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ORIGINAL PAPER

Rapid harvest schedules and fruit removal as non-chemical approaches for managing spotted wing Drosophila

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Abstract Spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), has caused significant economic losses to small fruit and berry growers throughout the USA and Europe since its invasion. This pest can lay many eggs over its lifetime within ripening and ripe berries, causing yield loss and the risk of fruit contamination. Zero tolerance for this pest has led to increased use of broad-spectrum insecticides to control it, which are costly and pose many other sustainability and pest management concerns. There is an urgent need to evaluate management strategies that can decrease reliance on chemical controls and mitigate economic losses. Over two growing seasons, we compared harvest schedules for their effect on infestation by *D. suzukii*, revealing that fruit harvested every 1 or 2 days had significantly fewer *D. suzukii* larvae than a 3-day harvest schedule. Furthermore, we found that yield per unit effort was highest on a 2-day schedule. Sanitation of the crop is another important component of a successful integrated pest management program, and we found that bagging infested waste berries killed 99% of larvae after 32 h, with higher fruit temperatures in clear bags than white or black bags. In combination, these methods can reduce the effects of this invasive pest on raspberry production. This study will provide guidance to growers on culturally based IPM tactics to decrease reliance on chemical management.

Keywords *Rubus idaeus* · Cultural control · Integrated pest management · Harvest frequency · Sanitation

Key message

- New management techniques for *Drosophila suzukii* are needed to decrease reliance on insecticidal control.
- Reducing the harvest interval of raspberries to 1–2 days decreases the prevalence of *D. suzukii* larvae.
- Harvesting every 2 days had higher marketable yield than plots harvested every day.
- Bagging infested berries in clear plastic for 32 h kills 99% of *D. suzukii* larvae.

Introduction

In the last eight years, berry production throughout North America and Europe has faced a new pest, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), a polyphagous frugivore native to East Asia. Adult females lay eggs into ripe and ripening fruit by means of a serrated ovipositor, causing direct damage to the fruit (Hamby et al. 2016). While *D. suzukii* has a large host range, the most affected crops are raspberries, blackberries, blueberries, and cherries (Lee et al. 2011a; Bellamy et al. 2013). *Drosophila suzukii* can develop from egg to adult in as little as 10 days and females can live for 1 month during the growing season and lay over 300 eggs (Kinjo et al. 2014; Tochen et al. 2014). The resulting multiple

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generations developing each summer can create intense pest pressure, particularly for crops ripening late in the growing season.

Estimated economic losses from *D. suzukii* in the western US for raspberries, blackberries, blueberries, strawberries, and cherries were predicted to be up to $500 million annually (Bolda et al. 2010; Goodhue et al. 2011; Farnsworth et al. 2016). In 2014, *D. suzukii* caused estimated economic losses of $159 million in US raspberry production (Burrack et al. unpublished data). Fewer reports on the economic losses from this pest are available from Europe, though estimated losses increased by $2.5 million in one province in Italy from 2010 to 2011 (De Ros et al. 2015). Additionally, complete crop loss has been noted in Europe on organic strawberries, raspberries, and cherries (Cini et al. 2012; Weydert and Mandrin 2013). While control of this pest has improved (Bruck et al. 2011; Van Timmeren and Isaacs 2013; Diepenbrock et al. 2016), economic losses have continued because of reduced nursery sales, increased pest management costs, and downgraded or culled fruit. Revenue losses from California raspberry growers have been almost eliminated in recent years due to effective chemical management strategies, but the sustainability of these control measures still threaten long-term berry production and are more challenging for organic growers (Farnsworth et al. 2016).

Current management of *D. suzukii* is insecticide based, with limited options for alternative controls (Van Timmeren and Isaacs 2013; Asplen et al. 2015; Diepenbrock et al. 2016; Wiman et al. 2016). These insecticide programs are intensive, with optimum control given by applications every 5–7 days during the fruit ripening season (Diepenbrock et al. 2016). This is costly both in terms of material and labor, and growers are challenged to meet maximum residue limits (MRLs) and pre-harvest intervals while maintaining control of this pest. Furthermore, frequent applications increase the risk of insecticide resistance, especially because *D. suzukii* has both high fecundity and generation turnover (Asplen et al. 2015; Diepenbrock et al. 2016; Wiman et al. 2016). Frequent insecticide applications pose risks to beneficial arthropods, including natural enemies that keep these secondary pests below damaging levels (Desneux et al. 2007; Biondi et al. 2012). Secondary pests of the affected crops have emerged recently in many berry production systems (R. Isaacs, unpublished), likely due to the overuse of broad-spectrum insecticides. In other crops with high inputs of insecticides, similar secondary pest outbreaks have been noted (Beers et al. 2016; Yang et al. 2016). It has become imperative to develop alternative tactics for control within an integrated pest management framework. This will be critical for delaying the development of insecticide resistance. Cultural control can work well in combination with insecticide applications making it a promising area to expand *D. suzukii* management options (Haye et al. 2016).

Raspberries and blackberries ripen over a long period, and adjusting the frequency of fruit harvest is a potential way to reduce *D. suzukii* infestation (Haye et al. 2016; Farnsworth et al. 2016). Growers have typically harvested a few times each week, thereby preventing berries from getting overripe (Bolda et al. 2012). However, the short development period of *D. suzukii* and grower experience suggests that shorter harvest intervals could reduce infestation, particularly of the most detectable third instar larvae, in harvested fruit.

Additionally, little guidance is given on what to do with the infested fruit after it is collected. Recommended options for disposal include fruit burial, freezing, or bagging (Haye et al. 2016). Of these options, bagging the unsaleable fruit is likely to be preferred by most growers since burial requires labor and freezing requires both equipment and space. Furthermore, burial has shown to not sufficiently reduce *D. suzukii* pressure in small field trials (Lee et al. 2011b). Guidance should be provided on whether bagging fruit is necessary to reduce *D. suzukii* populations and how long the bags should be left in the sun before further disposal. Bags are likely to split or become unsealed, so ensuring the death of *D. suzukii* larvae in these bags is important.

To understand the potential of cultural control approaches to reduce the risk of infestation by *D. suzukii,* we compared infestation and yield from plots with different harvest frequencies. We also investigated waste fruit removal by quantifying the emergence of *D. suzukii* from fruit held in different colored plastic bags compared with fruit left in the open. The aim of this paper is to provide guidelines for the integration of these cultural control strategies into existing pest management programs for this challenging fly pest.

Materials and methods

Harvest frequency

This study was conducted at the Horticultural Teaching and Research Center in East Lansing, MI in mid-August in 2015 and 2016, when raspberries were ripe and *D. suzukii* populations were high. A planting of raspberries (*Rubus idaeus* cv. 'Himbo Top') managed for primocane harvest was used for this experiment. The plants were managed under organic standards and were located in multiple 7.6 × 122 m Haygrove high tunnels (Haygrove Ltd, Herefordshire, UK) covered with Luminance® plastic (Visqueen, Stevenston, UK). Twelve plots consisting of single rows 10 m long were established in the planting, arranged in a randomized complete block design with each of three harvest treatments replicated four times. At the beginning of the trial in each year, all ripe fruit was harvested from each plot, and then, plots were harvested every 1, 2, or 3 d by a crew of pickers trained to pick only ripe fruit (fully colored berries that detached readily). In both years, there were nine harvests conducted every day, four harvests made every 2 d, and three harvests made every 3 d. For each sampling date, additional fruit was collected from a plot of the same variety within 10 m of the research plots and incubated to confirm that the larvae were *D. suzukii*.

For each harvest, the number and weight (g) of berries harvested from each plot was recorded. These berries were then put into a salt test (Leach et al. 2016) to extract *Drosophila* eggs and larvae. The berries and liquid were filtered through a coarse screen and the remaining liquid was then put through a reusable coffee filter (4-Cup Universal Coffee Filter, Medelco Incorporated, Bridgeport, CT) and the contents left were examined under a SZX10 stereomicroscope (Olympus America, Inc., Center Valley, PA). The number of intact *Drosophila* eggs and larvae in each sample were counted and categorized as first instars (approximately 1 mm long), second instars (larger than 2 mm), or third instars (about 4 mm). First and second larval instars were grouped together for statistical analysis, which distinguishes these undetectable smaller instars from the visibly detectable third instars. In 2016, the total weight of fruit harvested from each plot during each harvest and the amount of time spent harvesting each plot were also recorded, in order to calculate harvest rate (hours per kg). Yeast–sugar traps (Van Timmeren and Isaacs 2013) were used to capture adult *D. suzukii* for an estimation of adult populations. Eight traps were deployed in 2015 and twelve traps were deployed in 2016 and the number of *D. suzukii* captured was counted weekly. Traps were within 10 m of the research plots.

Fruit bagging for disposal

In September 2016, overripe raspberries that were infested with *D. suzukii* were collected from the Horticulture Teaching and Research Center in East Lansing, MI. Samples of 0.7 L were placed into four different containers: clear, white, or black plastic bags (0.1 m^3, 0.02 mm thick, Array, Gordon Food Service, Grand Rapids, MI) or unsealed 5.7 L clear plastic storage containers as a control. The bag treatments each used two bags, one inside the other, so that the white and black bags were entirely opaque. A temperature probe (Hobo Pendant® Temperature Data Logger, Onset Computer Corporation, Bourne, MA) was placed in the center of the fruit within each bag or container to record the temperature every minute. Each treatment was replicated four times, and the containers were placed in an open grassy field without shade for 5 d in a randomized complete block design with the location of each treatment separated by 5 m. A temperature probe inside of a radiation shield (Spectrum® Technologies, Inc., Aurora, IL) was also placed in the center of the block design, 1 m above ground to record ambient temperature.

From each bag or container, 0.06 L of fruit was collected at 0 (initial), 1, 4, 8, and 30 h after placement. Each sample was weighed and put into a 0.5-L plastic container (Gordon Food Service, Grand Rapids, MI) fitted with a mesh top and a yellow sticky panel at the top to capture emerging *D. suzukii* adults. A salt test was also done (as described above) on the initial samples to identify the age composition of the larvae. At each sampling period, surface temperature of the fruit or the bags was also recorded using a non-contact infrared thermometer (LaserGrip 630, Etekcity Co., Anaheim, CA) that took readings three times on each container. After the fruit samples were held for two weeks in an environmental chamber at 24 °C, 65% RH, and a photoperiod of 16:8 L:D, the number of trapped adult *D. suzukii* was counted.

Statistical analyses

Egg and larval infestation data from the harvest frequency study satisfied normality assumptions and were analyzed using a two-way analysis of variance. This was done using the GLM function with a negative binomial distribution and the link function set to log transform. This was followed by Tukey's Honest Significant Difference for post hoc comparisons. The egg and larval infestation data were the response variables. Data from the fruit removal experiment were analyzed using analysis of variance followed by Tukey's Honest Significant Difference for post hoc comparisons among the mean values. Data were analyzed using R (3.2.2., R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Harvest frequency

There were significantly fewer eggs and larvae when fruits were harvested every 1 or 2 d compared to every 3 d in 2015 (Fig. 1a) (eggs: d.f. = 2, 61; $F = 5.96$; $p = 0.022$, first and second instars: d.f. = 2, 61; $F = 10.74$; $p = 0.004$, third instars: d.f. = 2, 61; $F = 6.85$; $p = 0.015$). In 2015, there was no significant difference between picking daily and picking every 2 days among all life stages of *D. suzukii*. In 2016, the trends were similar to the previous year, with significantly lower infestation by

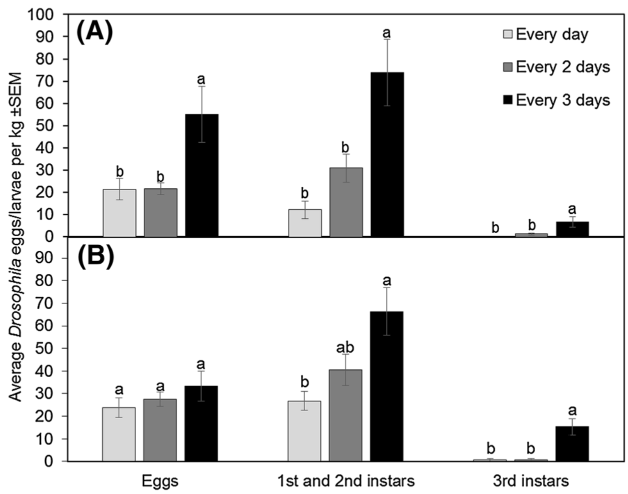


Fig. 1 Average number of eggs, undetectable larvae (first and second instars), and detectable larvae (third instar) (±SEM) per kilogram of raspberries when harvested every 1 d, every 2 d, and every 3 d in 2015 (**a**) and 2016 (**b**). *Bars* marked with the same letter within each life stage are not significantly different at $\alpha = 0.05$

smaller and larger larvae in the 1-d treatment compared to the 3-d treatment (Fig. 1b) eggs (d.f. = 2, 61; $F = 0.80$; $p = 0.45$), first and second instars (d.f. = 2, 61; $F = 8.92$; $p < 0.0001$), and third instars (d.f. = 2, 61; $F = 27.49$; $p < 0.0001$). In the second year, we found no statistical differences in the number of eggs found in fruit among the three harvest treatments.

In 2015, 100% of the *Drosophila* larvae reared from collected fruit were identified as *D. suzukii*. In 2016, 80.2% of the *Drosophila* larvae reared from collected fruit were identified as *D. suzukii*, and the remaining were other common *Drosophila* species, including *Drosophila simulans* Sturtevant and *Drosophila melanogaster* Meigen. *Zaprionus indianus* Gupta was also found in low numbers in these samples, which is an emerging invasive pest in the Drosophilidae family (Van Timmeren and Isaacs 2014). It is important to note that during our salt-testing procedure, no clusters of *Drosophila* eggs were seen, which would indicate oviposition by other *Drosophila* species (Mitsui et al. 2006). There was an average of 17.4 ± 4.6 adult *D. suzukii* flies caught per trap per week in 2015, and an average of 29.3 ± 5.2 adult flies caught per trap per week in 2016.

The average daily yield (kg) per hectare was highest in plots harvested every 2 d (134.52 ± 11.76 kg) followed by every 3 d (89.59 ± 7.42 kg) and every 1 d (88.31 ± 9.30 kg), and the 2-d plots had significantly higher yield than the 1-d plots (d.f. = 2, 61; $F = 5.16$; $p = 0.008$). The harvest rate was significantly higher from plots picked every 2 d (0.22 ± 0.02 kg/hr) than those harvested every 1 d (0.17 ± 0.01 kg/hr), but every 3-d treatment (0.20 ± 0.02 kg/hr) was not different from the 1- or 2-d treatments (d.f. = 2, 61; $F = 4.71$; $p = 0.013$).

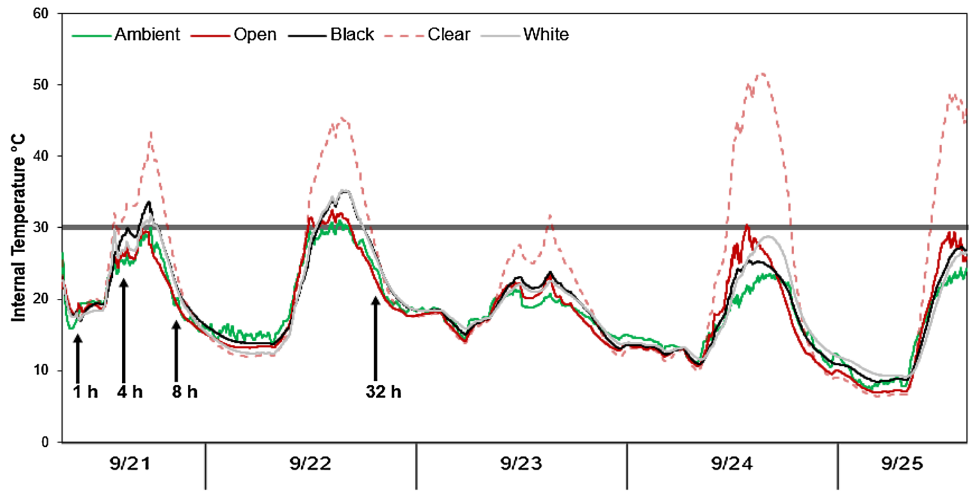
Fruit bagging for disposal

The initial salt test indicated fruit contained mostly second instars (106.76 ± 31.0 larvae/kg) followed by first instars (75.89 ± 21.35), third instars (67.87 ± 32.16), and eggs (49.93 ± 20.67). The average surface temperature was highest in the black treatment (27.55 ± 2.34 °C), and lower in the clear (24.99 ± 1.88 °C), open (22.19 ± 1.18 °C), and white (20.91 ± 1.13 °C) treatments. The black bags were significantly hotter than all other treatments except for the clear, and the other treatments were not statistically different (d.f. = 3, 60; $F = 4.95$; $p = 0.004$). Maximum surface temperatures for each of the treatments also followed the same trend with the black treatment (47.6 ± 2.34 °C) being statistically similar to the clear treatment (39.8 ± 1.88 °C) but higher than the white treatment (29.6 ± 1.13 °C) and the open fruit (37.9 ± 1.18 °C). The clear, white, and open fruit treatments did not statistically differ from each other (d.f. = 3, 60 $F = 4.58$; $p = 0.005$).

Container type also had a significant effect on the internal temperature of the raspberries (Fig. 2). On average, fruit temperatures from 11 am to 4 pm were significantly higher in the clear bags (37.19 ± 1.69 °C) compared to the black (26.49 ± 1.25 °C), open (25.96 ± 1.14 °C), or white (25.59 ± 1.78 °C) (d.f. = 3, 11; $F = 15.29$; $p < 0.001$). Maximum internal temperature for the same time period had a similar trend with the clear treatment reaching significantly higher temperatures (51.66 ± 4.61 °C), compared to the white (35.27 ± 2.31 °C), black (35.18 ± 2.11 °C), and the open treatments (32.50 ± 1.93 °C) (*d.f.* = 3, 11; $F = 20.29$; $p < 0.001$). On average, the clear bag had the greatest duration of internal temperatures surpassing the 30 °C thermal limit for *D. suzukii* larvae (Tochen et al. 2014) with an average of 5.5 ± 0.2 h of temperature readings exceeding 30 °C per day, followed by the white (2.2 ± 0.5 h), and the black and open (1.5 ± 1.5 h). Ambient temperatures exceeded 30 °C for 0.3 h per day for the duration of the study. At the 8-h sampling period, the clear (3.2 ± 0.6 h) and black (1.9 ± 1.1 h) treatments had accumulated the most time above the lethal temperature threshold, followed by the white (0.9 ± 0.5 h) and the open (0.4 ± 0.4 h). At the 32-h sampling period, the clear bag treatment had accumulated the most time surpassing 30 °C (11.2 ± 0.8 h), followed by the white (6.5 ± 0.6 h), the black (5.1 ± 1.8 h), and the open (4.1 ± 0.8 h).

In the initial sample used for rearing flies, an average of 615.0 ± 225.3 flies emerged per kg of raspberries. Emergence was statistically similar among all treatments at 1 and 4 h of exposure to sunlight (Fig. 3) (1 h: d.f. = 3,11; $F = 0.34$; $p = 0.79$, 4 h: d.f. = 3,11; $F = 1.19$; $p = 0.36$). After 8 h of exposure, raspberries held in the

Fig. 2 Average internal temperature of raspberries held within three different *colors* of plastic bags or in an open plastic container, compared to the ambient temperature. *Arrows* indicate when the fruit sampling periods took place, and the dark horizontal bar indicates the upper temperature threshold (30 °C) of *D. suzukii*. Temperature was recorded every minute. (Color figure online)

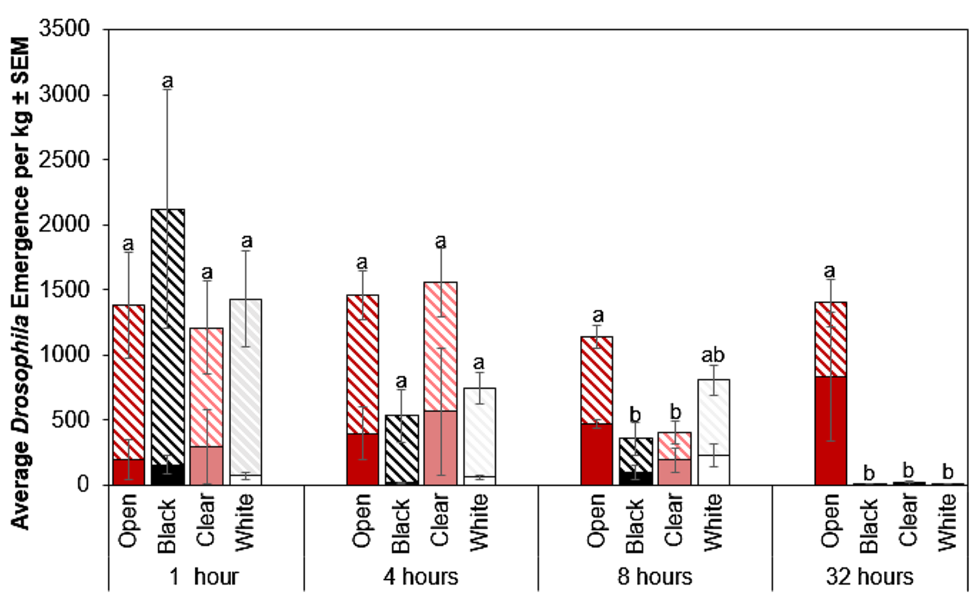


black bag and clear bag had significantly lower emergence of *D. suzukii* than the open container treatment, and the white bag was not different from any of the treatments (d.f. = 3,12; $F = 5.15$; $p = 0.02$). After 32 h, the bagged treatments had significantly lower emergence compared to the open treatment (d.f. = 3,12; $F = 4.32$; $p = 0.03$). None of the bag treatments were significantly different from each other at the 32-h sample, because emergence was close to zero.

Discussion

Increasing harvest frequency can be built into an integrated pest management plan for *D. suzukii* in raspberries and other small fruits to decrease the number of larvae infesting fruit, especially larger third instars. Fruits with large third instars are most likely to be rejected, so preventing this life stage is particularly important. When fruit is picked daily, larvae and eggs are still present but at low levels, which suggests that increasing harvest frequency should not be relied on for the sole management technique against *D. suzukii*. When berries are ripening quickly and pest pressure is high, we suggest using pesticides with short preharvest intervals in combination with a 1- or 2-d harvest frequency. We also suggest rapid postharvest cooling of the fruit to reduce damage and kill or arrest development of the larvae (Aly et al. 2016). Organic producers have limited chemical management options, and harvesting often gives them an opportunity to keep saleable fruit. While few examples of modifying harvest frequency for pest control exist, many important crop pests and diseases can be managed by changing the time of planting or harvesting early so that peak pest populations and harvest time do not

Fig. 3 Average number of *D. suzukii* (striped) and all other flies (solid) emerged (±SEM) from each bag color across the four sampling times. Other flies include *Drosophila* species and *Zaprionus indianus*. *Bars* marked with the same letter within each sample period are not significantly different at $\alpha = 0.05$ for the total number of flies emerged



intersect (Hawkins et al. 1979; Connell et al. 1989; Borgemeister et al. 1998; Miller et al. 2002; Moegenburg and Levey 2003).

Both yield and harvest rate were highest when fruit was harvested every 2 d, indicating that growers may spend the least to pick fruit at 2-d intervals. Daily picking may have reduced yields because berries continue to gain weight after they attain optimum quality for fresh marketing (Ramsay 1983). Two- and three-day intervals may allow some fruit to gain weight. While we recorded actual picking time, commercial harvesters may find rates differ when they consider the time required to transport fruit from the field, sort out culls, and transfer berries to retail containers. Furthermore, this study was conducted on only one cultivar, and results may differ with other types that vary in bush architecture, fruiting habits, and fruit ripening rates. It can be useful to explore the relative costs of labor between these harvesting schedules despite the variation among growers with their harvesting and sorting systems, distances from plantings to a central receiving location, and other variables. Typically, harvesters are paid by the weight they pick, with an estimated \$2 per kg harvested (Bolda et al. 2012). If we assume a revenue of \$8 per kg, 1-d plots would provide a partial contribution margin of about \$530, 2-d plots about \$807, and 3-d plots about \$538 per day. This estimate only includes fixed revenue and labor estimates, and does not include equipment costs, transportation and cooling of fruit, or other expenses. Moreover, it does not incorporate the potential drop in quality of the fruit and thus rejected or lower priced fruit, from increased *D. suzukii* infestation on the 3-d schedule, or potential savings from reduced insecticide applications on the 1-d or every 2-d schedules. While the exact estimates given here are likely to vary widely from grower to grower, they may provide insights that can be adjusted for different labor and equipment costs, and they highlight the need for more detailed economic analysis of how varying cultural control can affect profit and the need for insecticide applications.

We found that culled fruit need to be contained or removed since flies continue to emerge and can potentially infest clean fruit. Letting these berries continue to ripen on the cane, or drop to the ground would exacerbate pressure from *D. suzukii* and may worsen disease incidence as well (Walsh et al. 2011). Culled fruits placed in clear bags were exposed to the highest temperatures, presumably due to a greenhouse-like effect. The upper extreme temperature for *D. suzukii* development is 30 °C (Tochen et al. 2014), and the surface and internal temperatures surpassed this threshold by at least 17.8 and 20.9 °C, respectively, in the clear bags. The duration above the lethal temperature was highest within the clear bags, followed by the white and black bags. However, none of the bagged treatments were significantly different from each other at 32 h. A small number of flies were able to survive and emerge to adulthood after being contained for 32 h in the bagged treatments, which may suggest that heat alone is not the only factor that contributes to the death of the larvae. Drowning or anoxia from fruit degradation may also play a role in larval mortality. Both internal and surface temperatures were also relative to weather conditions and cloud cover. This suggests that waste fruit should be left in a sealed container for at least 2 or 3 d in direct sun to ensure all larvae have been killed. A longer time may be required when weather conditions are cooler or with greater cloud cover.

Additionally, reducing the number of larvae in overripe or waste fruit may affect potential biological control against *D. suzukii*. Endemic parasitoids and predators have been found to attack *D. suzukii* larvae in both Europe and the USA (Chabert et al. 2012; Woltz et al. 2015; Mazzetto et al. 2016), and reducing larvae could further reduce population suppression that these natural enemies provide. Additionally, parasitoids from the native range of *D. suzukii* have been evaluated for classical biological control (Daane et al. 2016), and the success of these parasitoids could be disrupted by the sanitation measures suggested here. However, *D. suzukii* has a large host range and populations are present outside of the crop (Lee et al. 2015; Klick et al. 2016), which could help natural enemy populations establish. While the activity of natural enemies was not accounted for in this study, we suggest that maintaining waste fruit for biological control to establish may cause more harm than good, and removing this fruit should be a priority for growers.

In summary, we have evaluated two cultural control tactics that could be used in an integrated management plan in combination with existing spray programs to manage *D. suzukii* in raspberry. Furthermore, these control tactics may reduce the total number of sprays needed, which is important for insecticide resistance management, maximum residue limits, potential success of biological control agents, secondary pest management, and long-term environmental sustainability.

Author contributions

All authors designed the research and conducted the experiments. HL analyzed the data. All authors wrote, read, and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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