Dispersion, Distribution, and Movement of *Lygus* spp. (Hemiptera: Miridae) in Trap-Cropped Organic Strawberries

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ABSTRACT Alfalfa (*Medicago sativa* L.) is a highly attractive plant host to *Lygus* spp. and is used as a trap crop in California organic strawberries to influence the dispersion and dispersal of these pests, particularly *Lygus hesperus* Knight. The abundance and distribution of *Lygus* spp. nymphs between two trap crops separated by 50 strawberry rows was analyzed in 2008 and 2010. Nymphs demonstrated a bimodal distribution in strawberries between trap crops, where nymphs were most abundant and aggregated in alfalfa, when compared with interior strawberry rows, where nymphs were less abundant. The majority of nymphs were concentrated in trap crops and nymphal densities in interior strawberry rows were well below economic thresholds. The movement of *Lygus* spp. from a marked alfalfa trap crop into adjacent strawberry rows or trap crops was also studied in 2008 and 2009 using a chicken egg albumin enzyme-linked immunosorbent assay mark-capture technique. The majority of marked-captured *L. hesperus* adults and *Lygus* spp. nymphs remained in alfalfa trap crops, rather than dispersing out into strawberry rows at 24 h, 48 h, and 2 wk, postprotein application. The attenuation of *Lygus* spp. movement in alfalfa associated with organic strawberries is a key component of successful trap cropping. A small percentage of marked adults and nymphs were captured in neighboring alfalfa trap crops, located 62 m from the point of protein application, highlighting the dispersal capacity of this key pest.

KEY WORDS organic, strawberry, *Lygus hesperus*, alfalfa trap crop, ELISA

California produced 89% of all commercial strawberries (*Fragaria ananasa* Duchesne) grown in the United States in 2009 (California Department of Food and Agriculture [CDFA] 2011). In the same year, California organic strawberry growers claimed a sales value of US$55.1 million on 884 ha (Klonsky and Richter 2011). This comprised 4.6% of California's total strawberry hectares (California Strawberry Commission [CSC] 2011). From 2005–2009, the declared value of California organic strawberries more than doubled and registered planted hectares increased by over 60% (Klonsky and Richter 2011). In 2011, the Salinas–Watsonville strawberry growing region, located in Monterey and Santa Cruz Counties, accounted for 42% of all fall-planted conventional strawberry hectares and 74% of organic fall-planted strawberry hectares in California (CSC 2011).

Lygus spp. in the Monterey Bay region are composed primarily of *Lygus hesperus* Knight (Hemiptera: Miridae) or western tarnished plant bug, but also include the less abundant *Lygus shulli* (Pickett et al. 2009). These polyphagous plant bugs distort strawberry fruit by feeding on, and destroying, immature embryos located inside achenes, thereby preventing proper development of the surrounding fruit tissue (Handley and Pollard 1993). Consequently, *Lygus* spp. are the key cosmetic strawberry pests in the Central Coast production region (Zalom et al. 2012).

Pest distributions that are aggregated in monocultures often generate greater crop losses than pest populations that are uniformly or randomly distributed (Hughes and McKinlay 1988). However, in diversified cropping systems, potentially aggregated populations can be purposefully attracted to a preferred plant host with little or no harvest value, thereby reducing their damage potential. This aggregation also creates an opportunity for spatial targeting, which efficiently focuses treatments to pest clusters (Weisz et al. 1995, Sylvester–Bradley et al. 1999).

On the central coast of California, vacuumed alfalfa (*Medicago sativa* L.) trap crops are interplanted with (primarily organic) strawberries to control *Lygus* spp. A trap crop is an economically manageable preferred host of a key pest intercropped with a more valuable commercial crop. Swezey et al. (2007) demonstrated

that western tarnished plant bug adults and nymphs were much more abundant in alfalfa when compared with nearby strawberries, and that strawberry damage associated with western tarnished plant bug feeding was reduced in nonvacuumed strawberries interplanted every 50 rows with vacuumed alfalfa. The use of trap cropping in organic strawberries in the Salinas–Watsonville growing region is increasing. We estimate that in this region, ≈27% of certified organic strawberry hectares were planted with alfalfa trap crops in 2011.

How alfalfa trap crops affect the dispersion of *Lygus* spp. in the 50 strawberry rows located in between two trap crops has not been documented. For example, in strawberry rows equidistant from two trap crops, *Lygus* spp. nymphal abundance could be highest away from the trap crops (bell-shaped pattern), abundance could be lowest away from the trap crops (U-shaped or bimodal pattern) or nymphal abundance may be random (straight line or no pattern). These patterns could in turn produce clumped, uniform, or random pest distributions, which would affect potential yield losses in strawberries. Documenting pest dispersion patterns in this system would complement our current understanding of arrested movement by *Lygus* spp. in polycultures (Sevacherian and Stern 1975, Bancroft 2005).

The strong attraction to alfalfa shown by western tarnished plant bug may not only influence distribution, but also physical movement (dispersal). Blackmer et al. (2004) and Williams et al. (2010) have documented the basis of attraction by identifying volatile plant odors in alfalfa that are highly attractive to western tarnished plant bug females, which could affect movement. For instance, western tarnished plant bug was historically observed moving to an alfalfa trap crop, when compared with the less-preferred cotton crop (Sevacherian and Stern 1975). Once in the trap crop, *Lygus* spp. movement may also be attenuated, such that movements from the trap crop into a strawberry crop would be delayed or less likely (Potting et al. 2005). Arrestment behavior of *L. hesperus* and *L. rugulipennis* has been previously demonstrated in alfalfa and scentless mayweed, *Tripleurospermum inodorum* (L.), respectively (Sevacherian and Stern 1975, Hannunen and Ekbom 2001).

The manner in which *Lygus* spp. move and are distributed in trap-cropped strawberry systems influences their potential to cause economic damage. High spatial concentrations of *Lygus* spp. in alfalfa trap crops are useful in developing effective and targeted management programs (Swezey et al. 2007). Removal efforts (via tractor-mounted vacuum) that can focus on aggregated *Lygus* spp. distribution patterns in the trap crop area are thereby more efficient.

We hypothesized that strips of alfalfa intercropped with organic strawberries affect *Lygus* spp. spatial pattern development, distribution type and movement. We tested the following: 1) *Lygus* spp. nymphal abundance adopts a bimodal distribution between trap crops, where abundance is greatest in alfalfa; 2) *Lygus* spp. movement is not random in trap-cropped straw-

berry fields; and 3) when *Lygus* spp. movement out of trap crops does occur, it is mostly limited to the strawberry rows in closest proximity to alfalfa.

Materials and Methods

Experimental Sites. This study was conducted from 2008 to 2010 at two certified organic strawberry farms in Prunedale, CA. In 2008 and 2010, experiments were performed at the 40 ha Eagle Tree Farm. In 2009, a marking experiment was conducted at the adjacent 20 ha Deadwood Farm. These farms were registered and certified under the provisions of the California Organic Products Act of 2003 (the California regulatory and enforcement mechanism for the federal Organic Food Production Act of 1990), and inspected and certified by an accredited third party certifier (Quality Assurance International, Inc., San Diego, CA).

Alfalfa trap crop rows were established in both study areas, one every 50 strawberry rows, comprising 2.0% of the farm's total area. In late October of each sample year, Ameristand 802 or Ameristand 901 nondormant alfalfa varieties were planted by hand in single-row beds as bare-root strawberry crowns were also being planted in adjacent beds. Alfalfa and first-year 'Albion' strawberry beds were planted on 122 cm centers, were 46 cm wide, and 30 cm tall, and were drip irrigated and fertilized with a single subsurface drip tape.

Between May and June of each experimental year, the collaborating grower began treating the alfalfa, strawberries, or both with a 65 hp tractor, mounted with three rectangular vacuum collector inlets (15 by 61 cm) as described in Swezey et al. 2007. Typically, trap crops (and two adjacent strawberry rows) were vacuumed once every 7–10 d from June through September. All other strawberry rows were infrequently vacuumed by the grower and only when necessary.

***Lygus* spp. Nymph Dispersion.** Dispersion is the spatial arrangement of individuals, in this case based on density per unit measure. To determine the in-field spatial patterns of *Lygus* spp. nymphs in strawberries interplanted with alfalfa trap crops, transects were established to record nymphal densities at various distances from a trap crop in 2008 and 2010. Transect plots of first year strawberry plants were 0.28 ha in area. Each of four replicated transect plots consisted of two alfalfa trap crops separated by 49 rows of strawberries. Strawberry rows 1, 12, and 25 were selected based on their respective position relative to the nearest trap crop (adjacent, near, or equidistant). Five strawberry rows and two trap crop rows were sampled per replicate. Each row measured ≈46 m in length. Adjacent plots were separated by a 4 m farm road.

Lygus spp. nymphs were collected using a handheld vacuum suction device (modified reversed Stihl BG75 leaf blower) fitted with a 13 cm insect-netted intake orifice. A sample consisting of 200 one-second suction points directed at flowers was taken from a continuous line walked along a trap crop or strawberry row. We made the assumption that timed suction collections on alfalfa and strawberry

flowers were comparable. Samples were collected approximately every 2 wk from June to October to record nymphal abundance, totaling nine sample dates per year.

Mean nymphal abundance was pooled over sample dates within sample years and compared by row using a randomized complete block analysis of variance (ANOVA) model. To meet assumptions of normality, nymphal counts were analyzed using a log $(n + 1)$ transformation for 2008 data. Nymphal data from 2010 were ranked after failing to meet assumptions of normality. All post hoc multiple comparisons were done using Tukey's Studentized Range (honestly significant difference [HSD]) Test (SPSS Inc. 2010).

***Lygus* spp. Nymph Distribution.** A distribution is a mathematical description of a dispersion pattern. To test the normality of *Lygus* spp. nymphal distributions, a χ^2 goodness-of-fit test was used to compare observed and expected frequencies based on insect counts. To identify nymphal distribution types in years where observed and expected frequencies were significantly different, Lloyd's Index of Patchiness (LIP) was used. LIP values of <1, 1, and >1 indicate poisson, normal, and aggregated distributions, respectively. If LIP was >1, the degree of nymphal aggregation was determined using a negative binomial K using Ruesink (1980), such that smaller K values represent a greater degree of aggregation. Nymphal density data were pooled over all alfalfa and strawberry sample locations and dates.

***Lygus* spp. Movement.** A protein mark-capture technique (Hagler 1997a, b) was used to determine the within-field movement patterns of western tarnished plant bug adults and *Lygus* spp. nymphs in strawberry fields with alfalfa trap crops in August of 2008 and 2009. Alfalfa trap crops were sprayed with a 12% chicken egg-albumin solution, as described by Hagler and Jones (2010). Applications were made using a gas-powered backpack sprayer (Mist Duster MD155DX, Maruyama U.S., Inc., Denton, TX) at a rate of 1 liter/6 m.

In 2008, protein sprays were applied on 19 August to the center alfalfa trap crop of four block replicates. Insects can be marked either directly, via protein application (i.e., spraying), or indirectly, through subsequent contact (i.e., walking or feeding) with marked vegetation (Hagler and Jones 2010). *Lygus* spp. were collected on 20 August, 21 August, and 9 September. In 2009, protein sprays were similarly applied on 25 August and *Lygus* spp. were collected on 26 August, 27 August, and 9 September. Replicates were ≈46 m in length and were separated by a 4 m farm road. Samples were taken from the adjacent strawberry rows and alfalfa trap crops of each replicate (Table 1).

Lygus spp. were collected from strawberries using a hand-held vacuum suction device (as described above) to generate 200-suction samples from each strawberry row. Sweep nets were used to collect *Lygus* spp. from the entire length of each trap crop (200 sweeps per sample). The comparability of these two sampling methods in collecting mirids on the same plant has been verified (Byerly et al. 1978, Buffington

Table 1. Experimental design for protein mark-capture study

Sample location	No. rows from center trap crop	Mean distance from center trap crop (m)	Direction
Alfalfa trap crop	50	62.30	West
Strawberry row 10	10	12.24	West
Strawberry row 3	3	3.85	West
Strawberry row 2	2	2.60	West
Strawberry row 1	1	1.40	West
Alfalfa trap crop (marked)	0	0	Center
Strawberry row 1	1	1.23	East
Strawberry row 2	2	2.37	East
Strawberry row 3	3	3.57	East
Strawberry row 10	10	12.30	East
Alfalfa trap crop	50	62.58	East

Four replicates were marked as shown. Egg albumin protein sprays were applied to center alfalfa trap crop in 2008. Insects were then collected 24 h, 48 h, and 2 wk postapplication from Eagle Tree Farm, in Prunedale, CA.

and Redak 1998, Doxon et al. 2011). However, to our knowledge, no published studies have dealt with interplant sampling comparability in strawberries. Collection nets (both sweep and suction) were machine-washed before each sample date and were only used once per sample location to minimize the likelihood of contamination. After each sample was collected, insects were put in Ziploc bags (S. C. Johnson and Son, Inc., Racine, WI) and were immediately placed on dry ice to avoid in-bag mark contamination via insect movement. While unlikely, there is a small chance (≈1%) of obtaining a false positive reaction because of surface contact either in a net during collection or in a plastic bag before immobilization (J.R.H., unpublished data). Samples were then stored in a freezer (−80°C) until individual adults and nymphs were sorted and separated.

Each western tarnished plant bug adult or *Lygus* spp. nymph was then examined for the presence of the marker at the U.S. Department of Agriculture Laboratory in Maricopa, AZ, using an anti-chicken egg albumin enzyme-linked immunosorbent assay (ELISA) described by Jones et al. (2006). If a field-collected lygus bug scored positive by ELISA for the presence of egg albumin, we assumed that it originated from the sprayed center trap crop.

Before the alfalfa trap crops were marked with protein, insects serving as negative controls were collected and assayed for the presence of chicken egg albumin as described above. Mean (±SD) absorbance values were calculated for the negative controls; post-spray field-collected insects were scored positive for a protein if the absorbance value was 6 SDs above the negative control mean (Hagler 2011, Slosky et al. 2012). It should be noted that we used this very conservative critical threshold value (e.g., most studies to date have used 3 SD; Jasrotia and Ben–Yakir 2006, Baker et al. 2007, Buczkowski and Bennett 2007) to reduce likelihood of falsely scoring a bug positive for the presence of the mark. Insects serving as positive controls were collected from the marked alfalfa shortly after the marker had been applied. These in-

Table 2. Randomized complete block design ANOVA testing differences in *Lygus* spp. nymph abundance between all rows (alfalfa trap crops and strawberry rows 1–25)

	df	MS	F	p
2008[a]				
Treatment	6	4.188	38.359	0.006
Block	3	0.833	7.630	0.002
Treatment × block	18	0.109	0.893	0.588
Error	215	0.122		
2010[b]				
Treatment	6	111,498.836	70.933	0.003
Block	3	14,268.074	9.077	0.001
Treatment × block	18	1,571.882	0.607	0.893
Error	224	2,590.303		

[a] Log transformation used after not meeting normality.
[b] Data ranked after not meeting normality.

sects ($n = 25$ per species) were assayed by ELISA to determine the proportion of marked insects at the time the study was initiated. Mean protein-marked *Lygus* spp. abundance by plant (alfalfa vs. strawberries) was compared using the nonparametric Kruskal–Wallis test after data would not meet assumptions of normality.

Average Distance Traveled. The average distances traveled by *Lygus* spp. from marked alfalfa to unmarked strawberry rows or neighboring trap crops were based on global positioning system (GPS) coordinates recorded on 19 August 2008, before protein application. Measurements were taken between the center of marked trap crops and the centers of adjacent strawberry beds and neighboring alfalfa. Between-row coordinates established in 2008 were used to generate distance traveled averages during both sample years. Marking data from 2008 to 2009 were pooled to establish mean distance-traveled estimates from a central marked trap crop to adjacent strawberry rows. All analyses were performed on SPSS 18 (SPSS Inc. 2010).

Results

Lygus spp. Nymph Dispersion. *Lygus* spp. nymphs were not evenly distributed in trap-cropped strawberries. There were significant differences in nymphal abundance by row in 2008 and 2010 (Table 2). In 2008, nymphs were significantly more abundant in both alfalfa trap crops than in all sampled strawberry rows (Fig. 1). Nymphs were eight times more abundant in these trap crops, relative to interior strawberry rows (12 and 25). *Lygus* spp. nymphs were also significantly more abundant in the eastern row 1 when compared with rows 12 and 25. Of the 4,193 nymphs collected in 2008, 63% were collected from alfalfa, 24% were collected from row 1, and 13% were collected from rows 12 and 25.

In 2010, *Lygus* spp. nymphal abundance in alfalfa trap crops was significantly greater than in all sampled strawberry rows, except for eastern row 1 (Fig. 1). Nymphs were 12 times more abundant in these trap crops, relative to interior strawberry rows (12 and 25). Nymphs were also significantly more abundant in both
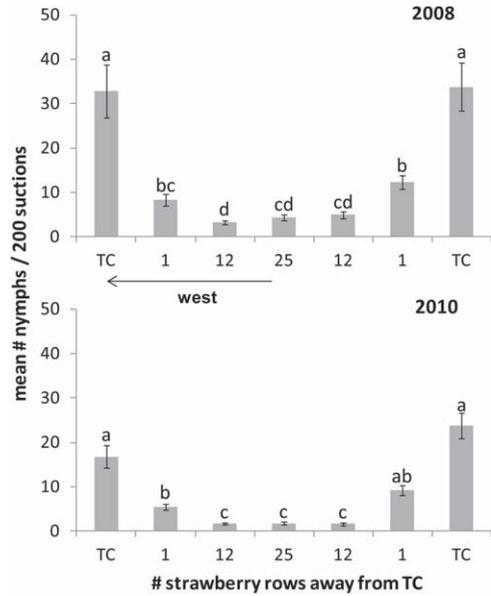

Fig. 1.   Mean *Lygus* spp. nymph abundance in alfalfa trap crops (TC) and adjacent strawberry rows, numbered based on proximity to the closest trap crop. The left half of all three graphs represents west-facing upwind sample locations, while the right half represents east-facing downwind locations. Data collected from June through October in 2008 and 2010 at Eagle Tree organic strawberry farm, Prunedale, CA. Different letters represent significant differences (ANOVA; $P < 0.05$) between rows within a sample year.

row 1 sample locations, when compared with rows 12 and 25. Of the 2,169 nymphs collected in 2010, 67% were collected from a trap crop, 24% were collected from row 1, and 8% were collected from rows 12 and 25.

Lygus spp. Nymph Distribution. *Lygus* spp. nymphs in trap-cropped strawberries were not normally distributed. Observed nymphal frequencies did not fit expected χ^2 goodness-of-fit distributions in 2008 ($\chi^2 = 117.29$; df = 15; $P < 0.001$) and 2010 ($\chi^2 = 120.29$; df = 12; $P < 0.001$). Using a calculated LIP, nymphal distributions showed a high degree of aggregation in trap-cropped strawberries in 2008 (2.21) and 2010 (2.37). Similarly, calculated negative binomial K values also demonstrated *Lygus* spp. nymph aggregation in trap-cropped strawberries: K values for 2008 and 2010 were 1.04 and 0.81, respectively. The nymphal variance exceeded the mean in all 20 sample dates during this study. When K values were regressed against sample means, there were no significant relationships in 2008 (slope = -0.025; $r^2 = 0.29$; $P = 0.109$). However, in 2010, there was a significantly negative linear relationship (slope = -0.101; $r^2 = 0.66$; $P = 0.014$) between nymphal densities and K.

Lygus spp. Nymph Movement. Of the 810 *Lygus* spp. nymphs collected in 2008, 244 (30.1%) had an egg albumin critical threshold optical density value exceeding 0.065, which was six times above the variation of the negative control lygus specimens. Captured
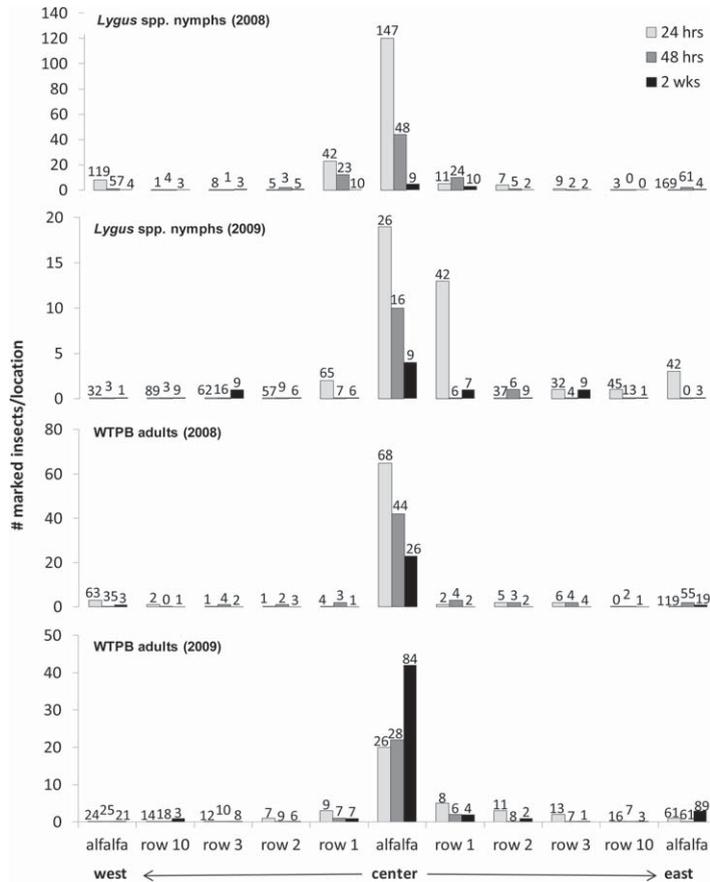
Fig. 2. Total number of *Lygus* spp. nymphs and western tarnished plant bug (WTPB) adults recovered with an egg albumin mark per time interval at each location. WTPB adults and *Lygus* spp. nymphs were collected from center (marked) alfalfa trap crops, (strawberry) rows 1, 2, 3, and 10 adjacent to the marked location, east/west alfalfa trap crops (50 rows from center trap crops). Numbers above data points refer to sample size (*n*) of all specimens collected from a given location per sample date. Data collected in 2008 and 2009 from Eagle Tree Farm and Deadwood Farm, respectively, in Prunedale, CA.

Lygus spp. nymphs with a protein mark were also significantly more abundant in the central marked trap crop, when compared with all combined adjacent strawberry rows (Kruskal–Wallis; $n = 108$; df $= 1$; $\chi^2 = 24.92$; $P < 0.001$) (Fig. 2). Of the 161 *Lygus* spp. nymphs captured with a protein mark 24 h after the egg albumin was applied, 74.5% were from the central marked trap crop, 17.4% were taken from row 1, 3.1% were from rows 2 and 3, none were found in row 10 and 5.0% were from east/west trap crops. Of the 72 *Lygus* spp. nymphs collected with an egg albumin mark 48 h after application, 61.1% were from the central marked trap crop, 30.5% were taken from row 1, 4.2% were from row 2, none were found in rows 3 and 10, and 4.2% were from east/west trap crops. Of the 11 nymphs captured with a protein mark 2 wk after the egg albumin was applied, five were from the central marked trap crop, three were taken from row 1, two were from rows 2 and 3, none were found in row 10, and one was from east/west trap crops.

Of the 683 *Lygus* spp. nymphs collected in 2009, 57 (8.3%) had an egg albumin critical threshold optical density value exceeding 0.065. Captured *Lygus* spp. nymphs with a protein mark were also significantly more abundant in the central marked trap crop, when compared with all combined adjacent strawberry rows (Kruskal–Wallis; $n = 108$; df $= 1$; $\chi^2 = 33.08$; $P < 0.001$) (Fig. 2). Of the 35 *Lygus* spp. nymphs captured with a protein mark 24 h after the egg albumin was applied, 48.7% were from the central marked trap crop, 38.5% were taken from row 1, 5.1% from strawberry rows 3 and 10, and 7.7% were from east/west trap crops. Of the 11 *Lygus* spp. nymphs collected with an egg albumin mark 48 h after application, 90.9% were from the central marked trap crop and 9.1% were from strawberry row 2. Of the seven nymphs captured with a protein mark 2 wk after the egg albumin was applied, four were from the central marked trap crop, one was from in strawberry row 1, and two were found in strawberry row 3. No nymphs were found in strawberry row 10 or east/west alfalfa.

Western Tarnished Plant Bug Adult Movement. Of the 532 western tarnished plant bug adults collected in 2008, 154 (28.9%) had an egg-albumin critical thresh-

old optical density value exceeding 0.065. Captured western tarnished plant bug adults with a protein mark were significantly more abundant in the central marked trap crop, when compared with all combined adjacent strawberry rows (Kruskal–Wallis; $n = 108$; df = 1; $\chi^2 = 44.82$; $P < 0.001$) (Fig. 2). Of the 74 western tarnished plant bug adults captured with a protein mark 24 h after the egg albumin was applied, 87.8% were from the central marked trap crop, 8.1% were from strawberry rows 1–3 and 10, and 4.1% were from east/west trap crops. Of the 55 western tarnished plant bug adults collected with an egg albumin mark 48 h after application, 76.4% were from the central marked trap crop, 20.0% were from strawberry rows 1–3, none were found in row 10, and 3.6% were from distant trap crops. Of the 25 adults captured with a protein mark 2 wk after the egg albumin was applied, 92.0% were from the central marked trap crop and 8.0% were from east/west trap crops.

Of the 617 western tarnished plant bug adults collected in 2009, 110 (17.8%) had an egg albumin critical threshold optical density value exceeding 0.065. Captured western tarnished plant bug adults with a protein mark were significantly more abundant in the central marked trap crop, when compared with all combined adjacent strawberry rows (Kruskal–Wallis; $n = 108$; df = 1; $\chi^2 = 33.53$; $P < 0.001$) (Fig. 2). Of the 35 western tarnished plant bug adults captured with a protein mark 24 h after the egg albumin was applied, 57.1% were from the central marked trap crop, 40.0% were from strawberry rows 1–3, none were found in row 10, and 2.9% were from east/west trap crops. Of the 25 western tarnished plant bug adults collected with an egg albumin mark 48 h after application, 88.0% were from the central marked trap crop and 12.0% were from strawberry row 1. Of the 50 adults captured with a protein mark 2 wk after the egg albumin was applied, 84.0% were from the central marked trap crop, 10.0% were from strawberry rows 1, and 10 and 6% were from east/west trap crops.

Average Distance Traveled. The average distance traveled by western tarnished plant bug adults from marked alfalfa into strawberries was 0.48 ± 0.14, 0.38 ± 0.10, and 0.27 ± 0.18 m at 24 h, 48 h, and 2 wk after protein application, respectively. *Lygus* spp. nymphs traveled an average distance into strawberry rows of 0.46 ± 0.08, 0.52 ± 0.09, and 1.10 ± 0.35 m at 24 h, 48 h, and 2 wk post protein-application, respectively.

Discussion

In 9 of 10 comparisons during 2008 and 2010, *Lygus* spp. nymphs were significantly more abundant in alfalfa trap crops, when compared with strawberry rows. This result illustrates nymphal attraction for alfalfa described in Blackmer and Cañas (2005). The concentration of *Lygus* spp. in alfalfa trap crops also demonstrates relative plant host preference over commercially relevant crops, such as cotton (Sevacherian and Stern 1975) and strawberry (Swezey et al. 2007).

Lygus spp. nymphs in strawberry rows between alfalfa trap crops displayed a U-shape or bimodal spatial pattern in 2008 and 2010. Three of four strawberry row 1 sample locations during this time had significantly greater nymphal abundance when compared with interior rows (12 and 25). Of the total collected nymphs in 2008 and 2010, 87 and 91% were concentrated in alfalfa and strawberry row 1, respectively. These data indicate that ≈90% of the *Lygus* spp. nymph population was concentrated in only 6% of total acreage of the study site (one row of alfalfa and two adjacent strawberry rows). This phenomenon lends itself to selectively targeting segments of a field for management. This efficiency is further improved by the compatibility of common tractor configurations (three vacuuming hoods) to this three-row area, so that these three rows can be vacuumed simultaneously during a single pass.

The bottom of this spatial "U" was encompassed by low nymphal abundance in a sample representing 26 interior strawberry rows. Interior strawberry rows (rows 12 and 25) averaged 4.20 and 1.70 nymphs per 200 suctions in 2008 and 2010, respectively. As economic thresholds are often set at one nymph per 10 suctions (Zalom et al. 2012), these nymphal densities per 10 suctions (0.21 nymphs in 2008 and 0.09 nymphs in 2010) fell well below this threshold. Consequently, pest management costs incurred by the grower were greatly reduced in this portion of the field: interior rows required vacuuming approximately once every 2 wk in 2008 and only three times in all of 2010.

Trap crops can generate aggregated herbivore distributions (Potting et al. 2005, Shelton and Badenes–Perez 2006). In trap-cropped strawberry fields, *Lygus* spp. nymphs demonstrated a highly aggregated distribution. The K of the negative binomial of *Lygus* spp. nymphs in strawberries and alfalfa during 2008 and 2010 was 1.0 and 0.8, respectively, compared with *Lygus* spp. nymph K values in cotton (4.3) and lentils (4.9) (Sevacherian and Stern 1972, Schotzko and O'Keeffe 1989). In this diversified organic strawberry system, aggregation is associated with host preference, such that nymphs are aggregated in and around alfalfa. This may be because of differing capacities for strawberry and alfalfa to feed and nourish *Lygus* spp. nymphs. For instance, *L. lineolaris* nymphs caused a decreasing proportion of damaged strawberry fruit when exposed to increased fruit densities, thereby exhibiting a saturating functional response to a non-preferred host (Rhainds and English–Loeb 2003).

The alfalfa-strawberry system has also created a unique set of circumstances for lygus bug aggregation with regard to mean population size. In a lentil monoculture, greater *Lygus* spp. densities were positively correlated with the negative binomial K (Schotzko and O'Keeffe 1989). In trap-cropped strawberries, however, the attractiveness of a preferred host drives aggregation; hence, larger population sizes in this system were correlated with higher degrees of clumping, which were observed in 2010 when there was a significant negative linear correlation between K and mean nymphal abundance.

High spatial concentrations of lygus bug nymphs may also improve the impact of biological control

agents, which is particularly important in organically managed systems. The recently introduced *Lygus* spp. parasitoid *Peristenus relictus* (Ruthe) displays density-dependent responses to greater host abundance present in alfalfa (Pickett et al. 2009). In addition, generalist predators, including *Nabis*, *Geocoris*, and *Orius*, which are all common in this strawberry growing region, increase consumption rates when exposed to greater prey densities (Propp 1982, Shrestha et al. 2004, Desneux and O'Neil 2008) that are generated through spatial aggregation (Mangan and Wutz 1983).

The majority of egg albumin-marked *Lygus* spp. nymphs were captured in the central trap crop area (west row 1, alfalfa trap crop, and east row 1). Collectively, these three rows accounted for 91 and 86% of captured-marked nymphs in 2008 and 2009, respectively. These limited movement patterns correspond with the nymph dispersion patterns (in which 87 and 91% of nymphs were collected in these three rows in 2008 and 2010, respectively) and reinforce the strategy of efficiently targeting these three rows with a tractor-mounted vacuum.

Nymphs were much more likely than adults to migrate into the adjacent row of strawberries. For instance, of the total marked nymphs captured in 2008 and 2009, 22 and 28% were collected in strawberry row 1, respectively. Two weeks after protein application, the mean nymphal distance traveled from a marked trap crop into strawberries was equivalent to one row. This spill-over of nymphs out of trap crops and into the adjacent strawberry row was an expected outcome (Potting et al. 2004, Swezey et al. 2007). We speculate that this short-distance dispersal behavior may be attributable to excessive competition and/or predator/parasitoid avoidance.

While a majority of *Lygus* spp. nymphs remained in the central three row area (west row 1, alfalfa trap crop, and east row 1) during this study, a small percentage of nymphs dispersed east and west. Of the captured-marked nymphs that dispersed from this central area, 55% (12 nymphs) and 38% (3 nymphs) were collected 50 rows away, in neighboring trap crops in 2008 and 2009, respectively. Detecting additional nymphs may have been affected by molting. Nymphs will likely lose their mark at their next molt (J. R. Hagler, personal observation). Without the marked alfalfa in close proximity to facilitate additional self-marking, nymphs would be less likely to be captured with a positive mark. Nonetheless, the ability of these flightless nymphs to travel 62 m in as little as 24 h was unexpected and underscores their dispersal potential. Previously, marked *L. lineolaris* nymphs were recaptured 50 m away in potatoes from the point of release in pigweed, after 48 h (Khattat and Stewart 1980).

In route to east or west alfalfa trap crops, *Lygus* spp. nymphs dispersed through a suitable, albeit less-preferred host, until they reached alfalfa. This behavioral response: 1) provides an example of habituation, where previous feeding establishes plant host preferences and can influence movement (Bancroft 2005), 2) illustrates that nymphs with relatively rapid dis-

persal speeds have a greater likelihood of coming in contact with a trap crop (Potting et al. 2005), and 3) lends credence to Blackmer and Cañas (2005), who hypothesized that nymphs may rely more on volatile cues than visual cues at further distances, which may help explain how these small insects moved through 50 rows of vision-obstructing strawberry plants to arrive at alfalfa.

Similar to *Lygus* spp. nymphs, western tarnished plant bug adult movement out of, or away from, a central marked alfalfa trap crop was infrequent during this study. The majority of marked captured adults were collected from central alfalfa at 24 h, 48 h, and 2 wk after albumin application. Ninety-two percent and 84% of albumin-marked adults were still found in alfalfa 2 wk after marking in 2008 and 2009, respectively. This plant host is well suited for western tarnished plant bug feeding (Strong 1970), reproduction (Cave and Gutierrez 1983), and trapping (Swezey et al. 2007).

Small recorded dispersal distances from central trap crops also reinforce the notion that the western tarnished plant bug adults were largely arrested on this plant host. At every sample interval, pooled data produced average western tarnished plant bug adult dispersal distances into strawberries of <0.5 m (<1 row). Arrestment may be strengthened by host preference that is limited to small spatial areas (2% of total acreage) in a broader heterogeneous system. In homogeneous alfalfa fields, Bancroft (2005) estimated that marked western tarnished plant bug adults dispersed 7.3 m/day. A GPS/geographic information system-based study estimated western tarnished plant bug movement to cotton from managed forage alfalfa up to 375 m away during 1 mo (Carrière et al. 2006). While direct comparisons between studies may be difficult because of the influence of experimental design on potential dispersal outcomes, the comparatively low-level dispersal recorded in this study independently suggests strong arrested behavior of western tarnished plant bug adults in trap-cropped strawberries.

The arrested movement displayed by western tarnished plant bug adults contributes to its retention in alfalfa. Pest retention in trap crops has not been extensively studied, yet may strongly influence a trap crop's effectiveness (Holden et al. 2012). Sevacherian and Stern (1975) documented trap crop retention by releasing marked western tarnished plant bug adults in alfalfa trap crops interplanted with cotton: ≈85% of marked adults were recaptured in alfalfa 8 d postrelease.

A small number of western tarnished plant bug adults dispersed away from the central marking-zone. Of the total marked-captured western tarnished plant bug adults in this study, 16 and 24% dispersed from the central trap crops in 2008 and 2009, respectively. Of these migrating adults, 29% (5% of total) and 15% (4% of total) traveled to neighboring alfalfa trap crops, 50 strawberry rows from the central marking zone in 2008 and 2009, respectively. The remaining adults that were collected from adjacent strawberry rows constituted 11 and 20% of all collected protein-positive adults.

Within the 10 sampled strawberry rows to the east and west of a central trap crop, marked adults were slightly more numerous in row 1, when compared with rows 2, 3, and 10.

For western tarnished plant bug adults that are dispersing within a network of trap crops, it is unclear how this movement is facilitated. A combination of alfalfa-based odors and visual cues may help distinguish single rows of alfalfa among large blocks of strawberry rows. At further distances, however, visual cues may be more useful than volatile signals for western tarnished plant bug adults (Blackmer and Cañas 2005), as volatiles may be less easily detected (Finch and Collier 2000). Cues to this preferred plant host likely generate the basis of attraction, which is a critical component of trap cropping and strongly contributes to improved pest control efficacy (Banks and Ekbom 1999).

Swezey et al. (2007) documented the economic success of trap cropping in California organic strawberries. The current study provides indications of the behavioral elements important to trap cropping: *Lygus* spp. show strong aggregation in alfalfa and are attenuated in their movements out of alfalfa. These *Lygus* spp. behaviors are highly compatible with an alfalfa-strawberry trap cropping system and importantly allow for optimized targeting of this key pest.

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References Cited

Baker, P. B., J. R. Hagler, R. Marchosky, S. A. Machtley, J. M. Brown, M. A. Riehle, and D. E. Bellamy. 2010. Utilizing rabbit immunoglobulin G protein for mark-capture studies on the desert subterranean termite, *Heterotermes aureus* (Snyder). Insect. Soc. 57: 147–155.

Bancroft, J. S. 2005. Dispersal and abundance of *Lygus hesperus* in field crops. Environ. Entomol. 34: 1517–1523.

Banks, J. E., and B. Ekbom. 1999. Modelling herbivore movement and colonization: pest management potential of intercropping and trap cropping. Agric. For. Entomol. 1: 165–170.

Blackmer, J. L., C. Rodriguez–Saona, J. A. Byers, K. L. Shope, and J. P. Smith. 2004. Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a y-tube olfactometer. J. Chem. Ecol. 30: 1547–1564.

Blackmer, J. L., and L. A. Cañas. 2005. Visual cues enhance the response of *Lygus hesperus* (Heteroptera: Miridae) to volatiles from host plants. Environ. Entomol. 34: 1524–1533.

Buczkowski, G., and G. Bennett. 2007. Protein marking reveals predation on termites by the woodland ant, *Aphaenogaster rudis.* Insect. Soc. 54: 219–224.

Buffington, M. L., and R. A. Redak. 1998. A comparison of vacuum sampling versus sweep-netting for arthropod biodiversity measurements in California coastal sage scrub. J. Insect Conserv. 2: 99–106.

Byerly, K. F., A. P. Gutierrez, R. E. Jones, and R. F. Luck. 1978. A comparison of sampling methods for some arthropod populations in cotton. Hilgardia 46: 257–282.

(CDFA) California Department of Food and Agriculture. 2011. California Agricultural Resource Directory 2010–2011. (http://www.cdfa.ca.gov/statistics/).

(CSC) California Strawberry Commission. 2011. California strawberry 2011 acreage survey. (http://www.calstrawberry.com/fileData/docs/2011_Acreage_Survey.pdf).

Carrière, Y., P. C. Ellsworth, P. Dutilleul, C. Ellers–Kirk, V. Barkley, and L. Antilla. 2006. A GIS-based approach for areawide pest management: the scales of *Lygus hesperus* movements to cotton from alfalfa, weeds, and cotton. Entomol. Exp. Appl. 118: 203–210.

Cave, R. D., and A. P. Gutierrez. 1983. *Lygus hesperus* field life tables studies in cotton and alfalfa (Heteroptera: Miridae). Can. Entomol. 115: 649–654.

Desneux, N., and R. J. O'Neil. 2008. Potential of an alternative prey to disrupt predation of the generalist predator, *Orius insidiosus*, on the pest aphid, *Aphis glycines*, via short-term indirect interactions. Bull. Entomol. Res. 98: 631–639.

Doxon, E. D., C. A. Davis, and S. D. Fuhlendorf. 2011. Comparison of two methods for sampling invertebrates: vacuum and sweep-net sampling. J. Field Ornithol. 82: 60–67.

Finch, S., and R. H. Collier. 2000. Host-plant selection by insects: a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol. Exp. Appl. 96: 91–102.

Hagler, J. R. 1997a. Field retention of novel mark-release recapture method. Environ. Entomol. 26: 1079–1086.

Hagler, J. R. 1997b. Protein marking insects for mark-release-recapture studies. Trends Entomol. 1: 105–115.

Hagler, J. R. 2011. An immunological approach to quantify consumption of protein-tagged *Lygus hesperus* by the entire cotton predator assemblage. Biol. Control 58: 337–345.

Hagler, J. R., and V. P. Jones. 2010. A protein-based approach to mark arthropods for mark-capture type research. Entomol. Exp. Appl. 135: 177–192.

Handley, D. T., and J. E. Pollard. 1993. Microscopic examination of tarnished plant bug (Heteroptera: Miridae) feeding damage to strawberry. J. Econ. Entomol. 86: 505–510.

Hannunen, S., and B. Ekbom. 2001. Host plant influence on movement patterns and subsequent distribution of the polyphagous herbivore *Lygus rugulipennis* (Heteroptera: Miridae). Environ. Entomol. 30: 517–523.

Holden, M. H., S. P. Ellner, D. H. Lee, J. P. Nyrop, and J. P. Sanderson. 2012. Designing an effective trap cropping strategy: the effects of attraction, retention and plant spatial distribution. J. Appl. Ecol. 49: 715–722.

Hughes, G., and R. G. McKinlay. 1988. Spatial heterogeneity in yield-pest relationships for crop loss assessment. Ecol. Modell. 41: 67–73.

Jasrotia, P., and D. Ben–Yakir. 2006. An immuno-marking technique for thrips. Entomol. Exp. Appl. 120: 155–160.

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ. Entomol. 35: 827–836.

Khattat, A. R., and R. K. Stewart. 1980. Population fluctuations and interplant movements of *Lygus lineolaris.* Annu. Entomol. Soc. Am. 73: 282–287.

Klonsky, K., and K. Richter. 2011. Statistical review of California's Agriculture 2005–2009. Agricultural Issues Center, University of California. (http://aic.ucdavis.edu/publications/Statistical_Review_05-09.pdf).

Mangan, R. L., and A. Wutz. 1983. Aggregation patterns of meadow spittlebugs, *Philaenus spumarius* L. (Homptera: Cercopidae), on old-field alfalfa plants. Environ. Entomol. 12: 151–157.

Paukku, S., and J. S. Kotiaho. 2008. Female oviposition decisions and their impact on progeny life-history traits. J. Insect Behav. 21: 505–520.

Pickett, C. H., S. L. Swezey, D. J. Nieto, J. A. Bryer, M. Erlandson, H. Goulet, and M. D. Schwartz. 2009. Colonization and establishment of *Peristenus relictus* (Hymenoptera: Braconidae) for control of *Lygus* spp. (Hemiptera: Miridae) in strawberries on the California Central Coast. Biol. Control 49: 27–37.

Potting, R.P.J., J. N. Perry, and W. Powell. 2005. Insect behavioural ecology and other factors affecting the control efficacy of agro-ecosystem diversification strategies. Ecol. Modell. 182: 199–216.

Propp, G. D. 1982. Functional response of *Nabis americoferus* to two of its prey, *Spodoptera exigua* and *Lygus hesperus.* Environ. Entomol. 11: 670–674.

Rhainds, M., and G. English–Loeb. 2003. Testing the resource concentration hypothesis with tarnished plant bug on strawberry: density of hosts and patch size influence the interaction between abundance of nymphs and incidence of damage. Ecol. Entomol. 28: 348–358.

Ruesink, W. G. 1980. Introduction to sampling theory, pp. 61–78. *In* M. Kogan and D.C. Herzog, (eds.), Sampling Methods in Soybean Entomology. Springer, New York, NY.

Schotzko, D. J., and L. E. O'Keeffe. 1989. *Lygus hesperus* distribution and sampling procedures in lentils. Environ. Entomol. 18: 308–314.

Sevacherian, V., and V. M. Stern. 1972. Spatial distribution patterns of lygus bugs in California cotton fields. Environ. Entomol. 6: 695–704.

Sevacherian, V., and M. Stern. 1975. Movements of lygus bugs between alfalfa and cotton. Environ. Entomol. 43: 163–165.

Shelton, A. M., and F. R. Badenes–Perez. 2006. Concepts and applications of trap cropping in pest management. Annu. Rev. Entomol. 51: 285–308.

Shrestha R. B., M. N. Parajulee, and C. Blanco. 2004. Functional response of selected cotton arthropod predators to boll worm eggs in the laboratory, pp. 1652–1659. *In* 2004 Beltwide Cotton Conferences, 5–9 January 2004, San Antonio TX, National Cotton Council, Washington, DC.

Slosky, L. M., E. J. Hoffmann, and J. R. Hagler. 2012. A comparative study of the retention and lethality of the first and second generation arthropod protein markers. Entomol. Exp. Appl. 144: 165–171.

SPSS. 2010. PASW statistics, version 18. SPSS, Chicago, IL.

Strong, F. E. 1970. Physiology of injury caused by *Lygus hesperus.* J. Econ. Entomol. 63: 808–814.

Swezey, S. L., D. J. Nieto, and J. A. Bryer. 2007. Control of western tarnished plant bug *Lygus hesperus* Knight (Hemiptera: Miridae) in California organic strawberries using alfalfa trap crops and tractor-mounted vacuums. Environ. Entomol. 36: 1457–1465.

Sylvester–Bradley, R., E. Lord, D. L. Sparkes, R. K. Scott, J.J.J. Wiltshire, and J. Orson. 1999. An analysis of the potential of precision farming in Northern Europe. Soil Use Manage. 15: 1–8.

Weisz, R., S. Fleischer, and Z. Smilowitz. 1995. Site-specific integrated pest management for high value crops: sample units for map generation using the colorado potato beetle (Coleoptera: Chrysomelidae) as a model system. J. Econ. Entomol. 88: 1069–1080.

Williams, L. III., J. L. Blackmer, C. R. Rodriguez–Saona, and S. Zhu. 2010. Plant volatiles influence electrophysiological and behavioral responses of *Lygus hesperus.* J. Chem. Ecol. 36: 467–478.

Zalom, F. G., M. P. Bolda, and P. A. Phillips. 2012. UC IPM pest management guidelines: strawberry. University of California. Division of Agriculture and Natural Resources Publication 3468. Oakland, CA.