Open Access Articles

Distribution and activity of Drosophila suzukii in cultivated raspberry and surrounding vegetation

Oregon State UNIVERSITY

ORIGINAL CONTRIBUTION

Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation

J. Klick[1,2], W. Q. Yang[3], V. M. Walton[1], D. T. Dalton[1], J. R. Hagler[4], A. J. Dreves[5], J. C. Lee[2] & D. J. Bruck[2,]*

1 Department of Horticulture, Oregon State University, Corvallis, OR, USA
2 Horticultural Crops Research Unit, USDA-ARS, Corvallis, OR, USA
3 North Willamette Research and Extension Center, Oregon State University, Aurora, OR, USA
4 Arid-Land Agricultural Research Center, USDA-ARS, Maricopa, AZ, USA
5 Department of Crop and Soil Sciences, Oregon State University, Corvallis, OR, USA

Abstract

Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), may utilize wild 'Himalaya' blackberry (HB) *Rubus armeniacus* Focke or other non-crop plants as refugia and possibly exploit adjacent field margins before colonizing cultivated fruiting crops. Studies were conducted to determine the role of field margins containing HB and their effect on *D. suzukii* activity, density and distribution in an adjacent commercial red raspberry crop. One-ha plots adjacent to field margins containing HB or known non-host (NH) grass crops were established in 2011 and 2012 and replicated three times. Each plot contained two transects with monitoring traps for *D. suzukii* in the field margin (0 m) and spaced approximately 10 (crop boundary), 40, 70 and 100 m into the adjacent crop (n = 10 traps/plot). Field margin vegetation was treated with a 10% chicken egg white mark solution weekly from pre-harvest until the end of harvest using a cannon sprayer. Adult *D. suzukii* were collected from traps weekly and analysed for the presence of the egg white mark using an egg white-specific enzyme-linked immunosorbent assay (ELISA). During both years, marked flies and total flies were captured in higher numbers in HB field margins, whereas virtually no flies were captured in field margins containing no known alternative host. Similarly, more flies were captured in the crop near HB than near NH. Spatial Analysis by Distance IndicEs (SADIE) and mean *D. suzukii* trap captures additionally displayed significantly higher fly densities in the raspberry field near HB than near NH. These results suggest that HB may contribute to elevated *D. suzukii* populations and pest pressure in comparison with field margins containing no known alternate host vegetation for *D. suzukii*. Having closely adjacent non-crop alternate host landscapes may result in increased *D. suzukii* pest pressure.

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a pest of small and stone fruits in all major production regions throughout North America and Europe (Walsh et al. 2011; Cini et al. 2012). Biological attributes that favoured its population growth and widespread establishment include mobility (Mitsui et al. 2010), high reproductive capacity, overlapping generations (Tochen et al. 2014; Wiman et al. 2014), limited natural enemies (Chabert et al. 2012; Cini et al. 2012) and an ability to utilize a wide range of ripe and intact fruit (Walsh et al. 2011; Burrack et al. 2013), including non-crop hosts and suitable alternative habitats (Dalton et al. 2011; Atallah et al. 2014; Lee et al. 2015). The globalization of fruit markets and recent expansion of susceptible fruit production likely resulted in the rapid spread of *D. suzukii* and

contributed to the rise in its economic impact (Bolda et al. 2010; Goodhue et al. 2011; Cini et al. 2012). In 2009, up to 80% of the annual value ($421.5 million) of cherry, blueberry, caneberry and strawberry was estimated as the worst-case scenario loss in western US production regions (Bolda et al. 2010).

Non-crop plants adjacent to cultivated commercial crops may exacerbate the economic impact of pests (Lafleur and Hill 1987; Boina et al. 2009; Basoalto et al. 2010) by providing an alternate host source or an association with special environmental conditions (e.g. protection, shelter, humidity, temperature). Nearby 'Himalaya' blackberry (HB), *Rubus armeniacus* Focke and seedling cherry, *Prunus* spp., habitats (Poyet et al. 2014) may be possible refugia and sources of *D. suzukii* invasion (Lee et al. 2015). Movement of *D. suzukii* from field margins to commercial crops is, however, largely unknown. Major cultivated *D. suzukii* host crops tested in a laboratory include *Rubus idaeus* L. (raspberry), *Fragaria* x *ananassa* Duchesne (strawberry), *Rubus ursinus* Chamisso & Schlechtendal (blackberry), *P. avium* L. (sweet cherry), *P. persica* L. (peach), *Vaccinium* spp. (blueberry) and *Vitis* spp. (grape) (Lee et al. 2011a; Bellamy et al. 2013; Tochen et al. 2014). Wild hosts in the same genera include *R. armeniacus*, HB (Caplan and Yeakley 2006; Fierke and Kauffman 2006), *P. avium* and *P. cerasus* L. (sour cherry) (Thilenius 1968; Poyet et al. 2014), all of which can be found in unmanaged field margins of the Pacific Northwest and could serve as refuge or overwintering sites.

Drosophila species are highly mobile and opportunistic to optimize seasonal survival (Taylor et al. 1984; Coyne and Milstead 1987; Bell 1990; Iliadi et al. 2002). Provided unfavourable conditions, *D. pseudoobscura* (Frolova and Astaurov) can disperse from a few metres to several kilometres per day (Crumpacker and Williams 1973; Coyne et al. 1982, 1987; Iliadi et al. 2002). *Drosophila suzukii* has high potential to disperse, a likely factor contributing to its rapid spread following introduction to suitable production regions across North America and Europe (Hauser 2011). When resources decline or population densities exceed optimal levels, *D. suzukii* are believed to migrate to more favourable habitats (Mitsui et al. 2010). These dispersal capabilities, coupled with the close proximity of cultivated and wild hosts, suggest that the impact of surrounding habitat on *D. suzukii* crop invasion needs to be closely examined.

A better understanding of seasonal activity and distribution of *D. suzukii* between non-crop areas and proximate commercial crops will aid to formulate future integrated pest management strategies (Bruck et al. 2011; Lee et al. 2011b, 2015; Cini et al. 2012; Wiman et al. 2014). Various protein mark–capture methods have been used to study insect movement (Jones et al. 2006; Boina et al. 2009; Horton et al. 2009; Basoalto et al. 2010; Krugner et al. 2012; Swezey et al. 2013, 2014; Lessio et al. 2014). In mark–capture studies, as opposed to mark–release–recapture studies, resident populations self-mark. This technique provides several advantages to track *D. suzukii* within a crop. Protein mark–capture techniques are inexpensive, easily applied, environmentally benign, persistent and clearly identifiable (Jones et al. 2006; Hagler and Jones 2010; Hagler et al. 2011; Sivakoff et al. 2012; Klick et al. 2014).

The overall goal of this work was to conduct mark–capture studies to determine the activity levels of *D. suzukii* within non-crop field margins and cultivated raspberry crop fields. Our specific objectives were to determine: (i) whether *D. suzukii* utilize HB-containing field margins as a refuge, (ii) whether crops adjacent to HB-containing field margins have higher population densities compared to crops near non-host (NH) field margins, and (iii) the distribution pattern of *D. suzukii* in cultivated raspberries adjacent to field margins containing HB in comparison with NH field margins.

Materials and methods

Study site and design

A 33.4-ha cultivated red raspberry (*R. idaeus* L.) study site with field margins containing either HB (Himalaya blackberry) or NH (non-host) was located near Jefferson, OR (44°40′01″N, 122°58′00″W). The NH area in 2011 was planted with a soft-white winter wheat (*Triticum aestivum* L. 'Madsen') monoculture, and the same NH area in 2012 was planted with tall fescue (*Lolium arundinaceum* Darbyshire ex. Schreb.) for turf seed production. The commercial raspberry crop was grown using conventional management practices. Three 1-ha plots were systematically selected near each of the two field margin types. Each plot contained two transects with *D. suzukii* traps in the field margin (0 m) and spaced approximately 10 (crop boundary), 40, 70 and 100 m into the adjacent crop (n = 10 traps/plot) (fig. S1). The plots with HB in the field margin were spaced 70–180 m from each other. The plots with NH in the field margin were spaced approximately 50 m from each other. These two plots were spaced 350–500 m from each other. Monitoring traps for *D. suzukii* were a modified clear trap (Lee et al. 2012) made of a bottomless 946-ml

plastic deli container (DM32R; Solo Cup Company, Lake Forest, IL) with ten 3.5-mm holes near the top and nested over a plastic cup (D32; Solo, Urbana, IL) containing ~150 ml apple cider vinegar (ACV) (5% acidity, Fred Meyer Apple Cider Vinegar; Kroger Co., Cincinnati, OH) (fig. S2). A 6.5 × 9 cm double-sided yellow sticky card (ASTO103; Alpha Scents Inc., West Linn, OR) was placed above the mesh that was positioned between the two cups. This trapping method attracted flies inside the baited cup onto a yellow sticky card and prevented flies from drowning and potentially losing the protein mark.

Insect marking

The field margins were treated weekly with 10% liquid chicken egg white (All Whites®; Papetti Foods, Elizabeth, NJ) (Klick et al. 2014) using a cannon sprayer (AJ-401; Jacto Inc., Pompea, Brazil) at 282 L/ha from pre-harvest (27 June to 11 July 2011 and 14 June to 7 July 2012) through harvest (12 July to 12 August 2011 and 8 July to 15 August 2012). The cannon sprayer treated a field margin width of approximately 15 m. To minimize contamination with protein marker, the traps were removed from the field margins prior to protein application and returned to their designated locations within 0.5 h after application. On each insect collection date, sticky cards containing adult *D. suzukii* were removed from the traps, covered with wax paper and placed in a cooler. Traps were serviced during bi-weekly collection periods at each location by replacing trap contents with fresh ACV and a new sticky card. Collected sticky cards were returned to the laboratory and frozen at −80°C. Sticky cards were removed from the freezer, and each *D. suzukii* adult was carefully removed with a disposable toothpick and individually placed into a 1.5-ml microcentrifuge tube along with the tip of the toothpick to prevent cross-contamination. Trap location and number of *D. suzukii* captured per trap were recorded. All fly samples were returned to a −80°C freezer until adults were removed and analysed for the presence of egg albumin by enzyme-linked immunosorbent assay (ELISA).

Egg albumin ELISA

Drosophila suzukii fly samples were thawed, and 1.0 ml of Tris-buffered saline (pH 7.4) was added to each sample. The samples were placed on an orbital shaker at 100 rpm for ca. 1 h. After shaking, a 100-μl aliquot from each sample was added to an individual well of a 96-well ELISA plate. All samples were then assayed by the egg albumin ELISA procedure described in detail by Hagler and Jones (2010). Samples were scored positive for the presence of the mark using the positive ELISA reaction threshold criteria defined by Sivakoff et al. (2011).

Data analysis

Data collected from the field margins (HB and NH) and crop areas were divided into two distinct time periods to address the changes in fly abundance and crop phenology: 'Early Susceptible' and 'Late Susceptible'. Seasonal phenologies of the crop and *D. suzukii* counts were considered when determining the two time periods for each year of study. The Early Susceptible period was defined as the appearance of the first ripe berry to just before the first substantial *D. suzukii* population increase, which coincided with the 'first generation peak egg-laying' as defined and validated by a degree-day (DD) model for *D. suzukii* activity (Coop 2015). The Late Susceptible period was defined from first generation peak oviposition to the end of harvest. In 2011, the Early Susceptible period was 27 June to 22 July and Late Susceptible period was 26 July to 12 August. In 2012, the Early Susceptible period was 14 June to 7 July and Late Susceptible period was 12 July to 15 August.

Total numbers of *D. suzukii* captured in traps were compared between HB and NH field margins, and between crops adjacent to HB and NH, using repeated measures generalized linear models in Proc Glimmix (SAS 9.4; SAS Institute Inc., Cary, NC). Trap captures were pooled for all traps within the crop or field margin portion of each plot on a given collection period (twice per week during harvest season). Plot was the subject of the repeated measures across collection periods. Data were fitted to either a Poisson distribution or a generalized Poisson distribution as necessary to account for overdispersion in the data. Data were analysed separately for Early Susceptible and Late Susceptible periods in 2011 and 2012. Data are presented as number of flies marked, total number of flies assayed and percentage of marked flies. The chi-squared goodness-of-fit test was conducted using R v3.03 (R Core Team 2013) in RStudio v0.97.306 (RStudio 2012) to determine the significance between total marked flies in HB and NH plots, and in field margin or crop in both years.

Nonparametric spatial analysis was conducted to describe general spatial trends of *D. suzukii* activity levels as indicated by total trap counts. The analytical procedure, Spatial Analysis by Distance IndicEs (SADIE, Perry 1995), was used to determine the

overall index of aggregation, I_a, of *D. suzukii* for each time period (Early Susceptible 2011, 2012 and Late Susceptible 2011, 2012). When I_a is near to unity, random arrangement of fly capture is observed. Values larger than unity indicate aggregated arrangement of capture. Regular arrangement of capture is indicated by values smaller than unity (Perry 1996; Maestre and Cortina 2002; De Villiers 2006). The dimensionless index of clustering, v, measures the degree of clustering in areas with above-average density of *D. suzukii*, that is patches (levels at or above 1.5; traps near each other containing high counts of flies) or areas with below-average density, that is gaps (levels below −1.5; traps near each other with low counts of flies) (Winder et al. 2001, 2012; Maestre and Cortina 2002; De Villiers 2006). Values of v_i between −1.5 and 1.5 indicate randomness (Winder et al. 2001; Perry and Dixon 2002; De Villiers 2006). To test for non-randomness, the mean values of the clustering indices, $\bar{v}_i$ and $\bar{v}_j$, were used. Patches of relatively high fly density are indicated by SADIE coordinate output values larger than 1.5, randomness indicated by coordinate values between 1.5 and −1.5, and gaps are indicated by coordinate values <−1.5. Significant clustering, random association and gaps were visually illustrated by inputting SADIE cluster analysis coordinated into Surfer® v12 (Golden Software, Inc., Golden, CO) and using the inverse distance weighted method.

Results

Total trap captures in HB field margins were 16, 134, 53 and 312 *D. suzukii* adults during Early Susceptible 2011, Late Susceptible 2011, Early Susceptible 2012 and Late Susceptible 2012, respectively, and in NH field margins total trap captures were 0, 0, 1 and 7, respectively, in those same time periods (table 1). Trap captures in the crop adjacent to HB were 9, 53, 29 and 283, respectively, and in the crop adjacent to NH trap captures were 9, 21, 14 and 254 in the respective time periods. In 2011, statistical comparison of the field margin data set was not possible because the distributions did not fit the model due to no fly captures in NH field margins. However, sum of captured flies in the crop near HB was statistically higher than in the crop near NH during the Late Susceptible 2011 period (F = 15.94, d.f. = 13, P = 0.002) (fig. 1b). Sums of captured flies per plot in the HB field margin were statistically higher than NH during Early Susceptible 2012 (F = 15.42, d.f. = 8, P = 0.004) and Late Susceptible 2012 (F = 18.30, d.f. = 15, P = 0.001) (fig. 1a).

In 2011, field margins treated with egg albumin protein resulted in 22 marked flies in HB and zero marked flies in NH (table 1). In the adjacent crop, five marked flies were collected near HB and four marked flies near NH (table 1). In 2012, field margins treated with egg albumin resulted in 66 marked flies in HB and one in NH. In the adjacent crop, 21 marked flies were captured near HB and 16 marked flies captured near NH. Although there were numerically more marked flies in HB and the adjacent crop, the marked ratio was significant only in the field margin (2011: χ^2 = 22.00, d.f. = 1, P < 0.001; 2012: χ^2 = 63.06, d.f. = 1, P < 0.001).

Spatial analysis (table 2, fig. 2) indicated significant *D. suzukii* aggregation (I_a) and clustering in all time periods except Early Susceptible 2011. Contour maps overlaid with locations of monitoring traps show significant patches in three HB regions to the south and gaps near three NH regions to the north (fig. 2). In Late Susceptible 2012, a small patch is visible near NH to the north.

Table 1 Number of protein-marked (n_m), assayed (n_a), the percentage (%) of marked *Drosophila suzukii* and yearly marked ratio (i.e. the total marked flies between HB and NH in field margin or crop in 2011 and 2012) in 'Himalaya' blackberry (HB) and non-host (NH) field margins (area treated with egg marker) and in the crop near HB and NH field margins during Early Susceptible (ES) and Late Susceptible (LS) in 2011/2012

Location	ES 2011 n_m/n_a	%	LS 2011 n_m/n_a	%	ES 2012 n_m/n_a	%	LS 2012 n_m/n_a	%
Field margin: HB	2/16	12.5	20/134	14.9	3/53	5.7	63/312	20.2
Field margin: NH	0	0	0	0	0/1	0	1/7	14.3
Marked ratio[1] by year	22:0 (P < 0.001)[2]				66:1 (P < 0.001)			
Crop: near HB	0/9	0	5/53	9.4	1/29	3.5	20/283	7.1
Crop: near NH	2/9	22.2	2/21	9.5	1/14	7.1	15/254	5.9
Marked ratio by year	5:4 (P = 0.74)				21:16 (P = 0.41)			

[1]Ratio of total marked flies between HB and NH in field margin or crop in 2011 and 2012.
[2]P-values based on chi-squared goodness-of-fit test (d.f. = 1).
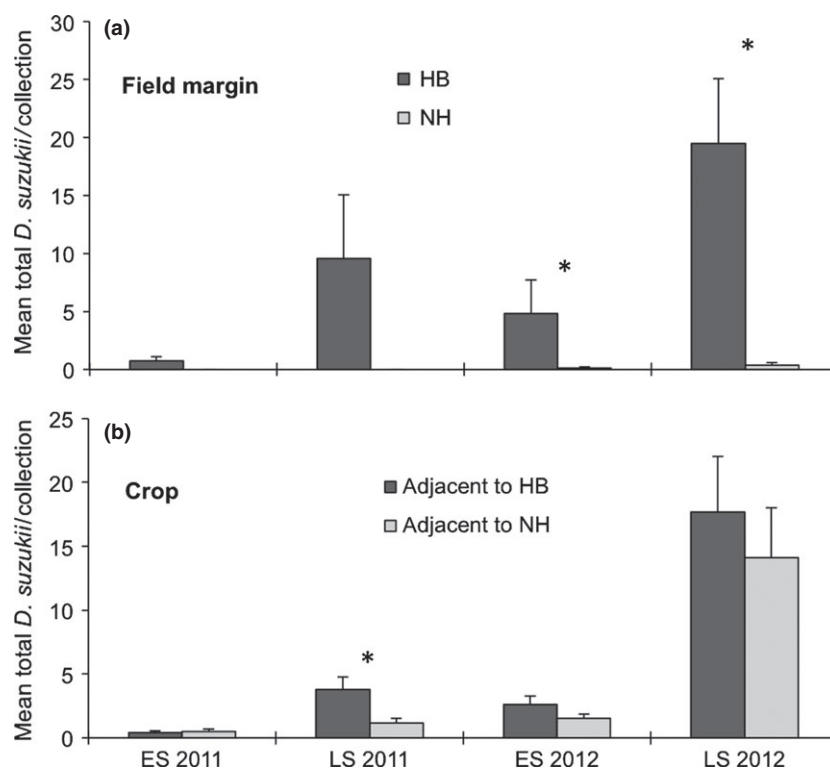
Fig. 1 Mean sum of *Drosophila suzukii* (±SE) captured per plot (pooled over collection period and trap location) in Early Susceptible (ES) and Late Susceptible (LS) 2011 and 2012 in (a) HB (Himalaya blackberry) and NH (non-host) field margins and (b) crop by HB and NH. Significant differences are denoted with an asterisk (*) (α = 0.05). Parametric statistical analysis of the 2011 field margin data was not possible because the large proportion of zeroes in the data set prevented fitting the data to an appropriate distribution. All means shown are taken on the original scale of the data.

Table 2 SADIE (Spatial Analysis with Distance IndicEs) summary statistics of Early Susceptible (ES) and Late Susceptible (LS) in 2011/2012. I_a = index of aggregation; $\bar{v}_j$ = mean index of gap clustering; $\bar{v}_i$ = mean index of patch clustering; P = probabilities associated with indices

Time	I_a[1]	P_a[2]	$\bar{v}_j$[3]	P_j	$\bar{v}_i$[4]	P_i
ES 2011	1.118	0.325	−1.057	0.327	1.142	0.259
LS 2011	3.141	<0.001	−3.988	<0.001	2.928	0.003
ES 2012	2.216	0.008	−2.114	0.018	2.174	0.015
LS 2012	1.866	0.034	−1.866	0.042	1.504	0.021

[1]Spatially random arrangement when the index of aggregation (I_a) is near to unity (i.e. between −1.5 and 1.5), aggregated arrangement when values are larger than unity and regular arrangement when values are smaller than unity.

[2]Probabilities associated with indices.

[3]Mean index of gap clustering (i.e. clustering gap, $v_j < −1.5$ or a low density of counts near each other).

[4]Mean index of patch clustering (i.e. clustering patch, $v_i > 1.5$ or high density of counts near each other).

Discussion

Understanding landscape complexity and pest activity are key elements of developing a pest management strategy (Schneider 1989; Holland and Fahrig 2000; Blackshaw and Vernon 2006; Carriere et al. 2006). This study is the first to demonstrate that *D. suzukii* may utilize nearby habitat containing HB (Himalaya blackberry) as a refuge and may migrate from this habitat and colonize a nearby crop. Several arthropods are known to seasonally colonize non-crop areas and adjacent cultivated crops (Duelli 1990; Pedigo 2002) including *Cydia pomonella* L. (Lepidoptera: Torticidae) (Basoalto et al. 2010), *Sitobion avenae* Fabricius (Hemiptera: Aphididae) (Longley et al. 1997), *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) (Lessio et al. 2014) and *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Boina et al. 2009). Focusing on field margins, management tools proven for other pests such as bait sprays (Vargas et al. 2001; Prokopy et al. 2003), mass trapping (Cohen and Yuval 2000) and non-crop host removal (i.e. ecological management) (Prokopy 2003; Suckling and Brockerhoff 2010) may significantly reduce *D. suzukii* populations, especially if implemented in area-wide programs. However, field margins may play an important ecological role by providing food and habitat for pollinators and natural enemies (Holland and Fahrig 2000; Marshall and Moonen 2002). Furthermore, field margins could provide a refuge to reduce *D. suzukii* insecticide resistance development as wild-type alleles may dilute selective pressure to develop insecticide resistance (Tabashnik and Croft 1985).

We demonstrated that elevated *D. suzukii* populations within the cultivated crops could be associated with high activity levels in the field margin containing HB and other host plants. When no host plants were

(a) (b) (c) (d)
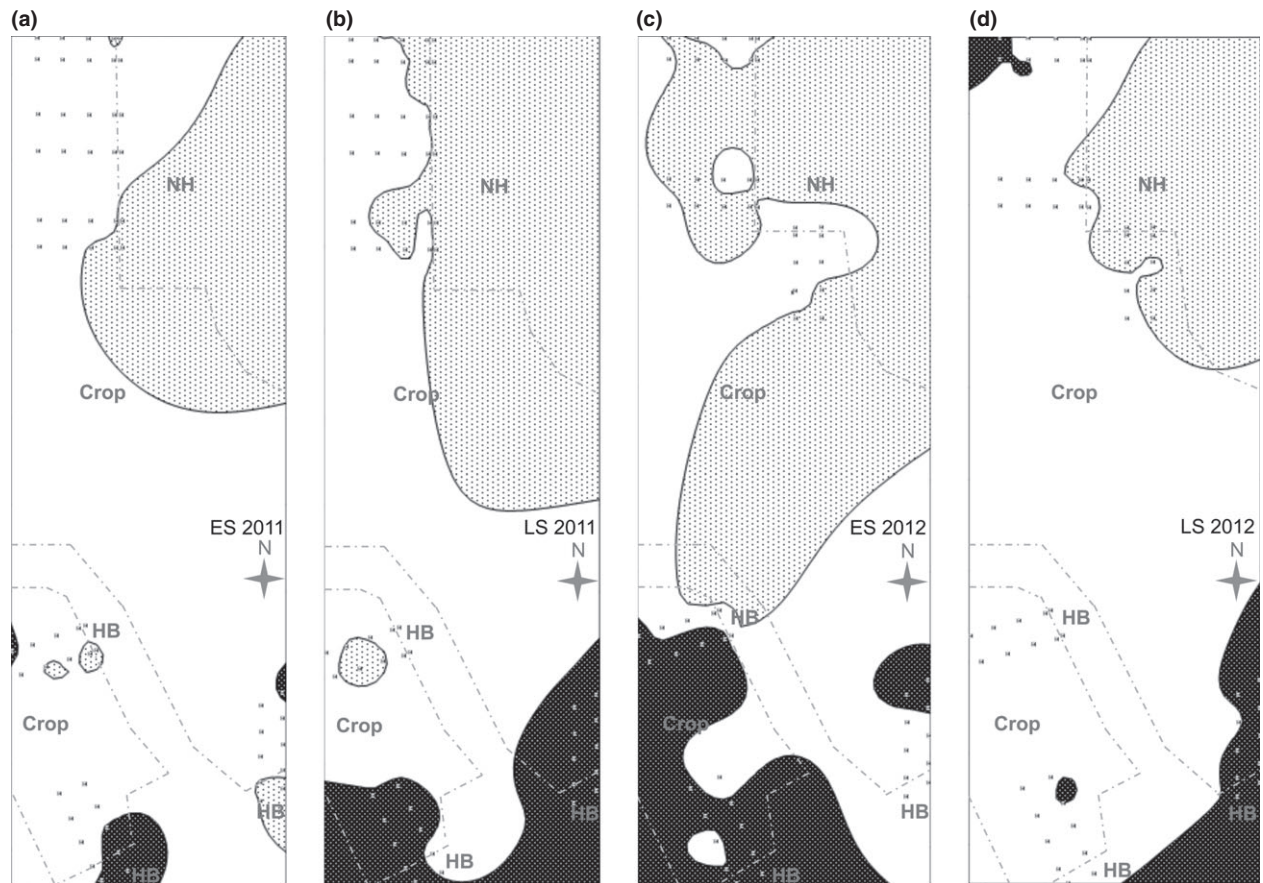
Fig. 2 SADIE (Spatial Analysis with Distance IndicEs) contour maps created in Surfer® overlaid with traps (small dots) of 3 plots near HB in the south and 3 plots near NH in the north and the crop boundaries denoted in a dotted line, as in fig. S1, in (a) Early Susceptible (ES) 2011, (b) Late Susceptible (LS) 2011, (c) ES 2012 and (d) LS 2012. The dark areas denote a clustering patch ($v_i > 1.5$) or high density of counts near each other. The light grey areas denote a clustering gap ($v_j < -1.5$) or low density of counts near each other. The white areas denote a random distribution pattern ($v_i < 1.5$ and $v_j > -1.5$).

present in the field margin, zero to low numbers of flies were captured. Three of four evaluated time periods had significantly more or numerically more trap catches in the crop adjacent to the HB, compared to crop adjacent to NH. We predict that more field sites and replicates would narrow the SE and detect statistical differences throughout all time periods evaluated.

For mark–capture research, recovery rates are often <1% (Hagler et al. 2014). In this study, the overall mean recovery rate was 8% (i.e. per cent marked *D. suzukii*). The field margin area marked with egg white protein was small compared to the total field margin area. Most of the field margin area in the southern half of the experimental site contained non-crop hosts. Future research efforts would benefit from a higher proportion of field margin area treated with egg white to improve the recovery rate.

We propose three hypotheses as to why a similar number of marked flies were caught in the crop near both field margin types. One, the greater influx of flies from HB field margins made it more difficult to detect marked flies, essentially diluting the proportion of marked flies near HB, whereas marked flies near NH (non-host) were not as strongly affected by these influxes due to presence of fewer flies overall. Two, the total adult fly population is most likely underestimated and marked flies from HB in the southern plots moved towards NH in the northern plots. *Drosophila obscura* Pomini (Diptera: Drosophiliade) and *D. subobscura* Collin dispersed up to 100 and 200 m per day, respectively (Taylor et al. 1984). A preliminary mark–release–recapture study using fluorescent dusts found that *D. suzukii* moved approximately 67–87 m in 36 h (JC Lee, unpublished data). Three, population density may have been inaccurately assessed because on poor trap design, bait attraction or placement (Knight and

Croft 1987; Cha et al. 2014; Kleiber et al. 2014). All NH traps and one trap from the HB field margin (from northernmost HB plot in fig. 2) lacked a forested overstorey and were exposed to full sun, a condition that seems to decrease habitat preference for *D. suzukii* (Tochen et al. 2014). These traps had low counts throughout both years of study. Improvements in monitoring and pest ecology knowledge will help understand population dynamics and resource usage in the field.

Evaluating the impact of vegetation containing early ripening hosts of *D. suzukii* populations adjacent to a commercial fruit crop needs further investigation. One such host is *P. avium* (Thilenius 1968), which provides important resources to *D. suzukii* (Lee et al. 2015) and was present in an HB field margin that had consistently high trap counts. The fruit-ripening period of *P. avium* is around the end of June to early July, and this species can thus provide an early ovipositional habitat for *D. suzukii*. In fact, peak oviposition by first generation females in mid-July, as estimated by degree-days (Coop 2015), coincided with the presence of susceptible cherry fruit and designated a key shift from low to high populations (also the cut-off between Early and Late Susceptible periods, as previously mentioned). Conversely, HB ripens near the end of raspberry harvest (typically early August) in the Pacific Northwest of the USA and may play a greater role in building overwintering *D. suzukii* populations as a favourable refuge for the winter period.

In 2012, the crop adjacent to the field margin containing *P. avium* and HB had low *D. suzukii* trap counts and substantial dieback, which reduced fruit and canopy load (i.e. unfavourable canopy architecture) and likely reduced humidity. Early Susceptible 2012 flies may have travelled further in search of the ideal crop area (Coyne and Milstead 1987; Iliadi et al. 2002). This could partially explain why a similar number of marked flies were found near HB and NH field margins. Spraying the NH field margin with a different protein marker, such as milk, could have determined which of the two field margins were visited by marked flies; however, the logistics of the project and uncertainties of the milk marker made this an unfeasible option (Klick et al. 2014).

In summary, mark–capture research using egg white protein documented movement of self-marked resident *D. suzukii* from field margins into the adjacent cultivated raspberry crop. Field margins containing possible host plants (i.e. *R. armeniacus* and *P. avium*) may have contributed to *D. suzukii* population build-up and resultant emigration to the cultivated crop. These conclusions were reached based on statistically and numerically higher *D. suzukii* trap counts in field margins containing HB, compared to field margins containing NH. Higher levels of *D. suzukii* activity were found in crop areas near fruit-bearing non-crop hosts, as illustrated by significant patching in close proximity to HB. Finally, our marking data indicated movement of *D. suzukii* into the crop from field margins.

These findings illustrate the potential risks associated with one such alternate host occurring in closely adjacent vegetation on pest pressure exerted by *D. suzukii* to cultivated fruit crops. Limited information is available on the relative importance of differing landscape types adjacent to cultivated crops, host plant usage and the impact of distance of non-crop hosts on pest pressure from *D. suzukii* into the crop. Overall, the activity of *D. suzukii* in non-crop host areas may be dependent on plant architecture, age, competition, number of hosts, water availability and seasonal fruit selection. A landscape with a combination of diverse and abundant overstorey and understorey plants that provide *D. suzukii* with a food source from spring to fall, overwintering refuge and satisfactory environmental conditions could increase potential risks of crop infestation by *D. suzukii*. Other effects of surrounding vegetation, such as *D. suzukii* insecticide resistance management, potential as *D. suzukii* trap crop and alternate resources for pollinators, and possible enhancement of biological control need to be considered when developing management strategies.

Acknowledgements

References

Atallah J, Teixeira L, Salazar R, Zaragoza G, Kopp A, 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proc Biol Sci 281, 20132840.

Basoalto E, Miranda M, Knight AL, Fuentes-Contreras E, 2010. Landscape analysis of adult codling moth (Lepidoptera: Tortricidae) distribution and dispersal within typical agroecosystems dominated by apple production in central Chile. Environ Entomol 39, 1399–1408.

Bell WJ, 1990. Searching behavior patterns in insects. Annu Rev Entomol 35, 447–467.

Bellamy DE, Sisterson MS, Walse SS, 2013. Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii*. PLoS ONE 8, e61227.

Blackshaw RP, Vernon RS, 2006. Spatiotemporal stability of two beetle populations in non-farmed habitats in an agricultural landscape. J Appl Ecol 43, 680–689.

Boina DR, Meyer WL, Onagbola EO, Stelinski LL, 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. Environ Entomol 38, 1250–1258.

Bolda MP, Goodhue RE, Zalom FG, 2010. Spotted wing drosophila: potential economic impact of a newly established pest. Agric Resour Econ 13, 5–8.

Bruck DJ, Bolda M, Tanigoshi L, Klick J, Kleiber J, DeFrancesco J, Gerdeman B, Spitler H, 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manag Sci 67, 1375–1385.

Burrack HJ, Fernandez GE, Spivey T, Kraus DA, 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore. Pest Manag Sci 69, 1173–1180.

Caplan JS, Yeakley JA, 2006. *Rubus armeniacus* (Himalayan blackberry) occurrence and growth in relation to soil and light conditions in western Oregon. Northwest Sci 80, 9.

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

Cha DH, Adams T, Werle CT, Sampson BJ, Adamczyk JJ, Rogg H, Landolt PJ, 2014. A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. Pest Manag Sci 70, 324–331.

Chabert S, Allemand R, Poyet M, Eslin P, Gibert P, 2012. Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, *Drosophila suzukii*. Biol Control 63, 40–47.

Cini A, Ioriatti C, Anfora G, 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull Insectol 65, 149–160.

Cohen H, Yuval B, 2000. Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. J Econ Entomol 93, 721–725.

Coop L, 2015. Online phenology and degree-day model for agricultural and decision-making in the US. Integrated Plant Protection Center, Oregon State University, Corvallis, OR. [WWW document]. URL http://uspest.org/cgi-bin/ddmodel.us?spp=swd (accessed on March 9, 2015).

Coyne JA, Milstead B, 1987. Long-distance migration of *Drosophila*. 3. Dispersal of *D. melanogaster* alleles from a Maryland orchard. Am Nat 130, 70–82.

Coyne JA, Boussy IA, Prout T, Bryant SH, Jones JS, Moore JA, 1982. Long-distance migration of *Drosophila*. Am Nat 119, 589–595.

Coyne JA, Bryant SH, Turelli M, 1987. Long-distance migration of *Drosophila*. 2 Presence in desolate sites and dispersal near a desert oasis. Am Nat 129, 847–861.

Crumpacker DW, Williams JS, 1973. Density, dispersion, and population structure in *Drosophila pseudoobscura*. Ecol Monogr 43, 499–538.

Dalton DT, Walton VM, Shearer PW, Walsh DB, Caprile J, Isaacs R, 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manag Sci 67, 1368–1374.

De Villiers M, 2006. Development of a pest management system for table grapes in the Hex River Valley. PhD thesis. University of Stellenbosch.

Duelli P, 1990. Population movements of arthropods between natural and cultivated areas. Biol Conserv 54, 193–207.

Fierke MK, Kauffman JB, 2006. Invasive species influence riparian plant diversity along a successional gradient, Willamette River, Oregon. Nat Area J 26, 376–382.

Goodhue RE, Bolda M, Farnsworth D, Williams JC, Zalom FG, 2011. Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manag Sci 67, 1396–1402.

Hagler JR, Jones VP, 2010. A protein-based approach to mark arthropods for mark-capture type research. Entomol Exp Appl 135, 177–192.

Hagler J, Mueller S, Teuber LR, Van Deynze A, Martin J, 2011. A method for distinctly marking honey bees, *Apis mellifera*, originating from multiple apiary locations. J Insect Sci 11, 1–14.

Hagler JR, Naranjo SE, Machtley SA, Blackmer F, 2014. Development of a standardized protein immunomarking protocol for insect mark–capture dispersal research. J Appl Entomol 138, 772–782.

Hauser M, 2011. A historic account of the invasion of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag Sci 67, 1352–1357.

Holland J, Fahrig L, 2000. Effect of woody borders on insect density and diversity in crop fields: a landscape-scale analysis. Agric Ecosyst Environ 78, 115–122.

Horton DR, Jones VP, Unruh TR, 2009. Use of a new immunomarking method to assess movement by generalist predators between a cover crop and tree canopy in a pear orchard. Am Entomol 55, 49–56.

Iliadi KG, Iliadi NN, Rashkovetsky EL, Girin SV, Nevo E, Korol AB, 2002. Sexual differences for emigration behavior in natural populations of *Drosophila melanogaster*. Behav Genet 32, 173–180.

Jones VP, Hagler JR, Brunner JF, Baker CC, Wilburn TD, 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ Entomol 35, 827–836.

Kleiber JR, Unelius CR, Lee JC, Suckling DM, Qian MC, Bruck DJ, 2014. Attractiveness of fermentation and related products to spotted wing drosophila (Diptera: Drosophilidae). Environ Entomol 43, 439–447.

Klick J, Lee JC, Hagler JR, Bruck DJ, Yang WQ, 2014. Evaluating *Drosophila suzukii* (Diptera: Drosophilidae) immunomarking for mark-capture research. Entomol Exp Appl 152, 31–41.

Knight A, Croft B, 1987. Regional population dynamics and seasonal spatial patterns of *Argyrotaenia citrana* (Lepidoptera: Tortricidae) as measured by a pheromone trap grid and larva sampling. Environ Entomol 16, 59–67.

Krugner R, Hagler JR, Groves RL, Sisterson MS, Morse JG, Johnson MW, 2012. Plant water stress effects on the net dispersal rate of the insect vector *Homalodisca vitripennis* (Hemiptera: Cicadellidae) and movement of its egg parasitoid, *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae). Environ Entomol 41, 1279–1289.

Lafleur G, Hill SB, 1987. Spring migration, within-orchard dispersal, and apple-tree preference of plum curculio (Coleopera: Curculionidae) in southern Quebec. J Econ Entomol 80, 1173–1187.

Lee JC, Bruck DJ, Curry H, Edwards D, Haviland DR, Van Steenwyk RA, Yorgey BM, 2011a. The susceptibility of small fruits and cherries to the spotted wing drosophila, *Drosophila suzukii*. Pest Manag Sci 67, 1358–1367.

Lee JC, Bruck DJ, Dreves AJ, Ioriatti C, Vogt H, Baufeld P, 2011b. In Focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Manag Sci 67, 1349–1351.

Lee JC, Burrack HJ, Barrantes LD, Beers EH, Dreves AJ, Hamby KA, Haviland DR, Isaacs Richardson TA, Shearer PW, Stanley CA, Walsh DB, Walton VM, Zalom FG, Bruck DJ, 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. J Econ Entomol 105, 1350–1357.

Lee JC, Dreves AJ, Cave AM, Kawai S, Isaacs R, Miller JC, Van Timmeren S, Bruck DJ, 2015. Infestation of wild and ornamental non-crop fruits by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Ann Entomol Soc Am 1–13 doi: 10.1093/aesa/sau014.

Lessio F, Tota F, Alma A, 2014. Tracking the dispersion of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) from wild to cultivated grapevine: use of a novel mark–capture technique. Bull Entomol Res 104, 432–443.

Longley M, Jepson PC, Izquierdo J, Sotherton N, 1997. Temporal and spatial changes in aphid and parasitoid populations following applications of deltamethrin in winter wheat. Entomol Exp Appl 83, 41–52.

Maestre FT, Cortina J, 2002. Spatial patterns of surface soil properties and vegetation in a Mediterranean semi-arid steppe. Plant Soil 241, 279–291.

Marshall EJ, Moonen A, 2002. Field margins in northern Europe: their functions and interactions with agriculture. Agric Ecosyst Environ 89, 5–21.

Mitsui H, Beppu K, Kimura MT, 2010. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol Sci 13, 60–67.

Pedigo LP, 2002. Entomology and pest management, 4th edn. Prentice Hall, Upper Saddle River, NJ.

Perry JN, 1995. Spatial analysis by distance indices. J Anim Ecol 64, 303–314.

Perry JN, 1996. Simulating spatial patterns of counts in agriculture and ecology. Comput Electron Agric 15, 93–109.

Perry JN, Dixon PM, 2002. A new method to measure spatial association for ecological count data. Ecoscience 9, 133–141.

Poyet M, Eslin P, Héraude M, Le Roux V, Prévost G, Gibert P, Chabrerie O, 2014. Invasive host for invasive pest: when the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. Agric For Entomol 16, 251–259.

Prokopy RJ, 2003. Two decades of bottom-up, ecologically based pest management in a small commercial apple orchard in Massachusetts. Agric Ecosyst Environ 94, 299–309.

Prokopy RJ, Miller NW, Piñero JC, Barry JD, Tran LC, Oride L, Vargas RI, 2003. Effectiveness of GF-120 fruit fly bait spray applied to border area plants for control of melon flies (Diptera: Tephritidae). J Econ Entomol 96, 1485–1493.

R Core Team, 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RStudio, 2012. RStudio: integrated development environment for R. Version 0.98.932, Boston, MA.

Schneider JC, 1989. Role of movement in evaluation of area wide insect pest management tactics. Environ Entomol 18, 868–874.

Sivakoff FS, Rosenheim JA, Hagler JR, 2011. Threshold choice and the analysis of protein marking data in long-distance dispersal studies. Methods Ecol Evol 2, 77–85.

Sivakoff FS, Rosenheim JA, Hagler JR, 2012. Relative dispersal ability of a key agricultural pest and its predators in an annual agroecosystem. Biol Control 63, 296–303.

Suckling DM, Brockerhoff EG, 2010. Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). Ann Rev Entomol 55, 285–306.

Swezey SL, Nieto DJ, Hagler JR, Pickett CH, Bryer JA, Machtley SA, 2013. Dispersion, distribution, and movement of *Lygus* spp. (Hemiptera: Miridae) in trap-cropped organic strawberries. Environ Entomol 42, 770–778.

Swezey SL, Nieto DJ, Pickett CH, Hagler JR, Bryer JA, Machtley SA, 2014. Spatial density and movement of the *Lygus* spp. parasitoid *Peristenus relictus* (Hymenoptera: Braconidae) in organic strawberries with alfalfa trap crops. Environ Entomol 43, 363–369.

Tabashnik BE, Croft BA, 1985. Evolution of pesticide resistance in apple pests and their natural enemies. Entomophaga 30, 37–49.

Taylor CE, Powell JR, Kekic V, Andjelkovic M, Burla H, 1984. Dispersal rates of species of the *Drosophila obscura* group: implications for population structure. Evolution 38, 1397–1401.

Thilenius JF, 1968. The *Quercus garryana* forests of the Willamette Valley, Oregon. Ecology 49, 1124–1133.

Tochen S, Dalton DT, Wiman N, Hamm C, Shearer PW, Walton VM, 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ Entomol 43, 501–510.

Vargas RI, Peck SL, McQuate GT, Jackson CG, Stark JD, Armstrong JW, 2001. Potential for areawide integrated management of Mediterranean fruit fly (Diptera: Tephritidae) with a braconid parasitoid and a novel bait spray. J Econ Entomol 94, 817–825.

Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O'Neal SD, Zalom FG, 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J Integr Pest Manag 2, 1–7.

Wiman NG, Walton VM, Dalton DT, Anfora G, Burrack HJ, Chiu JC, Daane KM, Grassi A, Miller B, Tochen S, Wang X, Ioriatti C, 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLoS ONE 9, e106909.

Winder L, Alexander CJ, Holland JM, Woolley JB, Perry JN, 2001. Modelling the dynamic spatio-temporal response of predators to transient prey patches in the field. Ecol Lett 4, 568–576.

Winder L, Alexander CJ, Woolley C, Perry JN, Holland JM, 2012. The spatial distribution of canopy-resident and ground-resident cereal aphids (*Sitobion avenae* and *Metopolophium dirhodum*) in winter wheat. Arthropod Plant Interact 7, 21–32.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Experimental design in 2011 (A) and 2012 (B) with traps (small dots) of three plots near 'Himalaya' blackberry (HB) in the south and three plots near non-hosts (NH) in the north and the crop boundaries denoted in a dotted line. Note: One of the plots near NH was moved to southeast in 2012 to account for environmental conditions (i.e. wind).

Figure S2. Monitoring trap for *Drosophila suzukii* trap captures was a modified 'clear' trap (Lee et al. 2012) made of a bottomless 946-ml clear plastic deli container (DM32R; Solo Cup Company) with ten 3.5-mm holes near the top and nested over a plastic cup (D32; Solo) containing ~150 ml apple cider vinegar (ACV) (5% acidity, Fred Meyer Apple Cider Vinegar, Kroger Co.). A 6.5 × 9 cm double-sided yellow sticky card (ASTO103, Alpha Scents Inc.) was placed on top of the mesh that was positioned between the two cups.