Characterization and manipulation of fruit susceptibility to Drosophila suzukii

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Characterization and manipulation of fruit susceptibility to *Drosophila suzukii*

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Abstract *Drosophila suzukii* (Matsumura) is an economic pest of small fruits and cherries that attacks intact ripening fruits. Host susceptibility may be influenced by characteristics such as flesh firmness, penetration force of the skin, total soluble solids (TSS, also known as °Brix), and pH. Improved knowledge of factors affecting fruit susceptibility is needed for developing thresholds and risk prediction models for IPM. A combination of laboratory and field studies was conducted to develop prediction and potential management tools. First, a direct bioassay was used to calculate the probability of oviposition in a given fruit based on various characteristics as determined across laboratory and field trials in Oregon and North Carolina, US. When multiple characteristics were evaluated simultaneously, oviposition probability consistently increased as penetration force decreased and pH increased. Oviposition probability sometimes increased as TSS increased. Second, raspberries and blueberries in unsprayed fields had substantially lower infestation in ripening fruit compared to ripe fruit. There was no or minimal infestation in green fruit. Third, given that skin penetration force influences oviposition, practices used to improve fruit quality were examined in laboratory no-choice cages for potential reduction of oviposition. Blueberry fruit sprayed with calcium silicate in the field had greater penetration force and firmness and reduced number of eggs laid by *D. suzukii* compared to untreated fruit. Other calcium-based treatments increased Ca content and firmness of fruit relative to untreated fruit. Timing of insecticide spray for *D. suzukii* might be delayed until fruit become susceptible.

Keywords Brix · Firmness · Fruit preference · pH · Spotted wing drosophila

Key message

- *Drosophila suzukii*, spotted wing drosophila, is a serious pest of small fruits and cherries.
- While fruits become more susceptible to *D. suzukii* as they ripen, the direct relationship between ripening characteristics and likelihood of oviposition was previously unknown.
- The probability of oviposition on a given fruit consistently increased as skin penetration force decreased, and pH increased.
- This knowledge is needed to develop thresholds and risk prediction models for IPM.

Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura), is an invasive pest from Asia that occurs in North America, Europe (Asplen et al. 2015; Cini et al. 2014) and recently in South America (Deprá et al. 2014). This pest

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infests blackberry, blueberry, cherry, raspberry, strawberry, and sometimes winegrape (Bellamy et al. 2013; Ioriatti et al. 2015; Lee et al. 2011), and also infests various wild and ornamental hosts (Lee et al. 2015). Given its rapid lifecycle (Tochen et al. 2014) and that 90–95 % of the population is estimated to be at immature life stages during blueberry harvest (Wiman et al. 2014), commonly used insecticides that target adult *D. suzukii* have limited impact on population trajectories. This situation necessitates repeated pesticide sprays to maintain pest control and fruit marketability.

Insecticide dependency can be reduced by improved knowledge of fruit susceptibility to *D. suzukii*. As fruit ripens, the percent of total soluble solids (TSS, also known as °Brix) and pH increases, and the skin and flesh soften. As expected, fruits increase in susceptibility to *D. suzukii* as the TSS and pH increase (Ioriatti et al. 2015; Lee et al. 2011), and as flesh firmness decreases, measured by pressing the fruit flesh (Lee et al. 2011). However, a fruit's resistance to skin puncture (henceforth referred to as penetration force) as opposed to flesh firmness is perhaps more representative of potential oviposition by *D. suzukii*. Oviposition in winegrape fruit appeared most dependent on penetration force compared to TSS or pH (Ioriatti et al. 2015). Studies have shown that fruit increases in susceptibility to oviposition as penetration force decreases in blueberry, as measured with a rheometer (Kinjo et al. 2013), and as penetration force decreases in various small fruits and winegrapes, as measured with a penetrometer (Burrack et al. 2013; Ioriatti et al. 2015). Moreover, *D. suzukii* would not oviposit on artificial diet exceeding 52 cN (Burrack et al. 2013), and winegrapes in the field were infested by *D. suzukii* when penetration force was generally below 40 cN (Ioriatti et al. 2015). These findings suggest that a penetration force threshold may exist for *D. suzukii* oviposition, but thresholds have not been definitively established for fruit crops.

Studies that have examined relationships between fruit characteristics and susceptibility to oviposition by *D. suzukii* used destructive methods that precluded examining one-to-one relationships (Burrack et al. 2013; Ioriatti et al. 2015; Kinjo et al. 2013; Lee et al. 2011). For example, one subset of fruit may have been used to measure *D. suzukii* infestation, while another subset may have been used to measure firmness, penetration force, TSS, or pH. To more precisely obtain a threshold, a non-destructive protocol was used in this study to draw direct relationships. For instance, the pH of each fruit could be directly correlated with having eggs laid on the same fruit. For growers, the presence or absence of infestation is of primary concern rather than how many eggs are laid, and a direct assay provides data for a predictive model and threshold. In this study, the first objective was to determine the probability of oviposition by *D. suzukii* on blueberry fruit of varying firmness, penetration force, TSS and pH using a direct bioassay.

Given that fruit ripening characteristics relate to susceptibility in controlled conditions, field studies are needed to confirm these trends caused by naturally occurring *D. suzukii*. In laboratory bioassays, blackberry, blueberry, cherry, raspberry, and strawberry fruit were found to increase in susceptibility as the fruit color changed from green to blush/pink (Lee et al. 2011). Grower observations validate these trends in the field, but to our knowledge, detailed field studies addressing these observations have not been published. Therefore, our second objective was to evaluate changes in susceptibility as fruit ripens and changes color in the field.

Given that fruit susceptibility is related to the skin penetration force, coatings that alter skin characteristics could affect oviposition by *D. suzukii*. An edible carnauba wax-based coating increased skin penetration force on blueberry (Swoboda-Bhattarai and Burrack 2014). In the same study, covering blueberry and raspberry fruit with various edible coatings reduced oviposition by *D. suzukii*, and both carnauba wax-based coating and carnauba plus kaolin coating reduced survivorship of immature *D. suzukii* in raspberry fruit in the laboratory. Applications of foliar calcium fertilizers targeting blueberry fruit increased skin penetration force in some cases (Ochmian 2012) but not others (Hanson 1995); investigation of their potential to reduce *D. suzukii* oviposition is thus warranted. In addition to potential reduction of *D. suzukii* infestation, applications of various compounds have other well-known benefits. Preharvest applications of calcium sulfate delayed postharvest softening of blueberry kept in storage (Angeletti et al. 2010), and hence may improve the marketability of fruit as postharvest freshness is related to firmness. Gibberellic acid (GA_3) is usually applied during bloom to enhance fruit set (NeSmith 2005). Anti-cracking biofilms are applied to improve elasticity of the skin and accelerate maturity (Kaiser et al. 2014). Given the potential of various fruit coatings to improve fruit quality as well as reduce infestation, our third objective was to evaluate the effects of spraying blueberry fruit with various compounds on *D. suzukii* oviposition rates.

To summarize, this study was designed to (1) determine the probability of oviposition by *D. suzukii* on blueberry fruit of varying firmness, penetration force, TSS and pH levels using a direct bioassay, (2) confirm increased susceptibility as fruits ripen in the field, and (3) evaluate how spraying blueberries with coatings may affect fruit characteristics and oviposition rates.

Materials and methods

Fruit characteristic trials

Oviposition by *D. suzukii* on fruits of varying characteristics was tested in one laboratory trial, as well as in complementary field trials in Oregon and North Carolina, US. Field-collected blueberry fruits for laboratory bioassays were verified by microscope to be free from damage and infestation prior to use. In Oregon, flies used in all laboratory and field experiments were from a laboratory colony that originated from locally collected flies in November 2009, to which wild flies were added each year. This colony was fed a yeast-based diet and maintained at 22 °C, 16:8 L:D photoperiod, and 60 % RH (Woltz et al. 2015). Flies used in experiments were ~2-week old because colony-reared flies are consistently ovipositing at this age (J.C.L., unpub. data). In North Carolina, flies used in field experiments were from a laboratory colony that originated from locally collected flies in October 2010. The colony was fed a standard cornmeal *Drosophila* diet and was maintained at 20 °C, 16:8 L:D, and 60 % RH. Flies used in experiments were 5–10-day old.

The laboratory trial conducted in Oregon, referred to as "OR-lab," tested oviposition on 'Duke' blueberry fruit while measuring the corresponding TSS and pH of each individual fruit. Five female and four male *D. suzukii* were exposed to five fully blue fruit, five pink-colored and five green-colored under-ripe fruit at 22 °C in a 23 × 23 × 25 cm plastic cage with a mesh sleeve. Ten cages were set up on 15 July 2011. Each cage had a wick saturated in a 20 % sucrose–water solution and a water-soaked sponge. Fruit were removed after a 24 h exposure period, and the numbers of oviposition sites per fruit were counted under the microscope. For further analyses, each fruit was individually tracked and stored frozen. The TSS and pH for each individually macerated fruit were determined using a digital refractometer (Hanna Instruments Inc., Woonsocket, RI) and digital pH meter (IQ Scientific Instruments, San Diego, CA), respectively. For this trial and others, TSS could not be measured on some small green fruit because they did not contain sufficient liquid for the refractometer.

A field study in Oregon, referred to as "OR-field," tested oviposition on 'Aurora' blueberry fruit while measuring the firmness, penetration force, TSS, and pH. Three female and two male *D. suzukii* were exposed to 20 hanging fruit that were in situ, within various clusters on a fruiting lateral. The fruiting lateral was enclosed in a mesh sleeve cage (23 × 33 cm) with a drawstring closure. Each cage contained at least five fruit of each ripeness stage with no more than 10 fruit per stage. Ten cages were set up per day (40 total) on 8, 12, 14, and 19 August 2013 at an experimental blueberry farm in Linn County, Oregon. Each cage contained a 1.5-ml microcentrifuge tube hung from the lateral, plugged with cotton, and filled with a 20 % sucrose solution. A water-soaked sponge was also placed at the bottom of the cage. After 24 h exposure, fruit were collected and taken to the lab where eggs were counted. Immediately after egg-counting, flesh firmness and diameter were measured using the deflect test option in a FirmTech2 (BioWorks, Wamego, KS). Firmness readings are reported as g/mm; a value of 150 indicates that 150 g of mass was needed to squeeze the fruit 1 mm. For this trial and others, firmness readings could not be taken on some smaller green and hard fruit. Immediately after flesh firmness testing, the penetration force of the fruit epidermis was measured with a penetrometer consisting of a gram-force gauge (Wagner Instruments, Greenwich, CT) with a No. 3 insect pin (Elephant Brand, Austria) attached 3 mm beyond the end with foam; readings from a penetrometer are reported in centiNewtons (cN) (Burrack et al. 2013; Ioriatti et al. 2015; Swoboda-Bhattarai and Burrack 2014). The ball of the pin was removed, and the blunt end was used to poke fruit. Three readings were taken per fruit on the girth and averaged. Fruit were then immediately frozen and later macerated for measuring TSS and pH as described above.

Two field studies in North Carolina, referred to as "NC-field 1" and "NC-field 2," tested oviposition on 'Premier' rabbiteye blueberry fruit while measuring penetration force and TSS. In NC-field 1, five females were exposed to five hanging fruit on a lateral enclosed in a mesh sleeve cage (12.7 × 7.6 cm) with a drawstring closure. Fruit clusters initially had one ripeness stage present. Nine cages were set up for this experiment on 10 July 2013. In NC-field 2, five females were exposed to 10 fruit of mixed ripeness stages. Nine cages were set up on 18 July 2013. In both trials, each cage provided flies with a yeast-sugar solution in a tube as described earlier. After 24 h exposure, fruit were taken to the laboratory where eggs were counted, penetration force was measured as described earlier, and TSS was measured using a hand-held refractometer (Fisher Scientific™ Fair Lawn, NJ).

First, the effect of multiple variables (firmness, penetration force, TSS and pH) on the probability of oviposition of a given fruit was evaluated using a generalized linear model with a binomial distribution and logit link function. Each fruit was an observational unit and scored for the binary outcome for the presence or absence of oviposition to estimate oviposition probability. Analyses were conducted separately for each trial, with fruits from cages pooled for analysis. Second, key variables identified in the first analyses were then tested individually as single

independent variables using a logistic regression (i.e., pH as an independent variable and presence/absence oviposition as a dependent variable). Third, to explore how attack density affects oviposition probability, logistic regressions were conducted for subsets of data within the OR-field trial by attack density. Low-density attack was defined as cages where an average of less than one egg was laid per fruit, and high-density attack included cages averaging one or more eggs laid per fruit. Analyses were done in JMP 11.0 (SAS 2013).

Field infestation among ripeness stages

Field infestation of fruit by naturally occurring *D. suzukii* populations was monitored in unsprayed raspberry and blueberry fields known to have infestations. On 20 September 2010, raspberries were collected from an experimental mixed-cultivar plot in Linn County, Oregon. Fifteen fruit were collected per sample, with 4–6 samples of each ripeness stage (green, pink/cream, ripe at full color) for red, black, and yellow-fruited cultivars. Fruit were incubated at room temperature and then dissected after 1 week to count the number of larvae and pupae on a per-sample basis. On 24 August, 1 and 8 September 2011, 'Blueray' blueberry fruit were collected from an unsprayed U-pick farm in Benton County, Oregon. Eight sample sets were collected across the farm per date. For each sample set, 25 green, 25 pink, and 25 fully blue fruit were collected concurrently in a location consisting of 2–4 bushes. Blueberry fruit were incubated at room temperature and then dissected after 2 weeks to count the number of larvae, pupae, and adult *D. suzukii*. Raspberry fruit were processed earlier than blueberry fruit, after 1 week rather than 2 weeks, because raspberry fruit often develop mold that prevents fly development.

For raspberry infestation, counts of *D. suzukii* were compared across three ripeness stages using a generalized linear model with a Poisson distribution. Each sample of 15 raspberries or 25 blueberries was an observational unit. Separate tests were conducted for the red, black, and yellow-fruited cultivars. For blueberry infestation, counts were compared for the effects of ripeness stage, date, and interactions with a generalized linear model with a Poisson distribution. For both raspberry and blueberry, means were separated using contrasts ($P < 0.05$). Analyses were done in JMP 11.0 (SAS 2013).

Host suitability manipulation

Five sprayable compounds were tested with the aim to reduce *D. suzukii* oviposition in blueberry; compounds were applied in the field and collected fruit were assayed in the laboratory. Compounds were applied during the ripening period of a conventionally managed 'Legacy' blueberry field near Salem, Oregon. Treatments included calcium borate, calcium chloride, calcium silicate, anti-cracking biofilm, and GA_3 (Table 3). Fruit treated with these five compounds were compared to control fruit that were not sprayed with compounds. Four sets of blueberry bushes were assigned one of the six treatments in a randomized block design within one row. Treatments were applied to three consecutive bushes, comprising a set, and the middle bush was designated as the sample bush. Fruiting laterals from each sample bush were flagged and covered using Tyvek® material (Du Pont, Wilmington, DE) prior to conventional pesticide sprays to eliminate pesticide exposure. Fruiting laterals were uncovered once pesticide sprays had dried. Compounds were applied 1–10 times during the ripening period depending on treatment (Table 3), but not during the week of 19–25 May 2013 due to rain. Fruit from flagged laterals were collected 1 day prior to laboratory assays and stored at 10 °C in the interim.

In the oviposition bioassay, each treatment was replicated in ten cages, with 15 fruit of varying ripeness stages, five females and four males in each cage as described in the fruit characteristics trial. Cages were set up from 10 July to 6 August 2013 using fruit collected from the field the previous day. Because the trial was set up over 5 weeks, it was difficult to present flies with a consistent number of blue, pink, and green fruit each week. However, the number of fruit at each stage did not differ between treatments (6×3 contingency table; Pearson $\chi^2 = 12.7$, $df = 10$, $P = 0.24$), and an average of 6.4 blue, 7.0 pink, and 1.6 green fruit was present in each replicate. As described earlier, deposited eggs were counted in fruit after 24 h exposure, and then each fruit was assessed for firmness, fruit diameter, penetration force, TSS, and pH. Additional ripe blue fruit were collected from the same plants and directly measured for firmness, diameter, and calcium (Ca) concentration; these fruit were not exposed to *D. suzukii*. On 10, 15, 23, and 31 July, and 6 and 14 August 2013, fruit were collected and directly measured for firmness and diameter at the farm site using FirmTech2. On 23 July, 8 and 20 August, ripe fruit were harvested from each plot and shipped overnight to Brookside Laboratories (New Bremen, OH) for determination of fruit Ca concentration using an inductively coupled plasma (ICP) spectrophotometer after wet-washing the samples in nitric/perchloric acid (Gavlak et al. 1994).

The number of eggs laid per fruit, with cage as the observational unit, was compared among spray treatments using a generalized linear mixed model with a Poisson distribution, and date as a random effect. Next, the

firmness, penetration force, TSS, pH, diameter, and arc-sin transformed Ca concentration of fruits were compared among treatments using a generalized linear mixed model with a normal distribution and date as a random effect and each fruit as the observational unit. Treatments were compared to the control with a Dunnett-Hsu multiple comparison ($P < 0.05$). Analyses are only shown for a subset of the data, blue-colored fruit, as this is the stage that is harvested, and any physiological changes may affect storage and marketability of fruit. Analyses were done using PROC GLIMMIX, SAS 9.3 (SAS Institute Inc. 2010).

Table 1 The probability of oviposition by *D. suzukii* as impacted by multiple fruit characteristics during trials in Oregon and North Carolina, US

Trial	Effect	χ^2	*P*	*N*	Eggs/berry[a]
OR-lab	TSS (or Brix°)	8.21	0.004	149	2.7
	pH	7.28	0.007		
OR-field	Firmness	1.30	0.255	467[b]	1.4
	Penetration force	9.72	0.002		
	TSS	1.40	0.237		
	pH	5.95	0.015		
NC-field 1	Penetration force	26.9	<0.001	20[c]	6.0
	TSS	0.29	0.592		
NC-field 2	Penetration force	5.68	0.017	28[c]	3.4
	TSS	0.95	0.330		

df = 1 in all analyses, *n* refers to number of fruits observed

[a] Background oviposition rate measured by the average number of eggs laid per fruit per cage of each trial

[b] Firmness could not be measured on green-colored berries with the equipment, and this analysis includes the pink to fully ripe berries

[c] TSS could not be measured on some green-colored fruit, and data from 4–5 cages were excluded because of weather-induced mortality of *D. suzukii* within the cage

Results

Fruit characteristics trials

In the single analysis that assessed firmness of flesh, firmness did not affect the probability of oviposition on blueberry fruit (Table 1). In contrast, penetration force consistently affected the oviposition probability in three out of three trials (Table 1). Oviposition probability increased as penetration force of the fruit decreased (easier to puncture) in all three trials (Table 2; Fig. 1a). TSS affected oviposition probability in one of the four trials in which it was assessed; only TSS and pH were assessed in the OR-lab trial (Table 1). Oviposition probability increased as blueberry fruit increased in TSS (became sweeter) (Table 2; Fig. 1b). The pH affected oviposition probability in two out of two trials (Table 1); probability increased as pH increased (became less acidic) (Table 2; Fig. 1c). Because r^2 values are typically low for logistic regressions having data points of 0 and 1 (Hosmer and Lemeshow 2000), the logistic regression curves are presented along with the proportion of fruit oviposited upon within a subset of fruit to show how the observed data fit the probability model (Fig. 1).

Oviposition probabilities were calculated under a range of background attack densities (Table 1). The OR-field trial had the lowest rate of 1.4 eggs laid per blueberry fruit, and the NC-field 1 trial had the highest rate of 6.0 eggs per blueberry fruit. Within the OR-field trial, trends were also compared among cages that had low-oviposition densities and among those with high-oviposition densities. Data are presented when oviposition trends were significant at both the low and high densities. The oviposition probability increased as the penetration force of blueberry fruit decreased in both the low- and high-density situations (Fig. 2). As expected, oviposition probability was lower under a low-density situation than under a high-density situation. Therefore, probabilities estimated by these models are mainly used to understand general trends and

Table 2 The probability of oviposition by *D. suzukii* as impacted by a single fruit characteristic during trials in Oregon and North Carolina, US

Effect	Trend	Trial	χ^2	*P*	*N*	Logistic regression equation
Penetration Force (pf)	↓	OR-field	137.1	<0.001	748	$\text{Prob.} = 1/(1 + e^{-(1.2 - 0.033 \times \text{pf})})$
	↓	NC-field 1	21.5	<0.001	29[a]	$\text{Prob.} = 1/(1 + e^{-(6.9 - 0.248 \times \text{pf})})$
	↓	NC-field 2	23.2	<0.001	32[a]	$\text{Prob.} = 1/(1 + e^{-(5.9 - 0.144 \times \text{pf})})$
TSS	↑	OR-lab	33.6	<0.001	150	$\text{Prob.} = 1/(1 + e^{-(-4.2 + 0.476 \times \text{TSS})})$
pH	↑	OR-lab	31.5	<0.001	149	$\text{Prob.} = 1/(1 + e^{-(-6.6 + 2.83 \times \text{pH})})$
	↑	OR-field	65.9	<0.001	748	$\text{Prob.} = 1/(1 + e^{-(-5.0 + 1.78 \times \text{pH})})$

df = 1 in all analyses, *n* refers to number of fruits observed, down arrow indicates that the probability of oviposition increased as the firmness of the blueberry fruit decreased

[a] Data from 4–5 cages were excluded because of weather-induced mortality of *D. suzukii* within the cage

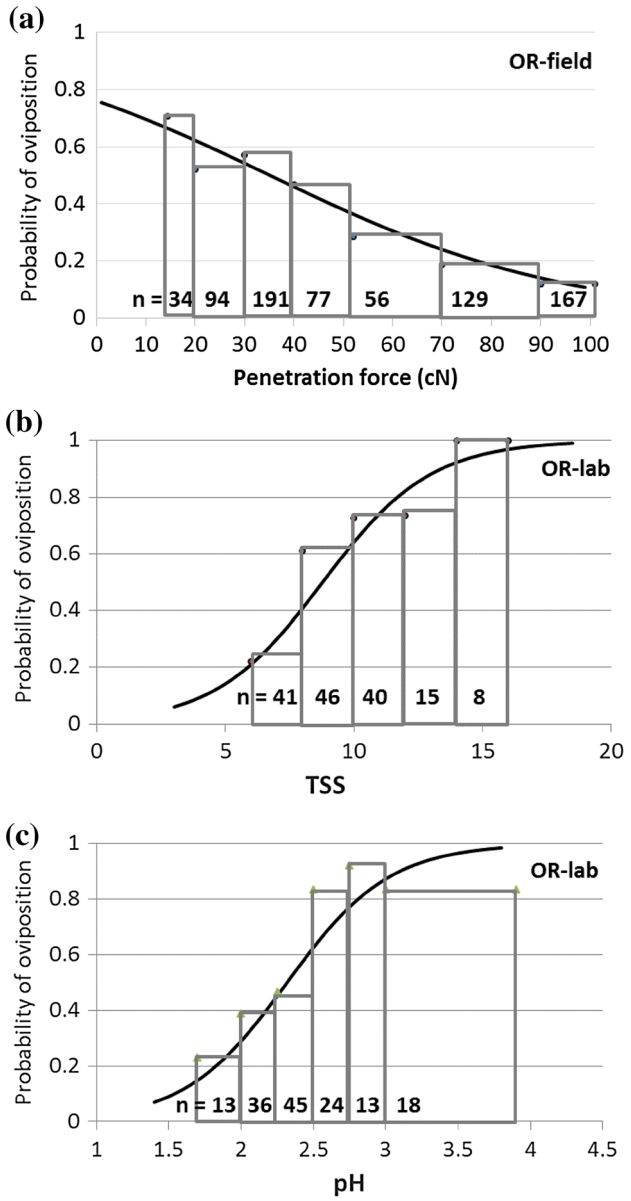

Fig. 1 Probability of oviposition by *D. suzukii* on blueberry as fruit varied by penetration force in OR-field (**a**), TSS (or °Brix) in OR-lab (**b**), and pH in OR-lab (**c**) trials. *Bars* show the observed proportions while *curves* describe the fitted relationship by logistic regression. First box in (**a**) indicates that 71 % of fruit with penetration force values of 14–20 cN had oviposition. Numbers inside the boxes refer to the number of fruit within each subset, and subsets in (**a**) are as low as 34 because fewer fruits at the lower cN values were available

the actual probability will depend on the background attack density.

Field infestation among ripeness stages

Infestation by naturally occurring *D. suzukii* varied significantly by fruit ripeness stage among various colored raspberry fruit and 'Blueray' blueberry fruit collected from

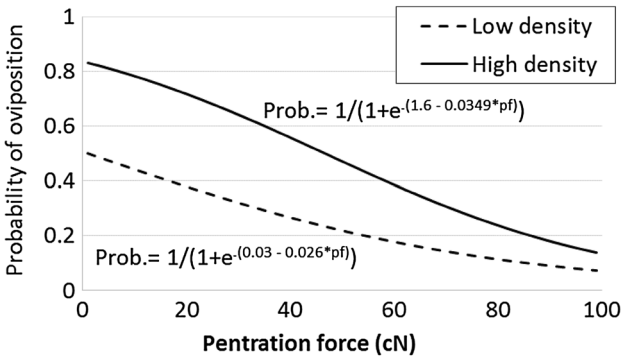

Fig. 2 Probability of oviposition by *D. suzukii* on blueberry fruit in replicates with low (<1 egg per fruit) and high attack densities (≥1 eggs per fruit) as fruit varies by penetration force in the OR-field trial. Logistic regression for penetration force: low density $\chi^2 = 21.3$, $P < 0.001$, $n = 265$; high density $\chi^2 = 110$, $P < 0.001$, $n = 483$. All $df = 1$

untreated fields (Fig. 3a, b). Very few or no *D. suzukii* were found developing on raspberry and blueberry fruit at the green unripe stage, and infestation in green fruit was lower than that of under-ripe or ripe raspberry and blueberry fruit. Infestation was also lower in under-ripe fruit compared to ripe fruit. Natural infestation among pink- or cream-colored raspberry was 79.5–83.7 % lower than among fully ripe red, black, or yellow raspberry. Natural infestation of pink-colored blueberry was 86.6–96.7 % lower than for ripe blue-colored blueberry.

Host suitability manipulation

Sprayable coatings on blueberry fruit significantly affected oviposition on fruit of mixed ripeness stages (Table 4). Blueberries treated with calcium silicate resulted in 52 % lower oviposition rates by *D. suzukii* compared to untreated control fruit. Other treatments did not result in oviposition rates different from the control. Sprayable coatings affected the physiological characteristics of the blueberry fruit in terms of firmness, penetration force, TSS, pH, diameter, and Ca concentration. Note, these fruit were treated in the field over the season (Tables 3, 4), but comparisons are presented only among fruit collected at the ripe blue harvestable stage, as this is most relevant to growers. Among fruit exposed to *D. suzukii*, fruit treated with chelated calcium or calcium silicate had 8–10 % greater firmness than untreated fruit. Among freshly collected fruit, fruit treated with calcium borate had 7.5 % greater firmness than untreated fruit. Fruit treated with calcium silicate had 10 % greater penetration force than untreated fruit. Sprayable coatings did not change the TSS or pH of fruit relative to untreated fruit. For effects on diameter, fruit treated with GA_3 were 5–15 % larger than untreated fruit. Fruit treated

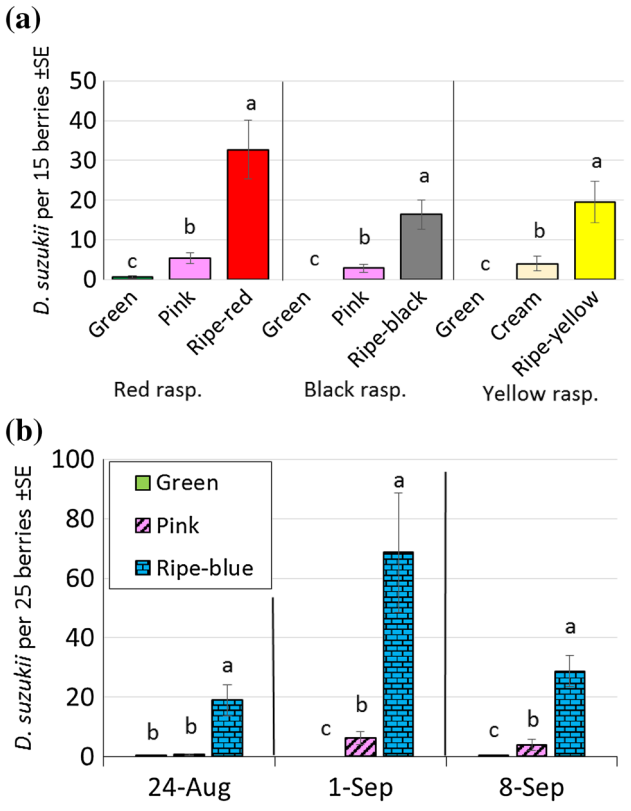

Fig. 3 Mean number of *D. suzukii* developing from naturally infested fruit collected from the field at different ripeness stages among various colored raspberry (**a**), and 'Blueray' blueberry (**b**). *Letters* denote significant difference within each date. General linearized model for red raspberry $\chi^2 = 284$, $df = 2, 15$, $P < 0.001$, *black* raspberry $\chi^2 = 131$, $df = 2, 12$, $P < 0.001$, *yellow* raspberry $\chi^2 = 151$, $df = 2, 12$, $P < 0.001$, and *blueberry* ripeness $\chi^2 = 1237$, $df = 2, 62$, $P < 0.001$, Date $\chi^2 = 5.3$, $df = 2, 62$, $P = 0.070$, ripeness x date $\chi^2 = 16.1$, $df = 4, 62$, $P = 0.0028$. (Color figure online)

with chelated calcium and calcium borate were 3.5 % smaller than untreated fruit among freshly collected fruit. Lastly, fruit treated with calcium borate and calcium silicate had higher Ca content than untreated fruit, and Ca increased by 0.014–0.022 % in concentration, representing a 26–42 % increase relative to the control.

Discussion

For growers, the risk of infestation is more important than the amount of infestation per berry, and a predictive model and threshold would be a helpful decision-making tool for timing of insecticide application. With a direct assay, the probability of oviposition on a blueberry fruit could be calculated and oviposition probability consistently increased as the fruit's penetration force decreased in all three trials where it was assessed in a multivariate model. This result is consistent with a Pearson correlation analysis and categorical grouping of winegrapes by infestation status showing that oviposition is highly dependent on penetration force (Ioriatti et al. 2015). In our trials, the probability of oviposition also increased as pH increased and sometimes increased as TSS increased (fruit became sweeter and less acidic), also consistent with Ioriatti et al. (2015). Notably, significant trends were found in datasets with a range of 20–467 observations, and with a range of attack rates of 1.4–6.0 eggs per fruit within a cage. These models help us to understand the behavior of *D. suzukii* under confined conditions, which can improve future efforts to predict risk in the open field. Natural attack rates in the field are expected to be lower than in the caged arenas, and therefore, the probability values should also be lower. For comparison, in the field study, fruit collected from an unsprayed field were naturally infested at 0.76–2.7 *D. suzukii* eggs per berry, and infestation on green and sometimes pink fruit was at or near zero (Fig. 3).

The trials presented here did not clearly delineate a threshold of when oviposition would not occur for the metrics of firmness, epidermal penetration force, TSS, or pH. In contrast, a threshold penetration force of 52 cN has been found on an artificial diet (Burrack et al. 2013), and 40 cN has been suggested for winegrape fruit based on observed field infestations (Ioriatti et al. 2015). The lack of thresholds in our trials might suggest that prediction of susceptibility on fruit versus artificial media is more complicated than relying on a single characteristic, or alternatively, our experimental design was not adequate for determining a threshold. Within a cage, *D. suzukii* have few ovipositional choices and may exhibit a lower degree of preference.

In the field, infestation by naturally occurring *D. suzukii* was highest on ripe fruit, lower on ripening fruit, and at or near zero on green fruit for mixed raspberry cultivars and 'Blueray' blueberry regardless of coloration at maturity. While this confirms previous laboratory assays, the magnitude of difference was more pronounced in the field. In this field study, infestation on raspberry was 5–6-fold higher among ripe raspberry fruit, and 7–30-fold higher among ripe blueberry fruit compared to ripening fruit. In a prior laboratory choice study, infestation was 1.5–2.5-fold higher among ripe 'Coho' raspberry, and 1–4-fold higher among ripe 'Duke,' 'Earliblue,' 'Jewel,' and 'Star' blueberry fruit than ripening fruit (Lee et al. 2011). Stronger preference for ripe fruit may be expected in the field because *D. suzukii* have a great quantity of ripe fruit available compared to a confined study with only 4 or 8 ripe fruits out of a total of 16–24 fruits.

Next, sprayable coatings applied in a 'Legacy' blueberry field affected the characteristics of the fruit as well as

Table 3 Coatings sprayed on blueberry plants during fruit development (from immediately after fruit set through early blue stage) in a mature field near Salem, Oregon, US

Coating	Spray dates	Rate	L/ha	Product information
Chelated calcium	23, 30 April, 8, 14, 31 May, 7, 15, 22, 27 June, 4 July 2013	1.5 % by volume	16	Biomin®, JH Biotech, Inc., Ventura, CA
Calcium borate	23, 30 April, 8, 14, 31 May, 7, 15, 22, 27 June, 4 July 2013	1.5 % by volume	16	Phyta-Set QC™, California Organic Fertilizers, Inc., Hanford, CA
Calcium silicate	30 April, 8, 14, 31 May, 7, 15, 22, 27 June, 4 July 2013	1.1 % by volume	12	Mainstay Calcium, Redox Chemicals, LLC, Burley, ID
Gibberellic acid (GA_3)	15 June 2013	30 ppm	0.03	ProGibb® Plus 2X, Valent BioSciences Corporation, Libertyville, IL
Biofilm	8, 31 May, 15, 27 June 2013	0.5 % by volume	5.4	Parka™, Cultiva, Portland, OR

Fruit were used in a laboratory assay for oviposition by *D. suzukii*

oviposition rates in a laboratory study. Blueberry sprayed with calcium silicate had a 52 % reduction in the number of eggs laid on it compared to untreated control fruit. One explanation for this reduction could be that females do not prefer the tactile cues of sprayed fruit, or alternately it could be due to a mechanism mediated through physiological changes in the fruit. Our trial was not designed to test the first explanation but support for the second mechanism was found: the same fruit sprayed with calcium silicate also had a 10 % higher penetration force and 10 % higher firmness compared to untreated fruit. In general, the calcium-based treatments increased fruit firmness, penetration force, and Ca concentration, while some treatments slightly reduced fruit diameter by 3.5 % relative to the control. Our results are consistent with reported effects of calcium fertilizer applications on 'Duke' (Ochmian 2012), 'O'Neal' and 'Bluecrop' blueberry (Angeletti et al. 2010). However, in previous studies (Ochmian 2012), fruit may also have been firmer as a result of a small average berry size. The small reduction in size associated with fruit treated with chelated calcium or calcium borate may be a response to plant stress or osmotic or turgor changes in treated fruit.

In our trial, application of GA_3 in mid-June when fruit were first showing color increased the size of blue-colored blueberry fruit relative to the control. Application of GA_3 at early bloom has been shown to increase fruit set and reduce berry size in rabbiteye blueberry (NeSmith 2005; NeSmith and Krewer 1999), but this growth regulator is not typically used during fruit development. The anti-cracking biofilm had no impact on fruit characteristics or oviposition behavior relative to the control. None of the coatings prevented *D. suzukii* oviposition but calcium silicate may reduce pest infestation. Some calcium-based coatings did improve fruit quality suggesting additional horticultural benefit separate from pest control.

The measurement of host potential consists of locating the host, success of oviposition, and larval development, and the studies presented here address various phases. The fruit characteristic trials measured oviposition success, and found penetration force and pH to consistently affect oviposition. The importance of penetration force is expected given that *D. suzukii* uses its serrated ovipositor to break the skin of the fruit before pushing the egg into the flesh further into the fruit (Stewart et al. 2014). How pH influences oviposition success is not known. The field infestation study examined the infestation status of fruit exposed to naturally occurring *D. suzukii*, and such observations would have been affected by all three phases of host selection. The host suitability manipulation study likewise measured oviposition success, and oviposition was notably reduced in fruit with higher firmness and skin penetration force. This result is consistent with Stewart et al. (2014), in which *D. suzukii* spent more time to complete oviposition on shaved peach than cherry, and this observation was attributed to peach having a denser flesh than cherry.

In summary, the timing of insecticide sprays should be delayed until fruit become susceptible to *D. suzukii* to maximize their utility. Our manipulated laboratory and field studies show a consistent increase in probability of oviposition as penetration force of fruit decreases, and as pH increases. These changes are associated with fruit ripening. Observations of infestation by naturally occurring *D. suzukii* in unsprayed blueberry and raspberry fields confirm that that fruits are most susceptible when ripe. Lastly, treating blueberry fruit with calcium silicate can increase fruit firmness and penetration force, which is beneficial for preserving postharvest fruit, and such treatment is associated with a reduction in oviposition by *D. suzukii*. Field-based work on *D. suzukii* susceptibility probability curves is needed and essential to allow growers

Table 4 Mean number (±SE) of eggs laid by *D. suzukii* and fruit characteristics of 'Legacy' blueberry treated with various coatings (see Table 3)

	Eggs laid per fruit per cage	Flesh firmness		Penetration force	TSS	PH	Diameter (mm)		Calcium % other
	All stages	Blue	Other blue[b]	Blue	Blue	Blue	Blue	Other blue[b]	Other blue[b]
Control	4.42 ± 0.62	245 ± 5.1	213 ± 2.3	22.8 ± 0.67	11.5 ± 0.17	2.81 ± 0.044	15.0 ± 0.2	17.0 ± 0.14	0.053 ± 0.11
Chelated Calcium	4.92 ± 1.15	265 ± 4.8[a]	212 ± 2.6	23.2 ± 0.87	10.9 ± 0.29	2.98 ± 0.047	15.0 ± 0.3	16.4 ± 0.14[a]	0.062 ± 0.008
Calcium Borate	3.31 ± 1.01	257 ± 6.0	229 ± 3.0[a]	24.1 ± 0.65	10.7 ± 0.22	2.85 ± 0.041	15.8 ± 0.3	16.4 ± 0.16[a]	0.067 ± 0.016[a]
Calcium Silicate	2.12 ± 0.40[a]	269 ± 4.7[a]	220 ± 2.7	25.0 ± 0.54[a]	12.2 ± 0.21	2.75 ± 0.038	15.2 ± 0.2	16.6 ± 0.13	0.075 ± 0.021[a]
Gibberellic acid	5.37 ± 1.07	243 ± 4.6	214 ± 2.6	22.5 ± 0.83	10.9 ± 0.23	2.98 ± 0.047	17.2 ± 0.2[a]	17.9 ± 0.15[a]	0.055 ± 0.009
Biofilm	3.51 ± 0.66	251 ± 4.2	214 ± 2.1	22.6 ± 0.61	11.3 ± 0.21	2.94 ± 0.057	16.1 ± 0.3	16.6 ± 0.14	0.058 ± 0.012
Statistics	$F_{5,50} = 3.57$	$F_{5,370} = 6.1$	$F_{5,853} = 8.0$	$F_{5,370} = 2.3$	$F_{5,370} = 6.0$	$F_{5,370} = 5.1$	$F_{5,370} = 12.1$	$F_{5,853} = 23.0$	$F_{5,64} = 6.2$
	$P = 0.008$	$P < 0.001$	$P < 0.001$	$P = 0.041$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

[a] Indicates difference from the control

[b] These berries were collected and directly measured, and not first stored in cold and exposed to *D. suzukii*

the option to delay and reduce dependence on insecticide sprays. Secondly, combinations of promising fruit sprays, pesticides, repellents, environmental manipulation, and cultural methods such as exclusion netting should be tested to develop more sustainable *D. suzukii* management options.

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