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Influence of edible fruit coatings on *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposition and development

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Abstract

Drosophila suzukii (Matsumura) is a highly invasive vinegar fly recently detected in the United States that severely threatens the viability of soft skinned fruit production. Insecticides mitigate some of this damage, but alternative methods to manage *D. suzukii* infestation are needed. We tested three edible coatings to determine if they could prevent or reduce oviposition by *D. suzukii* females or affect immature survivorship and development in two important host crops, blueberry and raspberry. None of the coatings prevented oviposition, but some reduced the number of eggs laid. Two carnauba wax-based coatings, PrimaFresh 45 and Raynox, dramatically reduced survivorship of immature *D. suzukii* in raspberries, but not in blueberries. Our results suggest that obtaining thorough, even coverage in the field will be essential if edible coatings are to be used as a management strategy for *D. suzukii*.

Keywords: invasive species, blueberry, raspberry, oviposition deterrent

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1. Introduction

Invasive insects are among the most significant management challenges for specialty crops, including small fruits, because established integrated pest management programs are often not robust to challenges from these novel pests and because they can often have large host ranges. *Drosophila suzukii*, which has commonly been referred to as the spotted wing drosophila, is an invasive pest of ripe and ripening soft skinned fruit, and has a broad crop and non-crop host range, including caneberries, blueberries, cherries, strawberries, grapes, and their wild relatives (Lee et al. 2011; Cini et al. 2012). *D. suzukii* was first detected in the continental United States in California in 2008 (Walsh et al. 2011), and in the southeastern United States in Florida in 2009. It has since been detected throughout the United States, Canada, Mexico, and Europe (Burrack et al. 2012; Cini et al. 2012). Left unchecked, *D. suzukii* infestations can reach 100%, particularly in later fruiting host crops. Insecticides have been employed to mitigate some of this damage, but there are limited insecticides effective against *D. suzukii* with sufficiently short preharvest intervals to allow their use during the period in which fruit is vulnerable to infestation (Bruck et al. 2011). Organically acceptable insecticides are even more limited than conventional tools (Van Timmeren & Isaacs 2013). Insecticide efficacy is further limited by environmental conditions and is lessened under rainy conditions, common during summers in the eastern United States (Van Timmeren & Isaacs 2013). Sanitation, in the form of thorough harvest and removal of fruit, is recommended in both conventional and organic systems, but the effectiveness of sanitation at reducing infestation is unclear.

The selection of fruit by *D. suzukii* females for oviposition appears to be influenced by fruit characteristics including substrate firmness, host plant odor, and other physical differences (Lee et al. 2011; Bellamy et al. 2013; Burrack et al. 2013). Edible coatings have been used to protect fruit post harvest and enhance shelf life (Pavlath & Orts 2009), and kaolin clay has been used to prevent sunscald and insect damage by tephritid fruit flies and stink bugs (Mazor & Erez 2004; Saour & Makee 2004; Lalancette et al. 2005; Villaneuva & Walgenbach 2007). Waxes such as those used in edible coatings, including beeswax, carnauba, candelilla, paraffin, and shellac (Pavlath & Orts 2009), may have a negative effect on oviposition (Neuenschwander et al. 1985; Kombargi et al. 1998). We conducted a series of experiments to determine if fruit coatings were effective in inhibiting oviposition or reducing immature survivorship of *D. suzukii* and, therefore, have potential for use in *D. suzukii* management programs either pre or post harvest.

2. Materials and methods

2.1. Coatings

We tested the effects of three edible coatings on the oviposition, immature survivorship, and development of *D. suzukii*. PrimaFresh® 45 (Pace International, LLC, Seattle, WA) is a carnauba-based protective coating used on stone fruit. It is generally applied undiluted post harvest to clean, dry fruit via overhead spray/drip systems (3.79 L per 6803–13,608 kg fruit). Raynox® Apple Sunburn Protectant (Pace International, LLC, Seattle, WA) is made from carnauba wax and organically modified kaolin clay. Suggested field application concentrations range from 1:40 to 1:20 (product:water ratio) depending on the desired spray volume to be applied per acre. REFLECTIONS™ Liquid Shade (Tiger Industries, Inc., Bristol, RI) is a calcium carbonate shade product used to reduce heat stress on fruits, vegetables, trees, and row crops. Suggested field application concentrations range from 1:20 to 1:5. We tested several potential application concentrations for each coating (PrimaFresh 45: 1:10, 1:5, 1:2, 1:1; Raynox: 1:20, 1:10, 1:5, 1:2, 1:1; REFLECTIONS: 1:20, 1:10, 1:5, 1:2). Dilutions of PrimaFresh 45 and REFLECTIONS were made using tap water to approximate field application conditions. Dilutions of Raynox were made with distilled water (365™ Brand, Whole Foods) to control solution pH.

2.2. Oviposition and immature survivorship in coated fruit

The effects of edible coatings on oviposition and immature survivorship were tested using raspberries, which are highly preferred by egg laying *D. suzukii*, and blueberries, which are less preferred (Burrack et al. 2013). A constant mass of approximately 20 g (±0.54 g) of either raspberries or blueberries was coated and presented to *D. suzukii* during no-choice assays. A quantity of 20 g was equal to an average of 4.71 raspberries and 13.10 blueberries. Uncoated fruit were used as standards. Organically grown fruit for use in assays was purchased from local grocery stores in Raleigh, NC. Because assays were performed over the course of several months, from February to November 2013, brands varied according to availability but were kept consistent when possible. In general, raspberries were from Driscoll's (Watsonville, CA) and blueberries were from Sunny Ridge (Winter Haven, FL). Because the use of edible coatings is not a standard practice in berry production and because even the lowest concentration of coatings used in our experiments were clearly visible to the naked eye, we do not believe that the purchased fruit used for assays had been previously coated. Before coatings were applied, raspberries were plugged with a small amount of cotton to prevent *D.*

suzukii females from laying eggs in the interior of fruit, which would be inaccessible under field conditions. Fruit were placed in an 800 diameter kitchen strainer positioned over a 1000 ml Pyrex beaker and coated using disposable plastic pipettes. Each berry was then picked up individually and gently rolled between the fingers to ensure that its entire surface was coated. Coated fruit were placed on wax paper and allowed to dry overnight. A 266 mL plastic GladWare® container vented on the bottom with thrips barrier mesh (Bioquip Products, Rancho Dominguez, CA) was positioned upside down over each fruit sample in order to protect it without preventing air circulation.

In each no-choice assay, 20 g of coated fruit were presented to 15 female and 15 male reproductively active *D. suzukii* in a 0.3 m^3 collapsible cage (Bioquip Products, Rancho Dominguez, CA) for 4 hours, from approximately 10 am – 2 pm, at 20 °C. Naive flies from a colony originating from flies collected at the Upper Mountain Research Station, Laurel Springs, NC, in October 2010 were used in each assay. The colony was maintained on a standard cornmeal *Drosophila* diet (Drosophila Species Stock Center 2013) and held at 20 °C and 16:8 light:dark cycle. The total number of eggs laid per 20 g fruit was counted immediately after exposure, using a stereomicroscope (Olympus SZ61; Olympus America, Center Valley, PA). Infested fruit were held at 20 °C in 266 mL plastic containers, as described above, and checked for pupal emergence for up to 21 days. Pupae were removed from fruit to allow for easier enumeration and to prevent potential re-infestation by emerging adults and were held in 60 × 15 mm polystyrene Petri dishes with a moistened paper towel square until adults emerged. Immature survivorship was calculated as (1) the proportion of eggs laid that survived to the pupal stage and (2) the proportion of pupae that emerged as adults. In general, two paired treated and control replicates of each concentration were conducted at a time. At least three replicates were obtained for each concentration of each coating on both types of fruit.

2.3. Development in coated fruit

The effect of fruit coatings on larval and adult developmental rates was tested in no-choice assays using raspberries, because *D. suzukii* larvae appear to perform better at high larval densities in raspberries compared to other fruits or diets (Burrack and Hardin, unpublished; Burrack et al. 2013). In each assay, 1:10, 1:5, and 1:2 concentrations of each coating were applied to 20 g of fruit and tested alongside an uncoated control. Directly following exposure, infested fruit were held at 20 °C and checked for pupal emergence daily for up to 21 days. Pupae were removed and housed as before, and adult emergence was recorded daily. Developmental times from egg to pupae and pupae to adult were calculated. Three replicates of each treatment were conducted during October and November 2013.

2.4. Effect of coatings on fruit firmness

Surface penetration force was measured in centinewtons (cN) on separate random samples of raspberries and blueberries using a Wagner gram force (gf) gage (Wagner Instruments, Greenwich, CT) fitted with a blunted No. 3 insect pin (Elephant Brand, Austria) (Burrack et al. 2013). For each raspberry, three firmness readings were taken from the center of haphazardly selected drupelets and averaged. For blueberries, three firmness readings were taken near the widest part of the berry and averaged. Firmness readings were taken from 10 berries for each coating and fruit combination and were compared with readings from uncoated fruit. Data were collected on the following dates: raspberries: PrimaFresh 45: 31 October and 20 December; Raynox: 31 October, 4 November, and 20 December; REFLECTIONS: 4 November and 20 December; blueberries: Raynox: 24 September; PrimaFresh 45 and REFLECTIONS: 1 November.

2.5. Effect of coatings on infestation in the field

To determine if coatings reduce *D. suzukii* infestation in the field, a small plot study was conducted at the Central Crops Research Station, Clayton, NC on 14 June 2013. All coatings were applied at 1:10 concentration to 20 strawberry plants in 0.00036 ha plots and compared with untreated controls. All treatments were replicated seven times in a randomized complete block design with rows serving as blocks. Coatings were applied using a CO^2-pressurized backpack sprayer fitted with a boom with 3 flat-fan nozzles at a spray rate of 72 ml/sec with 45 psi pressure in a spray volume of 935 l per ha. A single application of each material was made to plots, and 15 sound, marketable-looking berries were collected from each plot seven days after treatment. Flies were reared as described previously. After emergence, adults were sorted and identified as *D. suzukii* or other *Drosophila* species, and the average number of *D. suzukii* per berry was calculated for each plot. In addition, water sensitive cards were placed within the strawberry canopy near fruit in each plot to measure droplet size and spray coverage and were photographed for later analysis.

Average droplet size was calculated using ImageJ software (Ferreira & Rasband 2012). The diameters of 10 randomly selected droplets were measured and averaged to estimate average droplet size for each plot. Spray coverage was calculated using Adobe® Photoshop® CS6 (Adobe Systems Inc., San Jose, CA) by determining the percentage of each card that was covered.

2.6. Data analysis

Oviposition data were either log or square root transformed as necessary to meet assumptions of normality (log: Raynox on blueberries, REFLECTIONS on raspberries and blueberries; square root: PrimaFresh on blueberries). Several potential distributions were fitted to models for immature survivorship data for raspberries coated with PrimaFresh 45 and Raynox via PROC GLIMMIX, and a normal distribution provided the best fit for both data sets. Therefore, oviposition and survivorship in no-choice assays for all materials tested were analyzed using mixed-model ANOVA via PROC MIXED in SAS 9.4 with concentration as a fixed effect and replicate as a random effect. Developmental rates for pupae and adults were analyzed separately using mixed-model ANOVA with treatment (coating and concentration) as a fixed effect. Similarly, fruit firmness was analyzed using mixed-model ANOVA with concentration as a fixed effect. Field infestation rates, droplet size, and spray coverage were analyzed using a mixed-model ANOVA with coating as a fixed effect and replicate as a random effect. Droplet size data were log transformed to meet assumptions of normality. All means separations were conducted using Fisher's least significant difference (LSD) procedure.

3. Results

3.1. Oviposition and immature survivorship in coated fruit

While none of the coatings at any concentration prevented oviposition in either fruit, PrimaFresh 45 and Raynox reduced oviposition in raspberries (PrimaFresh 45: $F_{4,15} = 26.56$, $P < 0.0001$; Raynox: $F_{4,14} = 28.09$, $P < 0.0001$). For both coatings, females laid fewer eggs in coated fruit, regardless of concentration, than in uncoated control fruit. REFLECTIONS did not reduce oviposition in raspberries ($F_{4,9} = 0.63$, $P = 0.6519$) (**Table 1**).

Raynox and REFLECTIONS reduced oviposition in blueberries (Raynox: $F_{4,15} = 5.18$, $P = 0.0080$; REFLECTIONS: $F_{4,15} = 3.09$, $P = 0.0485$). Females laid fewer eggs in fruit coated with 1:2 and 1:1 concentrations of Raynox than in uncoated fruit. For REFLECTIONS, females laid fewer eggs in 1:2 coated fruit than in uncoated and 1:20 and 1:10 coated fruit. PrimaFresh 45 did not reduce oviposition in blueberries ($F_{4,15} = 1.91$, $P = 0.1606$) (Table 1).

PrimaFresh 45 and Raynox both reduced immature survivorship in raspberries by reducing the proportion of eggs that survived to the pupal stage (PrimaFresh 45: $F_{4,14} = 21.41$, $P < 0.0001$; Raynox: $F_{4,14} = 6.39$, $P = 0.0038$). Fewer eggs survived to the pupal stage in raspberries coated with 1:5, 1:2, and 1:1 concentrations of PrimaFresh 45 than in uncoated and 1:10 coated

Table 1. Mean* eggs laid in raspberries and blueberries coated with potential field application rates of PrimaFresh 45, Raynox, and REFLECTIONS.

		Concentration					
Fruit	Coating	Control	1:20	1:10	1:5	1:2	1:1
Raspberries	PrimaFresh 45	119.50a	–	45.00b	39.00b	37.75b	27.00b
	Raynox	93.00a	42.25b	32.50bc	25.75c	28.00bc	–
	REFLECTIONS	132.67	94.50	115.67	134.33	119.67	–
Blueberries	PrimaFresh 45	34.00	–	40.50	37.25	17.75	19.00
	Raynox	45.00a	–	41.00ab	34.75ab	15.50bc	7.75c
	REFLECTIONS	43.50a	32.00a	31.00a	19.50ab	11.75b	–

* Values within a row followed by the same letter are not significantly different at α = 0.05 via LSD adjustment.

fruit. Very similar results were observed for Raynox, in which fewer eggs survived to the pupal stage in raspberries coated with 1:10, 1:5, and 1:2 concentrations than uncoated and 1:20 coated fruit (**Table 2**). REFLECTIONS did not reduce immature survivorship in raspberries ($F_{4,9}$ = 0.87, P = 0.5202). In blueberries, Raynox reduced immature survivorship by reducing the proportion of eggs that survived to the pupal stage ($F_{4,14}$ = 3.39, P = 0.0388). Fewer eggs survived in blueberries coated with the 1:1 concentration than in uncoated, 1:10, and 1:5 fruit. Both PrimaFresh 45 ($F_{4,15}$ = 1.55, P = 0.2393) and REFLECTIONS ($F_{4,14}$ = 0.76, P = 0.5661) failed to reduce immature survivorship in blueberries.

For those concentrations in which some eggs survived to the pupal stage (1:20 and 1:10; Table 2), Raynox ($F_{2,8}$ = 8.72, P = 0.0098) reduced immature survivorship in raspberries by reducing the proportion of pupae that emerged as adults. Higher proportions of adults emerged from pupae reared

Table 2. Mean* proportion of *D. suzukii* eggs that survived to the pupal stage in raspberries and blueberries coated with potential field concentrations of PrimaFresh 45, Raynox, and REFLECTIONS.

		Concentration					
Fruit	Coating	Control	1:20	1:10	1:5	1:2	1:1
Raspberries	PrimaFresh 45	0.99a	–	0.73a	0.11b	0.00b	0.00b
	Raynox	0.61a	0.50a	0.15b	0.00b	0.00b	–
	REFLECTIONS	1.00	1.00	0.99	1.00	1.00	–
Blueberries	PrimaFresh 45	0.70	–	0.66	0.78	0.69	0.42
	Raynox	0.61a	–	0.58a	0.53a	0.35ab	0.17b
	REFLECTIONS	0.36	0.29	0.41	0.57	0.43	–

* Values within a row followed by the same letter are not significantly different at α = 0.05 via LSD adjustment.

Table 3. Mean* proportion of *D. suzukii* pupae that emerged as adults from raspberries and blueberries coated with potential field concentrations of PrimaFresh 45, Raynox, and REFLECTIONS.

		Concentration					
Fruit	Coating	Control	1:20	1:10	1:5	1:2	1:1
Raspberries	PrimaFresh 45	0.94	–	0.95	1.00	n/a	n/a
	Raynox	0.97a	1.00a	0.42b	n/a	n/a	–
	REFLECTIONS	0.95	0.97	0.93	0.96	0.97	–
Blueberries	PrimaFresh 45	0.93	–	0.85	0.88	0.84	0.85
	Raynox	0.91	–	0.91	0.92	0.98	1.00
	REFLECTIONS	0.99	0.95	0.87	0.92	0.94	–

* Values within a row followed by the same letter are not significantly different at α = 0.05 via LSD adjustment.

in 1:20 and uncoated fruit than in 1:10 fruit (**Table 3**). Conversely, PrimaFresh 45 did not reduce the proportion of pupae that emerged as adults; similar proportions of adults emerged from pupae reared in uncoated, 1:10, and 1:5 fruit ($F_{2,8}$ = 1.75, P = 0.2339). REFLECTIONS did not reduce immature survivorship in raspberries ($F_{4,8.02}$ = 0.54, P = 0.7106), while none of the coatings reduced the proportion of pupae that emerged as adults in blueberries (PrimaFresh 45: $F_{4,11.4}$ = 0.44, P = 0.7775; Raynox: $F_{4,11}$ = 1.24, P = 0.3503; REFLECTIONS: $F_{4,14}$ = 0.48, P = 0.7506).

3.2. Development in coated fruit

Pupae emerged from raspberries between 6.0 and 13.0 days after infestation. Larvae appeared to develop more slowly in raspberries coated with the 1:2 concentrations of Prima-Fresh 45 and Raynox (**Figure 1**). However, because of the small number of pupae that emerged from these treatments in general, these data could not be analyzed (PrimaFresh 45 1:2 concentration: 2 pupae emerged; Raynox 1:2 concentration: 1 pupa emerged). For the remaining treatments, larval developmental time ranged from 8.4 days (Raynox 1:5) to 9.0 days (uncoated controls) on average. Larval developmental time did not differ between uncoated and coated fruit, regardless of coating or concentration ($F_{7,15}$ = 0.62, P = 0.7289) (Figure 1). Adults emerged between 3.0 and 8.0 days after pupation, and adult developmental time did not differ between uncoated and coated fruit, regardless of coating or concentration ($F_{7,15}$ = 1.01, P = 0.4595). On average, adult developmental time ranged from 5.7 days (Raynox 1:10) to 6.6 days (Raynox 1:5).

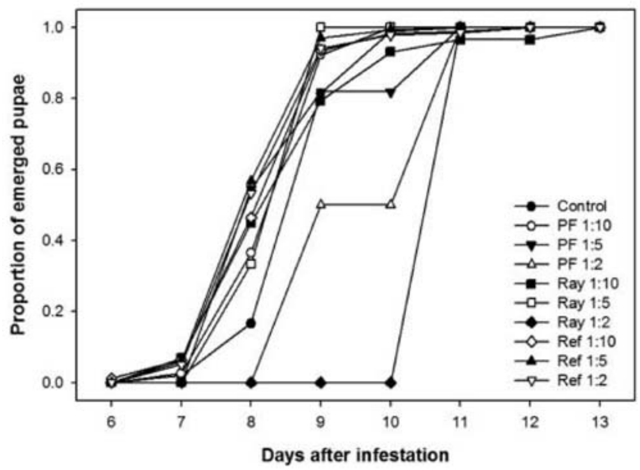

Figure 1. The proportion of *Drosophila suzukii* pupae that emerged daily between 6 and 13 days after infestation from raspberries coated with 1:10, 1:5, and 1:2 concentrations of Prima- Fresh 45 (PF), Raynox (Ray), and REFLECTIONS (Ref).

3.3. Effect of coatings on fruit firmness

Raynox and REFLECTIONS both affected the firmness of raspberries, but in opposite ways (**Figure 2**). REFLECTIONS increased the firmness of raspberries, and fruit coated with 1:2 and 1:5 concentrations were firmer than uncoated controls ($F_{4,45}$ = 56.88, P < 0.0001). Raynox decreased the firmness of raspberries, and fruit coated with 1:2, 1:5, and 1:10 concentrations were softer than uncoated controls ($F_{4,50}$ = 2.72, P = 0.0397). PrimaFresh 45 did not affect raspberry firmness ($F_{4,45}$ = 1.34, P = 0.2689).

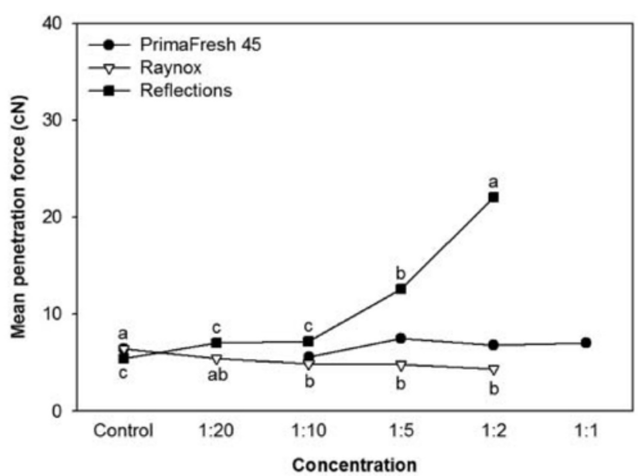

Figure 2. Firmness of raspberries coated with PrimaFresh 45, Raynox, and REFLECTIONS. Points sharing a letter within a coating are not different at a D 5%. The data point for PrimaFresh 45.

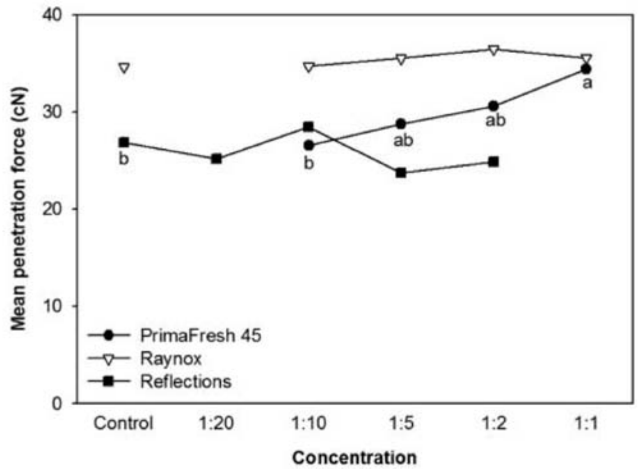

Figure 3. Firmness of blueberries coated with PrimaFresh 45, Raynox, and REFLECTIONS. Points sharing a letter within a coating are not different at a D 5%. The data point representing the control treatment for PrimaFresh 45 is hidden behind the data point representing REFLECTIONS controls.

PrimaFresh 45 did increase the firmness of blueberries (**Figure 3**). Blueberries coated with 1:1, 1:2, and 1:5 concentrations were firmer than uncoated controls ($F_{4,45}$ = 2.63, P = 0.0464). Neither Raynox nor RELECTIONS affected the firmness of blueberries (Raynox: $F_{4,45}$ = 0.07, P = 0.9919; REFLECTIONS: $F_{4,45}$ = 2.07, P = 0.1005).

3.4. Effect of coatings on infestation in the field

Coatings did not reduce *D. suzukii* infestation in strawberries in the field ($F_{3,29}$ = 1.04, P = 0.3890). Seven days after coatings were applied, untreated strawberries had 1.8 *D. suzukii* per berry on average, while PrimaFresh 45, Raynox, and REFLECTIONS had 0.48, 0.94, and 2.05 *D. suzukii* per berry, respectively (**Figure 4**).

Even though average droplet size ranged from 0.19 mm for one of the PrimaFresh 45 replicates to 0.51 mm for one Raynox replicate, there was not a significant difference in average droplet size between the three coatings ($F_{2,187}$ = 0.74, P = 0.4778). Average droplet size was 0.35 mm for PrimaFresh 45, 0.37 mm for Raynox, and 0.38 mm for REFLECTIONS. Similarly, percent coverage did not differ between the three coatings ($F_{2,12}$ = 1.77, P = 0.2111) and average percent coverage was equal to 55%, 38%, and 50% for PrimaFresh 45, Raynox, and REFLECTIONS, respectively.

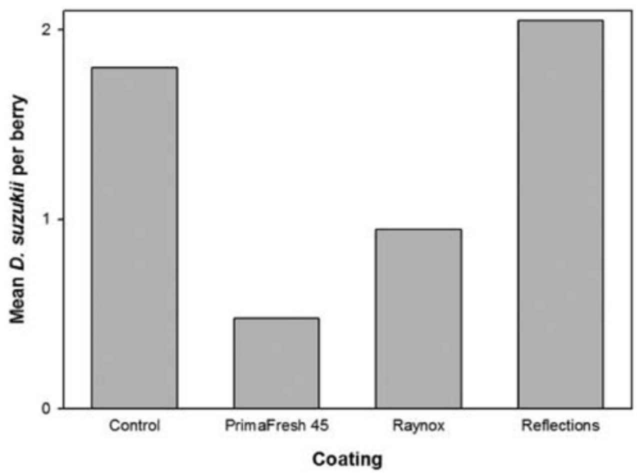

Figure 4. Mean *Drosophila suzukii* in strawberries seven days after fruit were coated with 1:10 concentrations of PrimaFresh 45, Raynox, or REFLECTIONS during a field trial in North Carolina in June 2013. Treatments were not different at α = 5%.

4. Discussion

4.1. Oviposition in coated fruit

The three edible coatings tested in this study reduced but did not prevent oviposition by *D. suzukii* females when applied to fruit, and this reduction varied by fruit type. Raynox, composed of both carnauba wax and kaolin clay, was the only coating that reduced oviposition in both raspberries and blueberries. PrimaFresh 45 reduced oviposition in raspberries, while REFLECTIONS reduced oviposition in blueberries. These differences may be due to adherence differences for materials on fruit surfaces.

Coatings varied in ease of application. Both Prima-Fresh 45 and Raynox, the two carnauba wax-based coatings, were easy to apply and full, fairly even coverage was achieved on both raspberries and blueberries. REFLECTIONS, the calcium carbonate coating, was difficult to apply to both raspberries and blueberries, especially at 1:20, 1:10, and 1:5 concentrations, which resulted in uneven coverage. For example, as it dried on raspberries, REFLECTIONS pooled in the center of individual drupelets. This "shrinking" effect left the margins of the drupelets exposed and allowed *D. suzukii* females to lay eggs as though the berries were uncoated, which is likely why all concentrations of REFLECTIONS failed to reduce oviposition in raspberries. On blueberries, REFLECTIONS also condensed as it dried and individual berries were left with areas of comparatively thick and thin coating. On such berries, *D. suzukii* females often laid more eggs in areas where the coating was thinner. Because REFLECTIONS did reduce oviposition in blueberries coated with the 1:5 and 1:2 concentrations, it is possible that females may have had to spend more time locating suitable oviposition sites with thinner coating. These results suggest that some edible coatings might reduce *D. suzukii* oviposition in some fruits by increasing the amount of time it takes for females to find suitable oviposition sites.

Another potential mechanism for how edible coatings might work to reduce or prevent oviposition is by increasing the firmness of fruit. It has been shown that female *D. suzukii* lay fewer eggs in firmer substrates and that a firmness may exist where *D. suzukii* will not lay eggs (Lee et al. 2011; Burrack et al. 2013). However, the effects of the coatings on the firmness of raspberries and blueberries were highly variable, and did not follow the same pattern as oviposition results. For example, REFLECTIONS was the only coating that increased the firmness of raspberries, but was also the only coating that failed to reduce oviposition in raspberries. Firmness readings in raspberries were taken from the center of drupelets, where REFLECTIONS pooled as it dried. Because REFLECTIONS did not increase firmness equally across the surface of each raspberry, they remained vulnerable to attack by *D. suzukii* females.

In addition, both Raynox and PrimaFresh 45 reduced oviposition in raspberries, but not because the coatings increased the firmness of the berries. The firmness of raspberries coated with PrimaFresh 45 stayed roughly the same, while berries coated with 1:10, 1:5, 1:2 concentrations of Raynox were not as firm as uncoated fruit. It has been shown that female *D. suzukii* prefer to lay eggs in ripe fruit and lay fewer eggs in firmer substrates such as green and ripening fruit (Lee et al. 2011). However, it is also possible that *D. suzukii* females avoid laying eggs in fruit that are too soft because it might signal to them that the fruit are or will soon become overripe. The fact that raspberries coated with Raynox were not as firm as uncoated berries might also be an artifact of the experimental design. It is possible that Raynox may have a different effect on the firmness of ripe raspberries that are still attached to a plant than on ripe berries purchased from a grocery store.

Our laboratory results suggest that obtaining thorough, even coverage in the field would be essential if edible coatings were to be used as a management strategy for *D. suzukii*. Because each fruit was coated individually, the level and evenness of coating obtained in the laboratory likely represents an optimum that might be difficult to replicate in a field setting. In fact, our limited field observations suggest that achieving the complete coverage necessary to deter *D. suzukii* oviposition will be very difficult, which is why further experiments were not conducted. Similar results have been observed for other coatings. In a similar field study of Surround® WP (Engelhard Co., Iselin, NJ), a kaolin clay particle film, coverage was not uniform throughout the canopy of apple trees. Inner leaves and fruit had lower amounts of Surround deposited on their surfaces than outer leaves and fruit (Villaneuva & Walgenbach 2007).

Another potential mechanism by which kaolin and other coatings work to deter oviposition is interference with host location. For example, it has been suggested that the bright white color of olive trees sprayed with kaolin may have impaired or disrupted the orientation of olive fruit flies, *Bactrocera oleae* (Rossi), resulting in significantly reduced infestation levels on trees coated with kaolin compared to untreated trees (Saour & Makee 2004). White was also reported to be the least attractive color for ovipositing females of the Mediterranean fruit fly, or medfly, *Ceratitis capitata* (Wiedemann) (Katsoyannos 1987). In one study, medfly females would not even approach white, kaolin-coated nectarines in both choice and no-choice laboratory assays (Mazor & Erez 2004).

Compared to tephritid fruit flies, the effects of color on host choice by *D. suzukii* are much less well understood. The results of a recent study designed to test the effectiveness of different trap colors at capturing *D. suzukii* adults were highly variable and may have been influenced by the crop type and the color of the host fruit itself (Lee et al. 2013). These observations may help to explain some of the results from this study. For example, both Raynox, composed of kaolin and carnauba wax, but especially REFLECTIONS, composed of calcium carbonate, left visible white residues on both blueberries and raspberries that increased with concentration. Female *D. suzukii* may have been confused or deterred by the white color of the coatings, especially when applied to dark-colored blueberries, which might be one reason why both REFLECTIONS and Raynox reduced oviposition in blueberries coated at higher concentrations. However, the strong white color of raspberries coated with REFLECTIONS did not deter females from laying eggs. We know that drosophilids use both visual and olfactory cues to locate hosts (Chow & Frye 2009). It is possible that chemical cues from coated raspberries were enough, at least at close range within the small flight cages, to overcome any deterrence that *D. suzukii* females may have had to the white appearance of the fruit. It is unknown if the same effects would be observed in a field setting, but it is likely that the effectiveness of any coating at reducing *D. suzukii* oviposition would depend on characteristics of the crop itself such as growth habit and leafiness, and fruit size, color, and odor.

In addition to potentially changing fruit appearance, edible coatings may also change surface characteristics. Both PrimaFresh 45 and Raynox, the two carnauba wax-based coatings, reduced oviposition in raspberries, regardless of concentration. Therefore, the waxy nature of the coatings may have deterred *D. suzukii* females from laying as many eggs in coated fruit as in uncoated berries. It has been shown that natural components of olive epicuticular waxes serve as deterrents to oviposition by female olive fruit flies, *Bactrocera oleae* (Gmelin). In one study, unusual "Kalamon" olives that lacked the typical waxy covering were more susceptible than normal olives to attack by *B. oleae* females (Neuenschwander et al. 1985). In another study, significant variation in oviposition was found when epicuticular waxes from five olive cultivars were collected and reapplied to olives from which the natural waxy coating had been removed; the highest numbers of eggs were laid in control olives that lacked the waxy covering. In addition, two major components of olive surface waxes, oleanolic and maslinic acids, had a negative effect on how susceptible fruit were to attack by olive fruit fly females; when the concentrations of the two components were high, oviposition was low (Kombargi et al. 1998). These findings raise the question of whether waxy coatings such as PrimaFresh 45 or Raynox could also be used to carry a repellant that would further reduce oviposition by *D. suzukii* in fruit crops. There is precedence for this type of use, as carnauba wax formulations have been used as carriers to apply antibacterial and antifungal agents to fruit crops (Narciso et al. 2012).

4.2. Immature survivorship and development in coated fruit

For most of the treatments, immature survivorship and developmental rate did not differ between uncoated and coated fruit, regardless of coating and concentration. However, PrimaFresh 45 and Raynox, the carnauba wax-based coatings, dramatically reduced the survivorship of immature *D. suzukii* in raspberries, results which were largely confirmed during the development assays in which very few flies emerged from raspberries coated with the 1:2 concentrations of PrimaFresh 45 and Raynox. These results could be due to physiological changes in the raspberries brought about by the coatings themselves. For example, some formulations of carnauba wax reduce oxygen transfer into and water vapor transfer out of fruit (Pavlath & Orts 2009). In a recent study, raspberries that were coated with beeswax-based coatings and stored in a cooler had reduced respiration, ethylene production, and oxygen transfer compared to uncoated fruit (Perez-Gallardo et al. 2012).

Significant variation in mortality was observed when several edible coatings were tested for their ability to kill Caribbean fruit fly larvae, *Anastrepha suspensa* (Loew), in grapefruits (Hallman et al. 1994). In a follow up study, high levels of mortality of the Mexican fruit fly, *Anastrepha ludens* (Loew), were observed in grapefruits coated with Citrus Lustr® 402 (DECCO US Post-Harvest, Inc., Monrovia, CA), a commonly used grapefruit coating that is composed of alkali soluble natural lac resins, fatty acids soaps, propylene glycol, silicone antifoam, and propylparaben as a wax preservative. Citrus

Lustr 402 did not affect survival when mixed into the diet used to rear Mexican fruit flies, indicating that the coating itself is not toxic to larvae. In addition, leaving one-third of each grapefruit uncoated reduced mortality levels considerably. All together, these results support the hypothesis that edible coatings act primarily by restricting gas exchange and creating a modified atmosphere where lowered oxygen and raised carbon dioxide levels kill insects (Hallman 1997). However, using coatings to reduce infestation is not always an optimal method and may also reduce fruit quality (Pavlath & Orts 2009). Further work is necessary to determine if fruit quality is lessened by pre or postharvest use of the materials we compared.

If PrimaFresh 45 and Raynox killed *D. suzukii* larvae by creating an inhospitable atmosphere within raspberries, it is interesting that the coatings did not have a similar effect in blueberries. Raynox did reduce immature survivorship in blueberries, but the effect was not as strong as in raspberries. Such a discrepancy could have resulted from the fact that it was difficult to completely coat the calyx area of many blueberries, or the fact that many blueberries are already covered in a protective coating of powdery epicuticular wax. Because coatings differ significantly in their ability to prevent fly emergence, it may be possible to develop coatings that could be used to maximize insect death in particular fruits (Hallman et al. 1995). For example, methyl cellulose and shellac, known to restrict gas exchange, were identified as the substances in NatureSeal® (NatureSeal, Inc., Westport, CT) that reduced the survival of Caribbean fruit fly in grapefruits (Hallman et al. 1994). Perhaps a waxy coating could be developed for use as a postharvest treatment for *D. suzukii* larvae or as a supplement to other postharvest treatments.

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