

# Rainfastness of Insecticides Used to Control Japanese Beetle in Blueberries

DANIEL HULBERT,<sup>1,2</sup> PABLO REEB,<sup>3</sup> RUFUS ISAACS,<sup>1</sup> CHRISTINE VANDERVOORT,<sup>4</sup>  
SUSAN ERHARDT,<sup>1</sup> AND JOHN C. WISE<sup>1</sup>

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**ABSTRACT** Field-based bioassays were used to determine the relative impact of rainfall on the relative toxicity of four insecticides, phosmet, carbaryl, zeta-cypermethrin, or imidacloprid, from different chemical classes on adult Japanese beetles, *Popillia japonica* Newman, in highbush blueberries, *Vaccinium corymbosum* L. Bioassays were set up 24 h after spraying occurred and Japanese beetle condition was scored as alive, knockdown or immobile 1, 24, and 48 h after bioassay setup. All insecticides were significantly more toxic than the untreated control and zeta-cypermethrin consistently had the greatest toxic effect against the Japanese beetles. All insecticides experienced a decrease in efficacy after simulated rainfall onto treated blueberry shoots, although the efficacy of zeta-cypermethrin was the least affected by rainfall. This study will help blueberry growers make informed decisions on when reapplications of insecticides are needed in the field with the aim of improving integrated pest management (IPM).

**KEY WORDS** wash-off, precipitation, *Popillia japonica*

In eastern and central North America, the Japanese beetle, *Popillia japonica* Newman, is an invasive pest first found in New Jersey that are pests of ornamentals, turfgrass, fruits, and vegetables causing \$450 million in damage annually to ornamental plants and turf alone (Fleming 1976, Potter and Held 2002). Japanese beetles were first discovered in the United States 1916 in New Jersey and are now a major pest in the eastern United States. During the periods of adult emergence, June to September in Michigan, Adult Japanese beetles feed in aggregations on the foliage of their host plants. Japanese beetle adults feed on >300 plant species including blueberry (*Vaccinium* spp.) (Fleming 1972, Potter and Held 2002, Van Timmeren and Isaacs 2009). Their phenology and behavior make Japanese beetles difficult to control in blueberry because of the high abundance of adults at the time of harvest. Mechanical over the row harvesters are commonly used for harvesting, but these are unable to effectively discriminate blueberries from Japanese beetles, creating a potential contamination risk. Japanese beetles can cause considerable economic damage in fields where they are not controlled at the time of harvest because there is zero tolerance for insect contamination in the final product. This stringent quality stan-

dard places pressure on growers to achieve very high levels of insect control.

There are a variety of ways in which Japanese beetle can be controlled in blueberries. Cultural practices such as tillage may be used to reduce populations of Japanese beetles in blueberries (Szendrei et al. 2005). Entomopathogenic nematodes can be used to effectively manage Japanese beetle larvae in the (Wright et al. 1988, Klein and Ramon 1992, Cappaert and Smitley 2002). Additionally, automated blueberry sorters are able to distinguish and separate Japanese beetle from blueberries on the basis of color on the packing line (R.I., unpublished data). Although these important tools exist for Japanese beetle management, they are all expensive and none of them is 100% effective, so conventional insecticides are still the preferred method for control of Japanese beetles in blueberries. In Michigan, 63% of blueberry growers surveyed said that application of foliar insecticides was the most important Japanese beetle control method (Szendrei and Isaacs 2006).

Michigan receives on average ≈60–80 mm of rain per month during the period of the blueberry growing season when adult Japanese beetles have emerged and are active (Michigan State University 2011). This has important implications for the fate of insecticides sprayed and their efficacy against Japanese beetle. Overestimation of wash off can cause unwarranted reapplications of insecticides, whereas underestimation may result in inadequate crop protection (Pimentel et al. 1992). The research on how rainfall affects insecticides during rainfall has been on older conventional insecticides such as organophosphates and car-

<sup>1</sup> Michigan State University, Department of Entomology, 206 Center for Integrated Plant Systems, East Lansing, MI 48824-1311.

<sup>2</sup> Corresponding author, e-mail: [hulbertd@msu.edu](mailto:hulbertd@msu.edu).

<sup>3</sup> Michigan State University, Department of Fisheries and Wildlife, 480 Wilson Road, Room 13 Natural Resources Bldg, East Lansing, MI 48824.

<sup>4</sup> Pesticide Analytical Laboratory, 206 Center for Integrated Plant Systems, East Lansing, MI 48824.

**Table 1.** Formulated compounds, field rates, and concentrations used for bioassay experiments on the effect of rainfall on insecticidal control of Japanese beetles in blueberries

Formulated name	Chemical class	Active ingredient	Rate/acre	g AI/ha	ppm	Company
Imidan 70 WP	Organophosphate	Phosmet	1.3 lbs	1,043	2,231	Gowan Company, Yuma, AZ
Sevin XLR 4L	Carbamate	Carbaryl	2 qt	4,481	9,586	Bayer CropScience, Pittsburgh, PA
Mustang Max 0.8 EC	Pyrethroid	Zeta-cypermethrin	4 oz	2	5	FMC Corp. Princeton, NJ
Provado 1.6 SC	Neonicotinoid	Imidacloprid	8 oz	9	19	Bayer CropScience, Pittsburgh, PA

All preparations were based on 468 liters/ha spray vol (50 gallons/acre).

bamates (McDowell et al. 1984; Willis et al. 1992, 1994, 1996; Zhou et al. 1997). Recent studies in apples have shown that foliar applications of insecticides results in various residue profiles depending on compound (Wise et al. 2006, 2007), but there are few studies on the implications of wash off for the newer chemical classes. Studying the efficacy of older and newer and reduced risk insecticides after rainfall could decrease unnecessary insecticide reapplication and the associated costs and risks. Hulbert et al. (2011) showed that the insecticides bifenthrin, thiamethoxam, and indoxacarb were all resistant to 12.7 and 25.4 mm of rainfall against Japanese beetle adults when sprayed on grapes, while other insecticides (phosmet and carbaryl) had significantly diminished efficacies after rainfall. It is important for a grower to be able to determine whether an insecticide spray is warranted after rainfall. Therefore, the results of investigation into how the efficacies of a wide range of insecticides are affected by rainfall will be valuable to growers as part of an integrated pest management (IPM) decision making process.

The purpose of this study was to evaluate the effects of rainfall and aging on the efficacy of insecticides used to control Japanese beetles, a late season pest of blueberries. The objectives were to: 1) Determine the inherent toxicity of these insecticides to the Japanese beetle and 2) to determine the effect of rainfall on the efficacy of four different insecticides representing major chemical classes of insecticides against Japanese beetles.

### Methods and Materials

**Insects.** Japanese beetle adults were collected from grass fields at the Michigan State University Trevor Nichols Research Center (TNRC) in Fennville, MI (42.5951° N, -86.1561° W), during July 2009. Beetles were captured using yellow and green canister traps with a floral and pheromone lure (Trécé Inc., Adaire, OK) during the 24-h period preceding each study. After collection, beetles were held in cages with non-sprayed *Sassafras* spp. foliage at ≈25°C and a photoperiod of 16:8 (L:D) h. Healthy beetles exhibiting mobility on the foliage were used in the experiments. Because a pheromone lure was used to capture Japanese beetle adults, male beetles were likely over-represented in the sample of captured beetles.

**Field Plots and Treatment Applications.** Each field plot consisted of a single mature Jersey blueberry (*Vaccinium corymbosum* L.) bush. There were four

different field plots for each of the four insecticide treatments and one control. A minimum of one mature blueberry bush in every direction separated each plot. Insecticide treatments were applied at labeled rates using an FMC 1029 airblast sprayer calibrated to deliver 467.5 liters of water/ha (50 gal/acre) (Table 1). These insecticides were chosen because they are all from different chemical classes and represent older more conventional chemicals as well as newer reduced risk insecticides. Insecticide applications were made on 7 July 2009 between the times 0700 and 1000. Average air temperature was between 11°C and 18.5°C and average wind speed was between 0.2 k/h and 1.3 k/h. These plots served as the source of foliage for use in bioassays. Untreated control plots were not sprayed. Daily high and low temperatures and precipitation volumes were recorded with an automated weather station (Michigan State University 2011) located within 1 km of the field plots.

**Bioassays.** Bioassays were used to compare the effects of rainfall on the efficacy of the four insecticides after they received rainfall. One shoot per field plot per rainfall treatment was collected. Shoots of at least 10 leaves with clusters of fruit were collected from the blueberry bushes 24 h after application. After 24 h of drying in the field a single shoot per plot per rainfall combination was randomly collected from each plot. Shoots were placed in water-soaked OASIS floral foam bricks (Smithers-Oasis Co., Kent, OH) and then placed in a Generation three Research Track Sprayer rainfall simulator (DeVries Manufacturing, Hollandale, MN). Shoots received 0, 12.7, 25.4, or 50.8 mm of simulated rain, that is, 0, 0.5, 1, or 2 inches of rain. In the rainfall simulator, 25.4 mm or 1 inch of rain takes ≈22 min to complete. All rainfall treatments were performed with a turbo floodjet VS5 nozzle (TeeJet Technologies, Wheaton, IL) when pressure of the rainfall simulator was set at 15 psi. Three rain gauges were placed around the inside of the rainfall simulator to accurately assess the amount and uniformity of simulated rain treatments. Shoots not assigned to receive rainfall were not placed in the rainfall simulator.

Each blueberry shoot collected from the rainfall simulator was pruned so that exactly 10 leaves remained on the shoot. The shoots were all pruned in the same manner: the shoots were pruned so that only the 10 most distal leaves on the shoot remained. This shoot was placed in water-soaked OASIS floral foam in a clear polypropylene 950-ml container (Fabri-Kal, Kalamazoo, MI) with lids making the completed bioassay chamber. The foam was covered with sealing

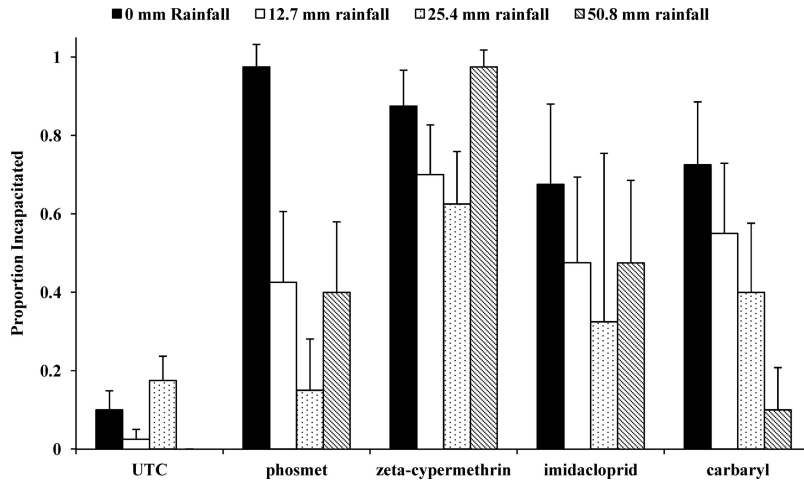


Fig. 1. Mean proportion (+SE) of the Japanese beetles in the incapacitated condition in bioassays containing foliage treated with each insecticide and at each level of rainfall tested.

wax (Gulf Wax, distributed by Royal Oak Sales, Inc., Roswell, GA) to preserve the integrity of the plant tissue by reducing evaporation of water. This also prevents Japanese beetles from digging into the foam. Holes were punched in the lid to reduce condensation of water vapor inside the container and minimize risk of fumigation effects. Each of these containers was considered an experimental unit in the bioassays.

As soon as bioassay arenas were prepared, 10 haphazardly selected Japanese beetle adults were placed in the bottom of each arena and the bioassay chambers were held in the laboratory at 21°C and a photoperiod of 16:8 (L:D) h. There was likely a bias toward collecting and selecting male beetles for the experiment because a pheromone lure was used, but this was not verified. There were four replicates for each treatment and rainfall amount combination. Each replicate was collected from a different field plot. The number of beetles that were alive, immobile, or in a knockdown condition were recorded after 4, 24, and 48 h of exposure. The knockdown condition was defined as beetles that were twitching in a nonupright position at the bottom of the container. Beetles were counted as alive if they behaved normally. For the analysis the categories knockdown and immobile were combined to a category called "incapacitated" to find an appropriate model to fit the data and will be referred to as the incapacitated condition. A separate analysis was conducted for the data corresponding to each of the insecticides tested. All insecticides were also compared against each other at 0 mm of rainfall. For all analyses time 0 was excluded because there were no differences (no uncertainty to the measure). For each insecticide, the data were fitted to a GLM with a binomial distribution and using the logit link function within the PROC GLIMMIX in SAS 9.3 (SAS Institute 2011). Pairwise mean comparisons were performed using Tukey test, slicing by main effects if interactions were significant. A  $P$  value less than  $\alpha = 0.05$  was considered significant.

The proportion of the leaf area defoliated by Japanese beetles was determined for each container in the bioassay. This was done using Photoshop Elements, version 8.0 (Adobe Systems; San Jose, CA). Images of the leaves were scanned into a computer using a Canon Image Runner c2880/c3380 (Canon U.S.A., Inc., Lake Success, NY). Different layers of the image were created for damaged and undamaged areas of leaf tissue using the Magic Wand Tool in Photoshop. The numbers of pixels comprising the two layers of the image were determined using the Histogram window in Photoshop and the proportion of the leaves defoliated was calculated. These data were arcsine square-root transformed before being analyzed by analysis of variance (ANOVA). A separate ANOVA was performed on the proportion of leaves defoliated for each insecticide to compare across rainfall levels. In addition, a separate ANOVA was performed to compare the proportion of leaves defoliated across insecticide at a particular rainfall level. These analyses were done using R version 2.14.2 (R Development Core Team, 2012).

## Results

**No-Rain Dose Effects.** When all insecticides were compared against each other at 0 mm of rain, the effect of insecticide was significant ( $F = 3.90$ ;  $df = 4, 45$ ;  $P = 0.0084$ ), but the effect of time ( $F = 0.41$ ;  $df = 2, 45$ ;  $P = 0.6675$ ) and the interaction of time and insecticide ( $F = 0.53$ ;  $df = 8, 45$ ;  $P = 0.8270$ ) were not significant. Phosmet ( $t = 3.44$ ;  $df = 45$ ;  $P = 0.0105$ ), zeta-cypermethrin ( $t = 4.49$ ;  $df = 45$ ;  $P = 0.0005$ ), imidacloprid ( $t = 3.59$ ;  $df = 45$ ;  $P = 0.0070$ ), and carbaryl ( $t = 3.16$ ;  $df = 45$ ;  $P = 0.0222$ ) were all significantly higher than the untreated control (Fig. 1).

Significantly less area of the blueberry leaves was defoliated in bioassays with leaves treated with each of the insecticides compared with the untreated control ( $F = 8.05$ ;  $df = 4, 15$ ;  $P = 0.0011$ ) (Fig. 2).

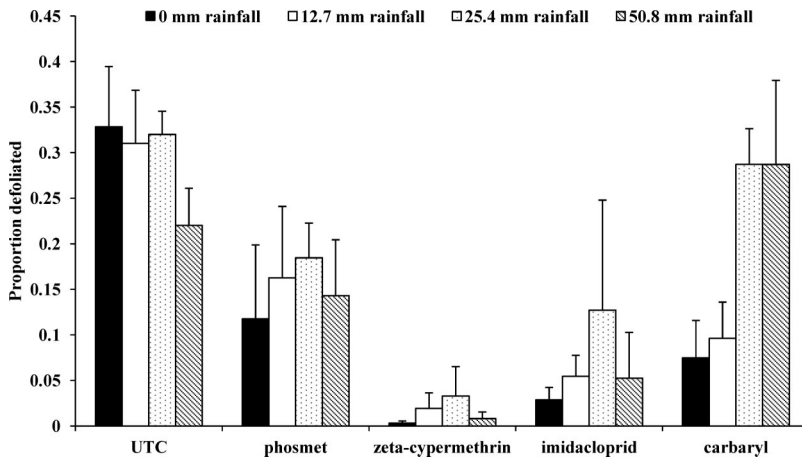


Fig. 2. Mean proportion (+SE) defoliation by Japanese beetles on blueberry foliage treated with four insecticides and at each level of rainfall tested.

**Effect of Rainfall.** For phosmet, there was not a significant effect for rain ( $F = 2.33$ ;  $df = 3, 36$ ;  $P = 0.0902$ ), time ( $F = 0.78$ ;  $df = 2, 48$ ;  $P = 0.4641$ ), or interaction ( $F = 2.08$ ;  $df = 6, 36$ ;  $P = 0.0837$ ).

For zeta-cypermethrin, there was not a significant effect of rain ( $F = 0.28$ ;  $df = 3, 36$ ;  $P = 0.8421$ ), time ( $F = 0.07$ ;  $df = 2, 36$ ;  $P = 0.9291$ ), but the interaction of the effect of rain and time was significant ( $F = 2.75$ ;  $df = 6, 36$ ;  $P = 0.0263$ ). The slice of beetle condition when 50.8 mm of rain was simulated was significant ( $F = 5.58$ ;  $df = 2, 36$ ;  $P = 0.0077$ ). Specifically, 1 h was significantly higher than 24 h ( $t = 2.72$ ;  $df = 36$ ;  $P = 0.0100$ ) and 24 h was significantly lower than 48 h ( $t = -2.46$ ;  $df = 36$ ;  $P = 0.0188$ ). The slice of beetle condition at 24 h across rainfall was significant ( $F = 4.76$ ;  $df = 3, 36$ ;  $P = 0.0068$ ). Specifically, 0 mm ( $t = 2.76$ ;  $df = 36$ ;  $P = 0.0090$ ), 12.7 ( $t = 2.53$ ;  $df = 36$ ;  $P = 0.0159$ ), and 24.5 mm ( $t = 2.85$ ;  $df = 36$ ;  $P = 0.0072$ ) of rain were all significantly higher than 50.8 mm of rain.

For imidacloprid, there was not a significant effect of rain ( $F = 0.77$ ;  $df = 3, 36$ ;  $P = 0.4877$ ), time ( $F = 0.08$ ;  $df = 2, 36$ ;  $P = 0.9196$ ), or the interaction of rain and time ( $F = 0.09$ ;  $df = 6, 36$ ;  $P = 0.9906$ ). For carbaryl, there was not a significant effect of rain ( $F = 2.66$ ;  $df = 3, 36$ ;  $P = 0.0626$ ), time ( $F = 0.23$ ;  $df = 2, 36$ ;  $P = 0.7964$ ), or the interaction of rain and time ( $F = 0.63$ ;  $df = 6, 36$ ;  $P = 0.7021$ ). For the untreated control, there was not a significant effect of rain ( $F = 0.83$ ;  $df = 3, 36$ ;  $P = 0.4866$ ), time ( $F = 0.00$ ;  $df = 2, 36$ ;  $P = 0.9996$ ), or interaction of rain and time ( $F = 0.12$ ;  $df = 6, 36$ ;  $P = 0.9929$ ) (Fig. 1).

Defoliation of blueberry leaves was also used to assess the toxicity of insecticides after they received rainfall. No significant difference was found across different rainfall levels in the level of defoliation in bioassays treated with phosmet ( $F = 0.35$ ;  $df = 3, 12$ ;  $P = 0.79$ ), zeta-cypermethrin ( $F = 0.26$ ;  $df = 3, 12$ ;  $P = 0.85$ ), imidacloprid ( $F = 0.19$ ;  $df = 3, 12$ ;  $P = 0.90$ ), or carbaryl ( $F = 2.49$ ;  $df = 3, 12$ ;  $P = 0.11$ ) (Fig. 2).

Defoliation was also compared across insecticide treatment at each rainfall level. There was a significant difference in proportion of leaf area defoliated at 0 mm of rain ( $F = 8.05$ ;  $df = 4, 15$ ;  $P = 0.0011$ ) with all insecticide treated bioassays having significantly lower proportions of defoliation than the untreated control. There was a significant difference in proportion of leaf area defoliated at 12.7 mm of rain ( $F = 6.56$ ;  $df = 4, 15$ ;  $P = 0.0029$ ) with zeta-cypermethrin and imidacloprid causing a significantly lower proportion of defoliation than in the untreated control. There was a significant difference in proportion of leaf area defoliated at 25.4 mm of rain ( $F = 3.84$ ;  $df = 4, 15$ ;  $P = 0.0241$ ) with zeta-cypermethrin causing a significantly lower proportion of defoliation than in the untreated control. There was a significant difference in proportion of leaf area defoliated at 50.8 mm of rain across insecticide treatments ( $F = 5.08$ ;  $df = 4, 15$ ;  $P = 0.0866$ ) with zeta-cypermethrin causing significantly lower defoliation than the untreated control (Fig. 2).

## Discussion

This study increases the understanding of the rainfastness of insecticides used to control Japanese beetles as well as the inherent efficacy and field residual activity of the compounds. Based on our analyses of the beetle conditions from bioassay chambers with freshly applied insecticides and no rain, the four tested insecticides showed evidence of having similar levels of toxicity (Fig. 1) all of which provided significant levels of plant protection.

None of the insecticides were significantly different in terms of the Japanese beetle physical condition they caused in the bioassays. Because of this, we looked at the proportion of the leaves defoliated to determine zeta-cypermethrin was the most effective insecticide tested in this study. At almost every level of rainfall, zeta-cypermethrin-treated shoots had significantly less defoliation than the other insecticides. The pro-

portion of defoliation caused by Japanese beetles did not change as rainfall increased for any of the insecticides, suggesting that this quality of performance is relatively unaffected by rainfall, but further investigation with greater statistical power is needed to draw further conclusions.

A physical attribute of pesticides that can affect their rainfastness is the octanol-water partitioning coefficient  $K_{ow}$ , which is defined as the ratio of a chemical's concentration in an octanol solution ( $[C_i]_{octanol}$ ) over its concentration in aqueous solution ( $[C_i]_{water}$ ) (Leo et al. 1971)

$$K_{ow} = \frac{[C_i]_{octanol}}{[C_i]_{water}}. \quad [1]$$

$K_{ow}$  varies from  $\approx 10^{-3}$  to  $10^7$  and is usually expressed as  $\log(K_{ow}) = P_{ow}$ . Compounds with lower  $P_{ow}$  are polar and have higher solubility in water and are hydrophilic. Compounds with higher  $P_{ow}$  are non-polar and have low water solubility and are considered to be lipophilic (Ragnarsdottir, 2000). The  $P_{ow}$  for phosmet, zeta-cypermethrin, imidacloprid, and carbaryl are 2.83, 4.47, 0.57, and 2.34, respectively (Chiou et al. 1977, Elbert et al. 1991, Noble 1993).

In a previous experiment, the insecticides phosmet and carbaryl were tested on Japanese beetles under identical rainfall conditions, but on grape (*Vitis labrusca* L.) leaves (Hulbert et al. 2011) and in this experiment we observed similar efficacy patterns. The differences observed between the two studies are likely because of differences between in the physical characteristics of grape and blueberry leaves.

There have been several studies done dealing with how rainfall affects insecticides. They have dealt with the practical aspect of the loss of efficacy because of rainfall. Rainfall has been found to significantly reduce the efficacy of foliar applied bifenthrin to mosquitoes (Allan et al. 2009) and a rainfall significantly reduced the efficacy of bifenthrin against Japanese beetles (*Popillia japonica* Newman) on grapes when the residues had been aged in the field (Hulbert et al. 2011). The index of persistence (the duration above which the insecticide kills <50% of larvae) of cypermethrin on cotton bollworm *Helicoverpa armigera* Hübner in cotton was significantly decreased because of rain after insecticide application (Brévault et al. 2009).

Other studies have dealt with the implication of insecticide runoff and the potential for damage to the environment. Sampling in the Choptank watershed on the Delmarva Peninsula showed that there were higher levels of insecticides detected in years with higher rainfall likely because of their more frequent application (compared with herbicides) and their physical properties (Goel et al. 2005). A recent investigation into the runoff of pyrethroids in urban environments showed that pyrethroid runoff is greatest when rainfall immediately after an application event (Jiang et al. 2012). In a study that sampled a drainage basin in northeastern Greece found that carbaryl, phosmet, and cypermethrin were frequently detected at high levels (Vryzas et al. 2011) indicating a risk of

these insecticides because of runoff, possibly because of rainfall. Imidacloprid, when applied to turf and concrete has been shown to wash off and pose a risk to aquatic vertebrates (Thuyet et al. 2012).

The results of this study will help blueberry growers and their crop protection advisors make informed decisions on the application and reapplication of insecticides before and after rainfall events. As part of an IPM decision making process, the inherent toxicity of insecticides and the likely behavior of their residues after rain are important to consider. Ultimately, this study will help growers plan insecticide use and react to rainfall to decrease unwarranted insecticide reapplication and improve agricultural efficiency. We have developed a "rainfastness decision chart" as a practical research-based guide for blueberry growers to use in their IPM program so that unnecessary pesticide sprays can be eliminated and increase the efficiency and sustainability of domestic fruit production (Wise et al. 2010).

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