Effect of simulated rainfall on the effectiveness of insecticides against spotted wing drosophila in
blueberries

Bal K. Gautam¹, Brian A. Little¹, Milton D. Taylor¹, James L. Jacobs², William E. Lovett³,
Renee M. Holland³, Ashfaq A. Sial^{1*}

¹413 Biological Sciences Building, Department of Entomology, University of Georgia
Athens, GA 30602; ²UGA Extension, Pierce County, Blackshear, GA 31516; ³UGA Extension,
Bacon County, Alma, GA 31510.

*Corresponding Author: ashsial@uga.edu

Phone: +17065421320

ABSTRACT

The current spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) management strategy relies on routine application of insecticides during the fruit ripening and harvesting period. The amount and frequency of insecticide spray needed to maintain continuous protection of fruits may depend on a variety of factors including the level of rainfall. Studies were conducted to determine the effects of simulated rainfall (0, 12.5, 25 and 37.5 mm) and adjuvant – Nu Film (with and without) on the residual efficacy of commonly used insecticides against *D. suzukii* in blueberries during the fruiting season of 2014 and 2015. Spray applications were made using an air-assisted backpack (2014) or airblast (2015) sprayer. Insecticide spray residues were allowed to dry for ~2 h before simulated rainfalls were applied using central pivot irrigation system. Semi-field bioassays were conducted to assess mortality at 1, 3 and 7 days after treatment (DAT). Results showed that simulated rainfall caused a significant reduction in *D. suzukii* mortality in all chemical treatments, particularly at 1 and 3 DAT in the 2014 study, and at 1 DAT in the 2015 study. In general, higher rainfall resulted in lower mortality in 2014, however such a clear trend was not observed in 2015. Addition of adjuvant, Nu Film 17, significantly increased mortality at 3 DAT in the zeta-cypermethrin treatment with 25 mm of simulated rainfall (2014). In 2015, the addition of Nu Film P significantly increased mortality at 1 DAT in the spinetoram and spinosad treatments with 12.5 mm, and in the malathion treatment with 25 mm of simulated rainfall. These results suggest that although frequent rainfall incidents increase the need for more frequent spray of insecticides, addition of adjuvants helps to prolong the residual efficacy of some insecticides.

Keywords. *Drosophila suzukii*, insecticides, rainfall, adjuvant, residual efficacy

1. Introduction

Blueberry is one of the most important agricultural commodities produced in Georgia. Based on the 2014 production, Georgia has become the number one blueberry producing state in the United States (Southeast FARM PRESS, 2014). Recently, more and more newly planted fields can be seen in southeast Georgia, where most of the blueberry producing counties are located, indicating the ongoing expansion of blueberry acreage in the state. The arrival and spread of the spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), in the state, however, has posed a huge challenge to the growers and researchers alike. *D. suzukii*, an invasive pest native to Eastern and Southeastern Asia, can lay eggs in the ripe and ripening berries with its sharp and highly sclerotized ovipositor (Walsh et al., 2011). This has caused significant economic and sociological impacts on the blueberry production as the flies can attack berries on the plants right before harvest and infested berries can go unnoticed until the berries are in the market. If the maggots/flies from the infested fruits are detected during the marketing phase, the producers might risk the rejection of the whole lot, or at least the price would be considerably reduced citing quality.

Given the nature of damage *D. suzukii* can inflict to soft-skinned fruits (Mitsui et al., 2006) and their fast multiplying capacity (Dreves et al., 2009), farmers have adapted a proactive approach to protecting blueberries from this invasive pest. This proactive approach typically consists of weekly applications of broad-spectrum insecticides from fruit ripening stage until the end of the harvest, as opposed to the need-based application of selective insecticides based on the monitoring of pest populations (Van Timmeren and Isaacs, 2013). The total number of sprays in a season and the exact days between two sprays, however, may vary considerably depending on the weather conditions, primarily the temperature and rainfall levels during this period. In

addition to the increased costs associated with increased products (chemicals) and labor, this chemically intensive protection strategy has inherent environmental and ecological concerns.

Insecticide deposits on crop plants must persist and withstand weathering for a required period of time in order to be effective against insect pests. However, rainfall can wash off insecticide deposits and reduce the efficacy requiring more frequent applications (Phillips, 1968; Taylor and Mathews, 1986; McDowell et al., 1987). The effect of rain on insecticide deposits varies depending on the intensity of rainfall, type of the insecticide and its formulation, and the properties of plant surfaces (Baker et al., 1983; Pick et al., 1984; Mashaya, 1993). Mashaya (1993) reported that monocrotophos SC and acephate SP were more affected by simulated rainfall as compared to fenvalerate EC and methomyl SP on tobacco plants. In some situations, even a small amount of rainfall can sufficiently remove deposits of an insecticide rendering it significantly less effective against the targeted insect pests (Pree et al., 1984).

Insecticide residue wash-off due to rainfall can be reduced with the addition of adjuvants in the spray preparations (Taylor and Mathews, 1986; van Zyl et al., 2010a). Adjuvants help to improve leaf wetting, spreading, adhesion and penetration into the leaves (Abbott et al., 1990; Ryckaert et al., 2007). Stickers in adjuvants work by reducing droplet surface tension thereby ensuring better droplet contact on the epicuticular wax layer of the plant surface (Hess and Foy, 2000).

Although significant level of rainfall (~40-50 cm) occurs during the blueberry harvest season in Georgia and spray residue wash-off is a major concern among blueberry growers, no studies have yet been conducted to evaluate the impact of rainfall on the insecticide efficacy against insect pests of blueberries. Being a relatively new insect pest, very little information is available on the efficacy of field-aged residual deposits on *D. suzukii* under Georgia weather

conditions. Since the blueberry ripening time coincides with the rainy season, understanding the effect of rainfall may help to optimize the control strategy by adjusting the timing and frequency of insecticide applications. The objectives of this study were to determine the effect of rainfall on residual efficacy of five insecticides most commonly used by blueberry farmers to control *D. suzukii*, and to evaluate the impact of an adjuvant on rainfastness of those insecticides.

2. Materials and Methods

2.1 Insects

The laboratory colony was established from *D. suzukii* adults collected from a home garden where blueberries were grown in Clarke County, Georgia during the summer of 2013. Stock cultures were reared on a standard cornmeal-molasses-yeast media according to Jaramillo et al. (2015) with slight modifications. Male and female adults were placed in 177 ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) filled with ~50 ml of media and capped with foam flugs (Genesee Scientific, San Diego, CA). The bottles were held in an environmental chamber (Model I36VLCB, Percival Scientific, Perry, IA) at 24 ± 1 °C; 60-70% RH and light:dark cycle of 13:11. Fly populations were maintained by routinely (usually in 2-3 weeks) transferring the adults to new media bottles and removing the old bottles that were unfit for rearing. Four to six day old adult (mature) males and females were used for bioassays.

2.2 Experimental plots

Field studies were carried out in commercial farms of rabbiteye blueberries (*Vaccinium ashei*) located in southeast Georgia during the blueberry harvest season (May – July) of 2014 and 2015. The blueberry cultivars used for the studies in 2014 and 2015 were ‘Powderblue’ and

‘Alapaha’, respectively. Both fields had mature blueberry bushes (5-6 yrs) and received regular maintenance such as pruning and irrigation. There was a center-pivot for the irrigation system that was used to generate different levels of simulated rainfall. Split plot design was used for both 2014 and 2015 studies where residual efficacy of each of the selected chemicals was evaluated at four levels of rainfall (0, 12.5, 25, 37.5 mm) and two levels of adjuvant – Nu Film 17® (2014) or Nu Film P ® (2015) (with and without). The field was divided into four sections (main plots), for four levels of rainfall, around the center point of the pivot and distanced at least 10 rows apart (~36 m) from each other as a buffer zone. Each main plot was divided into subplots to apply each chemical with and without the adjuvant. Each subplot comprised of at least four blueberry bushes. All the treatments were replicated three times. Depending on the length of a particular row covered by the pivot, two to three rows were used in each main plot to accommodate all the sub-plots. A few bushes at both ends of the pivot bar were not included to increase uniformity of the simulated rainfall treatment.

2.3 Chemical treatments

The insecticides and the rates used for the studies are presented in Table 1. A total of five chemical treatments and control (water only) were used in 2014 and six chemical treatments and control were used in 2015. Commercial formulations of all the chemicals were obtained from the manufacturers. The rates were calculated based on the label recommendations for spotted wing drosophila on blueberry and/or small fruits. The rates and chemicals tested were the same for both years except that a higher rate of cyantraniliprole (152.7 g AI/ha) was included in 2015. The main difference between the 2014 and 2015 studies were the types of adjuvant mixed with the chemicals (see Table 1) and the types of sprayer used. In 2014, an air-assisted backpack sprayer was used whereas in 2015 an airblast sprayer (tractor operated) was used. In 2014, there was no

difference between the water control and the water with the adjuvant (Nu Film 17) control, and the preliminary tests with Nu Film P also did not show any effect of Nu Film P mixed in water on *D. suzukii* mortality. Therefore, only water treated control was used in 2015. Insecticide spray was applied on both sides of the rows. Prior to this, the airblast sprayer was calibrated to deliver at the rate of 467 L/ha of spray volume at the tractor speed of 4.8 km/h and 1300 RPM. After insecticide application, the bushes were left to dry for ~2 h before simulated rainfall treatments were applied.

Table 1

Insecticides/adjuvants and rates used for the studies in 2014 and 2015.

Trade name	Chemical name	Manufacturer	Rate (g AI/ha)
<i>2014 Study</i>			
Delegate™ 25WG	Spinetoram	Dow AgroSciences LLC, Indianapolis, IN	105.3
Entrust® 2SC	Spinosad	Dow AgroSciences LLC, Indianapolis, IN	98.6
Exirel™10SE	Cyantraniliprole (Cyazypyr®)	E. I. du Pont de Nemours and Company, Wilmington, DE	100.5
Malathion 8F	Malathion	Gowan Company LLC, Yuma, AZ	2322.1
Mustang Max™ 0.8EC	Zeta-cypermethrin	FMC Corporation, Philadelphia, PA	28.2
Nu Film 17® ^e	Nu Film P	Miller Chemical and Fertilizer Corporation, Hanover, PA	300.0

2015 Study

Delegate™ 25WG	Spinetoram	Dow AgroSciences LLC, Indianapolis, IN	105.3
Entrust® 2SC	Spinosad	Dow AgroSciences LLC, Indianapolis, IN	98.6
Exirel™10SE	Cyantraniliprole	E. I. du Pont de Nemours and Company,	
	(Cyazypyr®)	Wilmington, DE	100.5
Exirel™10SE	Cyantraniliprole	E. I. du Pont de Nemours and Company,	
	(Cyazypyr®)	Wilmington, DE	152.7
Malathion 8F	Malathion	Gowan Company LLC, Yuma, AZ	2322.1
Mustang Max™ 0.8EC	Zeta-		
	cypermethrin	FMC Corporation, Philadelphia, PA	28.2
Nu Film P® ^ε	Nu Film P	Miller Chemical and Fertilizer Corporation,	
		Hanover, PA	293.8

Note: ^ε = Adjuvants

2.4 Rainfall Simulation

Simulated rainfall treatments were applied on the same day after ~2 h of the insecticide application. The four rainfall levels consisted of 0 mm (control for rainfall), 12.5 mm, 25.0 mm and 37.5 mm. These rainfall levels were determined based on the historical rainfall data during the blueberry harvest season (May – July) in southeast Georgia. The center-pivot system was programmed to simulate the rainfall over a period of 1-2 hours for each level. Five rain gauges were placed in each main plot to measure the amount of simulated rainfall. The gauge measurements showed that the average rainfall did not deviate by more than ± 3 mm in any of the rainfall levels. The control plots receiving 0 mm rainfall were covered with plastic sheets during the natural rain events whereas all other plots that received simulated rainfalls were not covered.

2.5 Semi-field Bioassays

Semi-field bioassays were conducted on samples collected at 1, 3 and 7 DAT. Each bioassay sample consisted of a single blueberry branch containing at least 5 leaves and 5 ripe berries, all placed in a 946 ml clear plastic container (Fabri-Kal®, Kalamazoo, MI), called “bioassay chamber” designed according to Van Timmeren and Isaacs (2013) with some modifications. The bioassay chamber consisted of a Jackson wire mesh (0.32 X 0.32 cm) boat to hold berries and a 30 ml soufflé cup (Solo Cup Co., Lake Forest, IL) wrapped with Parafilm® (Bemis Corporate, Neenah, WI) to hold water, both affixed to the base of the chamber. In addition, a 10 cm long single anchor water pick (No. 1932, Smithers-Oasis Co., Kent, OH) was inserted through a hole on the bottom of the chamber. The chamber lid consisted of two holes (~2 cm diameter each) made with a soldering iron, one covered with fine mesh fabric for ventilation and the other to release flies for the bioassay that was then plugged with cotton balls. The water pick was filled with water and the sample branch was inserted into the pick to prevent the branch from desiccation during the bioassay periods.

The chambers were placed in chamber boxes (trays) and transported to the laboratory. A non-sterile cotton roll (Defend®, Hauppauge, NY) and a strip of filter paper dipped in fly diet (1:2:3 sugar:yeast:water by wt) and frozen overnight were inserted through a cut made on the Parafilm® cover of the water cup. The cotton roll provided extra moisture inside the chamber and the diet strip provided diet for the flies. The water cups, wire mesh boats and the fine mesh fabric were affixed with a hot glue gun.

Ten *D. suzukii* adults (5 males and 5 females) were aspirated from the culture bottles and introduced into each bioassay chamber through the release hole and then the hole plugged with a cotton ball. The chambers were placed in the laboratory at 25 ± 1 °C and 60-70% RH.

Humidifiers were used to increase humidity when necessary. Mortality was recorded at 72 hours after exposure in the 2014 study and at 120 hours after exposure in the 2015 study. The reason we increased the exposure time in 2015 was to make sure that flies got sufficiently long exposure time to come in contact with the samples.

All mortality data were arcsine of the square root transformed to meet the assumption of normality. In order to compare the effect of adjuvant on the residual activity of different insecticides in the absence of rainfall, the mortality data at 1 DAT were subjected to two-way ANOVA in proc glm of SAS 9.3. To determine the effect of different levels of rainfall on the residual activity of each insecticide with and without the adjuvant, the mortality data at 1, 3 and 7 DAT were analyzed using two-way ANOVA, with rainfall as a main plot factor and adjuvant as a subplot factor. Fisher's protected LSD was used to separate means at $\alpha=0.05$.

3. Results

Chemical treatments had significant effect on *D. suzukii* adult mortality in both years (2014: $F = 44.53$; $df = 5, 24$; $P = <0.0001$; 2015: $F = 19.39$; $df = 6, 26$; $P = <0.0001$). In the absence of rainfall, adjuvant did not have significant effect on residual activity of chemical treatments in 2014 or 2015 (2014: $F = 0.02$; $df = 1, 24$; $P = 0.877$; 2015: $F = 0.95$; $df = 1, 26$; $P = 0.339$). However, there was an interaction effect of chemical treatments and adjuvant in 2015 but not in 2014 (2014: $F = 0.21$; $df = 5, 24$; $P = 0.956$; 2015: $F = 4.98$; $df = 6, 26$; $P = 0.0025$). All five chemicals, with and without the adjuvant, caused significantly higher mortality as compared to the controls at 1 DAT. When compared among the chemicals, spinosad had significantly lower mortality than malathion or zeta-cypermethrin for both with and without the adjuvant in 2014. In

2015, spinosad had significantly lower mortality than the rest of the chemicals without the
 adjuvant. Spinosad with the adjuvant in 2015 increased mortality to the level similar to those
 with spinetoram, malathion or zeta-cypermethrin, which are near 100% (Fig 1. A, B).

Fig. 1. Percent mortality (Mean $\pm$ SEM) of *D. suzukii* adults exposed to blueberry foliage and
 fruits collected from blueberry bushes in no rainfall plots at 1 DAT with various insecticides and
 controls in 2014 trials (A) and 2015 trials (B). Means with the same letters are not significantly
 different ($\alpha = 0.05$). ^ = higher rate.

Table 2 presents the percent mortality (Mean $\pm$ SEM) of *D. suzukii* adults after 72 h of
 exposure to foliage and fruits collected from the blueberry bushes sprayed with one of the five
 selected insecticides or the controls with and without the adjuvant (Nu Film 17) followed by the

application of four levels of simulated rainfalls in the 2014 trials. The results showed that application of simulated rainfalls had significant effect on *D. sukuzii* mortality in all the chemical treatments, especially at 1 DAT and 3 DAT tests. Means comparisons showed the lowest level of rainfall (12.5 mm) significantly reduced mortality as compared to no rainfall for the malathion treatments with no adjuvant at 1 DAT. The 25 mm level of rainfall reduced mortality in the spinosad treatment at 3 DAT, and in the zeta-cypermethrin treatments at 1 and 7 DAT, with and without the adjuvant. The highest level of rainfall (37.5 mm) resulted in the significant reduction of mortality for all the chemical treatments at 1 and 3 DAT tests as compared to the no rainfall application, with the exception of zeta-cypermethrin at 3 DAT with no adjuvant (see Table 2).

Addition of Nu Film 17 significantly increased *D. sukuzii* mortality only for the zeta-cypermethrin treatment with 25 mm rainfall at 1 DAT tests (Table 2). The impact of the adjuvant was more pronounced with the higher level of rainfall as the fly mortality held more or less constant even with the increase of rainfall levels. In the case of the cyantraniliprole treatments, however, addition of Nu Film 17 caused reduced mortality at 0 mm and 12.5 mm rainfall treatments at 3 DAT.

In the 2015 trials, applications of simulated rainfall significantly reduced mortality at 1 DAT tests but not so at 3 DAT and 7 DAT tests except for the cyantraniliprole higher rate at 3 DAT and spinosad or malathion at 7 DAT. Like in 2014, mortality was almost always higher in the no-rainfall plots than in the rainfall simulation plots. Within the rainfall levels, however, the data do not show a clear trend between the levels of rainfall and the percentage of fly mortality (Table 3).

Addition of Nu Film P with spinetoram or spinosad caused significantly higher mortality when compared with the respective chemical only treatments at the 12.5 mm rainfall simulation plots at 1 DAT. Similarly, addition of the adjuvant with malathion caused significantly higher

238 mortality than the malathion only treatment at the 25 mm rainfall simulation plots at 1 DAT.

239 Mixing of the adjuvant with the cyantraniliprole higher rate caused reduced mortality at 3 DAT

240 tests at 0 mm and 25 mm rainfall simulation plots. For the zeta-cypermethrin and the lower rate

241 of cyantraniliprole (100.5 g/ha) treatments, addition of the adjuvant did not have any significant

242 effect on mortality at any DAT for any rainfall levels. For the spinetoram or spinosad treatments,

243 there was significant interaction between the effects of rainfall and Nu Film P at 1 DAT (2015);

244 the effect of the adjuvant was pronounced at lower rainfall level than at the higher rainfall levels.

245 The interaction of the adjuvant with rainfall caused lower mortality in the cyantraniliprole

246 treatments in 2014 with 0 and 12.5 mm levels of rainfall. In 2015, however, similar interaction

247 effect was observed only at the higher rate of cyantraniliprole treatments with 0 and 25 mm

248 levels of rainfall (Table 3).

249

250 **Table 2**

251 Percent mortality (Mean $\pm$ SEM) of *D. suzukii* adults exposed to foliage and fruits collected from

252 blueberry bushes sprayed with various insecticides followed by application of 4 levels of

253 simulated rainfalls in 2014 trials. The samples were evaluated for three time periods, 1, 3 and 7

254 DAT. Each group is followed by the statistical parameters. For a given insecticide, means within

255 a column followed by the same letter are not significantly different. Similarly, means within a

256 row within the same DAT followed by a star shows significantly different. Statistical

257 significance was determined at $\alpha = 0.05$.

Insecticide	Rain (mm)	Statistical parameters	1 DAT		3 DAT		7 DAT	
			Without Nu-Film	With Nu-Film	Without Nu-Film	With Nu-Film	Without Nu-Film	With Nu-Film
Spinetoram	0		96.6 $\pm$ 3.3 a	100 $\pm$ 0 a	96.6 $\pm$ 3.3 a	90.0 $\pm$ 5.7 a	20.0 $\pm$ 10.0 a	36.6 $\pm$ 3.3 a

	12.5		96.6±3.3 a	93.3±6.6 ab	93.3±3.3 a	90.0±5.7 a	36.6±16.6 a	10.0±5.7 a
	25		86.6±3.3 ab	90.0±5.7 ab	86.6±8.8 a	83.3±3.3 ab	23.3±3.3 a	26.6±12.0 a
	37.5		76.6±3.3 b	86.6±3.3 b	63.3±3.3 b	60.0±10.0 b	13.3±3.3 a	26.6±3.3 a
	Rain	<i>F</i> ; df; <i>P</i>	6.71; 3, 16; 0.0038		6.86; 3, 16; 0.0035		0.29; 3, 16; 0.8311	
	Nu-Film-17	<i>F</i> ; df; <i>P</i>	1.25; 1, 16; 0.2805		1.18; 1, 16; 0.2932		0.04; 1, 16; 0.839	
	Interaction	<i>F</i> ; df; <i>P</i>	0.37; 3, 16; 0.7771		0.15; 3, 16; 0.9286		2.49; 3, 16; 0.0972	
Spinosad	0		90.0±5.7 a	90.0±5.7 a	76.6±3.3 a	73.3±8.8 a	26.6±6.6 a	20.0±5.7 a
	12.5		86.6±3.3 a	83.3±3.3 a	66.6±3.3 ab	66.6±6.6 ab	16.6±8.8 ab	26.6±6.6 a
	25		70.0±5.7 a	73.3±3.3 a	50.0±10.0 bc	46.6±3.3 bc	0±0 b	13.3±6.6 a
	37.5		50.0±10.0 b	53.3±8.8 b	40.0±11.5 c	36.6±8.8 c	10.0±0 ab	10.0±5.7 a
	Rain	<i>F</i> ; df; <i>P</i>	6.61; 3, 16; 0.0041		9.43; 3, 16; 0.0008		3.95; 3, 16; 0.0277	
	Nu-Film-17	<i>F</i> ; df; <i>P</i>	0.29; 1, 16; 0.5968		0.16; 1, 16; 0.6923		0.97; 1, 16; 0.3402	
	Interaction	<i>F</i> ; df; <i>P</i>	0.43; 3, 16; 0.7369		0.03; 3, 16; 0.9927		1.2; 3, 16; 0.3406	
Cyantraniliprole	0		93.3±6.6 a	93.3±3.3 a	100.0±0 a	83.3±6.6 a *	46.6±16.6 a	23.3±14.5 a
	12.5		93.3±3.3 a	90.0±5.7 a	96.6±3.3 a	66.6±8.8 ab *	43.3±12.0 a	20.0±5.7 a
	25		76.6±8.8 ab	40.0±15.2 b	70.0±5.7 b	63.3±6.6 ab	6.6±6.6 b	3.3±3.3 a
	37.5		46.6±8.8 b	60.0±10.0 b	40.0±15.2 b	53.3±13.3 b	3.3±3.3 b	10.0±5.7 a
	Rain	<i>F</i> ; df; <i>P</i>	11.37; 3, 16; 0.0003		15.71; 3, 16; <0.0001		4.82; 3, 16; 0.0141	
	Nu-Film-17	<i>F</i> ; df; <i>P</i>	0.99; 1, 16; 0.3335		11.18; 1, 16; 0.0041		2.48; 1, 16; 0.1349	
	Interaction	<i>F</i> ; df; <i>P</i>	1.35; 3, 16; 0.2924		5.00; 3, 16; 0.0123		1.14; 3, 16; 0.3637	
Malathion	0		100.0±0 a	100.0±0 a	83.3±8.8 a	80.0±5.7 a	13.3±8.8 a	16.6±12.0 a
	12.5		76.6±3.3 b	90.0±5.7 ab	60.0±20.0 ab	70.0±10.0 ab	3.3±3.3 a	0±0 a
	25		70.0±15.5 b	76.6±6.6 ab	60.0±15.2 ab	60.0±10.0 ab	6.6±3.3 a	0±0 a
	37.5		63.3±3.3 b	66.6±12.0 b	26.6±17.6 b	30.0±5.7 b	3.3±3.3 a	0±0 a
	Rain	<i>F</i> ; df; <i>P</i>	16.78; 3, 16; <0.0001		5.85; 3, 16; 0.0068		2.59; 3, 16; 0.0893	
	Nu-Film-17	<i>F</i> ; df; <i>P</i>	1.71; 1, 16; 0.2096		0; 1, 16; 0.9615		0.36; 1, 16; 0.5575	
	Interaction	<i>F</i> ; df; <i>P</i>	0.63; 3, 16; 0.6041		0.2; 3, 16; 0.8929		0.28; 3, 16; 0.841	
Zeta-cypermethrin	0		100.0±0 a	100.0±0 a	100.0±0 a	90.0±10.0 a	73.3±12.0 a	80.0±10.0 a
	12.5		100.0±0 a	96.6±3.3 ab	100.0±0 a	96.6±3.3 a	40.0±11.5 b	56.6±12.0 ab
	25		80.0±5.7 b	93.3±3.3 bc *	86.6±13.3 a	96.6±3.3 a	36.6±8.8 b	53.3±12.0 b
	37.5		76.6±3.3 b	90.0±0 c	90.0±0 a	70.0±5.7 b	26.6±3.3 b	43.3±6.6 b
	Rain	<i>F</i> ; df; <i>P</i>	22.21; 3, 16; <0.0001		4.43; 3, 16; 0.019		6.87; 3, 16; 0.0035	
	Nu-Film-17	<i>F</i> ; df; <i>P</i>	3.09; 1, 16; 0.098		1.62; 1, 16; 0.2209		3.53; 1, 16; 0.0787	
	Interaction	<i>F</i> ; df; <i>P</i>	3.36; 3, 16; 0.0449		0.88; 3, 16; 0.472		0.06; 3, 16; 0.9791	

258

259

260 **Table 3**

261 Percent mortality (Mean±SEM) of *D. suzukii* adults exposed to foliage and fruits collected from
 262 blueberry bushes sprayed with various insecticides followed by application of 4 levels of
 263 simulated rainfalls in 2015 trials. The samples were evaluated for three time periods, 1, 3 and 7
 264 DAT. Each group is followed by the statistical parameters. For a given insecticide, means within
 265 a column followed by the same letter are not significantly different. Similarly, means within a
 266 row within the same DAT followed by a star shows significantly different. Statistical
 267 significance was determined at $\alpha = 0.05$.

Insecticide	Rain (mm)	Statistical parameters	1 DAT		3 DAT		7 DAT	
			Without Nu-Film	With Nu-Film	Without Nu-Film	With Nu-Film	Without Nu-Film	With Nu-Film
Spinetoram	0		100±0.0 a	100±0 a	33.3±8.8 a	50.0±25.1 a	26.6±3.3 a	36.6±3.3 a
	12.5		66.6±17.6 b	100±0 a *	40.0±20.8 a	10.0±10.0 a	56.6±3.3 a	36.6±3.3 a
	25		96.6±3.3 ab	83.3±8.8 ab	53.3±29.0 a	46.6±18.5 a	50.0±5.7 a	60.0±15.2 a
	37.5		83.3±8.8 ab	73.3±3.3 b	46.6±14.5 a	70.0±0 a	16.6±8.8 a	30.0±25.1 a
	Rain	<i>F</i> ; df; <i>P</i>	3.73; 3, 16; 0.033		0.82; 3, 16; 0.5009		2.65; 3, 16; 0.0842	
	Nu-Film-P	<i>F</i> ; df; <i>P</i>	0.04; 1, 16; 0.848		0; 1, 16; 0.9471		0.29; 1, 16; 0.5971	
	Interaction	<i>F</i> ; df; <i>P</i>	3.26; 3, 16; 0.0489		0.89; 3, 16; 0.4689		0.94; 3, 16; 0.4452	
Spinosad	0		76.6±6.6 a	96.6±3.3 a *	50.0±20.0 a	66.6±24.0 a	23.3±14.5 ab	20.0±11.5 b
	12.5		23.3±8.8 b	80.0±5.7 ab *	20.0±11.5 a	46.6±8.8 a	53.3±3.3 a	46.6±3.3 ab
	25		33.3±18.5 b	46.6±14.5 b	36.6±14.5 a	16.6±8.8 a	30.0±11.5 ab	56.6±18.5 a
	37.5		90.0±10.0 a	33.3±8.8 b *	23.3±8.8 a	46.6±29.0 a	13.3±8.8 b	20.0±11.5 b
	Rain	<i>F</i> ; df; <i>P</i>	7.75; 3, 16; 0.002		1.27; 3, 16; 0.3167		3.97; 3, 16; 0.0271	
	Nu-Film-P	<i>F</i> ; df; <i>P</i>	0.83; 1, 16; 0.3765		1.29; 1, 16; 0.2727		0.62; 1, 16; 0.4426	
	Interaction	<i>F</i> ; df; <i>P</i>	10.91; 3, 16; 0.0004		0.63; 3, 16; 0.6036		1.04; 3, 16; 0.4024	
Cyantraniliprole	0		96.6±3.3 a	70.0±1.00 a	66.6±20.2 a	76.6±23.3 a	23.3±8.8 a	23.3±12.0 a
	12.5		73.3±6.6 ab	40.0±26.4 a	16.6±12.0 a	26.6±13.3 a	30.0±5.7 a	33.3±3.3 a
	25		60.0±20.8 ab	76.6±13.3 a	13.3±3.3 a	73.3±13.3 a	33.3±14.5 a	63.3±13.3 a
	37.5		46.6±13.3 b	40.0±5.7 a	53.3±24.0 a	60.0±20.0 a	33.3±12.0 a	36.6±14.5 a
	Rain	<i>F</i> ; df; <i>P</i>	3.3; 3, 16; 0.0476		2.56; 3, 16; 0.0917		1.87; 3, 16; 0.1749	
	Nu-Film-P	<i>F</i> ; df; <i>P</i>	2.37; 1, 16; 0.1436		1.91; 1, 16; 0.1856		1.5; 1, 16; 0.2383	
	Interaction	<i>F</i> ; df; <i>P</i>	1.12; 3, 16; 0.3692		0.83; 3, 16; 0.4979		0.91; 3, 16; 0.4575	
Cyantraniliprole^	0		96.6±3.3 a	96.6±3.3 a	96.6±3.3 a	53.3±12.0 a *	36.6±12.0 a	23.0±3.3 a

	12.5		43.3±17.6 b	73.3±8.8 b	13.3±6.6 b	40.0±30.5 a	43.3±18.5 a	43.3±13.3 a
	25		30.0±5.7 b	56.6±3.3 bc	80.0±10.0 a	30.0±15.2 a *	56.6±14.5 a	36.6±12.0 a
	37.5		63.3±14.5 b	30.0±10.0 c	30.0±10.0 b	50.0±5.7 a	30.0±15.2 a	26.6±13.3 a
	Rain	<i>F; df; P</i>	19.43; 3, 16; <0.0001		3.35; 3, 16; 0.0456		0.97; 3, 16; 0.4315	
	Nu-Film-P	<i>F; df; P</i>	0.42; 1, 16; 0.5284		1.99; 1, 16; 0.1774		1.04; 1, 16; 0.3236	
	Interaction	<i>F; df; P</i>	3.15; 3, 16; 0.0538		4.28; 3, 16; 0.0212		0.23; 3, 16; 0.8775	
Malathion	0		100.0±0.0 a	96.6±3.3 a	63.3±23.3 a	36.6±12.0 a	10.0±5.7 b	10.0±5.7 c
	12.5		43.3±14.5 b	56.6±21.8 a	40.0±20.0 a	20.0±20.0 a	43.3±12.0 a	56.6±6.6 ab
	25		40.0±17.3 b	83.3±16.6 a *	36.6±14.5 a	23.3±13.3 a	43.3±12.0 a	73.3±8.8 a
	37.5		36.6±14.5 b	76.6±20.2 a	36.6±14.5 a	40.0±20.8 a	40.0±5.7 a	40.0±20.8 bc
	Rain	<i>F; df; P</i>	5.11; 3, 16; 0.0114		0.8; 3, 16; 0.5139		7.16; 3, 16; 0.0029	
	Nu-Film-P	<i>F; df; P</i>	4.5; 1, 16; 0.0499		1.47; 1, 16; 0.2432		2.52; 1, 16; 0.132	
	Interaction	<i>F; df; P</i>	1.26; 3, 16; 0.3221		0.47; 3, 16; 0.7067		1.03; 3, 16; 0.4049	
Zeta-cypermethrin	0		100.0±0 a	96.6±3.3 a	56.6±8.8 a	60.0±23.1 a	36.6±13.3 a	16.6±3.3 a
	12.5		56.6±18.5 b	60.0±0.0 b	46.6±12.0 a	33.3±3.3 a	53.3±6.6 a	40.0±10.0 a
	25		46.6±14.5 b	70.0±15.2 ab	30.0±25.1 a	16.6±6.6 a	33.3±3.3 a	50.0±5.7 a
	37.5		50.0±15.2 b	40.0±11.5 b	36.6±21.8 a	40.0±5.7 a	50.0±20.0 a	23.3±13.3 a
	Rain	<i>F; df; P</i>	4.06; 3, 16; 0.0253		1.59; 3, 16; 0.2315		1.16; 3, 16; 0.3548	
	Nu-Film-P	<i>F; df; P</i>	0.79; 1, 16; 0.3864		0.05; 1, 16; 0.8279		2.07; 1, 16; 0.1693	
	Interaction	<i>F; df; P</i>	0.54; 3, 16; 0.6633		0.33; 3, 16; 0.804		1.61; 3, 16; 0.2253	

268 Note: ^ = higher rate

269

270 4. Discussion

271 These studies showed that simulated rainfall reduced the effectiveness of all the insecticides
272 tested by washing the chemical residues off the plant surface. The samples (foliage and berries)
273 collected at 1 DAT caused almost 100% fly mortality in each of the insecticide treatments in the
274 no rainfall plots. The application of simulated rainfall reduced the mortality to a varying degree
275 depending on the level of rainfall and in some cases the type of insecticide used. These results
276 are consistent with Hulbert et al. (2012) who reported that simulated rainfall (range: 12.5-50.8
277 mm) decreased the efficacy of the phosmet, carbaryl, imidacloprid or zeta-cypermethrin treated
278 blueberry shoots on adult Japanese beetle, *Papilio japonica* Newman. The data from the 2014

study showed lower mortalities with the higher levels of rainfall suggesting that higher levels of rainfall resulted in the removal of greater amount of residues from the plant surface. Such a trend, however, was not observed in the 2015 study. Hulbert et al. (2012) also did not observe the trend of higher wash-off with the higher levels of rainfall (range: 7.5-50.8 mm) on phosmet, carbaryl or zeta-cypermethrin but carbaryl.

Although intensity of a given rainfall event seems to be an important factor to determine the amount of insecticide residue wash-off, the relationship between the intensity of rainfall and the amount of insecticide residue wash-off from the plant surfaces are not clearly established. Mashaya (1993) reported that the intensity of rain did not affect the rainfastness of the insecticides, acephate SP, methomyl SP, fenvalerate EC and monocrotophos SC. Similarly, McDowell et al. (1987) did not observe any differences in fenvalerate concentration in wash-off from rain intensities that ranged from 7-51 mm/h and Pick et al. (1984) found no effect of rain intensity on WP and EC formulations of malathion. Pick et al. (1984) further reported that as small as 1 mm of simulated rainfall removed sufficient amount of deposits off cotton plants, and Pree et al. (1984) found similar effect of small amount of rain on phosmet deposits on peaches. Our 2015 data which did not show a correlation between the level of rainfall and the residual activity in some cases (as suggested by the fly mortality data) was therefore not a surprise. These results suggest that even a small amount of rainfall in some situations is enough to wash an appreciable amount of deposits off plant surfaces and reduce the insecticide efficacy significantly.

In the 2015 trials, the addition of the adjuvant (Nu Film P) resulted in significantly higher fly mortality in the spinetoram, spinosad and malathion treatments than without the adjuvant for at least one rainfall level. The higher fly mortality observed in this study is attributed to higher

persistence of the insecticide deposits on plant surfaces as a result of the adjuvant (van Zyl et al., 2010b). The present results with the three chemicals, i.e., spinetoram, spinosad and malathion support this assertion. The results of the semi-field bioassays conducted during 2014, however, were not as promising since only one chemical, zeta-cypermethrin, showed improved persistence with the addition of the adjuvant, Nu Film 17. It is important to note that both the adjuvants (Nu Film P and Nu Film 17) were mixed at the lowest rate of recommendation, so it would be interesting to see the results when mixed at a higher rate. Nevertheless, at their respective lower rates, Nu Film P seemed to work better with different classes of insecticides.

One possible explanation for the lower performance of the adjuvant in 2014 could be the use of backpack sprayer, as the backpack sprayer may not have created high enough agitation to mix the adjuvant in the spray preparation as the airblast did. The results from airblast are more relevant as it is the one most commonly used by farmers to spray in blueberry fields. It is also possible that the different formulations of the adjuvant have different performance results. In addition, the blueberry cultivars tested were different in 2014 and 2015, so the role of cultivar specific physical properties in the persistence of residues also cannot be ruled out. Surprisingly, mixing of either adjuvant with cyantraniliprole resulted in reduced performance in both years' trials at 3 DAT tests, especially at lower levels of rainfall. The reasons behind these results are unclear. Previous studies have reported varied performance results of the adjuvant, Nu Film 17, depending on the types of pesticides and their concentrations. Talekar et al. (1977) found that Nu Film 17 improved the persistence of malathion but not cyanofenphos. Addition of Nu Film 17 has also improved the persistence of NeemAzal (Olenici and Olenici, 2006).

The effectiveness of the insecticides declined rapidly as the chemicals aged in the field even in the absence of rainfall (0 mm simulated rain). Mortality remained high at 3 DAT in 2014 but

a significant reduction occurred during the same period in 2015. At 7 DAT, mortality was low (below 50%, except for the zeta-cypermethrin treatment in 2014) for both years indicating that the residual efficacy did not last a week in Georgia weather conditions. This is consistent with the findings by Van Timmeren and Isaacs (2013) who reported that by one week after treatment *D. suzukii* adult mortality was not significantly different from the untreated controls for most insecticides that had been exposed to just over 24 mm rainfall. Mason and Isaacs (2013) studied the residual activity of pyrethroid insecticides and found that the number of eggs by grape berry moth was not significantly reduced in 3 DAT as compared to controls suggesting a short residual effect of the insecticides applied in the field. Unfortunately, in 2015, ~37 mm rainfall occurred on 6 DAT and hit all the plots including controls as the attempt to cover the control plots was futile due to strong winds. However, we are unsure of the impact of this rain on the 7 DAT results.

The removal of appreciable amount of deposits on or after 3 days of spraying could be due to the combined effect of environmental conditions, including rainfall, temperature, wind, dew and UV light. In 2015, the average maximum temperature during the trial period ranged from 35-38°C whereas in 2014 it was 32-35°C (US Climate Data). The higher temperatures in 2015 may have played a role in reducing the efficacy of the chemicals at a faster rate than in 2014. This suggests that, where possible, sprayings should not only avoid rains but also extremely hot day(s). This is important from *D. suzukii* adult activity point of view as well since they are less active at temperatures above 30°C (Walsh et al., 2011). In fact, 32.6°C was estimated as a temperature that brings about 50% mortality in the laboratory tests (Kimura 2004). Although *D. suzukii* adults may not die in the field even if the temperatures are high as they may adapt survival strategy by finding cooler places in shades and grounds, their activities are noticeably

reduced during the daytime when the temperatures are high (Hamby et al., 2013; Evans and Sial, unpublished data). So, simply by adjusting the spray time to avoid high temperatures and rain events, where possible, may increase the probability of direct exposure of flies to the spray applications and prolong the residual efficacy of insecticides. In areas where significant amount of rainfall occurs during blueberry harvest season, addition of suitable adjuvants may help to prolong the residual efficacy of some insecticides against SWD and other insect pests of blueberries. Our findings are not only relevant to southeastern United States but also to other regions with similar climatic conditions.

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