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Abstract

BACKGROUND: Fruit growers largely depend on chemical control to reduce populations of the economically damaging invasive fly, *Drosophila suzukii* (Matsumura). *Drosophila suzukii* is susceptible to high temperatures and low humidity; therefore, it may be possible to implement cultural control practices that create microclimates unfavorable for its development and survival. In addition to other fruit production benefits, in-row mulches may impede the development of *D. suzukii* immatures when larvae leave the fruit to pupate in the soil. This study compared the effects of different mulches (black polypropylene fabric weedmats, sawdust, and wood chips) on temperature and relative humidity (RH), and on adult emergence of *D. suzukii* from larvae in blueberries and pupae, both above and below the ground surface in blueberry plantings (*Vaccinium corymbosum L.).*

RESULTS: Across regions, both lower larval survival and longer periods with high suboptimal temperatures occurred above the ground in comparison to buried below the ground, regardless of mulch type. Fewer *D. suzukii* adults emerged from larvae on weedmat mulch at one site, but there was no effect of mulch type on temperature, RH, or *D. suzukii* emergence at most sites. The relationships between temperature, RH, and the emergence of adults from larvae and pupae varied by region. Natural infestation by *D. suzukii* in blueberries was lower over weedmat compared to wood-based mulches at one site. Greenhouse experiments showed that larvae burrowed to pupate underneath sawdust mulch, but were unable to pupate underneath a weedmat mulch.

CONCLUSIONS: Although weedmats may not modify temperatures or RH enough to consistently affect *D. suzukii* emergence, they can reduce field suitability for *D. suzukii* by providing a barrier that prevents larvae from reaching favorable pupation microhabitats underground.

Keywords: Berry crops, integrated pest management, invasive species, Spotted-wing drosophila, *Vaccinium corymbosum*, weedmats.

1. Introduction

Integrated pest management (IPM) depends on the integration of chemical, biological and cultural controls to reduce insect pest populations below economic thresholds.[1] By potentially changing microhabitat conditions, cultural control practices that were originally implemented to improve the horticultural aspects of crop production can also contribute to insect pest management. For example, in berry crops, selective pruning,[2] mulching,[3] and different irrigation systems[4] that increase yield may also help reduce insect pest populations.

As part of cultural control tactics, ground manipulation and mulching can have positive effects for IPM in various crops. For instance, the installation of landscape fabric in citrus trees can reduce root weevil (*Diaprepes abbreviates* Linnaeus, Coleoptera: Curculionidae) emergence.[5] Plastic mulches of different colors can influence the number of aphids (Hemiptera: Aphididae), thrips (Thysanoptera), and whiteflies (Hemiptera: Aleyrodidae)[6] in tomatoes (*Lycopersicon esculentum* Mill.). Furthermore, combining reflective mulches and insecticides can suppress silverleaf whiteflies (*Bemisia argentifolii* Bellows and Perring) and melon aphids (*Aphis gossypii* Glover, Hemiptera: Aphididae) in zucchini squash (*Cucurbita pepo* L.) crops.[7] Similarly, incorporation of metallized mulches can delay the colonization of Asian citrus psyllid (*Diaphorina citri* Kuwayama, Hemiptera: Psyllidae) in hybrid orange.[8] Black plastic mulch reduces foliar injury by Mexican bean beetles (*Epilachna varivestis* Mulsant, Coleoptera: Coccinellidae), compared to bare soil in snap beans (*Phaseolus vulgaris* L.).[9] Finally, mulch type and soil moisture content can affect pupation depth and emergence of blueberry maggot (*Rhagoletis mendax* Curran, Diptera: Tephritidae).[10,11] It is thus evident that different types of mulches can contribute to suppress insect pest populations.

Polypropylene fabric mats (also known as weedmats or weedmat mulch) are used to suppress weed growth, and to retain water and nutrients in multiple cropping systems.[12] Together, these effects can increase fruit yield and quality. For example, blueberry plants of various cultivars grown on a black weedmat mulch produce higher yields and larger berries than plants grown on sawdust without weedmat.[13] Additionally, weedmats can improve sanitation, as fallen fruit can be more easily removed, thereby minimizing favorable conditions for the development of disease and insect pest populations. The deployment of weedmats into blueberry crops can also increase soil temperatures and moisture levels,[14] and modify light intensity in the plant canopy.[15] These modifications of abiotic factors can affect insect survival, and it may therefore be possible to use weedmats to create unfavorable habitats for insect pest colonization, reproduction and survival.

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive pest of berries and small fruit. Native to Asia, *D. suzukii* was first detected in North America and Europe in 2008,[16,17] and in South America in 2013,[18] spreading rapidly within and across these continents.[19,20] In blueberries, female flies can lay eggs in the ripening fruit after the green-pink stage.[21,22] Current *D. suzukii* management programs primarily depend on multiple applications of broad-spectrum insecticides before and during the harvest period when fruit are susceptible to attack.[23,24] Such pest management strategies are environmentally and economically unsustainable, thus there is an urgent need to develop complementary IPM components to mitigate *D. suzukii* crop damage and reduce dependence on insecticide-based approaches.[25] *Drosophila suzukii* is susceptible to unfavorable environmental conditions,[26] and it may be possible to manipulate these conditions in order to reduce populations and infestation in berry crops.

Abiotic factors such as high temperature and low humidity can negatively affect *D. suzukii* oviposition and survival.[27] Relative humidity (RH) levels at and above 80% are optimal for larval development, female egg production, and activity.[28] Additionally, optimal female fecundity occurs at around 22 °C, and larval developmental time is shortest between 22 °C and 28 °C, whereas temperatures above 30 ˚C reduce reproduction and larval development.[29,30] Adult *D. suzukii* tend to be more abundant and exhibit higher oviposition in the center of the plant canopy where RH is higher and temperatures are more moderate.[31,32] Furthermore, *D. suzukii* pupae have higher survival rates in humid environments.[33] Larvae within fruit are less able than adults to behaviorally avoid unfavorable conditions. Therefore, by modifying the architecture of crop landscapes in ways that increase the unsuitability of abiotic factors for *D. suzukii* immature survival, it could be possible to create a detrimental microenvironment that will reduce infestation of berry crops by *D. suzukii*.

A few effective cultural control practices have been studied for *D. suzukii* management. Exclusion netting[34,35] and regular harvests and removal of overripe fruit remaining on the trees and ground[36] can reduce *D. suzukii* populations in fruit crops. Reduction of ambient humidity through drip irrigation can also reduce *D. suzukii* emergence from pupae.[33] *Drosophila suzukii* populations consist mainly of immature life stages (eggs, larvae, and pupae) during the growing season[37] and control strategies should therefore aim to suppress these stages. Third instar larvae usually exit the fruit to pupate in the soil,[38,39] and in this plant/soil interface larvae are vulnerable to predation, extreme temperatures, and desiccation. Therefore, cultural control strategies that manipulate the soil surface hold some promise for minimizing survival of *D. suzukii* immature life stages. Although black weedmat is widely used for its horticultural benefits in northwestern United States berry crops,[13] its effects on *D. suzukii* survival have not been studied.

The goal of this study was to determine how mulching type can modify abiotic factors such as temperature and RH in blueberry crops, and consequently affect emergence of *D. suzukii* flies from larvae and pupae. We also compared naturally occurring *D. suzukii* infestation between different mulch types in blueberries in different regions of the United States.

2. Methods

2.1. Mulching trial field layout

Field experiments were conducted at blueberry plantings in five US states (Georgia GA, Maryland MD-Q and MD-K, Michigan MI, Minnesota MN, Oregon OR) during 2016 and 2017. Characteristics of the blueberry plantings are described in Table 1. Within each planting, 'weedmat' and 'grower standard' mulch treatments were applied to blueberry plant rows, such that each experimental unit consisted of a row section (plot) with each mulching treatment, replicated across different rows (experimental design at each site is summarized in Table 2). 'Weedmat' mulch treatments consisted of black woven polypropylene fabric mats (obtained from local farm suppliers, see Table 1) installed on top of mulch or soil on plant rows. Other mulch types including wood chips or sawdust were selected based on local grower standard practice (Table 1).

2.2. Abiotic factors and D. suzukii *emergence from artificial infestation in blueberry plots*

We explored the following variables as predictors of temperature, percent relative humidity (RH), and *D. suzukii* adult emergence from larvae or pupae in blueberry plots: (1) mulch type (local grower standard mulching, local mulching covered by black weedmat, or bare soil; Table 1), (2) location in relation to mulch (above or buried 1 - 3 cm below the mulch), and (3) time point within the harvest season (early, mid, or late season; specific trial dates are given in Table 2).

Temperature and RH were measured every 10 or 20 min in the blueberry plots using dataloggers (Table 2) during seven-day periods when artificially infested blueberries and *D. suzukii* pupae were exposed in the field (see below). Dataloggers were placed in the middle of each plot replicate, underneath a blueberry bush. From each treatment, we measured total time above 30 °C (developmental arrest threshold temperature),[30] and mean RH for a seven-day exposure period. In MI, temperatures were continuously recorded during the period when natural infestation was assessed (Jul – Aug 2016 and 2017). To allow categorical comparisons similar to the other sites, four seven-day periods (mid 2016: 2 Aug 2016, late 2016: 25 Aug 2016, mid 2017: 19 Jul 2017, and late 2017: 9 Aug 2017) were selected for analyses for MI.

To determine the effect of each variable on temperature and RH, a general linear model (GLM) was used to analyze the influence of mulch type, mulch location, and season as fixed effects, separately by site. We used a p-value of 0.05 on all tests to reject the null hypothesis that the independent variables tested did not have an effect on the outcome variables. The GA site did not have enough datalogger replicates, and was excluded from this analysis. Variables were transformed using a Box-Cox power transformation to meet assumptions of normality and heteroscedasticity.[40] All data analyses were performed in RStudio;[41] data were organized using the package “dplyr”,[42] and all figures generated using the package “ggplot2”.[43] Transformations and GLM analyses were performed using the R packages “MASS”[44] and “car”.[45]

To explore adult emergence from larvae in blueberries, unsprayed ripe blueberries were infested with *D. suzukii* eggs by exposing the blueberries to mature *D. suzukii* females from laboratory colonies and allowing flies to oviposit freely for 24 – 48h. On the same day after exposure, each blueberry was inspected under a dissecting microscope to count number of eggs laid; to avoid larval competition effects, only blueberries containing fewer than eight eggs were used. Infested blueberries were then distributed in white organza bags (5.5 x 10.5 x 1.5 cm), such that each bag contained a similar number of berries and eggs per bag for deployment in field treatments (see Table 2).

Adult emergence from pupae outside of fruit was determined by attaching twenty *D. suzukii* pupae (1 - 2 days after pupation) inside rectangular wire cages (5 cm x 5 cm x 1 cm, referred to as ‘pupal cages’) using double-sided clear tape. The clear tape was covered with sand or sawdust to prevent emerging flies from sticking to the tape. Pupal cages were then placed inside white organza bags to protect them from parasitism and predation during each trial period.

Organza bags containing infested blueberries and pupal cages were placed in the middle of mulch treatment blueberry plots 1 - 2 days following blueberry infestation or pupal cage setup. In each mulch type replicate plot, bags were placed above and buried 1 - 3 cm below the mulch. To account for losses due to predation or farm operations, in some plots there were two bags per treatment, and the values of these bags were averaged for each treatment replicate (such that each independent replicate was the plot, not each bag). No pesticides were applied in the blueberry plots while the infested blueberries and pupal cages were exposed.

Bags containing infested blueberries and pupal cages were removed from the field after seven days and placed in controlled laboratory conditions (22.5 ± 2.5 °C, 50 – 70 % RH) for an additional 2 - 3 weeks. Seven days in the field was long enough to potentially observe differences among treatments, but not lose many bags due to predation from mammals or ants. Additional control blueberries and pupal cages were kept in laboratory conditions during the 7-day field exposure period. This was done to confirm *D. suzukii* development, but controls were excluded from final data analyses, as we aimed to compare field treatments. Emerging flies were counted and removed several times per week, and the total number of emerged *D. suzukii* flies in each bag at the end of the incubation period was recorded to determine the proportion of emerged flies (compared to the total number of eggs in blueberries or pupae originally in each bag). Bags that were predated by mammals or ants were discarded from analysis. Artificial infestation trials were not performed in MI.

We performed a beta regression using mulch type, mulch location, and season as fixed effects, separately by site. Beta regressions are appropriate for outcome variables bound between 0 and 1 (in this case, proportion of flies emerged from total eggs or pupae), and are not subject to assumptions of heteroscedasticity.[46] Furthermore, a multiple regression was performed to determine whether temperature and RH jointly and uniquely were significant predictors of percent emerged *D. suzukii* adults from artificially infested blueberries or pupae cages for all sites pooled. For this multiple regression analysis, time above 30°C, RH, and *D. suzukii* emergence were averaged across treatment replicates, such that a single datapoint represented a mulch and location treatment for each site and trial date. Beta regressions were performed using the RStudio package “Betareg”.[47]

2.3. *Natural infestations of* D. suzukii

We assessed natural infestation in blueberry plots in GA (2017), MI (2016, 2017), and OR (2017) during June, July or August based on crop ripening in each region. Ripe berries were collected weekly from branches (GA, MI), or picked from the floor (GA, MI, OR) in each plot replicate with weedmat or grower standard mulch. In OR and GA, berries collected from the floor were kept in ventilated plastic containers in controlled laboratory conditions for an additional 3 - 4 weeks, and the total number of emerged adult *D. suzukii* flies in each container was recorded. In berries collected from bushes in MI, *D. suzukii* larvae were extracted and counted using a filter salt test method.[48] Because the number of berries collected varied by date and by site, we divided number of *D. suzukii* by number of berries collected to obtain the number of *D. suzukii* per berry. At the end of the season, the overall mean of *D. suzukii* per berry for each mulching treatment was used for analyses (pooling all sampling dates).

An analysis of variance (ANOVA) with post-hoc Tukey tests compared the effect of mulch type on the number of *D. suzukii* per berry. This was done separately for berries collected from branches or fallen on the mulch, by year and site. Variables were log-10 or inverse transformed where appropriate to meet assumptions of normality.

2.4. Drosophila suzukii *pupation in greenhouse enclosures*

Experiments in greenhouse enclosures were done to determine whether mulch type affects *D. suzukii* pupation location. Greenhouse experiments were carried out at Oregon State University (Corvallis, OR). Sixteen potted blueberry plants (cv. ‘Liberty’, planted in 2009) were kept in greenhouse conditions (21 – 25 °C, 75 – 85% RH). In each plant, two separate branches with 20 ripe blueberries each were selected and enclosed in 5 gallon paint strainer mesh bags (one branch for control and one for experimental trials). In 30 Aug 2017, ten mature *D. suzukii* females and five males were released inside each mesh bag, and left inside for 48h to allow oviposition in fruit. After the oviposition period, flies and mesh bags were removed from the blueberry cluster branches. To serve as a control and confirm oviposition, one branch with an infested cluster of 20 blueberries was removed from each plant (N = 16) and keep in the laboratory for one week to count total number of larvae found in all 20 berries using the salt flotation method. The second infested branch in each blueberry plant was positioned straight above a black plastic tray (42.6 x 42.6 x 4.8 cm) filled with (1) Douglas fir sawdust (N = 8), or (2) Douglas fir sawdust covered by a layer of black weedmat (N = 8). To prevent larvae from escaping the trays, the edges of each tray were coated with a thin layer (~1cm wide) of sticky coating (Tangle Foot, Grand Rapids, MI).

Every day we recorded in each tray number of immature *D. suzukii* (pupae, prepupae and larvae) on the mulch (sawdust or weedmat) or attached to fruit (fruit location was either fallen on the mulch or still on the branch), until no more larvae were found (only pupae were found). Only larvae and pupae visible on top of the mulch or protruding from the fruit were counted. An analysis of variance (ANOVA) compared the effect of mulch type on the number of immature *D. suzukii* observed on the mulch or attached to the fruit by date.

3. **Results**

3.1. Abiotic factors and D. suzukii emergence by region

The relationships among temperature, RH, season, mulch type, and *D. suzukii* emergence varied among states, and are described in Tables 3, S1, and Figures 1 - 6. In most regions, we found that temperatures remained above 30 °C for longer periods, and fly emergence from infested blueberries was lower above the mulch compared to below the mulch, regardless of mulch type. There was a non-significant trend in GA for fly emergence to be lower above than below the mulch. Likewise, fly emergence from pupal cages was lower above the mulch at all sites, except for GA. RH was lower above the mulch than below the mulch in OR, but similar in MN. Mulch type influenced temperatures in MD-Q and MI, where temperatures remained above 30 °C for longer periods on weedmats compared to standard grower mulch. Mulch type did not affect RH at any of the sites. Mulch type in general did not affect *D. suzukii* emergence from infested blueberries or pupal cages at most sites, except for one site (MD-Q), where emergence from both infested blueberries and pupal cages was lower on weedmat compared to wood chip mulch.

Pooling results from all sites, we found that temperature and RH jointly were significant predictors of *D. suzukii* emergence from blueberries ($R^2 = 0.20$, df = 2, 31, F = 3.99, p = 0.02), but temperature (time above 30 °C) was a stronger predictor of *D. suzukii* emergence from infested blueberries, although the correlation coefficient (R^2) was low (Table 4, Figure 7a, b). Neither temperature nor RH were significant predictors of *D. suzukii* emergence from pupal cages ($R^2 = 0.17$, df = 2, 15, F = 1.55, p = 0.24, Table 4, Figure 7c, d).

3.2. Natural infestation

In GA and OR, there was no significant effect of mulch type on *D. suzukii* emergence from berries collected on top of the mulch or from branches (Table 5, Figure 8). In MI, there was no significant effect of mulch type on *D. suzukii* larvae extracted from berries in 2016, but in 2017, berries collected from weedmat sections had fewer *D. suzukii* larvae than berries collected on bare soil (for berries collected on top of the mulch) and woodchips (for berries collected on both branches and on top of the mulch; Table 5, Figure 8).

3.3. Drosophila suzukii *pupation in greenhouse enclosures*

More *D. suzukii* immatures (larvae, prepupae and pupae) were visible on the mulch in trays with weedmat mulch compared to trays with sawdust mulch (df = 1, 38, $F = 6.996$, $p = 0.011$). More immatures were also found lying on the weedmat than protruding from the berries (df = 2, 38, $F = 8.134$, $p < 0.01$). Some larvae were observed burrowing through the sawdust to pupate underneath, but no larvae were observed burrowing through the weedmat, and they remained above the weedmat. We did not find any larvae stuck to the adhesive tray lining, suggesting that larvae did not wander a long distance after they left the fruit to pupate.

4. **Discussion**

Weedmats had variable effects on the blueberry ground microclimate, and on *D. suzukii* adult emergence from larvae and pupae. In a majority of the test sites, weedmats did not affect temperatures or RH enough to negatively affect larval and pupal emergence. In MD-Q however, high temperatures were recorded for longer periods on weedmat compared to woodchip mulch, and fewer *D. suzukii* survived from infested blueberries and pupal cages. This suggests that in certain regions and crops, incorporating weedmat can arrest the development of immature *D. suzukii*, and reduce population build-up from the plantings. Differences in canopy size, irrigation, and climate among the different research sites likely affected the range in temperature and RH observed in these experiments, and their effects on *D. suzukii* emergence.

In general, temperatures were high for longer periods and RH was lower above the mulch compared to below the mulch, and as expected, *D. suzukii* emergence tended to be lower above the mulch. According to previous studies, very few *D. suzukii* larvae develop when reared at constant temperatures above 30 °C.[29,30] Pooling all sites in our study, we found that time above 30 °C had a weak significant correlation with low adult emergence from larvae, and no effect on emergence from pupae. However, when examining each individual site, we found that *D. suzukii* emergence did not always fluctuate according to temperature changes by season, or mulch type. As an example, the early and mid-2017 trials in Oregon had longer periods with high temperatures and lower RH compared to early 2016, yet *D. suzukii* adult emergence from infested blueberries and pupal cages was not significantly lower. Likewise, in MD-K, late 2017 trials had shorter periods with high temperatures compared to 2016 trials, but *D. suzukii* emergence from infested blueberries and pupae was not higher. In periods of cooler temperatures, the presence of a weedmat may not significantly raise the temperatures for long enough to kill *D. suzukii* larvae or pupae.

Sites where weedmat had an effect on temperature and *D. suzukii* emergence or natural infestation (MD-Q and MI) also had young, small plants. This results in less shade over the weedmat and increased solar radiation, which may have contributed to increased *D. suzukii* mortality. In sites with larger plants (i.e., GA and OR), canopy shade may attenuate high temperatures over the weed fabric. Light penetration and sun radiation exposure are abiotic factors that should be explored in future studies to determine the horticultural settings in which weedmat can raise temperatures sufficiently to reduce *D. suzukii* emergence.

We expected that higher RH would lead to higher *D. suzukii* emergence, as has previously been reported in laboratory[28] and field conditions[33]. However, when examining all sites together, RH did not significantly correlate with *D. suzukii* adult emergence from larvae and pupae. Adult *D. suzukii* have similar survival rates between 71 and 94% RH[28], and the RH among all our sites ranged between 58% and 97%, therefore it is possible that this variation in RH was within the tolerance limits of *D. suzukii* immatures. We expected that sites with overhead sprinkler irrigation (i.e., GA and MI) would have less pronounced differences in RH between mulch types compared to sites with drip irrigation (i.e., MN and OR). We found however that RH was not affected by mulch type at any site. We also expected that sites with drip irrigation would have different RH above and below the ground; we found this effect in OR, but not in MN. Some mulch types may retain more moisture than others, and this could be the reason why fine sawdust mulch in OR had higher RH below the ground compared to coarse wood chip mulch in MN. Our data showed that temperature had a stronger effect than RH on *D. suzukii* emergence. Similarly, Enriquez and Colinet (2017) found that in long exposure periods at

30°C (more than 80h), low RH (5-10%) or high RH (80-100%) did not affect pupal mortality,[49] suggesting that prolonged periods of high temperatures can overshadow RH mortality effects. In contrast, Rendon and Walton (2018) found that low RH affects pupae mortality more than temperatures in the field.[33] It has been shown that Diptera larvae pupation behavior and depth is affected by the type and moisture content of the substrate.[10] The effect of RH on pupation behavior of *D. suzukii* therefore warrants further investigation.

Drosophila suzukii 3rd instar larvae usually leave the fruit to pupate under the soil,[38,39] which as shown above, increases their chances of survival. Impeding larvae from reaching protection underground may be a valuable strategy to reduce *D. suzukii* population pressure. Greenhouse experiments showed that larvae were unlikely to burrow through a weedmat to pupate underneath, or travel distances greater than 40 cm to escape the weedmat surface. As such, black polypropylene weedmat offers an effective barrier to prevent access to suitable pupation sites and microclimates. If fallen fruit remain on a weedmat, there is a lower probability that larvae developing on it will be able to survive by pupating underground after they leave the fruit, therefore reducing potential reservoirs for *D. suzukii*.[50]As part of farm sanitation, fallen fruit is also sometimes buried to prevent females from laying eggs, and to kill any existing larvae. Our results however illustrate the importance of burial depth; with the favorable climate just below the mulch surface, infested fruit buried at depths of 1 - 3 cm resulted in greater *D. suzukii* adult emergence compared to fruit left above the mulch. *Drosophila suzukii* emergence from infested fruit can be greatly reduced by burying at depths greater than 10 cm (Hooper and Grieshop; Rodriguez-Saona, unpublished data).

As we observed, exposed larvae and pupae are susceptible to abiotic factors, but they may also succumb to natural enemies such as predators and parasitoids. In blueberries, compost mulch did not significantly affect predation of blueberry maggot pupae by ground beetles compared to bare soil,[51] but an impermeable mulch such as a weedmat may facilitate predation. Insect larvae are usually more protected from predators once they are buried underground, but are susceptible to predation while they are above the soil.[52] In *D. suzukii*, the only predators observed digging up pupae from underground are ants ,[39] but a more diverse guild of predators (beetles and crickets[38]) and parasitoids may attack *D. suzukii* immatures above the ground. The synergistic interactions between cultural control and biological control need to be further studied, as mulching can potentially affect predation and parasitism of *D. suzukii* larvae and pupae.

Although the artificial infestation trials assessed *D. suzukii* immature survival only, it is possible that mulching may also affect female oviposition choice, but we did not observe a clear trend to support this. In MI in 2017, there were fewer *D. suzukii* larvae in blueberries collected from the branches on weedmat plots compared to wood chip and bare soil. Female flies may select fruit hanging above suitable substrates for laying eggs, which will allow their offspring to successfully pupate underground and increase their chances of survival. *Drosophila* females can assess the local landscape to make oviposition decisions and presumably can select sites where larvae will have to travel only a short distance to find ideal microclimate conditions for development. For example, Abbott and Dukas (2016) showed that *D. melanogaster* females laid fewer eggs when an oviposition substrate was further away and physically separated from a media ideal for larval growth.[53] Another study showed that female *D. suzukii* females lay fewer eggs on blueberries placed on a surface at 30°C compared to blueberries at 22°C.[54] Larvae developing in fruit on weedmat would have to travel a longer distance to find suitable pupation substrates than larvae that develop on fruit directly above mulch under which they can burrow. Further research on the effect of cultural control and female oviposition choice across different ecoregions and crops is needed.

5. Conclusion

To effectively use weedmat mulching as an IPM strategy, the costs of implementing and maintenance need to be considered.[55] Because of its benefits (organic weed control, water retention, increased yield) relative to its costs, black weedmats have been widely adopted in some regions for modern blueberry production (i.e., in Oregon,[14]) but in other regions berry crops are still planted on soil or plant mixture mulches. This study has shown that, besides plant production benefits, the use of weedmat may reduce *D. suzukii* emergence and proliferation by preventing larvae from reaching suitable pupation habitats underground. Overall, our data illustrate that although this cultural technique is not a stand-alone strategy, mulching can be used within the whole production systems to lower *D. suzukii* pressure.

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Table 1. Blueberry plots characteristics for *Drosophila suzukii* artificial and natural infestation experiments in five states.

State, location	**Cultivar**	**Year planted**	**Average canopy size (Height x width, m)** [a]	**Irrigation**	**Mulch types** [c]
Georgia (GA) Blueberry research and demonstration farm (Bacon county) 31.534°N 82.509°W	Brightwell	2009	Wood chips: 1.90 x 1.70 Weedmat: 1.79 x 1.63 Bare soil: 1.92 x 1.75	Overhead sprinkler	Wood chips: Pine bark (5 – 8 cm deep layer) Weedmat: Weed block, Easy Gardener Products Inc., Waco TX Bare soil
Maryland – Keedysville (MD-K) Western Maryland Research and Education center 39°30'40.3"N 77°43'50.9"W	Bluecrop	2013	Late 2017: 0.56 x 0.46 Bed width = 0.7	Drip No irrigation needed	Wood chips: Plant mixture, locally sourced Weedmat: Landmaster Landscape Fabric, 6' x 100' polypropylene, Home Depot

Maryland – Queenstown (MD-Q) Wye Research and Extension center 38°54'58.5"N 76°08'18.7"W	Bluecrop	2013	Late 2017: 0.84 x 0.58 Bed width = 0.6	Drip Less than 25 cm^2 per week	Wood chips: Plant mixture of various sizes, locally sourced Weedmat: Landmaster Landscape Fabric, 6’ x 100’ polypropylene, Home Depot
Michigan (MI) Trevor Nichols Research Center (Fennville, MI) 42.603°N 86.153°W	Bluecrop	2010	Late 2017: Wood chips: 0.44 x 0.41 Weedmat: 0.43 x 0.43 Bare soil: 0.42 x 0.42	Overhead sprinkler	Wood chips: Consumers Energy, East Lansing MI, 5-6 cm layer Weedmat: Black woven ground cover, Cherokee Manufacturing, South St. Paul, MN, product # GC4300 Bare soil
Minnesota (MN) Little Hill Blueberry Farm (Northfield,	Jersey	2011	0.90 x 1.43	Drip	Wood chips: Various trees, Castle Rock Contracting & Tree, Castle Rock MN. Weedmat: Yardworks® 4’ x 80’ Standard

MN) 44.458° N, 93.161° W					Landscape Fabric
Oregon (OR) [b] Lewis Brown Farm (Linn County) 44.553°N, 123.218°W	Elliott	2004	June 2017: 1.56 x 1.01 July 2017: 1.51 x 1.28 Bed width = 1.57	Drip Volume according to evapotranspiration rate	Sawdust: Douglas fir, The Bark Place, Corvallis, OR. 5 -6 cm layer Weedmat: Weed Barrier Fabric 4' x 300', Wilson Orchard and vineyard supply, The Dalles, Oregon

Footnotes:

[a] Canopy size was estimated at each research plot using either a meter stick and visually measuring bushes, or by analyzing pictures and using a scale of known length. There was variability on the measurement recording among sites; in some sites they were measured on each mulch, and in other sites they were representative of the whole block.

[b] Oregon was the only site that received insecticides (three applications in 2016, none in 2017): Malathion on 5 July, Zeta-cypermethrin on 21 July, Malathion on 4 August. All of the artificial infestation trials took place outside of the pre-harvest interval (PHI) of these applications. No insecticides were applied in any other site.

[c] In OR and MN, weedmats were placed and removed only for trials, and in the other sites, they were permanently incorporated into planting beds. In GA, weedmats were laid on top of soil, in all other sites weedmats were laid on top of grower standard mulch.

Table 2. Trial characteristics for artificial infestation and pupal cages.

Site	Artificial infestation trial dates and growing season	Experimental plot length (m)[a]	Replicates per treatment[b]	Berries / eggs per bag	Pupae per cage[e]	Abiotic factors measured[f]
GA[c]	25 Jun 2016 (Mid 2016) 30 Jun 2016 (Late 2016 a) 13 Jul 2016 (Late 2016 b)	3.7	N = 3	20 ± 3 berries / 60 eggs	N/A	Temperature and RH
MD–Q	28 Jul 2016 (Mid 2016) 11 Aug 2016 (Late 2016) 06 July 2017 (Early 2017) 20 Jul 2017 (Late 2017)	7.3	N = 3	10 – 15 berries/ 41 - 53 eggs	20	Temperature
MD-K	21 Jul 2016 (Early 2016) 19 Aug 2016 (Late 2016) 28 Jun 2017 (Early 2017) 14 Jul 2017 (Late 2017 a) 22 Jul 2017 (Late 2017 b)	7.3	N = 3	10 – 15 berries/ 41 - 53 eggs	20	Temperature

MI[d]	N/A	2016: 14 2017: 7	N = 4	N/A	N/A	Temperature and RH
MN	5 Aug 2016 (Mid 2016) 16 Aug 2016 (Late 2016) 16 Aug 2017 (Mid 2017) 1 Sep 2017 (Late 2017)	3	2016: N = 4 2017: N = 3	12- 15 berries / 25 – 30 eggs	N/A	Temperature and RH
OR	20 May 2016 (Early 2016) 1 Sep 2016 (Late 2016) 1 June 2017 (Early 2017) 27 July 2017 (Mid 2017)	6	N = 8 (Temperature and RH data was only replicated in 3 rows)	20 ± 2 berries / 50 ± 2 eggs	20	Temperature and RH

Footnotes:

[a] Plot size represents one row width by the length of a weedmat fabric section. 1 - 2 bags with blueberries, pupae, and a datalogger were placed in the middle of each plot under a blueberry bush.

[b] In MD-Q, MD-K and MI (2016), each treatment was on separate rows. In all other sites, each treatment was in the same row separated by at least one bush buffer, and replicated across different rows.

[c] There were not enough datalogger replicates in GA for GLM.

[d] Artificial infestation trials were not done in MI. RH was recorded only above the mulch for the different mulch types.

[e] Pupae were not tested in GA, MI, and MN.

[f] Datalogger model for GA, MI, MN and OR was HOBO U23 Pro V2 (Onset Computer Corps, Bourne MA). Datalogger model for MD-K and MD-Q was HOBO pendant® Temperature / Alarm waterproof Dataloggers (Onset Computer Corps, Bourne MA). Temperature and RH on bare soil treatments were measured only above, not below.

Table 3. Variance parameters for the effects of mulch type, mulch location, and season on temperature (time above 30 °C), RH, and *D. suzukii* survival from artificially infested blueberries or pupal cages split by site. Effects on temperature and RH were calculated using a general linear model with Box-Cox lambda transformations, and effects on *D. suzukii* survival were calculated using a Beta regression.

	Outcome variables	**Temperature (time above 30 °C)**			**RH (%)**			***D. suzukii* emergence (infested blueberries)**			***D. suzukii* emergence (pupal cages)**		
Site	**Fixed effects**	**df**	**F**	**p**	**df**	**F**	**P**	**df**	**X^2**	**p**	**df**	**X^2**	**p**
GA	Mulch type	-	-	-	-	-	-	2	1.61	0.44	2	1.67	0.21
	Mulch location	-	-	-	-	-	-	1	3.32	0.06	1	0.48	0.49
	Season	-	-	-	-	-	-	5	31.00	< 0.01	2	3.08	0.21
MD- K	Mulch type	1, 53	0.27	0.60	-	-	-	1	1.20	0.27	1	1.05	0.30
	Mulch location	1, 53	25.65	< 0.01	-	-	-	1	30.57	< 0.01	1	4.21	0.04
	Season	4, 53	43.49	< 0.01	-	-	-	4	32.37	< 0.01	4	7.06	0.13
MD- Q	Mulch type	1, 42	35.04	< 0.01	-	-	-	1	27.20	< 0.01	1	11.96	< 0.01
	Mulch location	1, 42	113.99	< 0.01	-	-	-	1	34.46	< 0.01	1	15.45	< 0.01

	Season	3, 42	26.00	< 0.01	-	-	-	3	23.10	< 0.01	3	16.15	< 0.01
MI	Mulch type	2, 63	23.89	< 0.01	2, 20	0.16	0.84	-	-	-	-	-	-
	Mulch location	1, 63	41.31	< 0.01	-	-	-	-	-	-	-	-	-
	Season	3, 63	8.61	< 0.01	1, 20	17.34	< 0.01	-	-	-	-	-	-
MN	Mulch type	1, 36	0.70	0.40	1, 13	0.04	0.82	1	0.24	0.61	-	-	-
	Mulch location	1, 36	41.62	< 0.01	1, 13	1.01	0.31	1	37.83	< 0.01	-	-	-
	Season	3, 36	11.17	< 0.01	1, 13	6.46	0.02	3	67.28	< 0.01	-	-	-
OR	Mulch type	1, 53	1.24	0.26	1,53	1.28	0.26	1	1.22	0.26	1	0.01	0.91
	Mulch location	1, 53	30.38	< 0.01	1, 53	56.26	< 0.01	1	97.03	< 0.01	1	16.76	< 0.01
	Season	3, 53	65.35	< 0.01	3, 53	18.09	< 0.01	3	24.08	< 0.01	2	3.54	0.16

Table 4. Multiple regression parameters using temperature (time above 30 °C), RH, and site as predictors of *D. suzukii* survival from infested blueberries or pupae, pooling all sites.

***D. suzukii* survival**	**Infested blueberries**			**Pupal cages**		
Fixed effect	**B**	**t**	**p**	**B**	**t**	**p**
Intercept	16.70	0.87	0.38	-6.27	-0.89	0.38
Temperature (time above 30 °C)	-0.005	-2.82	< 0.01**	-0.000	-0.15	0.88
RH (%)	0.091	0.38	0.70	0.15	1.75	0.09

Table 5. Analysis of variance parameters (ANOVA) testing the effect of mulch type on natural infestation by *D. suzukii* (counts) at three blueberry sites.

Site	**Year**	**Berry location**	**df**	**F**	**p**
GA	2017	Branch	2, 24	1.35	0.27
		Mulch	2, 24	0.44	0.64
MI	2016	Branch	1, 60	0.47	0.49
		Mulch	1, 62	0.88	0.35
	2017	Branch	2, 81	3.74	0.02
		Mulch	2, 45	7.94	< 0.01
OR	2017	Mulch	1, 83	0.69	0.4

Figure captions

Figure 1. Effects of mulch type and location on *D. suzukii* adult emergence from (a) infested blueberries and (b) pupae in Georgia (GA).

Figure 2. Effects of mulch type and location on (a) temperature (time above 30 °C), and *D. suzukii* adult emergence from (b) infested blueberries and (c) pupae in Maryland – Keedysville (MD-K).

Figure 3. Effects of mulch type and location on (a) temperature (time above 30 °C), and *D. suzukii* adult emergence from (b) infested blueberries and (c) pupae in Maryland – Queenstown (MD-Q).

Figure 4. Effects of mulch type and location on (a) temperature (time above 30 °C) and (b) relative humidity in Michigan (MI).

Figure 5. Effects of mulch type and location on (a) temperature (time above 30 °C), (b) relative humidity, and (c) *D. suzukii* adult emergence from infested blueberries in Minnesota (MN).

Figure 6. Effects of mulch type and location on (a) temperature (time above 30 °C), (b) relative humidity, and *D. suzukii* adult emergence from (c) infested blueberries and (d) pupae in Oregon (OR).

Figure 7. Relationship *between D. suzukii* adult emergence from infested blueberries and (a) temperature (mean time above 30°C) or (b) RH (%), and adult emergence from pupal cages and (c) temperature or (d) RH. Each dot represents the mean value for one site and date, shaded area is 95% C.I.

Figure 8. *Drosophila suzukii* found on blueberries (mean ± SE) collected from branches or on top of the mulch (natural infestation) on different mulch types in Georgia, Michigan, and Oregon.

Figure 1. Effects of mulch type and location on *D. suzukii* emergence from (a) infested blueberries and (b) pupae in Georgia (GA).

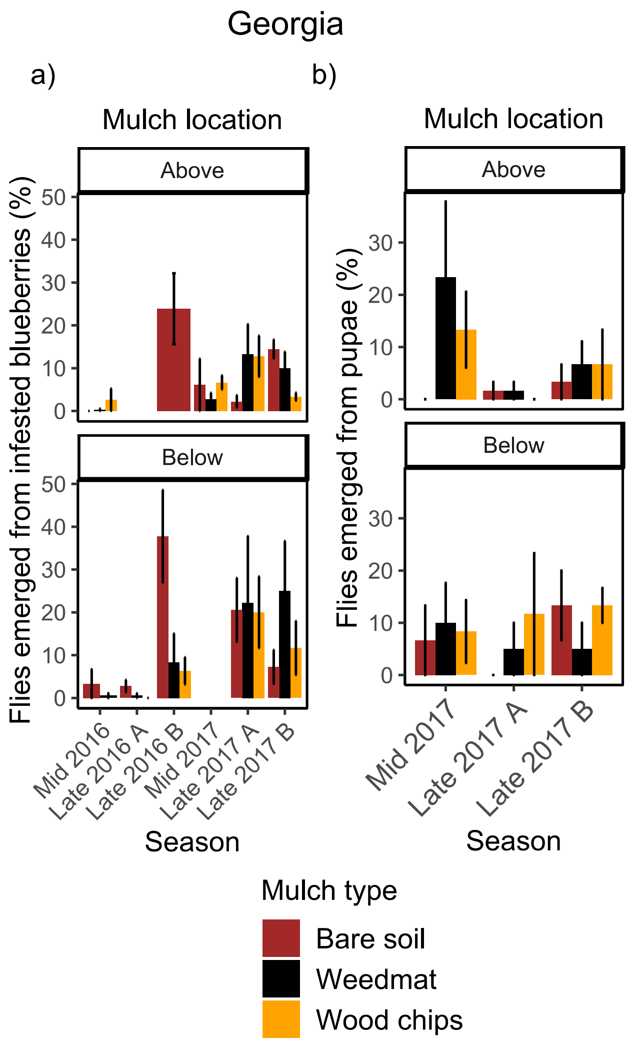


Figure 2. Effects of mulch type and location on (a) temperature (time above 30 °C), and *D. suzukii* emergence from (b) infested blueberries and (c) pupae in Maryland – Keedysville (MD-K).

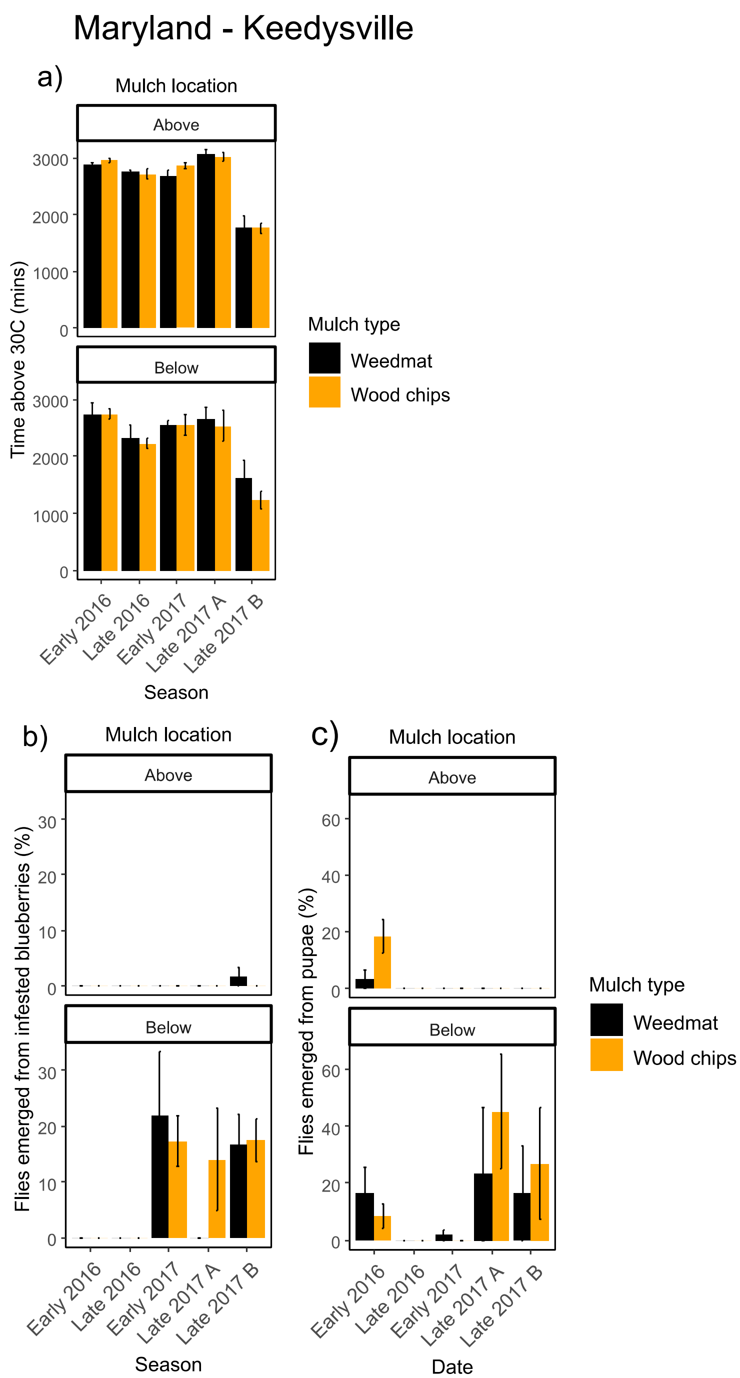


Figure 3. Effects of mulch type and location on (a) temperature (time above 30 °C), and *D. suzukii* emergence from (b) infested blueberries and (c) pupae in Maryland – Queenstown (MD-Q).

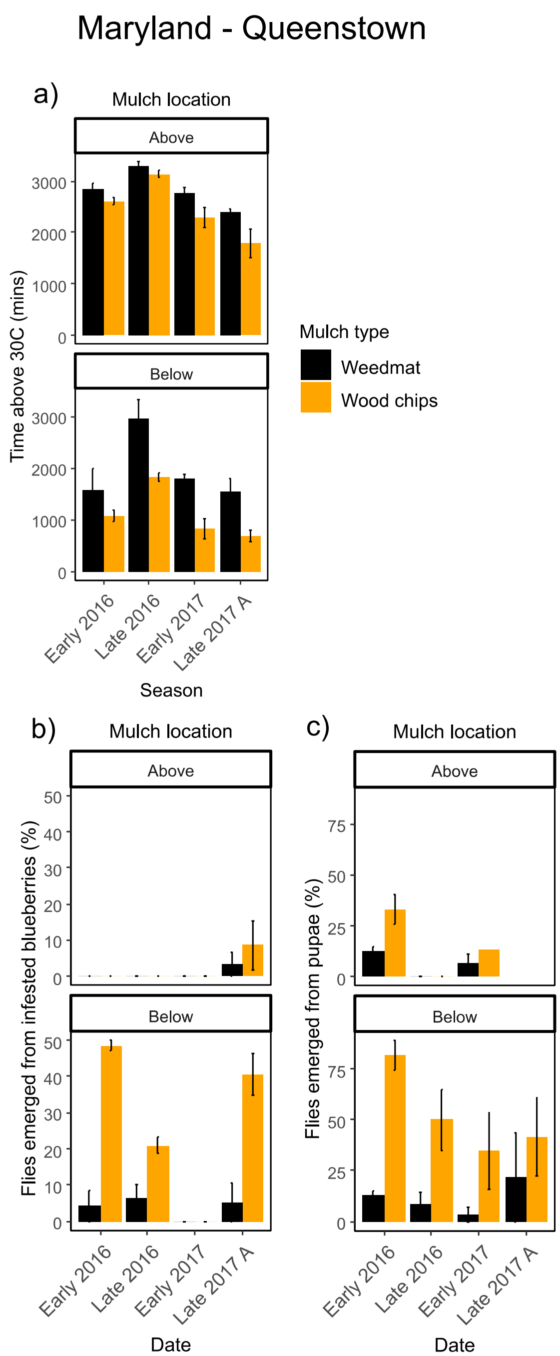


Figure 4. Effects of mulch type and location on (a) temperature (time above 30 °C) and (b) relative humidity in Michigan (MI).

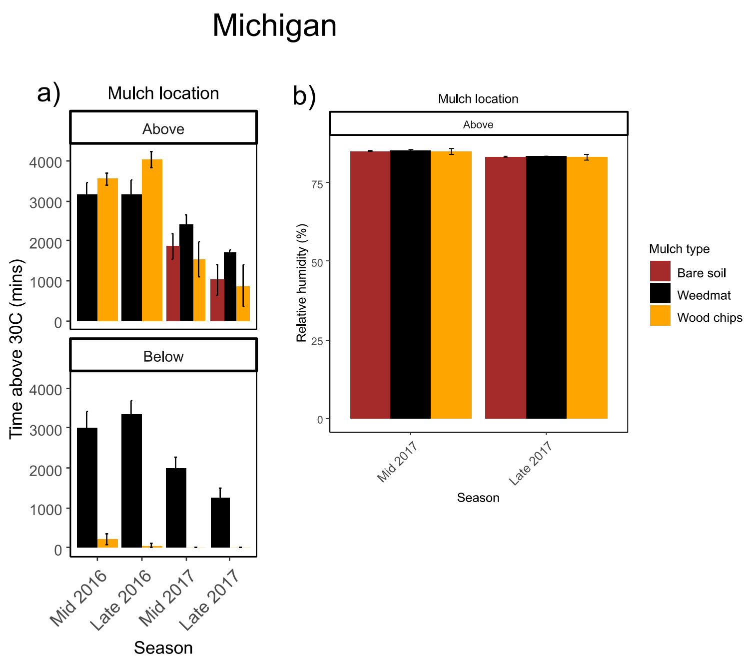


Figure 5. Effects of mulch type and location on (a) temperature (time above 30 °C), (b) relative humidity, and (c) *D. suzukii* emergence from infested blueberries in Minnesota (MN).

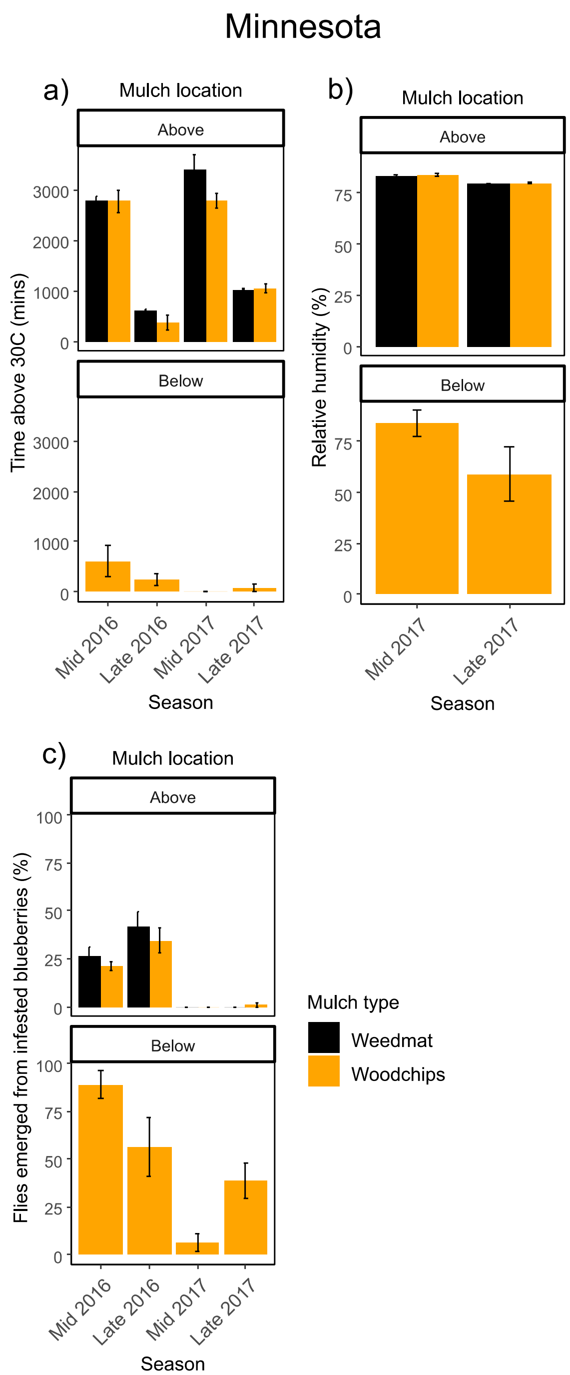


Figure 6. Effects of mulch type and location on (a) temperature (time above 30 °C), (b) relative humidity, and *D. suzukii* emergence from (c) infested blueberries and (d) pupae in Oregon (OR).

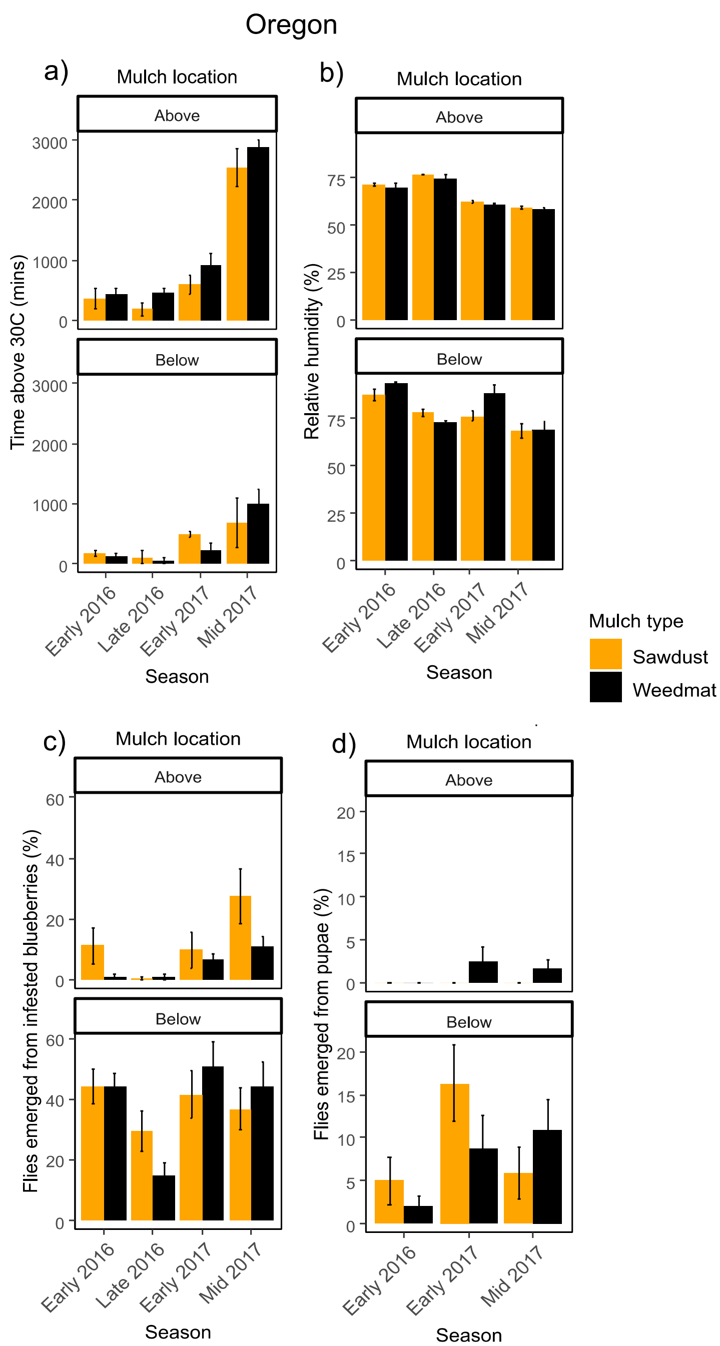
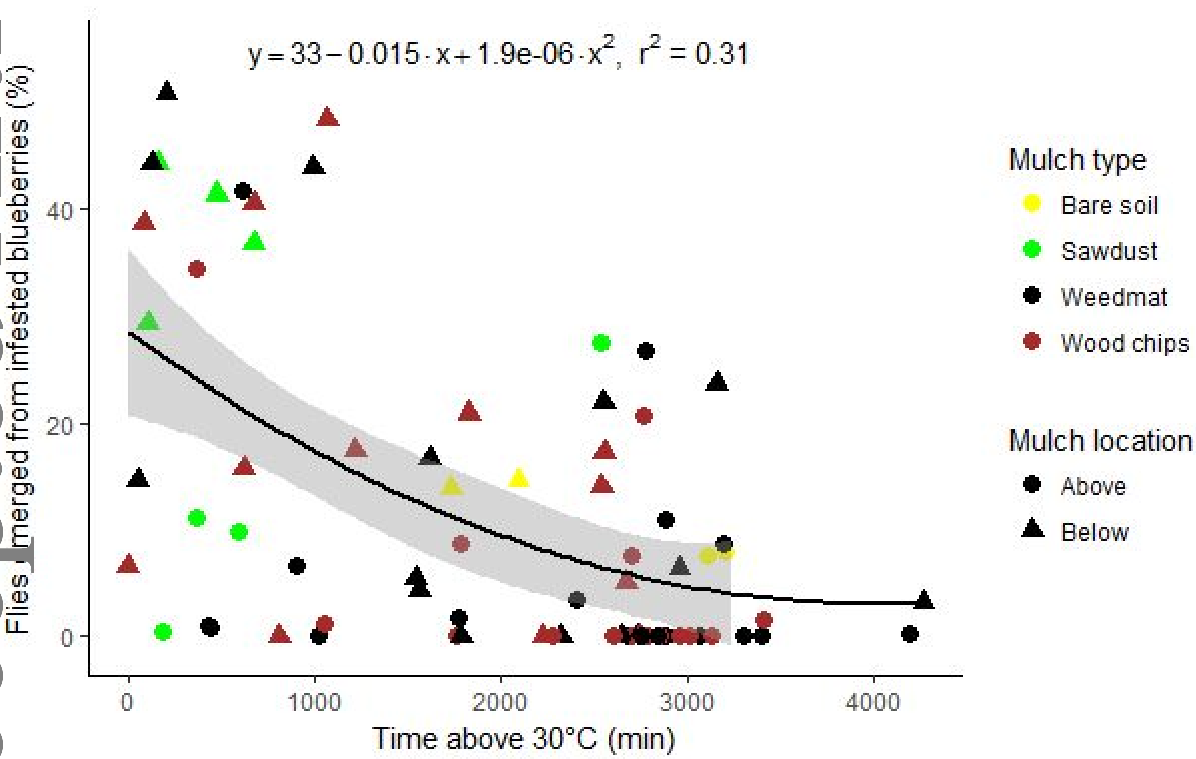
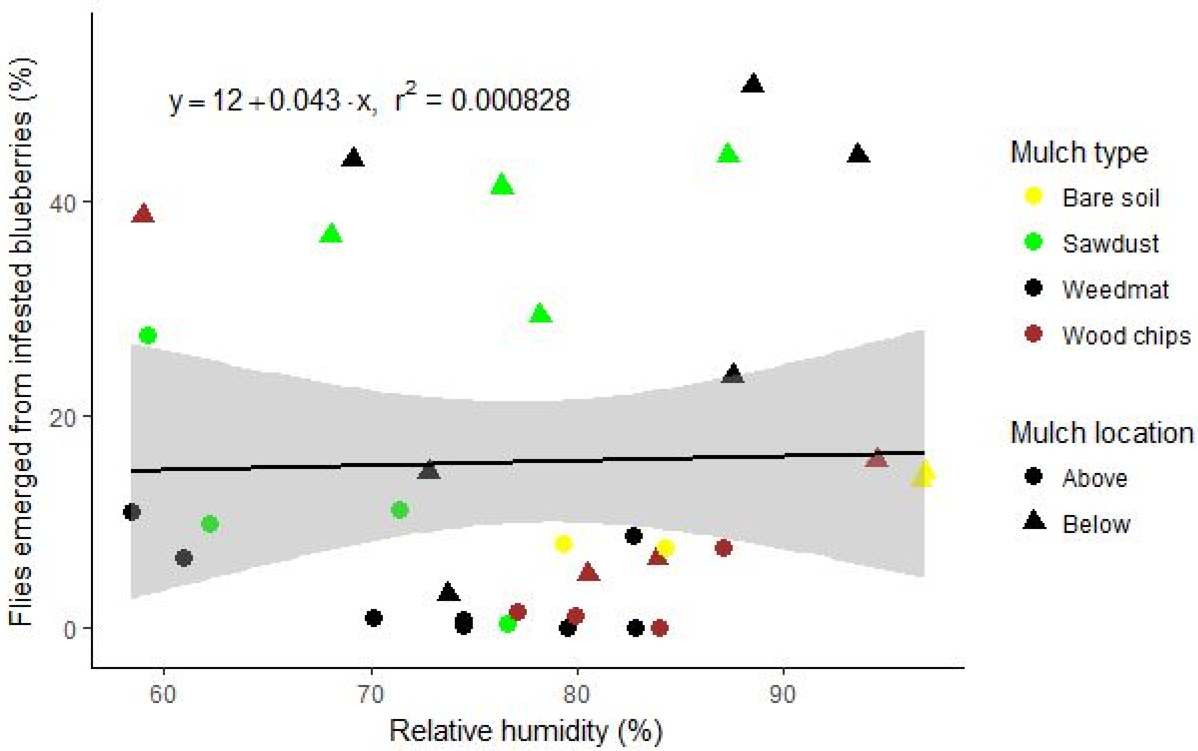


Figure 7. Relationship between *D. suzukii* emergence from infested blueberries and (a) temperature (mean time above 30°C) or (b) RH (%), and emergence from pupal cages and (c) temperature or (d) RH. Each dot represents the mean value for one site and date, shaded area is 95% C.I.

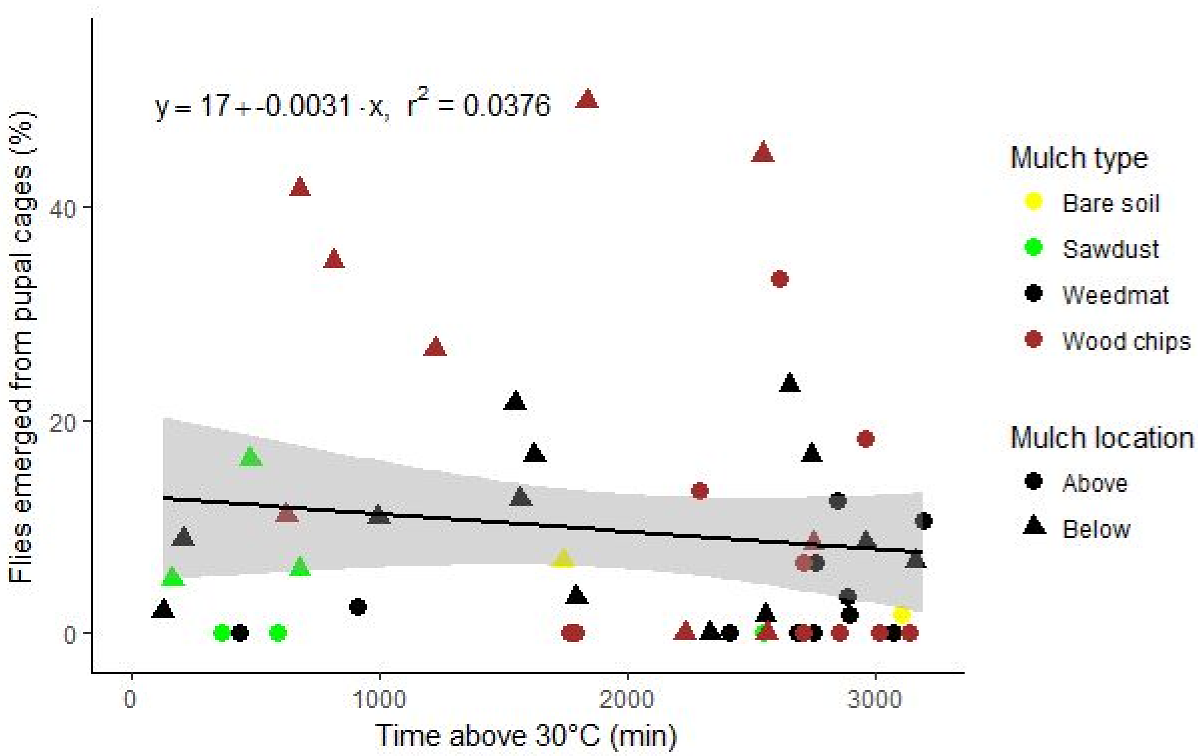
a)



b)

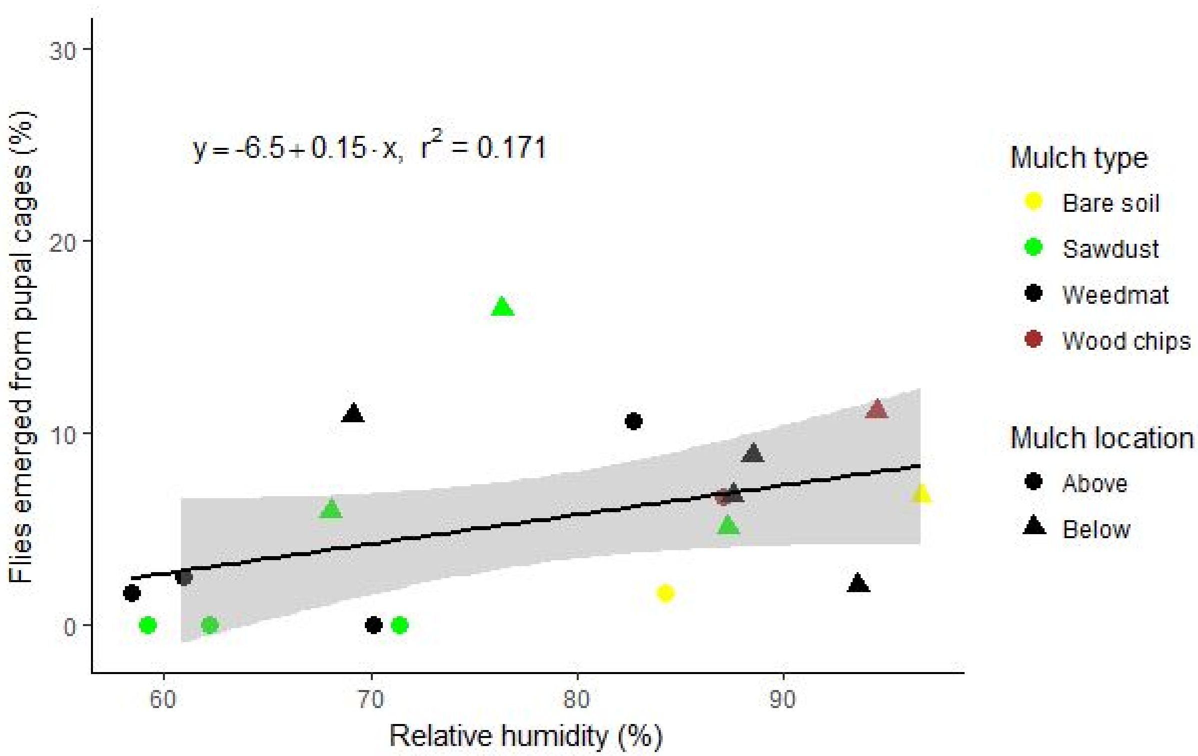

y = 12 + 0.043 · x, r^2 = 0.000828
Flies emerged from infested blueberries (%)
0
20
40
60
70
80
90
Relative humidity (%)
Mulch type
Bare soil
Sawdust
Weedmat
Wood chips
Mulch location
Above
Below

c)



y = 17 + -0.0031 · x, r2 = 0.0376
Flies emerged from pupal cages (%)
0
20
40
0
1000
2000
3000
Time above 30°C (min)
Mulch type
Bare soil
Sawdust
Weedmat
Wood chips
Mulch location
Above
Below

d)



$y = -6.5 + 0.15 \cdot x, \; r^2 = 0.171$
Flies emerged from pupal cages (%)
30
20
10
0
60
70
80
90
Relative humidity (%)
Mulch type
Bare soil
Sawdust
Weedmat
Wood chips
Mulch location
Above
Below

Figure 8. *Drosophila suzukii* found on blueberries (mean ± SE) collected from branches or on top of the mulch (natural infestation) on different mulch types in Georgia, Michigan, and Oregon.