Influence of fruit coatings on *Drosophila suzukii* oviposition and development and implications for field use

Final report

2013-02

Research proposal

Name, Mailing and Email Address of Principal Investigator(s):
Hannah J. Burrack
Department of Entomology
North Carolina State University
Raleigh, NC 27695-7634

Katie Swoboda
Department of Entomology
North Carolina State University
Raleigh, NC 27695-7634

OBJECTIVES

1. To determine if *Drosophila suzukii* lay fewer eggs in coated fruit.
2. To determine if coatings can be applied to fruit preharvest.
3. To determine if field applications of fruit coatings reduce *Drosophila suzukii* infestation under field conditions.

JUSTIFICATION

Invasive insect pests have emerged as one of the most significant management challenges for specialty crops, including small fruits. Spotted wing drosophila (SWD, *Drosophila suzukii*), an invasive pest of ripe and ripening soft skinned fruit, has a broad crop and non crop host range, including blueberries, caneberries, grapes, cherries, strawberries, grapes, and their wild relatives. SWD was first detected in the southeastern United States in Florida in late 2009 and has since been detected throughout the eastern United States. With support from the Southern Region Small Fruit Consortium support (2011 E-01 and 2010 E-01), we developed a volunteer based SWD early detection network. This network was responsible for the first SWD detections in NC, VA, WV, MD, and AR and first detection at agricultural sites in SC. Since these initial detections, SWD has been found throughout the eastern United States and caused significant damage in blueberries, caneberries, strawberries, and grapes. Left unchecked SWD infestations can reach 100%, particularly in later fruiting host crops. Insecticide applications can mitigate some of this damage, but there are limited insecticides effective against SWD with sufficiently short preharvest intervals to allow their use during the period which fruit is vulnerable to SWD infestation. Organically acceptable control options are even more limited (essentially spinosad and pyrethrins). Sanitation is recommended in both conventional and organic systems, but it is unclear how effective sanitation is at reducing infestation. Insecticide efficacy is also limited by environmental conditions and is lessened under rainy conditions, common during summers in the southeast (Van Timmeren and Isaacs 2013). Despite limited options and variable efficacy, insecticides are the primary tool used against SWD, and up to 11 harvest-period insecticide applications are being made in significantly affected crops, such as caneberries. In caneberries in particular, few, if any, harvest period insecticide applications were made prior to the detection of SWD.

Edible coatings are used to protect fruit post harvest and enhance shelf life (Pavlath and Orts 2009), and kaolin clay has been used to prevent sunscald and insect damage by tephritid fruit flies and stink bugs (Mazor and Erez 2004, Saour and Makee 2004, Villaneuva and Walgenbach 2007, Lalancette et al. 2005). Waxes used in coatings include beeswax, carnauba, candelilla, paraffin, and schellac (Pavlath and Orts 2009). Some coatings are also organically acceptable. We assessed the potential of several conventional and organically acceptable fruit coatings to reduce or prevent SWD infestation preharvest.

METHODOLOGIES

Coatings

We tested the effects of three coatings on the oviposition and development of *D. suzukii*. PrimaFresh® 45, manufactured by Pace International, is a carnauba-based protective coating for stone fruit. It is generally applied post harvest to clean, dry fruit via overhead spray/drip at full strength (1 gallon per 15,000-30,000 lbs of fruit). Raynox® Apple Sunburn Protectant, manufactured by Pace International, is made from carnauba wax and organically modified kaolin clay. Suggested field application rates range from 1:40 to 1:20 (product:water) depending on the desired spray volume to be applied per acre. REFLECTIONS™ Liquid Shade, manufactured by Tiger Industries, is a calcium carbonate shade product used to reduce heat stress on fruit, vegetables, trees, and row crops. Suggested field application rates range from 1:20 to 1:5. We tested four potential field application rates for each coating based on manufacturer suggestions. Dilutions of PrimaFresh 45 and REFLECTIONS were made using tap water in order to best approximate field conditions. However, dilutions of Raynox were made with distilled water (365™ brand from Whole Foods) in order to avoid the need to buffer the resulting solution.

Objective 1: Oviposition and immature survivorship in coated fruit

The effects of fruit coatings on oviposition and immature survivorship were tested using raspberries, which are highly preferred by *D. suzukii*, and blueberries, which are less preferred (Burrack et al. 2013). A constant mass of approximately 20 g of fruit was coated and presented to *D. suzukii* during in a no-choice assay. Certified organic fruit purchased from local grocery stores in Raleigh, NC was used for assays. 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 (raspberries were generally from Driscoll's, blueberries were generally from Sunny Ridge, Winter Haven, FL).

Before coatings were applied, raspberries were plugged with a small amount of cotton (non-sterile absorbent cotton balls) to simulate the presence of the receptacle which would be present under field conditions. Fruit samples were placed in an 8' diameter kitchen strainer positioned over a 1000 ml Pyrex beaker and coatings applied using disposable plastic pipettes. Each berry was then rolled between the fingers to ensure that its entire surface was coated. Coated fruit samples 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 sample in order to protect it without preventing air circulation.

In no-choice assays in the laboratory, coated fruit were presented to fifteen female and fifteen 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. Uncoated fruit were used as controls. The total number of eggs laid in each 20 g fruit sample was counted immediately after exposure, using a stereomicroscope (Olympus SZ61; Olympus America, Center Valley, PA). Fruit samples were then held at 20°C and checked for pupal emergence for up to 21 days. Pupae were collected and held in 60 x 15 mm polystyrene Petri dishes with a moistened paper towel square until emergence. Each treatment was replicated 3-4 times.

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) following Burrack et al. (2013).

Data analyses

In no-choice laboratory assays, oviposition was measured as the total number of eggs laid in a 20 g fruit sample. Immature survivorship was calculated as the proportion of eggs laid that survived to the adult stage. Oviposition and immature survivorship were analyzed using mixed-model ANOVA via PROC MIXED in SAS 9.3 with application rate as a fixed effect and replicate as a random effect. Some data sets were either log or square root transformed to meet the assumptions of normality (log: Raynox, blueberries; REFLECTIONS, raspberries and blueberries; square root: PrimaFresh, blueberries). A normal distribution was the best fit (lowest (AIC) via SAS Proc GLIMIXX) for both PrimaFresh 45 and Raynox data sets. Fruit firmness was analyzed using mixed-model ANOVA (via PROC MIXED in SAS 9.3) with application rate as a fixed effect.

Objectives 2 and 3: Feasibility of field application and efficacy

Dilutions of 1:10 (product:water) of each material were applied to 20-plant strawberry plots with known SWD infestation on 25 April 2013 using a CO_2 pressurized backpack sprayer fitted with 3 flat fan nozzles at 45 psi pressure in a spray volume equivalent to 100 gpa. Each treatment was replicated at least four times. Fruit samples (15 per plot) were collected 7 and 14 days after treatment and held for 7 days at 20°C after which all *Drosophila* spp. larvae and pupae were counted.

RESULTS

Objective 1: Oviposition and immature survivorship in coated fruit

PrimaFresh 45 reduced oviposition in raspberries (F = 26.56; df = 4, 15; P < 0.0001). Females laid fewer eggs in coated fruit, regardless of application rate, than in uncoated control fruit (Fig. 1). Raynox reduced oviposition in raspberries (F = 28.09; df = 4, 14; P < 0.0001). Again, females laid fewer eggs in coated fruit, regardless of application rate, than in uncoated fruit (Fig. 2). REFLECTIONS did not reduce oviposition in raspberries (F = 0.46; df = 4, 9; P = 0.7669) (Fig. 3).

PrimaFresh 45 did not reduce oviposition in blueberries (F = 1.91; df = 4, 15; P = 0.1606) (Fig. 4). Raynox reduced oviposition in blueberries (F = 5.18; df = 4, 15; P = 0.0080). Females laid fewer eggs in fruit coated with 1:2 and 1:1 application rates than in uncoated fruit

(Fig. 5). REFLECTIONS reduced oviposition in blueberries ($F = 3.09$; df = 4, 15; $P = 0.0485$). Females laid fewer eggs in fruit coated with the 1:2 application rate than in uncoated, 1:20, and 1:10 fruit (Fig. 6).

PrimaFresh 45 reduced immature survivorship in raspberries ($F = 21.94$; df = 4, 14; $P < 0.0001$). Fewer eggs survived to adulthood in fruit coated with 1:5, 1:2, and 1:1 than in uncoated and 1:10-coated fruit. Very similar results were observed in Raynox, which also reduced immature survivorship in raspberries ($F = 6.41$; df = 4, 14; $P = 0.0038$). Fewer eggs survived to adulthood in fruit coated with 1:10, 1:5, and 1:2 application rates than uncoated and 1:20-coated fruit. REFLECTIONS did not reduce immature survivorship in raspberries ($F = 0.49$; df = 4, 8.03; $P = 0.7423$), while none of the coatings reduced immature survivorship in blueberries (PrimaFresh 45: $F = 2.26$; df = 4, 15; $P = 0.1108$; Raynox: $F = 2.71$; df = 4, 14; $P = 0.0730$; REFLECTIONS: $F = 0.68$; df = 4, 14; $P = 0.6167$).

Effect of coatings on fruit firmness

Both Raynox and REFLECTIONS affected the firmness of raspberries, but in opposite ways (Fig. 9). REFLECTIONS increased the firmness of raspberries ($F = 25.21$, df = 4, 20, $P < 0.0001$). Fruit coated with 1:2 and 1:5 application rates were firmer than uncoated controls. Raynox decreased the firmness of raspberries ($F = 11.67$, df = 4, 25, $P < 0.0001$). Fruit coated with 1:2, 1:5, and 1:10 application rates were softer than uncoated controls. PrimaFresh 45 did not affect raspberry firmness ($F = 2.15$, df = 4, 25, $P = 0.1127$).

Only PrimaFresh 45 increased the firmness of blueberries (Fig. 10). Blueberries coated with 1:1, 1:2, and 1:5 application rates were firmer than uncoated controls ($F = 2.63$, df = 4, 45, $P = 0.0464$). Neither Raynox nor RELECTIONS affected the firmness of blueberries (Raynox: $F = 0.07$, df = 4, 45, $P = 0.9919$; REFLECTIONS: $F = 2.07$, df = 4, 45, $P = 0.1005$).

Objectives 2 and 3: Feasibility of field application and efficacy

There were no issues with nozzles clogging during application for any of the materials at 1:10 dilutions. However, spray cards placed in the strawberry canopy suggested that droplet size and coverage were highly variable between treatments (Fig. 11). Perhaps because coverage was less than complete, none of the materials reduced SWD infestation 7 or 14 days after application. However, this period was quite rainy, and it is possible that the material also washed off plants.

CONCULSIONS

Raynox, composed of both carnauba wax and kaolin clay, was the only coating that reduced oviposition in both raspberries and blueberries. In blueberries, coatings only reduced oviposition when applied at higher application rates (1:2 and 1:1). In raspberries, female *D. suzukii* laid fewer eggs in berries coated with PrimaFresh 45 and Raynox regardless of application rate, suggesting that the presence of the coating deterred females from laying eggs. Conversely, female *D. suzukii* laid as many eggs in berries coated with REFLECTIONS, regardless of application rate, as they did in uncoated controls. PrimaFresh 45 and Raynox, the two carnauba wax-based coatings, were very easy to apply and we were able to get full and fairly even coverage across the berries. REFLECTIONS, the calcium acetate coating, was difficult to apply to both raspberries and blueberries, especially at 1:20, 1:10, and 1:5 application rates, which resulted in uneven coverage. On raspberries, REFLECTIONS shrunk as it dried and pooled in the center of each druplet, which left the edges free of coating and allowed females to lay eggs as if the coating was not present. On blueberries coated with REFLECTIONS, more eggs were observed in areas where the coating was thinner; REFLECTIONS did reduce oviposition in blueberries coated with 1:5 and 1:2 applications rates, suggesting that females may have to spend time trying to find suitable spots to lay eggs.

These results suggest that getting good coverage in the field will be essential for edible coating to work as a management strategy for *D. suzukii*. Because each fruit was coated individually, the level and evenness of coating obtained in this experiment probably represents a maximum. It be difficult to replicate this level of coating or to get uniformly distributed, even coverage in a field setting. For example, in a study with Surround, a kaolin clay particle film, Villanueva and Walgenbach (2007) observed that Surround was not distributed uniformly in the apple tree canopy. Inner leaves and fruit had lower amounts of Surround deposited on their surfaces compared with outer leaves and fruit.

The effects of the coatings on fruit firmness were highly variable. Only PrimaFresh 45 increased fruit firmness in blueberries, which is interesting because it was the only coating that failed to reduce oviposition. The firmness of blueberries coated with Raynox stayed roughly the same, while the firmness of blueberries coated with REFLECTIONS was highly variable. Again, this variability was likely due to the patchiness of the coating itself and the fact that firmness readings were taken at random. These results suggest that fruit firmness was probably not the most important factor determining how many eggs females laid in coated blueberries. Perhaps females were confused or deterred by white coatings, Raynox and REFLECTIONS, on the dark fruit.

Similarly, only REFLECTIONS increased fruit firmness in raspberries, but this was the only coating that failed to reduce oviposition. The firmness of raspberries coated with Raynox stayed roughly the same, while PrimaFresh 45 made the raspberries softer. We know that female *D. suzukii* lay fewer eggs in firmer substrates (Burrack et al. 2013), but perhaps female *D. suzukii* also avoid laying eggs in fruit that are too soft. It is possible that it might be harder to make an incision in fruit that is less firm (like trying to cut raw chicken with a serrated knife versus trying to cut semi-frozen chicken--it is much easier to cut frozen chicken because you can get some traction with the knife). Conversely, perhaps female *D. suzukii* associate softer fruit with overripe fruit, which they do not prefer to oviposit in.

PrimaFresh 45 and Raynox, the carnauba wax-based coatings, dramatically reduced the survivorship of immature *D. suzukii*. This could have resulted from physiological changes in the raspberries brought about by the coatings themselves. Some formulations of carnauba wax reduce oxygen transfer into and water vapor transfer out of fruit (Pavlath and Orts 2009). In addition, Perez-Gallardo et al. (2012) observed that raspberries coated with beeswax-based coatings and stored in a cooler had reduced respiration, ethylene production, and oxygen transfer compared to uncoated fruit.

IMPACT STATEMENT

We demonstrated that two edible coatings, PrimaFresh 45 and Raynox, have the potential to reduce but not prevent SWD oviposition in raspberries and blueberries. These materials may also reduce survivorship of SWD in treated fruit. Because thorough coverage appears necessary to reduce infestation, field use of these materials may not be feasible, but post harvest treatment may prevent development of young, undetectable larvae present in fruit. Additional work is necessary to confirm field observations.

References

Burrack, H.J., G.E. Fernandez, T. Spivey, and D.A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), an invasive frugivore. Pest Manage. Sci. 69: 1173-1180.

eFly: The Spotted Wing Drosophila Working Group. 2012. Spotted wing drosophila impacts in the eastern United States. (http://www.sripmc.org/WorkingGroups/eFly/index.cfm).

Lalancette, N., R.D. Belding, P.W. Shearer, J.L. Frecon, and W.H. Tietien. 2005. Evaluation of hydrophobic and hydrophilic kaolin particle films for peach crop, arthropod and disease management. Pest Manage. Sci. 61: 25-39.

Lee, J.C., D.J. Bruck, H. Curry, D. Edwards, D.R. Haviland, R.A. Van Steenwyk, and B.M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii.* Pest Manage. Sci. 67: 1358-1367.

Mazor, M. and A. Erez. 2004. Processed kaolin protects fruits from Mediterranean fruit fly infestations. Crop Protect 23: 47-51.

Pavlath, A.E., and W. Orts. 2009. Edible films and coatings: why, what, and how? pp. 1-23. *In* M.E. Embuscado and K.C. Huber (eds.), Edible Films and Coatings for Food Applications, 1. Springer Science + Business Media, LLC, New York, NY.

Perez-Gallardo, A., S.D. Mattinson, A. Lazcano-Peralta, J.K. Fellman, G. Barbosa-Canovas, B. Garcia-Almendarez, and C. Regalado. 2012. Effect of native and acetylated-crosslinked waxy corn starch-beeswax coatings on quality attributes of raspberries during storage. Starch 64: 665-673.

Saour, G. and H. Makee. 2004. A kaolin-based particle film for suppression of the olive fruit fly *Bactrocera oleae* Gmelin (Dip., Tephritidae) in olive groves. J. Appl. Entomol. 128: 28-31.

Van Timmeren, S.V., and R. Isaacs. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection systems. Crop Prot. 54: 126-133.

Villaneuva, R.T., and J.F. Walgenbach. 2007. Phenology, management and effects of Surround on behavior of the apple maggot (Diptera: Tephritidae) in North Carolina. Crop Prot. 26: 1404-1411.

Figures

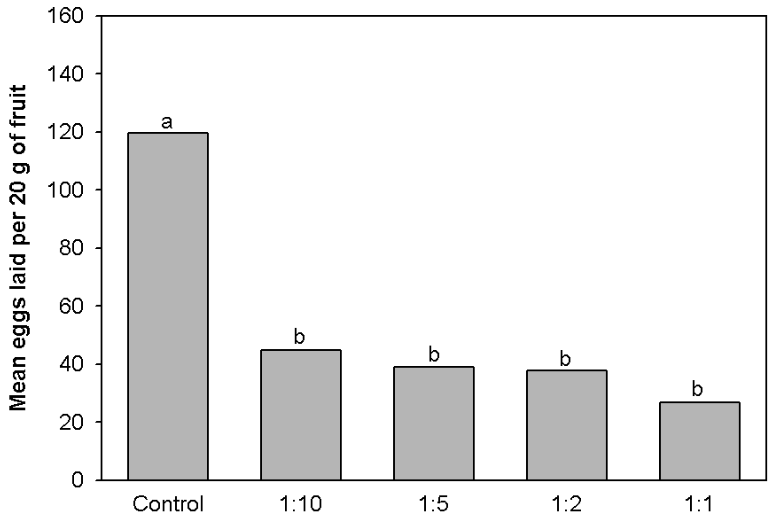

Fig. 1. Oviposition in raspberries coated with PrimaFresh 45. Bars sharing a letter are not different at α = 5%.

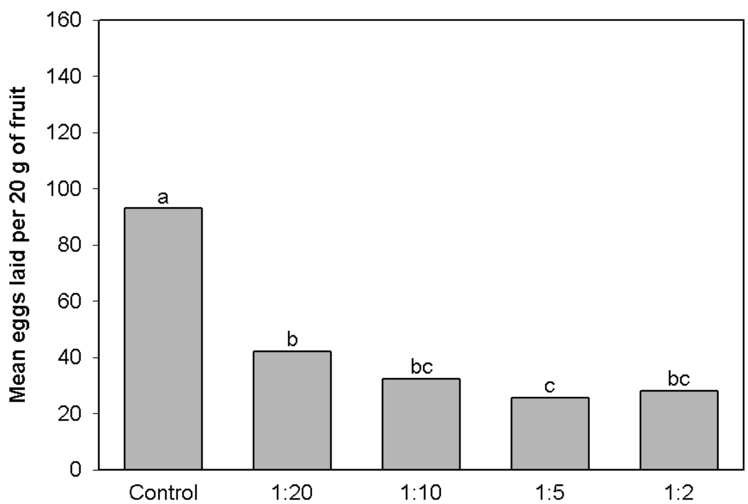

Fig. 2. Oviposition in raspberries coated with Raynox. Bars sharing a letter are not different at α = 5%.

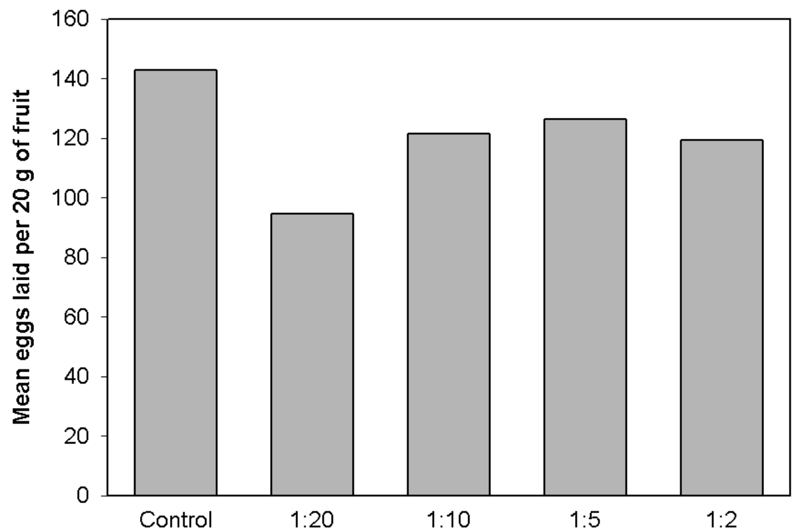

Fig. 3. Oviposition in raspberries coated with REFLECTIONS.

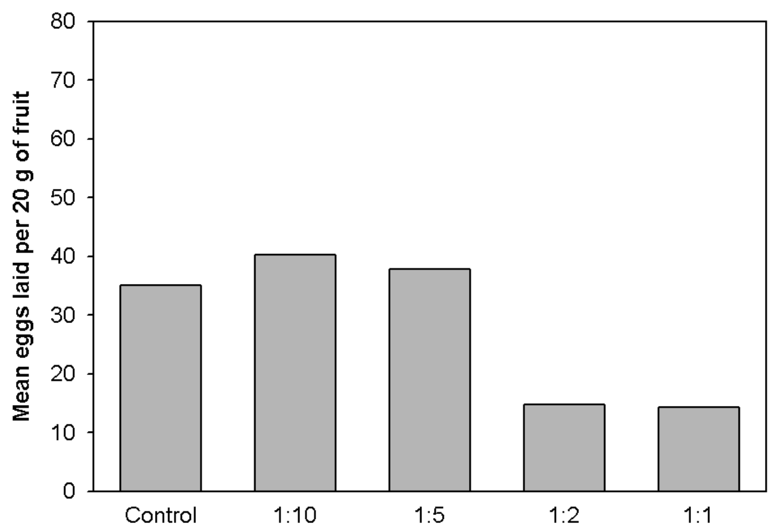

Fig. 4. Oviposition in blueberries coated with PrimaFresh 45.

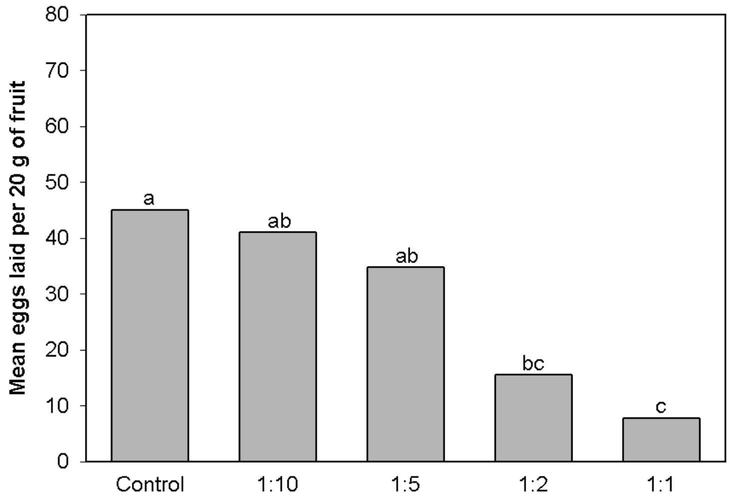

Fig. 5. Oviposition in blueberries coated with Raynox. Bars sharing a letter are not different at α = 5%.

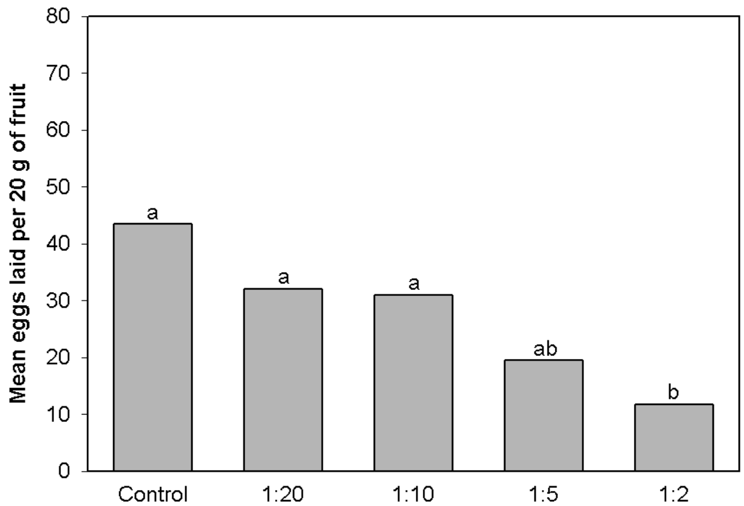

Fig. 6. Oviposition in blueberries coated with REFLECTIONS. Bars sharing a letter are not different at α = 5%.

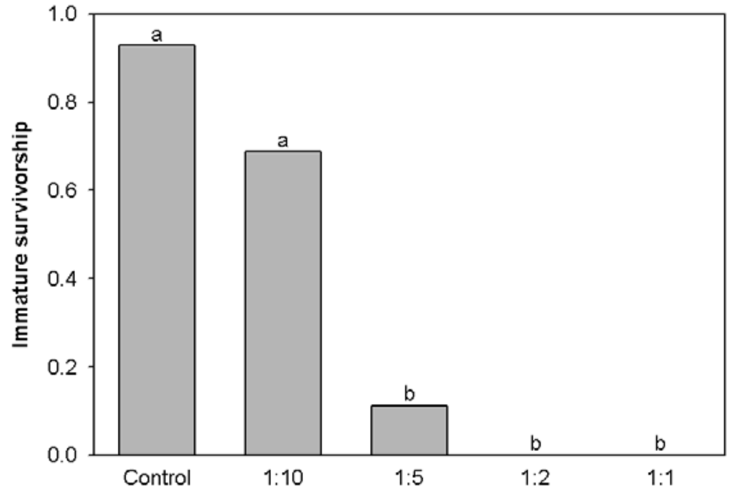

Fig. 7. Immature survivorship in raspberries coated with PrimaFresh 45. Bars sharing a letter are not different at α = 5%.

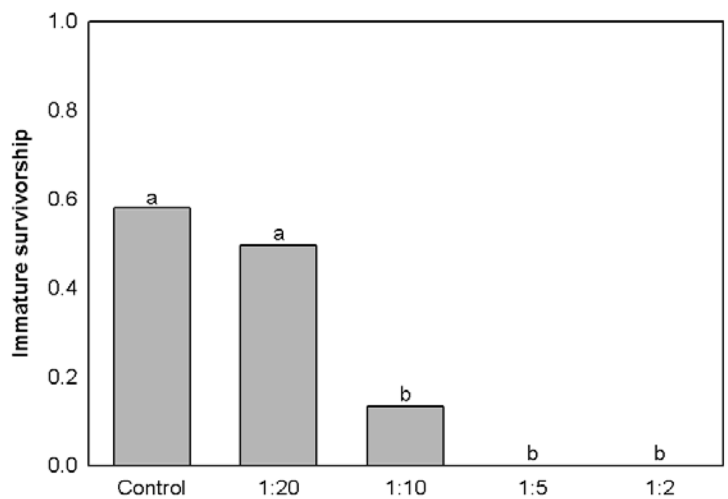

Fig. 8. Immature survivorship in raspberries coated with Raynox. Bars sharing a letter are not different at α = 5%.

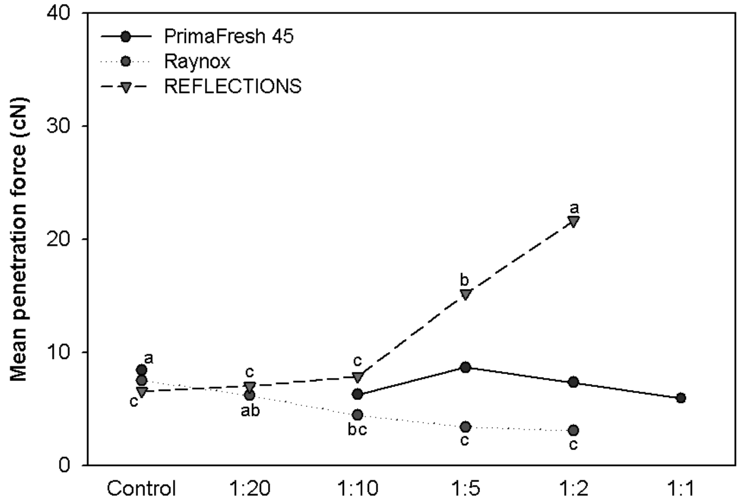

Fig. 9. Firmness of raspberries coated with PrimaFresh 45, Raynox, and REFLECTIONS. Bars sharing a letter within a coating are not different at α = 5%. There is not a circle for PrimaFresh 45 at the 1:20 application rate because it was not tested.

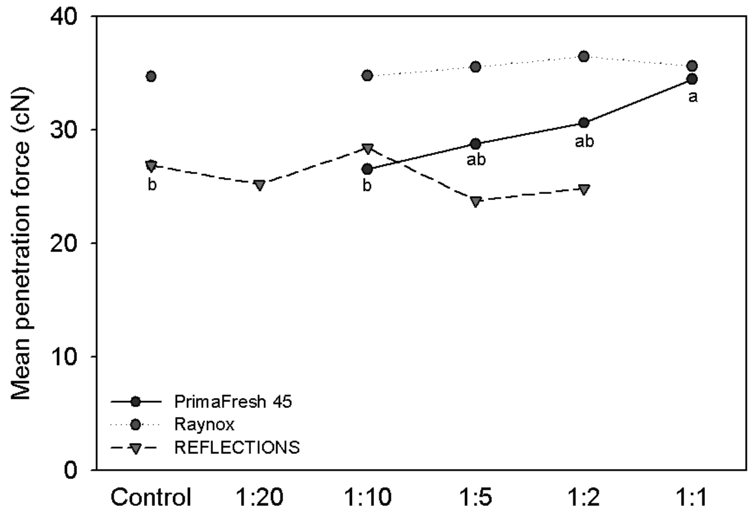

Fig. 10. Firmness of blueberries coated with PrimaFresh 45, Raynox, and REFLECTIONS. Bars sharing a letter within a coating are not different at α = 5%. The circle representing the control treatment for PrimaFresh 45 is hidden behind the triangle representing REFLECTIONS controls.

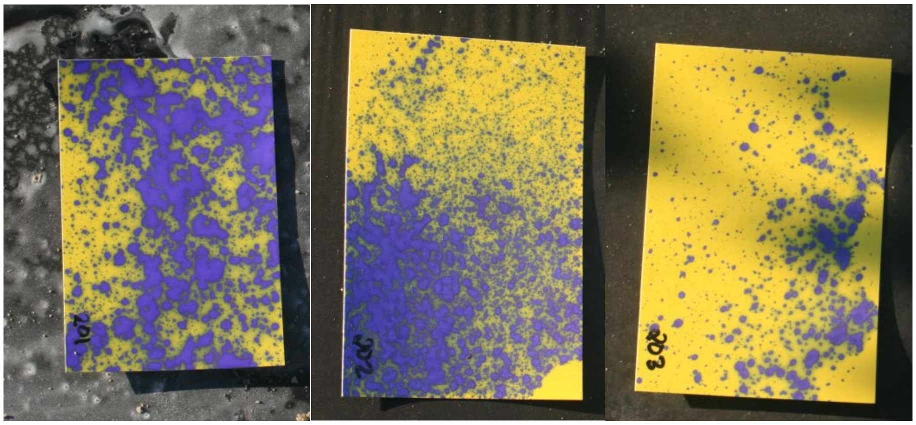

Fig. 11. Water sensitive spray cards placed in strawberry plots treated with 1:10 dilutions of REFLECTIONS (left), PrimaFresh 45 (center), and Raynox (right).