Spotted wing drosophila (Drosophila suzukii) utilization and dispersal from the wild host Asian bush honeysuckle (Lonicera spp.)

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Abstract
1 Wild host plants in the landscape surrounding fruit farms may significantly influence the movement and population of the polyphagous and invasive pest Drosophila suzukii.
2 Across 2 years, we sampled wild hosts adjacent to 10 blueberry farms in Michigan, U.S.A. We found five commonly infested wild fruits.
3 Honeysuckle was a particularly abundant early season reproductive host. Consequently, six blueberry farms with honeysuckle at the margin were evaluated further. At each farm, nonhost plants and honeysuckle were monitored for larval and adult D. suzukii. The season-long abundance of D. suzukii adults and early-season infestation was highest near and within honeysuckle.
4 In 2017, we tracked the movement of D. suzukii between honeysuckle and blueberries in early and late season using a protein immunomarking technique. Of the 1881 flies captured in our study, 7.1% were marked and their distribution pattern was even throughout the farm. Moreover, early season flies were less likely to remain in the marked host compared with late season flies.
5 The findings of the present study highlight the importance of wild hosts on local pest pressure from D. suzukii and suggest that wild host management should be considered as part of integrated strategies for reducing the economic impacts of this pest.

Keywords Immunomarking, integrated pest management, mark–capture, spatial distribution.

Introduction
Habitats surrounding crops can provide insects with refugia, overwintering habitat, food sources or areas for reproduction (Lewis et al., 1997; Thies et al., 2003; Schmidt & Tscharntke, 2005; Gardiner et al., 2009; Isaacs et al., 2009). Generally, a higher abundance of semi-natural habitat has been found to reduce insect pest pressure in adjacent agricultural crops (Thies et al., 2005; Bianchi et al., 2006; Ricci et al., 2009; Veres et al., 2013). However, these studies are often conducted on pests that are either native or naturalized to the area and are influenced by natural enemies in the surrounding landscape. For invasive species that are often free of natural enemies (Liu et al., 2006; Colautti et al., 2014), the relationship with surrounding landscapes is not well understood. Moreover, this is likely highly dependent on the behaviour, number of hosts and life cycle of the invasive pest.

For insect pests that are highly polyphagous, such as the spotted wing drosophila [Drosophila suzukii (Matsumura)], we might expect that, as the abundance of non-managed land increases, there is greater opportunity for the pests to thrive (Lee et al., 2015; Pelton et al., 2016). Supporting this, Pelton et al. (2016) found that landscapes with greater woody habitat near berry farms allowed for earlier seasonal arrival of D. suzukii in monitoring traps, although season-long catches were unchanged. The abundance of D. suzukii may not be driven by wooded landscapes alone, whereas the presence of wild hosts within those landscapes may be important at the local scale. Lee et al. (2015) reported on multiple reproductive hosts for D. suzukii, including honeysuckle, autumn olive, wild blackberries and raspberries. Many of these hosts are invasive, commonly establishing at the edge of woodlots, in opened canopies or in other disrupted areas (DeMars & Runkle, 1992; Luken & Goessling, 1995; Buckley et al., 2007).

The influence of these wild hosts on the severity of D. suzukii in the adjacent crops is not well understood. Klick et al. (2016) found that D. suzukii utilize large stands of wild
blackberry adjacent to raspberry crops and then subsequently move into and infest the raspberry field. However, the role of the sparse wild hosts distributed throughout habitats surrounding berry crop fields in the Midwest has not been evaluated. Of interest is the abundant Asian bush honeysuckle (Lonicera maackii Rupr., L. morrowii Gray, L. tatarica), which bears ripe fruit before blueberries are harvested and continues to ripen fruit throughout the summer. This could offer a host for early population expansion of D. suzukii before they infest blueberries and other commercial crops. These plants were introduced to the U.S.A. as a potential landscape species (Dirr, 1983; Schierenbeck, 2004) in the early 1800s (King, 1966) and have subsequently established widely throughout North America. Honeysuckle and other wild hosts that bear fruit before berry crops ripen (e.g. mistletoe, Briem et al., 2016) may serve as important drivers in the local abundance of D. suzukii and should be evaluated for their role in the ecology of this new pest on farmland.

Although understanding the utilization of these wild hosts is critical, we also need to evaluate the behaviour and movement of D. suzukii to determine the risk of infestation in wild hosts moving into crop fields. The maximum dispersal distance of D. suzukii in tart cherry orchards has been estimated at 90 m (Kirkpatrick et al., 2018). This indicates that it is capable of movement at scales that allow for travel from the crop edge to crop interior. However, we have an inadequate understanding of how often this movement occurs and the degree to which wild hosts are influencing this movement. Insect movement can be tracked in a variety of ways (Hagler & Jackson, 2001), including using dyes (Einkerlin et al., 1996), fluorescent powders (Kirkpatrick et al., 2018) and immunomarking (Hagler et al., 2014). Although there are advantages to each technique, immunomarking represents a cost-effective method for monitoring the movement of a self-marked population of insects. Quantifying the movement patterns of D. suzukii on farms in both early-season and late-season will provide insights into its dispersal and interactions between wild hosts and the adjacent crop. The management of these wild hosts may provide an ecological approach to control D. suzukii through a better understanding of its behaviour and biology, potentially restricting population development adjacent to crops and reducing infestation. An improved understanding of how this pest uses the surrounding landscape could allow us to limit the local carrying capacity (Clementine et al., 2005; Saeed et al., 2015), reduce the probability of insecticide resistance (Mallet & Porter, 1992; Livingston et al., 2004) and target areas of release for potential biological control agents (Daane et al., 2016; Wang et al., 2018). Using cultural control techniques on farms in combination with chemical control represents a realistic way of reducing the expansive population pressure on a local scale (Prokopy & Kogan, 2003). Increasingly, research and management techniques for this pest are focused in this direction (Iglesias & Liburd, 2017; Leach et al., 2017; Wallingford et al., 2018) with recognition that successful control of this pest will require use of diverse approaches (Asplen et al., 2015).

In the present study, we evaluated: (i) the wild hosts of D. suzukii in habitats surrounding blueberry fields; (ii) the influence of honeysuckle on the arrival and abundance of D. suzukii in adjacent blueberry fields; and (iii) the local movement of D. suzukii between honeysuckle and blueberry fields using a protein immunomarking technique.

Materials and methods

Wild fruit collections

Across 10 blueberry farms in five counties in the central and west-central regions of the lower peninsula of Michigan, wild plants with fruits were sampled in the blueberry farms and 15 m into the surrounding woodlot in 2015 (June to August) and 2016 (June to September). At least 120 mL of wild fruit was collected when available. All farms that were sampled were either managed organically or received very little management for insect pests. The plants were identified in the field, and then berry samples were brought back to the laboratory where they were weighed and put in containers to rear out flies. The rearing containers consisted of deli cups (0.47 L) (Gordon Food Service Inc., Grand Rapids, Michigan) with dental wicking (Absorbal Inc., Wheat Ridge, Colorado) in the bottom to soak up excess liquid, and the containers were fitted with a mesh-top lid to allow air circulation. The containers were placed in a rearing chamber under an LD 16 : 8 h photocycle at 25 °C and 60–70% relative humidity. Every second day, the emergence of flies was checked and flies were removed and stored in 75% EtOH. The flies were later identified as D. suzukii or other drosophilid flies. The rearing containers were held for 2 weeks after the last fly emerged.

Honeysuckle monitoring

Six blueberry farms were selected that had multiple sections of their bordering unmanaged land containing honeysuckle (Lonicera spp.). All honeysuckle plants were checked for a hollow pith, confirming them as one of the invasive Asian bush honeysuckles (L. maackii, L. morrowii, L. tatarica, or a hybrid). In 2015, four farms were located in Allegan County and two in Ottawa County in west Michigan. This was repeated in 2016 with the same farms except that one in Allegan County was removed, and an additional farm was added in Van Buren County in southwest Michigan. All farms were either organically or minimally managed, and all blueberry bushes produced berries. At each farm, two productive honeysuckle bushes at the wood edge, adjacent to the crop, were selected. Likewise, two areas with nonhost plants at the wood edge were selected. The nonhost plants did not produce berries, and they were usually a small tree (e.g. Quercus, Acer, Pinus spp.), ranging from 3 m to 6 m in height. Each sampling location was at least 20 m away from the other sampling locations. Additionally, a 15-m radius of each location was checked for the presence of other potential wild hosts, such as blackberry or raspberry. When found, these were removed to prevent influence from other potential hosts. In each wood edge (containing honeysuckle or nonhost), we placed a single yeast-sugar baited monitoring trap containing a yellow sticky insert (Van Timmeren & Isaacs, 2013), in early May of each year. An additional trap was also placed in the blueberry bushes directly adjacent each of these locations. Adult D. suzukii in the traps were counted weekly and the traps were replaced...
until September. Two locations in the interior of each blueberry planting, at least 20 m away from each other, also contained a trap that was monitored in the same way. The berry growth stages of the blueberries and honeysuckle were monitored season-long in both years. Additionally, at least 120 mL of honeysuckle and blueberry fruits were collected from these bushes weekly from first ripening until no more fruit was available to collect. The honeysuckle fruits were used to rear out D. suzukii (as described above) and the blueberries were lightly crushed, put into a salt solution (312.6 g of salt per 3.79 L of water) for 1 h and later filtered to count larvae that floated out of the fruit (Van Timmeren et al. 2017a). Different methods to assess larval abundance were used so that we could confirm larvae as D. suzukii in wild fruits. In both years, a subset of the blueberries from each site was left to rear out to confirm the larvae as D. suzukii. In all cases, the flies that emerged from the blueberries were D. suzukii. In September of both years, the vegetation type and density within a 10-m radius of each trapping location was recorded.

Fly dispersal

In 2017, at a 4.3-ha blueberry farm located in Allegan County, we used a protein immunomarking technique to track the movement of D. suzukii between honeysuckle in the surrounding habitat and the blueberry crop. A 0.4-ha plot in the centre of the blueberry field was selected for marking and then four transects were identified that led from inside this central plot in four directions to the wood edge where there was a honeysuckle bush in the adjacent habitat. Each transect was 120 m in length and we placed a yeast-sugar baited monitoring trap at six intervals of 24 m. The traps were made as described above, except that a second 0.47-L deli-cup with a mesh bottom was placed into the 0.95-L deli-cup. This allowed flies to enter the trap but prevented them from going into the solution and losing their protein mark, or contaminating unmarked flies. The four honeysuckle bushes were marked with a 25% milk protein solution (TGS Nutrition LLC, Las Vegas, Nevada) using a 3-L CO₂-powered backpack sprayer (R&D Sprayers; Bellspray Inc., Opelousas, Los Angeles). The blueberry plot was sprayed with a 10% egg protein solution (Great EGGSpectations Liquid Egg White; Meijer, Grand Rapids, Michigan) (Klick et al., 2014, 2016) using a 94.6-L 12-V sprayer (25-Gallon Deluxe ATV Boomless Sprayer; County Line Equipment Sales LLC, Vandalia, Missouri) powered by a Gator utility vehicle (John Deere, Moline, Illinois). Each of the protein solutions was mixed with NuFilm P (Miller Chemical, Hanover, Pennsylvania) at a rate of 0.58 L/hectare to increase the spread of the markers on the plant surfaces. Two to 4 days before the protein application, traps were put out and collected just before the protein spray to collect unmarked flies to serve as negative controls. On 17 July (early blueberry ripening season) and 8 September (late season), the proteins were applied. An application on 9 August was also made but this trial was abandoned as a result of unexpected rain immediately after the treatment. As soon as the proteins were dry, traps were placed along each transect. Traps were collected and replaced with fresh traps 12 and 36 h after marking, and collected again at 84 h. Blueberry and honeysuckle leaf samples were collected prior to the protein applications, immediately after the application (once they dried), and at 12, 36 and 84 h after the application. Ten to 15 leaves were collected from 0, 48 and 120 m within each transect. Leaf samples were placed in plastic bags and put in a cooler with ice packs as soon as possible, and then placed in a −80°C freezer until analysis. Traps were brought back to the laboratory where the yellow sticky card was removed, wrapped in parchment paper, placed in a plastic bag and then put into the −80°C freezer. Later, the yellow sticky cards were removed from the freezer and the total number of male and female D. suzukii flies was counted. Next, 15 males and 15 females, when available, were individually removed from the sticky cards with a clean toothpick for each insect and placed in individual 1.6-mL microcentrifuge tubes (4445.X; Biotix Inc., San Diego, California). Likewise, leaf samples were removed from the freezer and five 0.8-cm diameter sub-samples were taken from each leaf by shutting the lid of the 1.6-mL tube over the leaf. The leaf was then pushed to the bottom of the tube using a clean toothpick. Tubes were placed in the −80°C freezer until being shipped overnight with freezer packs to the USDA-ARS Arid Land Agricultural Research Center in Maricopa, Arizona, for protein analyses.

For each fly and leaf sample, two immunoassays were carried out, both performed using the indirect enzyme-linked immunosorbent assay (ELISA) procedures described by Klick et al. (2014). Fly and leaf samples were considered marked using the maximum negative control threshold method described by Sivakoff et al. (2011).

Statistical analysis

All data analyses were conducted using R, version 3.3.3 (R Core Team, R Foundation for Statistical Computing, Austria). In the wild fruit collections, we compared the average number of D. suzukii larvae by fruit type using a generalized linear mixed effect model (GLMM) with site, date of collection and year as random effects. The date of collection was nested within the year and then crossed with site. A Poisson distribution was used for this model because the frequency of larvae in the fruit was skewed towards zero. From adult trap data, 2016 and 2017 had dramatically different levels of pest pressure by adult D. suzukii and were separated for analysis. Adult counts were also analyzed with a GLMM using a Poisson distribution and with site and date of collection as random effects and nested as described above. Infestation in blueberries was similar for both years and so these were analyzed together, divided by early season (all July sample dates) and late season (August and September sample dates). Larval infestation in the blueberries was analyzed using a GLMM using a negative binomial distribution (R package ‘lme4’; Bates et al., 2015, 2017), with the same random effects as described above.

For the D. suzukii dispersal analysis, all time periods (12, 36 and 84 h) were combined for polynomial regression analysis because trends were the same across days. A GLMM with a binomial distribution was used and Tukey’s honestly significant difference was used for all post-hoc comparisons.

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Table 1 The most common wild fruits sampled across six blueberry farms in west Michigan in 2016 and 2017 that supported emergence of *Drosophila suzukii*

<table>
<thead>
<tr>
<th>Type of fruit</th>
<th>Collection time</th>
<th>Total samples</th>
<th>Percentage detection at farms sampled</th>
<th>Average percentage infestation</th>
<th>Mean ± SE <em>D. suzukii</em> per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Himalayan blackberry</td>
<td>Rubus armeniacus</td>
<td>Mid-June to September</td>
<td>54</td>
<td>70</td>
<td>74.1</td>
</tr>
<tr>
<td>Wild raspberry</td>
<td>Rubus strigosus, Rubus idaeus</td>
<td>Late June to September</td>
<td>26</td>
<td>70</td>
<td>61.5</td>
</tr>
<tr>
<td>Asian bush honeysuckle</td>
<td>Lonicera maackii, Lonicera morrowii, Lonicera tatarica</td>
<td>Early June to August</td>
<td>135</td>
<td>100</td>
<td>49.6</td>
</tr>
<tr>
<td>Autumn olive</td>
<td>Elaeagnus umbellata</td>
<td>August to September</td>
<td>8</td>
<td>30</td>
<td>62.5</td>
</tr>
<tr>
<td>American pokeweed</td>
<td>Phytolacca americana</td>
<td>August to September</td>
<td>16</td>
<td>40</td>
<td>75</td>
</tr>
</tbody>
</table>

Different lowercase letters denote fruit types that had significantly different levels of *D. suzukii* per gram.

Figure 1 Mean *Drosophila suzukii* adults per trap in (A) 2016 and (B) 2017 across each location. Years are separated as a result of very different levels of pest pressure. Different lowercase letters within each year denote significant differences among locations.

**Results**

Wild fruit collections

Throughout 2016 and 2017, in total, 278 wild fruit collections were made to determine the level of *D. suzukii* infestation. Of those samples, 52.9% were infested with *D. suzukii*. The earliest fruit collected was from honeysuckle (*Lonicera* spp.), which, in both years, was collected approximately 3 weeks before blueberries ripened (Table 1). Overall, 49.6% of the honeysuckle collections were infested with *D. suzukii*. Himalayan blackberry (*Rubus armeniacus* Focke) was also collected in the early season and 74% of all collections made contained *D. suzukii*. Late-season wild hosts, including wild raspberry (*Rubus strigosus* Michx., *Rubus idaeus* L.), American pokeweed (*Phytolacca americana* L.) and autumn olive (*Elaeagnus umbellata* Thunb.), all contained high levels of infestation of *D. suzukii* (> 60%). On average, Himalayan blackberry had the greatest number of *D. suzukii* emerging per gram, which was statistically higher than all fruit types except for wild raspberry (*F*₄,186 = 6.2, *P* < 0.001) (Table 1). Other fruits that had *D. suzukii* detected but where the sample size was less than five samples included crab apple (*Malus* spp.), silky dogwood (*Cornus anomum* Mill.) and elderberry (*Sambucus canadensis* L.). Other fruits, including black currant (*Ribes nigrum* L.), wild grape (*Vitis* spp.), cranberry cotoneaster (*Cotoneaster apiculatus* Medik.), bittersweet nightshade (*Solanum dulcamara* L.), Virginia creeper (*Parthenocissus quinquefolia* Planch.), Japanese barberry (*Berberis thunbergii* DC.), choke cherry (*Prunus virginiana* L.) and horsenettle (*Solanum carolinense* L.), were also sampled for *D. suzukii* infestation, although none were detected in these plants.

Honeysuckle monitoring

In both years, honeysuckle produced fruit on average 22.2 ± 7.2 d before blueberries produced fruit. The honeysuckle was usually finished fruiting around mid-late July, coinciding with ripening blueberries. Across both years, we caught significantly more *D. suzukii* adults in the honeysuckle bushes compared with the blueberry bushes adjacent to the non-host (2016: *F*₄,832 = 2.5, *P* = 0.04; 2017: *F*₄,521 = 16.2, *P* < 0.001) (Fig. 1). In 2016, all other adult trapping locations had statistically similar abundance of *D. suzukii* caught season-long. In 2017, more *D. suzukii* were caught in the honeysuckle bushes season-long than all other trapping locations. The interior treatment had the least amount of *D. suzukii* caught in 2017 and was significantly lower than all treatments except the blueberries adjacent to nonhosts (*F*₄,521 = 1.3, *P* = 0.27). In both years of the study, we found a significant effect of field location on infestation in the early season (*F*₂,199 = 5.24, *P* = 0.005), with higher infestation in the blueberries adjacent to the honeysuckle...
compared with the interior of the crop ($P = 0.002$) and no significant difference from the blueberries adjacent to a nonhost ($P = 0.17$) (Fig. 2). In the late part of the season, there was no difference among infestation across any of the locations ($F_{2,250} = 0.88$, $P = 0.41$). In our vegetation surveys, all sites had similar plant communities.

**Fly dispersal**

In both trials, all leaf samples from either the egg white or milk spray zone remained marked until the last sample date, which was $4$ days after the spray. Unsprayed areas remained unmarked throughout the study. In July, over $80\%$ of the flies captured in the traps at each sampling site were analyzed for the presence of the protein marks. In September, a slightly lower percentage of flies was assayed because there was a higher number of flies collected in the traps (Fig. 3). In July, the percentage of marked flies from the source mark zone found $120\,\text{m}$ away was statistically lower than the percentage found in September, regardless of mark type ($F_{1,402} = 5.9$, $P = 0.01$). Across both trials, there was a similar distribution of male and female flies at each trapping location ($F_{1,402} = 0.7$, $P = 0.41$). Likewise, there was no significant difference in distribution of marked flies by latitude ($F_{1,402} = 0.4$, $P = 0.54$) or longitude ($F_{1,402} = 0.5$, $P = 0.50$) across both trials.

In the July mark–capture trial, we analyzed $587$ flies for the presence of the two protein markers over the course of the trial (three sampling dates). Only $10$ flies were caught $12\,\text{h}$ after the proteins were applied in July and none of them tested positive for either the egg white or milk mark. At $36\,\text{h}$, $31$ flies were captured. Of these, none contained egg white protein and five contained milk protein, all of which were found within a
honesuckle bush. At 84 h, 546 flies were analyzed for the two protein marks. Of these, six contained the egg white mark and 16 contained the milk mark. Moreover, the protein-marked flies were evenly dispersed throughout all distances from the source mark (egg white: $F_{5,41} = 1.0, P = 0.4$; milk: $F_{5,41} = 0.8, P = 0.6$). Throughout the July trial, the slope for milk marked individuals from the source of the mark to 120 m away was steeper, although not significantly different from the slope of flies marked with egg ($F_{1,38} = 2.1, P = 0.08$) (Fig. 4A). The percentage of flies marked with milk in the honeysuckle (5.5 ± 3.8%) was higher but not statistically different from the percentage of flies marked with milk in the blueberry crop (2.8 ± 1.3%; $F_{1,6} = 0.6, P = 0.5$). The percentage of flies marked with egg in the egg-sprayed zone of the blueberries (0.6 ± 0.7%) was lower but not statistically different from the percentage marked with egg outside of the egg-marked zone (1.8 ± 1.4%) for each transect ($F_{1,6} = 0.8, P = 0.4$) (Fig. 5A). In this trial, maximum dispersal distance of marked flies was recorded at 120 m and this occurred at 84 h.

In the September mark–capture trial, we analyzed 1294 flies. Throughout the September trial, the source of the mark (0 m) had significantly more egg-marked individuals than all other sampling sites, although there was an even distribution (0 m) had significantly more egg-marked individuals than all other sampling sites, although there was an even distribution (0 m) had significantly more egg-marked individuals than all other sampling sites, although there was an even distribution throughout all distances from the source mark (egg white: $F_{4,41} = 1.0, P = 0.4$; milk: $F_{4,41} = 0.8, P = 0.6$). Throughout the September trial, the slope for milk marked individuals from the source of the mark to 120 m away was steeper, although not significantly different from the slope of flies marked with egg ($F_{1,38} = 2.1, P = 0.08$) (Fig. 4A). The percentage of flies marked with milk in the honeysuckle (5.5 ± 3.8%) was higher but not statistically different from the percentage of flies marked with milk in the blueberry crop (2.8 ± 1.3%; $F_{1,6} = 0.6, P = 0.5$). The percentage of flies marked with egg in the egg-sprayed zone of the blueberries (0.6 ± 0.7%) was lower but not statistically different from the percentage marked with egg outside of the egg-marked zone (1.8 ± 1.4%) for each transect ($F_{1,6} = 0.8, P = 0.4$) (Fig. 5A). In this trial, maximum dispersal distance of marked flies was recorded at 120 m and this occurred at 84 h.

In the September mark–capture trial, we analyzed 1294 flies. Throughout the September trial, the source of the mark (0 m) had significantly more egg-marked individuals than all other sampling sites, although there was an even distribution throughout all distances from the source mark (egg white: $F_{5,131} = 71.3, P < 0.0001$). This was consistent across the sampling intervals at 12 h ($F_{5,39} = 74.5, P < 0.0001$), 36 h ($F_{5,38} = 56.0, P < 0.0001$) and 84 h ($F_{5,42} = 9.3, P < 0.0001$). The same trend was observed for the milk-marked flies ($F_{5,131} = 48.1, P < 0.0001$), 12 h ($F_{5,39} = 14.6, P < 0.0001$), 36 h ($F_{5,38} = 27.3, P < 0.0001$) and 84 h ($F_{5,42} = 11.6, P < 0.0001$). In September, the slope for egg white marked flies with distance was significantly different from that for milk marked flies ($F_{1,206} = 10.6, P = 0.001$) (Fig. 4B). The percentage of flies marked with egg within the honeysuckle (29.4 ± 11.8%) was significantly higher than the percentage of flies marked with milk in the blueberry crop (7.2 ± 1.2%; $F_{1,43} = 13.9, P = 0.009$). Likewise, the percentage of flies marked with egg within the egg-sprayed zone of the blueberries (52.7 ± 17.4%) was significantly higher than the percentage of flies marked with egg outside of the egg-marked zone (16.5 ± 6.5%) for each transect ($F_{1,6} = 41.6, P < 0.001$) (Fig. 5B). In this trial, maximum dispersal distance was also recorded at 120 m, which occurred at all sampling times.

**Discussion**

*Drosophila suzukii* was found commonly in five wild hosts that are present in the habitats surrounding blueberry fields in central and west-central Michigan across two growing seasons (Table 1). This pest is highly polyphagous and is known to utilize a variety of wild hosts, many of which were not found in the present study (Lee et al., 2015; Poyet et al., 2015; Briem et al., 2016; Kenis et al., 2016; Thistlewood et al., 2018). We expect that the wild hosts sampled are only a fraction of the hosts available to *D. suzukii* in our landscape and these data do not represent the complete host range of wild fruits. However, our survey focused on wild fruits that were commonly infested by *D. suzukii* directly adjacent to blueberry farms and identified the plants most likely to influence populations of this fly in adjacent blueberry fields. As such, we found honeysuckle to be infested at all blueberry farms surveyed and producing ripe fruit approximately 3 weeks before blueberry ripened, potentially allowing one or two generations of *D. suzukii* to build up (Tochen et al., 2014) and subsequently infest the nearby blueberry.

Indeed, when honeysuckle was investigated further, we found more *D. suzukii* trapped season-long at sites with honeysuckle bushes than those without (Fig. 1). Subsequent infestation in the early fruiting period of blueberry revealed more larvae in the crop adjacent to honeysuckle compared with the interior planting (Fig. 2). Although there was no statistical difference of infestation between the crop adjacent to a honeysuckle bush and the crop adjacent to a nonhost, infestation was consistently higher near the wild host and may represent a spillover effect (Rand et al., 2006; Tonina et al., 2018). In the later part of the season, when fly populations were very high at these unmanaged sites, infestation was found throughout the blueberry field regardless of location. Our results suggest that honeysuckle may be an important early season resource for this pest. Measuring the effects of one plant in a complex landscape can be challenging, although the influence of honeysuckle on *D. suzukii* was
consistent across all farms evaluated. Moreover, honeysuckle can grow in a variety of environments (Laken & Thieret, 1995; Hutchinson & Vankat, 1997) and their presence across multiple farms does not necessarily indicate that these habitats are similar. Although the vegetation surveys showed little inter-site variation in plant composition, spatial and environmental factors, including proximity to water, overwintering habitat, amount of wooded or semi-natural area and other resources were not evaluated in the present study and may also affect D. suzukii movement. All farms used for this experiment were organically managed or received minimal pest management, such that we could evaluate the behaviour and movement of D. suzukii with minimal influence of insecticides. Populations of D. suzukii tend to be much higher in these farms compared with conventional farms (H. Leach, unpublished data) and so the dependence and influence of honeysuckle on populations could be more apparent in conventional farms, where flies may be more dependent on wild hosts and refugia.

In comparison with the other wild fruits surrounding blueberry farms, honeysuckle has one of the lowest average densities of D. suzukii per gram of fruit, indicating that it may not be a preferred host plant (Diepenbrock et al., 2016). Himalayan blackberry, wild raspberry and pokeweed, in comparison, have more fly larvae per gram and therefore may be preferable hosts. In part, this could be because of large D. suzukii populations in the late summer and autumn, resulting in the high utilization of all available hosts (Lee et al., 2015), whereas honeysuckle produces fruit in the early season. Importantly, although these other wild hosts were present, they were not abundantly surrounding each site, as with honeysuckle. If these preferred hosts were more abundant, we might expect to see an even greater influence of D. suzukii population expansion near these wild hosts and into the surrounding blueberry.

Using a protein immunomarking technique, we found that D. suzukii moved from honeysuckle plants into the surrounding blueberry. Klick et al. (2016) found similar results for D. suzukii dispersal in Oregon farms, with higher populations of this pest in stands of wild hosts and in cultivated raspberries near the wild host compared with nonhosts. In the early season trial, we found that more flies were moving from the honeysuckle into the blueberry, although this was not significantly different from flies moving throughout the blueberry (Fig. 5). Dispersal across all distances was even in July, whereas it was biased towards the mark source in September, indicating that flies are more mobile in July than in September. We can infer that, as a result of greater host availability during September, they do not need to be as mobile to find a resource for oviposition. Other variables, such as temperature, day length and mate finding may also influence the mobility of D. suzukii, although there is little published information on the magnitude of these effects (Van Timmeren et al. 2017b). Additionally, more flies were marked in September compared with July, which is likely a product of population size and detection ability.

The present study had different sized spray areas with a 50-fold larger egg white spray area in the field compared with the milk protein on the honeysuckle, which would increase the probability of capturing an egg white marked fly. However, the pattern of movement away from the sprayed plants should be unaffected by the sprayed size. Despite this difference in spray area, we found a higher proportion of milk marked flies compared with egg white marked flies recaptured throughout both trials. This may further support the degree to which honeysuckle not only is utilized, but also acts as a population source for these flies. The even dispersal pattern throughout the blueberry from the honeysuckle, however, was unexpected. Our first experiment from adult and larval infestation suggests that flies are moving from the honeysuckle to the directly adjacent blueberry. The trends in movement patterns observed in the present study could be explained by high populations of D. suzukii on the crop edge compared with the interior regardless of whether there is a presence of a wild host (Fig. 1). Although we have shown that D. suzukii are indeed using honeysuckle as a host (Table 1), their
likelihood of dispersal is high, especially in the early season (Fig. 4). Flies are likely coming from multiple sources and the presence of honeysuckle alone may not be sufficient to affect their movement patterns.

In the present study, we also found that the flies dispersed up to 120 m, which was the maximum flight distance tested. This is similar to the findings reported by Kirkpatrick et al. (2018) in Michigan cherries, with a maximum dispersal determined at 90 m. We have shown that movement of \( D. \) suzukii is frequent and evenly dispersed, and that they can move at least 120 m regularly and within short time periods. Interestingly, Klick et al. (2016) found that \( D. \) suzukii had a clumped distribution in the crop adjacent to the wild host compared with nonhosts. Tait et al. (2018) also found dispersal up to 9 km in a mountainous landscape. There may be regional differences in behaviour, and we suspect that propensity for this insect to move is dependent on many factors, including crop type, wild host type, environmental conditions, presence of pesticides, topographic features (Tait et al., 2018) and wind dispersal (Asplen et al., 2015). We have also shown that their movement patterns are different depending on the time of year and this is likely influenced by host availability. Tonina et al. (2018) also found that vertical and horizontal movement of \( D. \) suzukii is dependent on host availability in sweet cherry. Additionally, it is possible that the presence of our yeast-sugar traps may alter their behaviour or movement patterns. However, traps in the present study were 24 m apart from each other and, in previous studies, the estimated area of activity of similarly designed traps for \( D. \) suzukii was found to be only 3 m (Kirkpatrick et al., 2018).

The results of the present study support our hypothesis that \( D. \) suzukii moves from honeysuckle into the surrounding blueberry crop, especially in the early season, which has implications for management.

Other studies have focused on how increasing natural and semi-natural habitat adjacent to a farm can increase ecosystem services, namely increasing natural enemy abundance (Varchola & Dunn, 2001; Denys & Tschamkette, 2002; Bianchi et al., 2006; Hendrickx et al., 2007). However, the relationship between increased natural area and management of polyphagous pests is less understood. Panizzi (1997) found higher pest pressure from multiple species of stink bugs with the presence of wild hosts. For invasive species, this research is especially limited, although Bakken et al. (2015) found a greater abundance of the brown marmorated stink bug associated with adjacent wild hosts. Associations between diseases and invasive wild hosts are often considered to be beneficial for managing insecticide resistance (Mallet & Porter, 1992; Livingston et al., 2004). Given the current management tactics for \( D. \) suzukii (Diepenbrock et al., 2016), this may be an exceptionally important aspect for managing resistant populations of this multivoltine pest. We must also consider the role of biological control for \( D. \) suzukii, especially regarding the prospects of classical biological control (Daane et al., 2016; Knoll et al., 2017; Haro-Barchin et al., 2018; Wang et al., 2018). These natural habitats and presence of wild hosts may serve as areas where parasitoids can seek refuge from insecticide-sprayed crops (Hochberg & Hawkins, 1992; Landis et al., 2005) and then successfully attack \( D. \) suzukii. Indeed, Haro-Barchin et al. (2018) found a positive relationship between abundance of parasitoids attacking \( D. \) suzukii and landscape complexity.

The potential benefits of these host plants or their removal from the farmscape for managing \( D. \) suzukii have not been explored and deserve investigation. However, because the host range of \( D. \) suzukii is so expansive (Lee et al., 2015) and their dispersal is at least 120 m, removal of all wild hosts would likely be labour intensive, costly and potentially not feasible for some farms. Additionally, susceptible crops that produce fruit early, such as cherries and blueberries, are less likely to see a population effect from surrounding wild hosts and their removal is unlikely to benefit \( D. \) suzukii management (Santoiemma et al., 2018). Because \( D. \) suzukii dispersal can be up to 9 km (Tait et al., 2018) and few flies are needed to begin an infestation as a result of their rapid life cycle and fecundity (Tochen et al., 2014), any benefit from the removal of wild hosts is likely to be regionally and crop specific. Scouting the crop edge for the presence of wild hosts and monitoring these areas, however, may provide researchers and growers with a tool to be able to predict \( D. \) suzukii hot-spots where there is expected to be first spring activity, especially in locations with early-fruiting wild hosts.

In summary, as we continue to develop an integrated pest management framework for \( D. \) suzukii tailored to specific cropping systems, we should also understand and evaluate the ecological factors that may limit the elevation of \( D. \) suzukii populations on a landscape scale (Lewis et al., 1997). This includes evaluating alternate hosts within farm landscapes, especially given the potential of \( D. \) suzukii to regularly move at least 120 m.

The knowledge gained from this research will strengthen our understanding of the behaviour, movement and local carrying capacity within farms, enabling the continued implementation of farm-specific pest management strategies for this damaging invasive fruit pest.

Acknowledgements

We thank Emilie Cole, Elizabeth Espeland, Logan Rowe, Jaclyn Stone, Stacy Kempfer, Patrick Stillson and Steve Van Timmeren for their technical assistance. We are grateful to those growers who allowed us access to their farms. This project was supported by the USDA Specialty Crops Research Initiative under Agreement No. 2015-51181-24252 and the Michigan State University Department of Entomology Hutson Endowment Research Funds. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply a recommendation or endorsement by the United States Department of Agriculture. USDA is an equal opportunity provider and employer.

HL and RI conceived and designed the research. HL, JH, and SM conducted the protein-specific ELISAs. HL conducted the field experiments and analyzed the data. All authors have read, reviewed and approved the final version of the manuscript submitted for publication. The authors declare that they have no conflicts of interest.
References


Accepted 29 October 2018