comprising 86 and 79% of the focal arthropod fauna reported in 2008 and 2009, respectively. *Orius*, along with the other taxa collected in this study (e.g., heteropterans, coccinellids, and spiders), have been documented consuming *Lygus* in cotton (Hagler, 2011) and/or strawberry (Hagler et al., 2018a) by predator gut analyses. As generalist predators are capable of regulating *Lygus* populations in agroecosystems (Zink & Rosenheim, 2008), developing strategies that maximize predation services should be prioritized. For instance, gaining a better understanding of how habitats with high prey densities (i.e., trap-crops) affect predator movement can clarify predator source/sink dynamics and thereby inform conservation biological control strategies (Lavandero et al., 2004).

The protein mark–capture technique proved to be useful for uniquely tagging the predators inhabiting the central alfalfa rows. Overall, 62% (n = 550 out of 888) of all the focal predators captured from the centrally marked alfalfa contained the protein mark. Moreover, the protein mark–capture data revealed that relatively few predators captured beyond the centrally marked alfalfa trap-crop (n = 361 out of 7 058) were marked (5.1%). As such, the average dispersal of protein-marked arthropods within 48 h was less than 2.0 m, which equates to an average of about 1.5 rows from the centrally marked trap-crop. This low average dispersal rate suggests that the alfalfa trap-crop retains the predators within, or adjacent to, the trap-crop. These data are similar to the dispersal results reported for *Lygus* and the *Lygus*-specific parasitoid, *Peristenus relictus* (Ruthe), by Swezey et al. (2013, 2014).

More specifically, *Orius* were largely retained by alfalfa, as roughly 75% of protein-marked minute pirate bugs were collected from the central trap-crop, whereas the remaining 25% were collected within three adjacent strawberry rows on either side of the marked alfalfa. As a result, the average dispersal of protein-marked *Orius* was less than one row from the central trap-crop. *Orius* feed on *Lygus* eggs (Hagler et al., 1992) and small nymphs (Zink & Rosenheim, 2008), particularly in alfalfa trap-crops (Hagler et al., 2018a). Additional feeding opportunities (e.g., pollen, thrips, aphids, etc.) further bolster the prey reservoir provided by alfalfa, which,
in turn, creates a sink effect for Orius within strawberry fields.

In this study, alfalfa also created a strong sink for coccinellids, as 97% (n = 145 out of 150) of all the lady beetles collected over the course of the study were obtained from the alfalfa trap-crop rows. Hippodamia convergens has been shown to consume Lygus nymphs in cotton (Hagler, 2011), and it is possible that beetle retention in alfalfa was influenced by the high Lygus prey densities found in trap-crops. However, in this case, it seems more likely that coccinellid fidelity to alfalfa was instead driven by the moderately sized aphid populations (e.g., Acyrthosiphon pisum Harris) that were found in trap-crops, but not strawberry. The converse can also occur, however, where high aphid densities in strawberry (e.g., Chaetosiphon fragaefolii Huber) can produce a robust response by coccinellids in strawberry, but not alfalfa (DJ Nieto, pers. obs.). Bastola et al. (2014) similarly reported that although alfalfa acted as a sink for H. convergens (relative to cotton), bidirectional lady beetle movement between these two crops was common and influenced by relative prey availability.

Conversely, alfalfa acted as a source of some predators during this study that dispersed from trap-crops, into neighboring strawberry rows. For example, protein-marked Hemerobius sp. were captured from every sampled strawberry row in 2009, including row 10 to the east and west of the central trap-crop. This may have been in response to relatively high Lygus nymphal densities in associated strawberry rows and increased management (i.e., vacuuming) frequency in the alfalfa trap-crop. Hemerobius sp. are commonly recognized as predators of hemipterans, such as aphids (Neuenschwander & Hagen, 1980) and psyllids (Nickel et al., 1965). However, as part of the predation study conducted by Hagler et al. (2018a), field-collected Hemerobius sp. were shown to feed on Lygus as well (DJ Nieto, unpubl.). Specifically, 33.3 and 4.5% of assayed Hemerobius sp. collected from alfalfa (n = 9) and strawberry (n = 44) contained Lygus remains.

For other predators, the sink/source dynamics of trap-cropping were ambiguous, as differences in predator counts (abundance) between the alfalfa trap and strawberry rows were generally unremarkable and predator movement patterns were counterintuitive. For example, in 2009, protein-marked harvestman and Geocoris-plus-Nabis were collected from five of six adjacent strawberry rows; yet, curiously, none were recovered from the marked trap-crop.

A reassessment of our sampling methodology may clarify these discrepancies: As discussed in detail by Hagler et al. (2018b), differences in sampling efficacy between alfalfa (sweep netting) and strawberry (hand-held vacuum suctioning) likely obscured some predator-dispersal behavioral patterns pertaining to trap-cropping. For instance, the majority of a strawberry plant’s vertical canopy is sampled using the hand-held vacuum device. Conversely, only the upper third of the alfalfa canopy is sampled with the sweep net. Sweeping therefore likely led to underestimated predator abundance data for those taxa that are evenly distributed throughout the alfalfa canopy. Furthermore, sweep netting would severely underestimate predator abundance of taxa (i.e., some true bug and spider taxa) that preferentially inhabit the lower portion of the alfalfa canopy (Crocker & Whitcomb, 1980; Nyffeler & Benz, 1988; Fasola & Mogavero, 1995). If predator counts in alfalfa were in fact underestimated, then potential alfalfa sink effects would be masked and, instead, could be falsely interpreted as either having no discernable effect on predator movement or as constituting a source of predators into strawberry. Future studies that address potential sampling biases based on the vertical distribution of predators within the alfalfa canopy (e.g., whole plant sampling) are therefore needed.

In summary, a predator population census is discussed and included six primary taxa. Overall, Orius was the numerically dominant predator taxon encountered. The dispersal pattern of the predator complex inhabiting alfalfa trap-cropped strawberry fields is also described. The protein mark-capture method effectively marked over 60% of the resident arthropod fauna inhabiting centrally located alfalfa trap-crops. The recapture of protein-marked predators beyond the centrally marked alfalfa trap-crop was rare. When discernable, these data suggest that the predator population was largely retained in the alfalfa trap-crop. It is likely that the sinking of predators into the preferred host plant improves the biological control services rendered by the Lygus natural enemy complex.

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