RAINFASTNESS AND LEACHING OF INSECTICIDES USED IN BLUEBERRY PRODUCTION

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ABSTRACT

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USED IN BLUEBERRY PRODUCTION

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Blueberries are an economically important crop for Michigan with several substantial insect pests. Industry standards put considerable pressure on blueberry growers to maintain insect free berries at the time of harvest. Because of this, insecticides are a common method for their control. Michigan also receives on average about 50 to 100 mm of rain per month during the blueberry growing season which causes a risk of insecticide wash-off and leaching into the environment. Field-based bioassays were used to determine the efficacy of insecticides to control Japanese beetles (*Popillia japonica* Newman) and cranberry fruitworm (*Acrobasis vaccinii* Riley) after receiving simulated rain fall. Soil columns were also collected in order to measure leaching of some of these insecticides. None of the insecticides used to control cranberry fruitworm decreased in efficacy after receiving rainfall. Zeta-cypermethrin and imidacloprid were the most resistant to wash off of the insecticides used to control Japanese beetle. In the leaching experiment, carbaryl was the only insecticide to leach into the lower levels of soil at a concentration high enough to cause significant toxic effects on redworms (*Eisenia feoetida* Savigny) which were used to determine toxicity of the insecticides at different soil depths. The results of these experiments will ultimately help blueberry growers increase the efficiency of their insecticide use and make better decisions in regards to their insecticide use and their likely to cause contamination.

Dedicated to Jean Hulbert and David Scheer

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CHAPTER 1: INTRODUCTION

The invention and application of pesticides has had profound impact on civilization. Through aggressive use of pesticides, crop loss to pests can be greatly diminished. Intense pesticide use has also had negative effects on the natural environment. Pesticides have simultaneously increased the number of humans that the earth is able to support and increased the risk of environmental contamination. The value of agriculture and the pressure on modern agriculture to produce makes pesticides an attractive tool. Given the pest pressures, such as the introduction of invasive species and demand for clean fruit at harvest, it is likely that growers will rely on pesticide applications for the foreseeable future.

Rainfall shortly following pesticide application is an uncontrollable condition which can make pesticide use more risky for the growers and the general public. Growers worry that rainfall would cause the insecticide residues to wash off the plants so that pest protection is lessened. This can cause the grower to reapply a pesticide to minimize the risk of lost efficacy, regardless of whether or not it is needed. Agricultural runoff is an issue of public concern which can have damaging effects for people, the environment and the economy (Pimentel and Edwards 1982, Pimentel et al. 1992).

Pesticides have different physical properties which cause them to wash off of crops and leach through soils at different rates. For example, pesticides which are highly lipophilic have a tendency to bind to the waxy cuticle and thus be less likely to wash off. The lipophilic property of these chemicals will also make them less mobile in soil decreasing the risk of contamination. Research to date on the impact of precipitation on insecticides has primarily targeted older chemistries, such as organophosphates and carbamates, or is in the context of cotton and field crops (McDowell et al. 1984, Willis et al. 1992, 1994, 1996, Zhou et al. 1997). Thus, the information available for blueberry farmers about reapplication of insecticides after rainfall comes from either this limited research or “conventional wisdom”. Clearly there is merit in researching how different pesticides used under different circumstances wash off of crops and leach through different types of soils.

An increased use of pesticides is a subject of concern because around 1% of pesticides may actually reach the target organism with the rest reaching non target organisms or end up in other areas of the environment (PSAC 1965, Pimentel and Edwards 1982). Older insecticides presented much greater risks of contamination because of their stability in the environment. Older organochlorines such as DDT and dieldrin are far more persistent in the environment than current conventional insecticides such as organophosphates such as parathion and carbamates such as carbaryl (Eichelberger and Lichtenberg 1971). Modern insecticides are likely to degrade over days and weeks, whereas older compounds may persist in the environment long enough to affect years into the future. Insecticides pose the greatest risk if they do not readily degrade in soil and do not bind to the soil. These insecticides may eventually reach ground water or other larger bodies of water.

Blueberries are an economically important crop for the United States and Michigan (“USDA-NASS Quick Stats (Chem)” 2010) (Figure 1.1). There is a zero tolerance standard for insect contamination at pack-out for much of the food industry which places considerable management pressure on growers. Growers are most likely to use insecticides to control major pests of blueberries such as the Japanese beetle (*Popillia japonica* Newman) (Szendrei and Isaacs 2006, Wise et al. 2007). The cranberry fruitworm (*Acrobasis vaccinii* Riley) is another direct pest of the blueberry industry for which insecticides are an effective control (Murray 1990, Wise et al. 2010). The phenology of the cranberry fruitworm and Japanese beetle show they emerge at times when considerable amounts of rainfall is likely in Michigan (Figures 1.2-1.4). It is during these periods of high emergence that insecticide use against these insects would be the most likely. Therefore, it is likely that a rainfall event would take place shortly following insecticide application.

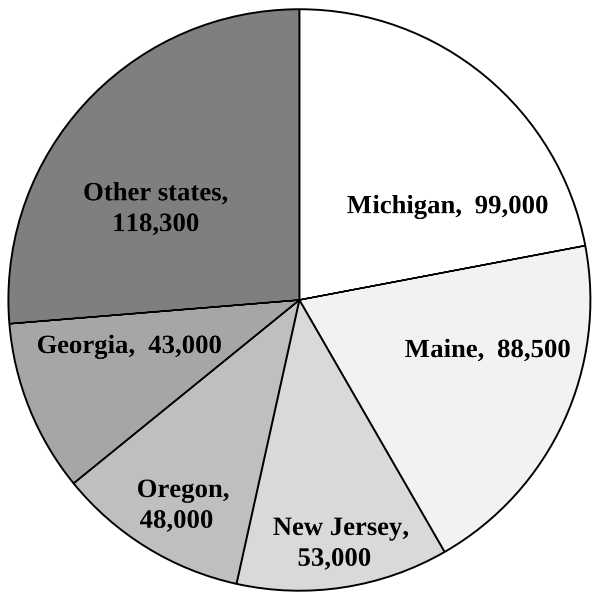

Figure 1.1 Total utilized U.S. blueberry production for 2009. Numbers are in thousands of pounds. ("NASS - National Agricultural Statistics Service" 2011).

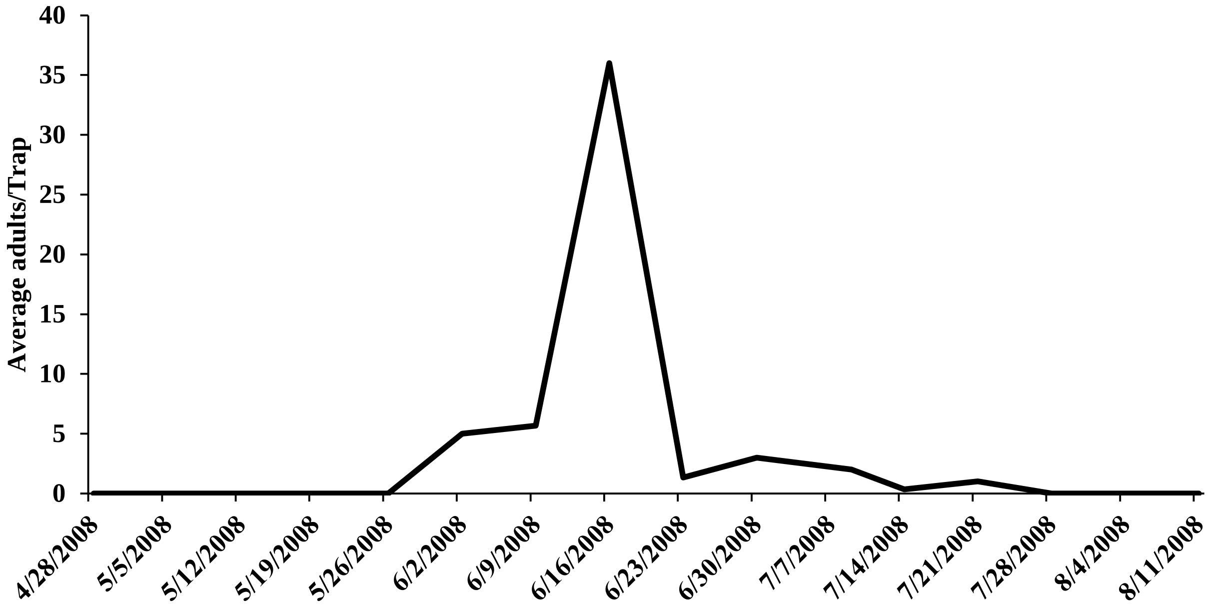

Figure 1.2 2008 pheromone trapline data for *A. vaccinii* adults at the Trevor Nichols Research Center . ("Trevor Nichols Research Center | Michigan State University | Michigan Agricultural Experiment Station" 2011).

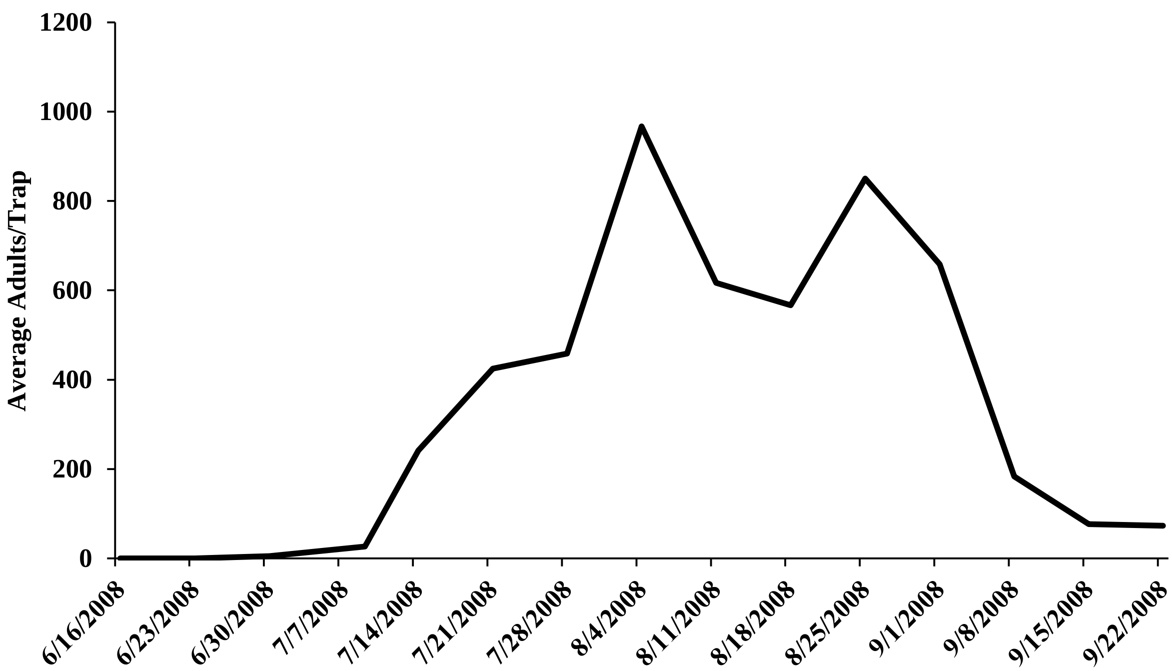

Figure 1.3 2008 pheromone trapline data for Japanese beetle adults at the Trevor Nichols Research Center ("Trevor Nichols Research Center | Michigan State University | Michigan Agricultural Experiment Station" 2011).

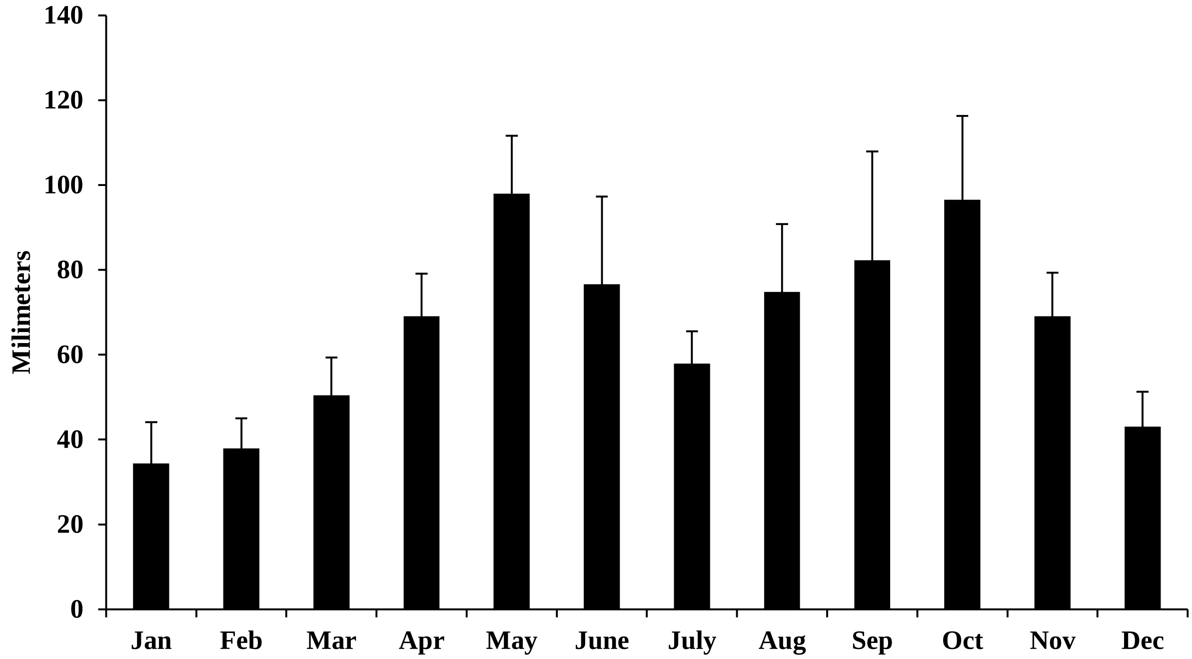

Figure 1.4 Average monthly precipitation from 2000 to 2009 at the Trevor Nichols Research Center ("Michigan Automated Weather Network" 2011).

The first major objective of this thesis was to examine how insecticides used control cranberry fruitworm (an early season pest) and Japanese beetle (a late season pest) perform as they are subjected to different rainfall conditions. The second major objective of this thesis was to examine the risk of leaching for a subset of these insecticides labeled for use in blueberries.

CHAPTER 2: RAINFASTNESS OF INSECTICIDES TO CONTROL CRANBERRY FRUITWORM IN BLUEBERRIES

Abstract

Field based bioassays and residue profile analysis were used to determine the relative toxicity and rainfastness of 6 insecticides from 6 different chemical classes on larval cranberry fruitworm, *A. vaccinii* Riley, in highbush blueberries, *Vaccinium corymbosum*. Bioassays assessed the larvae as alive or dead on the surface, and the fruit was evaluated for entry holes by the larvae. Bioassays were evaluated a week after set up. The most toxic compounds were found to be spinetoram and methoxyfenozide. The performances of the insecticides were not found to be significantly affected by rainfall in this study based on the fruit condition and mortality data. Insecticide residues on the surface and subsurface of blueberry leaves and fruit were quantified to determine the impact of rainfall on insecticide residues. 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 by increasing insecticide efficiency in blueberry production.

Introduction

In eastern North America, the cranberry fruitworm, *Acrobasis vaccinii* Riley, is a native pest of several species of the genus *Vaccinium* including cranberries and blueberries. As a native insect of North America there are a variety of natural agents which control *A. vaccinii* in less disturbed environments, but modern agricultural practices may have a role in serious infestations by this pest (Murray 1990). *A. vaccinii* are univoltine and in Michigan the adults generally fly from the end of May the end of July. Females oviposit in the newly exposed calyx soon after petal fall, which may occur in early to mid-June in Michigan (Franklin 1948). *A. vaccinii* hatch 4 to 5 days after oviposition and the newly hatched larvae will crawl on the berry for a period of time before penetrating the fruit near the stem (Hutchinson 1954). The best time to apply a controlling agent, such as an insecticidal spray, is between oviposition and fruit penetration, when the larvae are most vulnerable.

A. vaccinii is a relatively serious pest of blueberry production in part because it feeds directly on the fruit. In untreated fields, up to 50% of a blueberry crop can be lost to due to *A. vaccinii* damage (Murray 1990). Additionally, detection of *A. vaccinii* larvae in a harvested crop can cause buyers to reject the entire load of blueberries. Therefore, *A. vaccinii* is damaging to production of blueberries both as a direct pest to blueberry fruit and as a contaminant pest. Seventeen percent of Michigan blueberry growers from a 2006 survey rated *A. vaccinii* as at least as important as any other blueberry pest if not more important (Szendrei and Isaacs 2006). Because of the high pressure *A. vaccinii* places on blueberry growers, application of insecticide sprays is the preferred method of control. In 2007 azinphos-methyl was applied to 72.9% of Michigan blueberry acreage (Wise et al. 2010). While azimphosmethyl is being phased out by the EPA, there are several other insecticides registered for use on blueberries which have shown promise in controlling *A. vaccinii* larvae (Wise et al. 2010).

Michigan is the leading producer of higbush the blueberries in the United States. In 2006, Michigan produced more than 23.6 million kg which was valued at about $140 million (New Jersey Agricultural Statistics Service 2007). Because of the importance of blueberries to

Michigan economy and impact *A. vaccinii* are capable of inflicting on blueberry production, its effective control is crucial.

In light of insecticides being the preferred method of control for *A. vaccinii*, blueberry growers face considerable production challenges associated with their use. One challenge to growers who apply insecticides is the washoff of these materials due to rainfall. Michigan receives on average about 50 to 100 mm of rain per month during the blueberry growing season ("Michigan Automated Weather Network" 2011), which has important implications for the fate of insecticides sprayed. Overestimation of wash off can cause unwarranted reapplications of insecticides and underestimation may result in crop loss. Insecticide wash off also has important environmental and public safety implications (Pimentel et al. 1992). Research on the behavior of insecticides in cotton and field crops under rainfall conditions has been done primarily on older conventional insecticides such as the organophosphates and carbamates (McDowell et al. 1984, Willis et al. 1992, 1994, 1996, Zhou et al. 1997). Most of the information used by farmers about reapplication of insecticides after rainfall comes from either this limited research or "conventional wisdom." Recent studies in apples have shown that applications of organophosphate insecticides such as azinpho-smethyl result in primarily surface residues on the fruit and foliage (Wise et al. 2006).

Many of the newer insecticides have chemical properties different from the organophosphates and carbamates, and have not been studied under varying rainfall conditions. Newer and reduced risk insecticides have chemical properties which may result either in stronger bonding in the plant cuticle or translaminar movement into the tissue. Studying the behavior of newer reduced risk insecticides under rainfall conditions could decrease unnecessary insecticide reapplication and the associated costs and risks.

A. vaccinii is a serious pest to the blueberry industry in eastern North America and there is a limited time in which insecticide sprays are the most effective. There are also a variety of environmental factors which influence insecticide performance. Therefore, information on how the performance of a variety of insecticides is affected by field conditions would be important to blueberry growers and extension agents. This study was designed to evaluate the effects of rainfall on the efficacy of insecticides used to control *A. vaccinii*. The objectives were to: 1) determine the effect of rainfall on the efficacy against *A. vaccinii* of 6 different insecticides representing 6 different classes of insecticides, and 2) compare the relative performance of these insecticides after rainfall.

Methods and Materials

Insects

Cranberry fruitworm (*A. vaccinii*) eggs were collected at an unmaintained blueberry field in Douglas, MI (+42°37'48.16'', -86°12'13.87'') during June 2010. Eggs were collected by scouting for blueberries in which *A. vaccinii* eggs had been laid. Infested blueberries were brought back to the laboratory and kept in a refrigerator for up to several days in order to slow development of the embryo until bioassay set up.

Field Plots and Treatment Applications

Each field plot consisted of a single mature blueberry bush and there were four replicate plots for each of the six treatments and one control. A minimum of one mature blueberry bush separated each sprayed bush. 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 2.1). Insecticide applications were made on 4 June 2010. These plots served as the source of foliage for use in bioassays and residue analysis. Untreated control (UTC) plots were not sprayed. Daily high and low temperatures and precipitation volumes were recorded with an automated weather station ("Michigan Automated Weather Network" 2011) located within 5 km of the field plots.

Table 2.1 Formulated compounds, field rates and concentrations used for bioassay experiments and residue analysis. All preparations were based on 468 L/Ha spray volume (50 gallons/ acre).

Formulated name	**Chemical class**	**Active ingredient**	**Rate /acre**	**g AI / Ha**	**ppm**	**Company**
Guthion® 50 WP	organophosphate	azinphos-methyl	1.25 lbs	700.23	1497.83	Bayer CropScience, Pittsburgh, PA
Asana® 0.66 EC	pyrethroid	esfenvalerate	8 oz	47.06	100.65	DuPont, Wilmington, DE
Intrepid ®2 F	Insect growth regulator	methoxyfenozide	12 oz	189.9	406.21	Dow AgroSciences LLC, Indianapolis, IN
Delegate®25 WG	spinosyn	spinetoram	4 oz	70.02	149.78	Dow AgroSciences LLC, Indianapolis, IN
Assail® 30 WG	neonicotinoid	acetamiprid	5.2 oz	182.06	389.44	UPI, PA
Altacor® 35 WG	diamide	chlorantraniliprole	3 oz	73.52	157.27	Dupont, Wilmington, DE

Bioassays

Bioassays were used to compare the toxicity of the four insecticides and the impact of rainfall on their performance. Shoots from the blueberry bushes containing a cluster of berries were collected from the field plot 24 h after application.

Shoots were then randomly selected for exposure to different simulated rainfall regimes. Shoots were placed in water-soaked OASIS floral foam bricks (Smithers-Oasis Co., Kent, OH) which were placed in the Generation 3 Research Track Sprayer (DeVries Manufacturing, Hollandale, MN). Shoots received 0, 12.7 or 25.4 mm of simulated rain. Three rain gauges were placed around the inside of the Generation 3 Research Track Sprayer to accurately assess the amount and uniformity of simulated rain treatments. Shoots not assigned to receive rainfall were held out of direct sunlight under ambient conditions.

From each blueberry shoot, a single blueberry was selected and placed in water soaked OASIS floral foam in a small plastic cup with a lid making the completed bioassay chamber. 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 the bioassay chambers were assembled, an *A. vaccinii* egg was placed in them. Using a razor blade, one *A. vaccinii* egg and a sliver of fruit were shaved off and placed in the calyx of the berry in the bioassay chamber. The bioassay chambers were held in the laboratory at 21°C and a photoperiod of 16:8 (L:D) h. There were 8 replicates for each insecticide and rainfall combination.

The bioassay chambers were evaluated 7 d after set up. The fruit was inspected for larval entry holes and then cut open with a razor blade to determine if a living larva was present inside. The fruit was also inspected for dead larvae on the surface of the fruit.

The mean proportions of fruit and larvae condition were compared across insecticide treatment by an analysis of variance (ANOVA) on arcsine square root transformed values. Mean separation was done using Tukey's honestly significant difference (HSD) test. These data were analyzed by also analyzed by logistic regression to determine an whether rainfall significantly affected the number of alive larvae, dead larvae, and entries into the fruit. These analyses were conducted in R version 2.12.1 (R Development Core Team 2010).

Insecticide Residue Analysis

A parallel series of foliage samples were taken from field plots 24 h post application time and received the same rainfall regimen as the shoots used in the bioassays (0, 12.7 or 25.4 mm). There were three replicates for each treatment at both of the post application time intervals.

To determine the amount of residue on the leaf surfaces, 10-g samples of blueberry fruit were placed in 150 ml of high-performance liquid chromatography (HPLC)-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ) and sonicated for 10-15 s. The acetonitrile was decanted through 5 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water. The sample was dried via rotary evaporation and brought up in acetonitrile for HPLC analysis. The remaining fruit samples were ground in 50 ml of HPLC grade dichloromethane (Burdick & Jackson, Muskegon, MI). The extracts were passed through 5 g of anhydrous sodium sulfate. The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45-µm Acrodisc 13-mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for insecticide residue with a Waters 2690 Separator Module HPLC equipped with a Waters 2487 Dual Wavelength Absorbance Detector (Waters, Milford, MA) set at 270 nm, and a C18 reversed phase column (150 by 4.6mm bore, 5 µm particle size, Restek, Bellefonte, PA) (Bayer 1998). The mobile phase was water/ acetonitrile (80:20) at 55°C. The HPLC level of quantification was 0.457 µg/g (ppm) of active ingredient, and level of detection was 0.138 ppm.

Results

Bioassays

When no rainfall was simulated, there was a significant difference in the number of entries by *A. vaccinii* larvae across treatments ($F = 2.33$; df = 6, 49; $P = 0.046$). Specifically, there were significantly fewer larval entries on fruit treated with spinetoram than on untreated fruit (Figure 2.1).

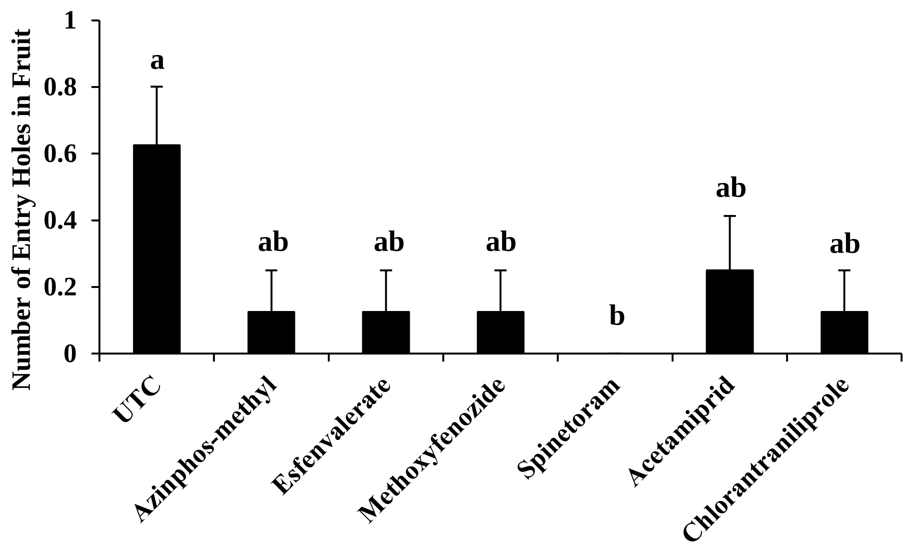

Figure 2.1 Mean proportion (+ SE) of fruit with entry holes by *A. vaccinii* larvae. Bars with different letters over them are significantly different from each other ($P < 0.05$). Data were arcsine square-root proportion-transformed before ANOVA. Mean separation calculated using the Tukey's honestly significant difference (HSD) test. Data shown are nontransformed means.

The result for the ANOVA in which the number of living *A. vaccinii* larva was recovered from the fruit when no rainfall was simulated was: $F = 2.02$; df = 6, 49; $P = 0.08$.

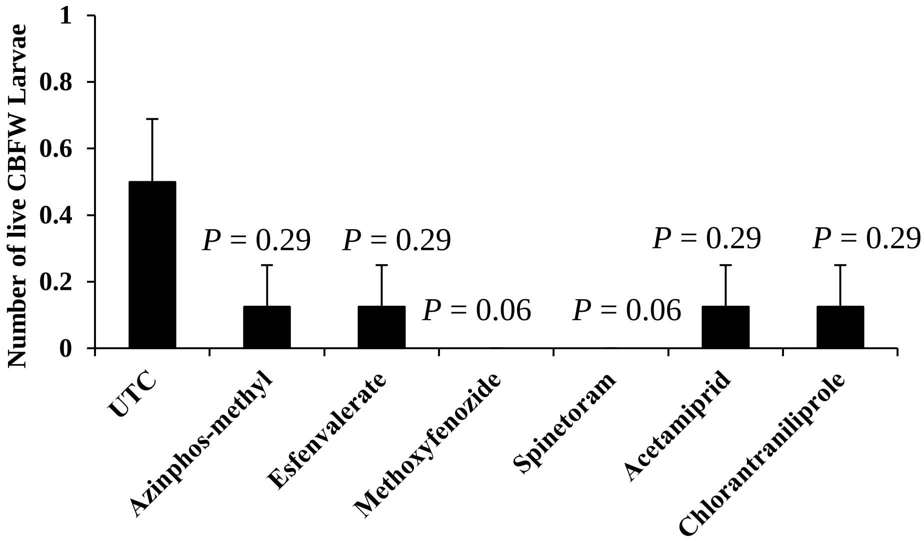

Figure 2.2 Mean proportion (+ SE) of live *A. vaccinii* larvae. The *P*-value associated with the comparison to the UTC is above each treatment bar. Data were arcsine square-root proportion-transformed before ANOVA. Mean separation calculated using the Tukey's honestly significant difference (HSD) test. Data shown are nontransformed means.

No significant difference was found between the number of dead larvae on the surface of the fruit between insecticide treatments when no rainfall was simulated ($F = 1.83$; df = 6, 49; $P = 0.11$) (Figure 2.3).

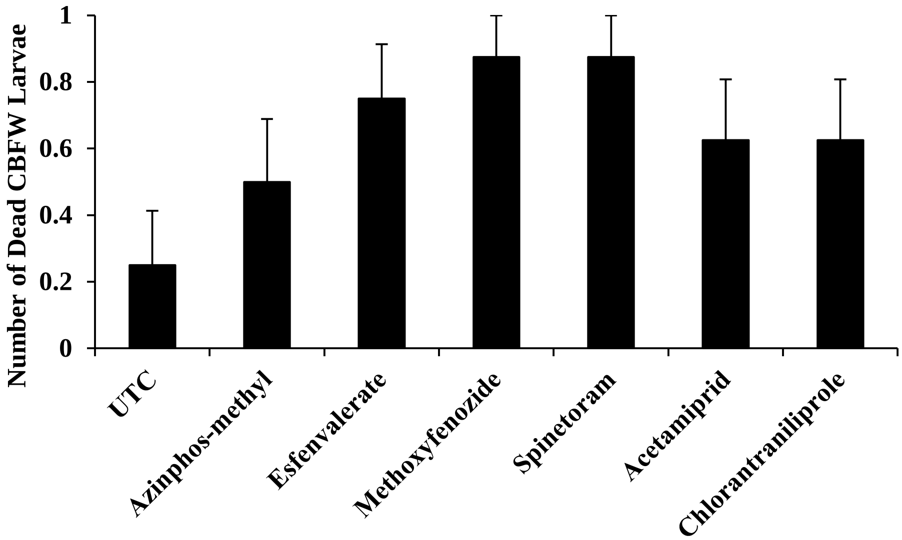

Figure 2.3 Mean proportion (+ SE) of observed dead *A. vaccinii* larvae on the surface of the fruit. Data were arcsine square-root proportion-transformed before ANOVA. There were no significant differences found at $P < 0.10$. Data shown are nontransformed means.

For the insecticides azinphos-methyl, esfenfvalerate, methoxyfenozide, spinetoram, acetamiprid, and chlorantraniliprole, there was no evidence that the number of CBFW larva alive in bioassays was significantly affected as rainfall increased (Table 2.2). There was also no evidence that the number of entries into the fruit by CBFW larva was significantly affected as rainfall increased in azinphos-methyl, esfenfvalerate, methoxyfenozide, spinetoram, acetamiprid, and chlorantraniliprole bioassays (Table 2.2). Additionally, there was no evidence that the number of CBFW larva found dead in azinphos-methyl, esfenfvalerate, methoxyfenozide, spinetoram, acetamiprid, and chlorantraniliprole bioassays was significantly affected as rainfall increased (Table 2.2).

Table 2.2 Results of the logistic regression analyses for the response variable of whether or not the larva in the bioassay was alive (Alive), whether or not there was an entry in the fruit in the bioassay (Entry), and whether or not a larva is dead in the bioassay (Dead).

Insecticide	Response	Slope (+ SE)	*z*- value	Pr(>|z|)
azinphos-methyl	Alive	0.04946 (+ 0.04583)	1.079	0.2805
	Entry	0.04946 (+ 0.04583)	1.079	0.2805
	Dead	2.536e-18 (+ 3.951e-02)	0	1
esfenvalerate	Alive	-0.07171 (+ 0.08381)	-0.856	0.3922
	Entry	0.05513 (+ 0.04896)	1.126	0.2601
	Dead	-0.04566 (+ 0.04372)	-1.044	0.2963
methoxyfenoxide	Alive	-5.445e-17 (+ 4.252e+03)	0	1
	Entry	2.516e-18 (+ 4.546e-02)	0	1
	Dead	-2.133e-17 (+ 4.546e-02)	0	1
spinetoram	Alive	-3.830e-18 (+ 5.952e-02)	0	1
	Entry	0.06398 (+ 0.05385)	1.188	0.2348
	Dead	-0.04946 (+ 0.04583)	-1.079	0.2805
acetamiprid	Alive	-1.439 (+ 414.182)	-0.003	0.9972
	Entry	-1.5036 (+ 409.8822)	-0.004	0.997
	Dead	0.06398 (+ 0.05385)	1.188	0.235
chlorantraniliprole	Alive	-1.439 (+ 414.182)	-0.003	0.9972
	Entry	-1.439 (+ 414.182)	-0.003	0.9972
	Dead	1.432e-17 (+ 4.331e-02)	0	1

Residue Analysis

The surface residues of azinphos-methyl decreased by approximately 75% when comparing residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). The subsurface residues of azinphos-methyl decreased by approximately 65% when comparing residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). The surface residues of esfenvalerate decreased by approximately 65% when comparing residues on the shoots receiving no rain to the shoots receiving 25.4 mm of rain (Table 2.3). The subsurface residues of esfenvalerate decreased by approximately 40% when comparing the residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). The surface residues of methoxyfenozide decreased by approximately 10% when comparing residues on the shoots receiving no rain to the shoots receiving 25.4 mm of rain (Table 2.3). There was little evidence for wash-off of subsurface methoxyfenozide residues (Table 2.3). The surface residues of spinetoram decreased by approximately 50% when comparing residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). The subsurface residues of spinetoram decreased by approximately 25% when comparing the residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). The surface residues of acetamiprid decreased by approximately 50% when comparing residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 3). There was not a pattern of wash-off for subsurface acetamiprid residues (Table 2.3). The surface residues of chloroantraniliprole decreased by approximately 50% when comparing residues on shoots receiving no rain to residues on shoots receiving 25.4 mm of rain (Table 2.3). There was no pattern of wash-off for subsurface chlorantraniliprole residues (Table 2.3).

Table 2.3 Insecticide residues recovered from blueberry fruit after 24 h field aging across rainfall level and insecticide treatment. Residues are measured in mean micrograms per gram (ppm) of active ingredient per fruit.

	Simulated Rainfall					
	0 mm		12.7 mm		25.4 mm	
Insecticide	surface	subsurface	surface	subsurface	surface	subsurface
Azinphos methyl	2.19	0.17	0.99	0.12	0.54	0.06
Esfenvalerate	0.23	0.16	0.12	0.10	0.09	0.09
Methoxyfenozide	0.43	0.03	0.36	0.02	0.39	0.03
Spinetoram	0.34	0.01	0.20	0.01	0.18	0.01
Acetamiprid	0.22	0.09	0.17	0.12	0.08	0.09
Chlorantraniliprole	0.49	0.05	0.31	0.04	0.28	0.06

Discussion

Bluebrerry protection against *A. vaccinii* has been historically dependent on spraying broad spectrum insecticides such as azinphos-methyl. As this insecticide is phased out by the US EPA, blueberry growers have begun to use specific insecticides. Understanding how these new chemicals perform under real-world field conditions, such as rainfall, is important information for blueberry production. This particular study was restricted to control of *A. vaccinii* at the first instar of the larval stage, whereas many of these materials are active on other life-stages as well (Wise et al. 2010).

In this experiment, the insecticides methoxyfenozide and spinetoram were the most toxic to the first instar larvae. This is based on the result of no living larva being recovered from the bioassay chambers containing fruit sprayed with these compounds (Figure 2.1) and no larval entries in these bioassay chambers (Figure 2.2). We did not find evidence that the other insecticides were significantly more toxic than the untreated control. However, with greater number of replicates, the other insecticides would likely have been found to be significantly toxic compared to the untreated control. Part of this problem may be due to, in part, to the relatively low level of robustness exhibited by the eggs and first instar larvae in the laboratory. This can be seen by the relatively low levels of living *A. vaccinii* larvae recovered from untreated bioassays (Figure 2.1, Figure 2.2). There is evidence for the toxicity of some of these insecticides to the first instar stage as well as other life stages in the literature (Wise et al. 2010).

The performance of these insecticides was not affected by rainfall (Table 2.2). The resistance of an insecticide to rainfall wash-off is governed by several factors including the amount sprayed, its partitioning coefficient, and the physical properties of the plant onto which the insecticide is sprayed. It is possible the resistance to rainfall these insecticides exhibited was masked by the weakness of the larvae in the lab, which is why residue data were obtained. The residue data from the fruit were intended to support the bioassay data.

A physical attribute of pesticides which 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)

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

K_{ow} varies from approximately 10^{-3} to 10^{7} and is usually expressed as $\log(K_{\mathrm{ow}}) = P_{\mathrm{ow}}$. Compounds with lower P_{ow} are polar and have high solubility in water. Compounds with higher P_{ow} are non-polar and have low water solubility and are lipophilic (Ragnarsdottir 2000). The octanol-water partitioning coefficient can also play an important role in insecticide toxicity. An insecticide with a higher P_{ow} would be able to penetrate the insect cuticle quicker and may have relatively fast action.

The P_{ow} of azinphos-methyl is 2.69 (Noble 1993) meaning that azinphos-methyl has relatively low water solubility and is somewhat lipophilic. In the residue analysis, azinphos-methyl was found to be mostly on the surface of the fruit and mostly surface, thus highly susceptible to wash-off when rainfall occurred (Table 2.3). Azinphos-methyl has been found to increase in rainfastness as time after application increases (Willis et al. 1994) and had more than 24 h elapsed before rainfall was simulated, a smaller decrease in azinphos-methyl residues may have been found.

The P_{ow} of esfenvalerate is 6.3 which is relatively high, indicating that the insecticide is relatively lipophilic. This quality allows the insecticide to adhere to the fruit and penetrate the fruit cuticle. This was observed in our residue profile analysis (Table 2.3). Esfenvalerate is also known to be relatively immobile in the environment (Adelsbach and Tjeerdema 2003) suggesting its relative safety to other insecticides.

The P_{ow} of methoxyfenozide is 3.7 which indicates that the insecticide is relatively lipophilic, which is unsurprising given its mode of action as a molting hormone mimic. The majority of the insecticide was recovered from the surface of the fruit and little wash off was observed (Table 2.3). A low level of subsurface residues for methoxyfenozide is an observation which could have important implications for use against internal feeding pests such as *A. vaccinii* because it would not be very effective as a curative agent.

The P_{ow} of spinetoram is 4.2 indicating the insecticide is relatively lipophilic. The residue data indicate that most of the compound was recovered from the surface of the fruit and that is also where most of the wash-off occurred (Table 3). It is also worth noting that most of the wash-off of spinetoram occurred after the first 12.7 mm of rain was simulated (Table 2.3).

The P_{ow} of acetamiprid is 0.8 and it is systemic. This insecticide is very water soluble which is responsible for its low P_{ow} and its systemic nature. This is evident by the high levels of acetamiprid found in the subsurface layer of the fruit (Table 2.3). The subsurface acetamiprid residues were also resistant to rainfall which suggests that this is a good insecticide to use where rainfall is common during *A. vaccinii* larval emergence.

The P_{ow} of chlorantraniliprole is 2.86 meaning that chlorantraniliprole has relatively low water solubility and is lipophilic. The majority of chlorantraniliprole that was recovered in the residue profile portion of the experiment was from the surface. On the surface, chlorantraniliprole residues decreased most after 12.7 mm of rain (Table 2.3).

Because of the inconclusive bioassay data, alterations to the methods might yield more robust results. The aim would be to increase the health of the larvae initially exposed to the insecticide. This might be accomplished by placing newly hatched larvae on treated fruit instead of the eggs because the eggs are relatively fragile in laboratory experiments.

The results of this and similar studies increase the knowledge available to blueberry growers and will help them make more informed decisions when managing insect pests. Growers have at their disposal more information to make scientifically validated decisions with regards to the threat rain poses to insecticide residues. With this information part of an IPM strategy, growers will be able to more efficiently use the tools at their disposal. Specifically, the insecticides spinetoram and methoxyfenozide are the most effective against *A. vaccinii*.

CHAPTER 3: RAINFASTNESS OF INSECTICIDES TO CONTROL JAPANESE BEETLE IN BLUEBERRIES

Abstract

Field-based bioassays and residue profile analysis were used to determine the relative toxicity and rainfastness of 4 insecticides from 4 different chemical classes of insecticides on adult Japanese beetles, *Popillia japonica* Newman, in highbush blueberries, *Vaccinium* spp. Bioassays assessed Japanese beetle condition as alive, knockdown or immobile when exposed for 24 h to 48 h field aged residues of phosmet, carbaryl, zeta-cypermethrin, and imidacloprid. All insecticides were significantly more toxic than the untreated control and zeta-cypermethrin was the most toxic insecticide under no-rainfall conditions. All insecticides experienced a decrease in efficacy after rainfall was simulated, although the efficacy of zeta-cypermethrin was the least affected by rainfall. Insecticide residues on the surface and subsurface of blueberry leaves and fruit were quantified to gain a better understanding of how the insecticide residues are 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.

Introduction

In eastern and central North America, the Japanese beetle, *Popillia japonica* Newman, is an invasive pest of ornamentals, turfgrass, fruits, and vegetables. Japanese beetles were first discovered in the United States 1916 in New Jersey and are now a major pest in the eastern United States causing $450 million in damage to ornamental plants and turf (Fleming 1976, Potter and Held 2002). During the periods of adult emergence, June to September in Michigan, Japanese beetles feed in aggregations on the foliage of their host plants. Japanese beetle adults feed on more than 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. Many commercial blueberry growers use mechanical over the row harvesters which are unable to effectively discriminate blueberries from Japanese beetles. Japanese beetles can cause considerable economic damage in fields where they are not controlled at the time of harvest. There is a zero tolerance standard for insect contamination at pack-out which places considerable pressure on growers.

There are a variety of ways in which Japanese beetle can be controlled in blueberries. Cultural practices such as tillage may be used to control Japanese beetles in blueberries (Szendrei et al. 2005). Entomopathogenic nematodes can be used to effectively manage Japanese beetle larvae in the soil (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. Although these important tools exist for Japanese beetle management, conventional insecticides are still the preferred method of control in blueberries. In Michigan, 63% of blueberry growers surveyed said that application of foliar insecticides was the most important Japanese control method (Szendrei and Isaacs 2006).

Michigan receives on average about 60 to 80 mm of rain per month during the period of the blueberry growing season when Japanese beetles are emerged ("Michigan Automated

Weather Network" 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 and underestimation may result in crop loss and environmental contamination (Pimentel et al. 1992). There has been research done on how some insecticides behave during rainfall, but this research has been done on older conventional insecticides such as organophosphates and carbamates (McDowell et al. 1984, Willis et al. 1992, 1994, 1996, Zhou et al. 1997). Recent studies in apples have shown that applications of organophosphate insecticides like azinphosmethyl result in primarily surface residues on the fruit and foliage (Wise et al. 2006, 2007). Therefore there is little scientific evidence for farmers to make insecticide application decisions in regards to rainfall on a range of crops and insecticides.

Many of the newer insecticides have chemical properties different from the organophosphates and carbamates and have not been studied under rainfall conditions. Newer reduced risk insecticides have chemical properties which may result either in stronger binding in the plant cuticle or translaminar movement into the tissue. These insecticides would be expected to be more rain-fast than older conventional insecticides. Studying the efficacy of newer and reduced risk insecticides under rainfall conditions could decrease unnecessary insecticide reapplication and the associated costs and risks.

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) Compare the inherent toxicity of these insecticides to the Japanese beetle against each other and 2) to determine the effect of rainfall on the efficacy against Japanese beetles of 4 different insecticides representing major chemical classes of insecticides.

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 lure (Great Lakes IPM Inc., Vestaburg, MI; 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 h (L:D). Healthy beetles exhibiting mobility on the foliage were used in the experiments.

Field Plots and Treatment Applications

Each field plot consisted of a single mature blueberry bush, four replicate plots for each of the four treatments and one control. A minimum of one mature blueberry bush separated each sprayed bush. 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 3.1). Insecticide applications were made on 7 July 2009. These plots served as the source of foliage for use in bioassays and residue analysis. Untreated control (UTC) plots were not sprayed. Daily high and low temperatures and precipitation volumes were recorded with an automated weather station (“Michigan Automated Weather Network” 2011) located within 1 km of the field plots.

Table 3.1 Formulated compounds, field rates and concentrations used for life-stage activity experiments. All preparations were based on 468 L/Ha spray volume (50 gallons/ acre).

Formulated name	Chemical Class	Active ingredient	Rate /acre	g AI / Ha	ppm	Company
Imidan® 70 WP	Organophosphate	phosmet	1.3 lbs	1043	2231	Gowan Company, Yuma, AZ
Sevin XLR® 4L	Carbamate	carbaryl	2 qt	4481	9586	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

Bioassays

Bioassays were used to compare the toxicity effects of the four insecticides and to determine the progression of these effects as the residues received rainfall. Shoots from the blueberry bushes of at least 10 leaves with clusters of fruit were collected from the field plot 24 h and 7d after application. Shoots were then randomly selected for exposure to different simulated rainfall regimes. Shoots were placed in water-soaked OASIS floral foam bricks (Smithers-Oasis Co., Kent, OH) which were placed in a Generation 3 Research Track Sprayer rainfall simulator (DeVries Manufacturing, Hollandale, MN). Shoots received 0, 12.7, 25.4 or 50.8 mm of simulated rain. 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 was pruned so that exactly 10 leaves remained on the shoot. 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 wax (Gulf Wax, distributed by Royal Oak Sales, Inc., Roswell, GA) to preserve the integrity of the plant tissue by reducing evaporation of water. 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, ten randomly 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 were four replicates for each treatment at each insecticide treatment and rainfall amount combination. 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 non-upright position at the bottom of the container. Beetles were counted as alive if they seemed to behave normally. The mean proportions of beetles in a particular condition were compared across insecticide treatment by an analysis of variance (ANOVA) on arcsine square root transformed values. Mean separation was done using Tukey's honestly significant difference (HSD) test. These data were analyzed by logistic regression to determine the time at which half of the organisms were in the immobile condition (LT_{50}) (Robertson et al. 2007). Logistic regression analyses were performed at every insecticide and rainfall combination. These analyses were conducted in R version 2.12.1 (R Development Core Team 2010) using the MASS library (Venables, W. N. & Ripley, B. D. 2002), the lmtest library (Achim Zeileis, Torsten Hothorn, 2002), and the doBy library (Søren Højsgaard, Ulrich Halekoh with contributions from Ulrich Halekoh, Jim Robison-Cox, Kevin Wright and Alessandro A. Leidi, 2011).

The proportion of the leaves defoliated by Japanese beetles was determined for each leaf used in the bioassay experiment. This was done by 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. 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 one-way ANOVAs comparing the proportion of leaves damaged for each insecticide across all rainfall treatments and comparing with the proportion of leaves eaten in the no rain controls.

Insecticide Residue Analysis

A parallel series of foliage and fruit samples were taken from field plots 24 h post application and received the same rainfall regimen as the shoots used in the bioassays (0, 12.7 25.4, or 50.8 mm). There were three replicates for each treatment at both of the post application time intervals for both leaves and fruit.

To determine the amount of residue on the leaf and fruit surfaces, 10-g samples of plant material were placed in 150 ml of high-performance liquid chromatography (HPLC)-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ) and sonicated for 10-15 s. The acetonitrile was decanted through 5 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water. The sample was dried via rotary evaporation and brought up in acetonitrile for HPLC analysis. The remaining leaf samples were ground in 50 ml of HPLC grade dichloromethane (Burdick & Jackson, Muskegon, MI). The extracts were passed through 5 g of anhydrous sodium sulfate. The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45-µm Acrodisc 13-mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for insecticide residue with a Waters 2690 Separator Module HPLC equipped with a Waters 2487 Dual Wavelength Absorbance Detector (Waters, Milford, MA) set at 270 nm, and a C18 reversed phase column (150 by 4.6mm bore, 5 µm particle size, Restek, Bellefonte, PA)(Bayer 1998). The mobile phase was water/acetonitrile (80:20) at 55°C. The HPLC level of quantification was 0.457 µg/g (ppm) of active ingredient, and level of detection was 0.138 ppm.

Results

Inherent Toxicity

Japanese beetles exposed to all 24 h field-aged insecticide residues for 48 h exhibited significantly lower numbers in the alive condition than beetles exposed to untreated blueberry foliage (F = 8.62; df = 4, 15; P > 0.001) (Figure 3.1). Japanese beetles exposed to all 24 h field-aged insecticide residues except imidacloprid exhibited significantly higher numbers of beetles in the immobile condition (F = 6.06; df = 4, 15; P < 0.004) (Figure 3.1). No significant difference was found between the numbers of beetles in the knockdown condition across treatments, (F = 0.28; df = 4, 15; P = 0.89) (Figure 3.1). Significantly less area of the blueberry leaves was defoliated in bioassays with leaves treated with each of the insecticides than in the untreated control (F = 8.05; df = 4, 15; P = 0.0011) (Figure 3.2).

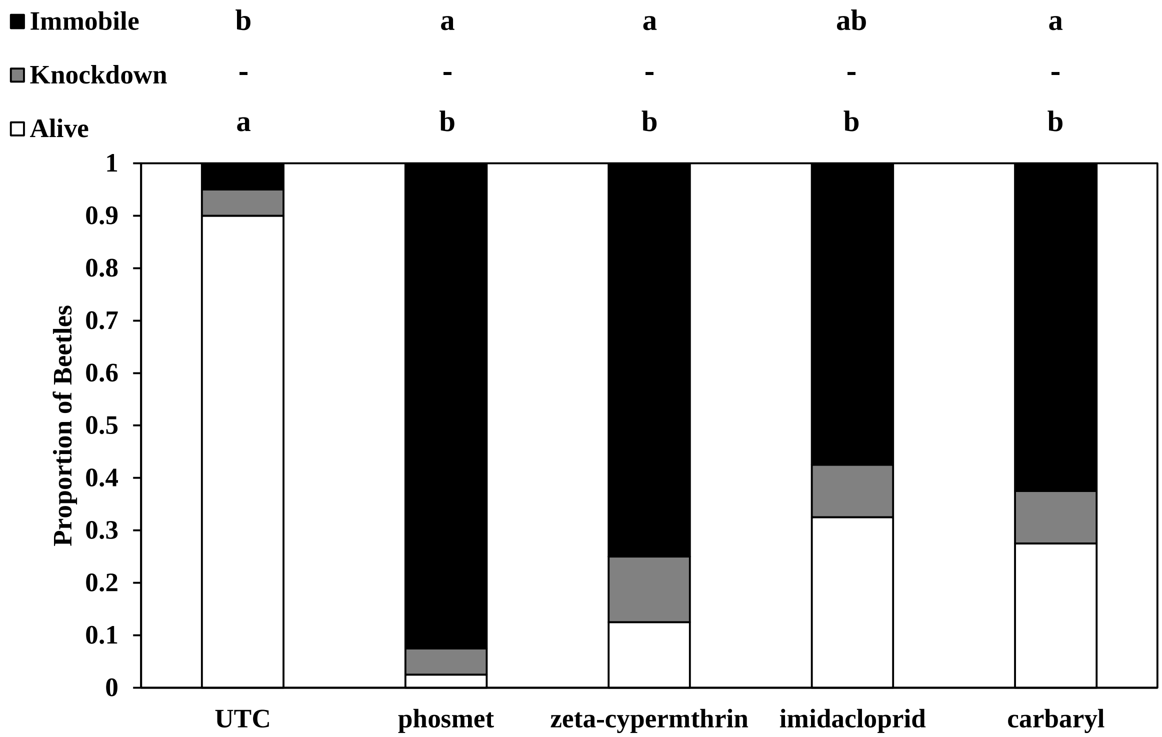

Figure 3.1 Average proportion of Japanese beetles in the alive, knockdown, and immobile condition. Letters above bars show significant differences across insecticide within condition. Fractions of bars corresponding to the same beetle condition associated with the same letter are not significantly different ($P < 0.05$). Data were arcsine square-root proportion-transformed before ANOVA. Mean separation calculated using the Tukey's honestly significant difference (HSD) test. Data shown are nontransformed means.

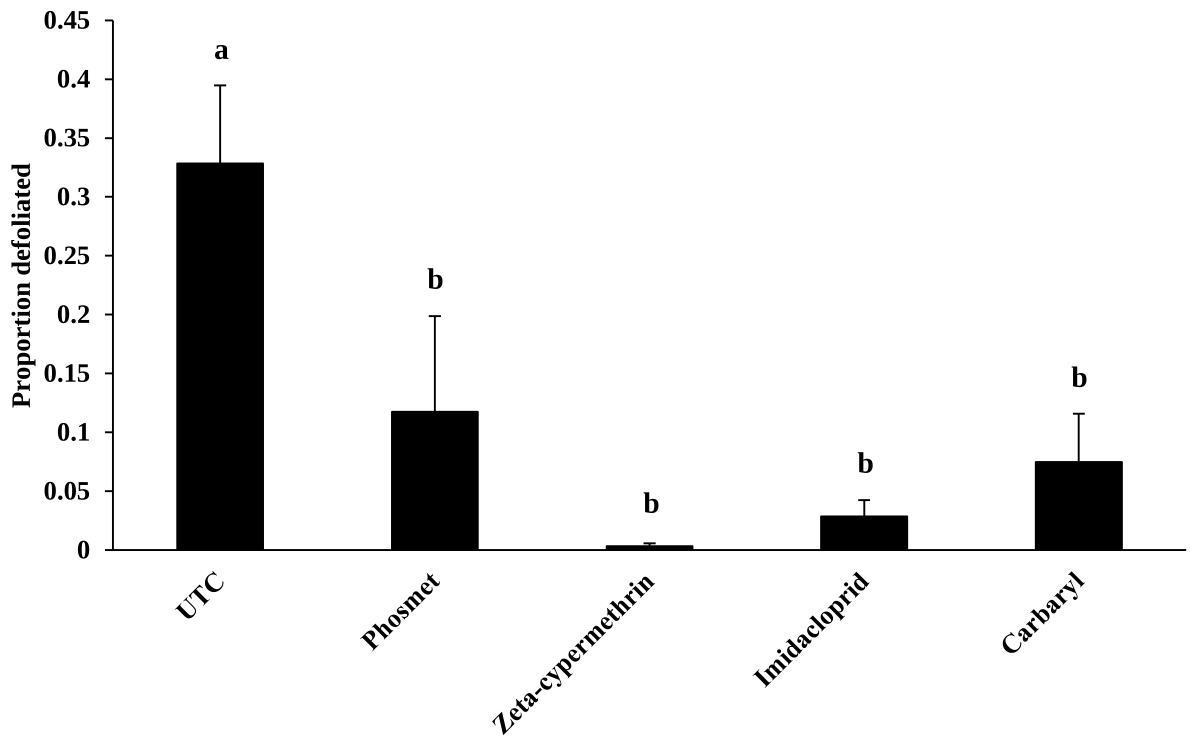

Figure 3.2 Average proportion (+ SE) of the blueberry leaves defoliated. Bars with different letters over them are significantly different from each other ($P < 0.05$). Data were arcsine square-root proportion-transformed before ANOVA. Mean separation calculated using the Tukey's honestly significant difference (HSD) test. Data shown are nontransformed means.

Effect of Rainfall

The time at which half of the Japanese beetles were killed by insecticides (LT_{50}) was calculated as a measure of toxicity for each insecticide on blueberry leaves at different rainfall and aging combinations. An overlap test of the 95% confidence intervals was used to determine significant differences in insecticide toxicity. There is evidence that the toxicity of phosmet significantly decreased as rainfall increased, specifically when comparing the LT_{50} values from zero mm of rain to 50.8 mm of rain (Table 3.2). The LT_{50} values of zeta-cypermethrin were not significantly affected by 12.7 and 25.4 mm of rain, but there is evidence that the LT_{50} of zeta-cypermethrin was significantly higher at 25.4 mm of rainfall than at 0 mm of rainfall (Table 3.2). The LT_{50} values for imidacloprid were not significantly affected by rainfall at 12.7, 25.4 or 50.8 mm of rainfall (Table 3.2). The LT_{50} values for carbaryl were not significantly affected by rainfall at 12.7 or 25.4 of rainfall (Table 3.2).

Table 3.2 LT_{50} values for insecticides sprayed on blueberry leaves at different rainfall levels for Japanese beetle adults.

Insecticide	Rainfall (mm)	n	Slope (+ SE)	LT_{50} (h)	95% CI	Chi square
phosmet	0	40	-0.097 (+ 0.01)	15.65	(11.29, 20.01)	<0.0001
	12.7	40	-0.002 (+ 0.01)	213.85	(-1502.35, 1930.05)	0.8224
	25.4	40	-0.004 (+ 0.01)	364.52	(-1262.43, 1991.48)	0.677
	50.8	40	-0.044 (+ 0.01)	32.95	(23.68, 42.22)	<0.0001
zeta-cypermethtin	0	40	-0.063 (+ 0.01)	-1.12	(-8.31, 6.07)	<0.0001
	12.7	40	-0.047 (+ 0.01)	16.95	(9.74, 24.16)	<0.0001
	25.4	40	-0.020 (+ 0.01)	-11.79	(-41.62, 18.03)	0.02765
	50.8	40	-0.046 (+ 0.01)	15.02	(7.69, 22.35)	<0.0001
imidacloprid	0	40	-0.034 (+ 0.01)	24.46	(13.73, 35.18)	0.0003
	12.7	40	-0.035 (+ 0.01)	43.85	(29.22, 58.47)	0.0001
	25.4	40	-0.0140 (+ 0.01)	85.98	(1.95, 170.01)	0.1104
	50.8	40	-0.021 (+ 0.01)	48.96	(21.78, 76.15)	0.01354
carbaryl	0	40	-0.065 (+ 0.01)	24.73	(18.73, 30.73)	<0.0001
	12.7	40	-0.027 (+ 0.01)	35.83	(20.44, 51.22)	0.0014
	25.4	40	-0.021 (+ 0.01)	64.84	(24.10, 105.57)	0.021
	50.8	40	-0.006 (+ 0.01)	357.81	(-998.89, 1714.50)	0.625

When there was no simulated rainfall, zeta-cypermethrin had a significantly lower LT_{50} value than the other insecticides, none of which were significantly different (Table 3.2). Zeta-cypermethrin had a lower LT_{50} value than imidacloprid at 12.7 mm of rainfall, and phosmet and carbaryl did not have significantly different LT_{50} values than the other insecticides (LT_{50} value).

Zeta-cypermethrin had a significantly lower LT_{50} value than any of the other insecticides (which were not significantly different from each other) at 25.4 mm of rain (Table 3.2). Zeta-cypermethrin had a significantly lower LT_{50} value than phosmet at 50.8 mm of rain, and imidacloprid and carbaryl were not significantly different from any of the other insecticides (Table 3.2).

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; $P = 0.79$). No significant difference was found in the level of defoliation in bioassays treated with zeta-cypermethrin across different rainfall levels ($F = 0.26$; df = 3, 12; $P = 0.85$). No significant difference was found in the level of defoliation in bioassays treated with imidacloprid across different rainfall levels ($F = 0.19$; df = 3, 12; $P = 0.90$). No significant difference was found in the level of defoliation in bioassays treated with carbaryl across different rainfall levels ($F = 2.49$; df = 3, 12; $P = 0.11$).

Insecticide Residue Analysis

There is evidence for the wash off of insecticide from the fruit and leaves of blueberries based on the data from the residue analysis portion of the experiment. Phosmet residues on blueberry leaf surfaces decreased by approximately 75% when comparing 0 mm of rain 50.8 mm of rain (Table 3.3). In the subsurface of blueberry leaves, phosmet residues were unaffected across rainfall levels (Table 3.3). Phosmet residues on the surface of the fruit decreased by approximately 90% when comparing 0 mm of rain to 50.8 mm of rain (Table 3.4). Phosmet residues for the subsurface of the fruit decreased by approximately 50% when comparing 0 mm of rain to 50.8 mm of rain (Table 3.4).

Table 3.3 Insecticide residues recovered from blueberry leaves after 24 h field aging across rainfall level and insecticide treatment. Residues are measured in mean micrograms per gram (ppm) of active ingredient per leaf.

	Insecticide							
	phosmet		carbaryl		zeta-cypermethrin		imidacloprid	
Rainfall (mm)	surface	subsurface	surface	subsurface	surface	subsurface	surface	subsurface
0	45.03	1.48	5.83	62.48	1.35	3.26	0.65	1.32
12.7	33.12	2.09	7.93	26.97	0.57	3.71	0.56	0.70
25.4	13.67	0.96	6.20	28.40	0.91	4.38	0.42	0.79
50.8	11.66	1.52	6.16	22.31	0.96	2.48	0.42	0.31

Table 3.4 Insecticide residues recovered from blueberry fruit after 24 h field aging across rainfall level and insecticide treatment. Residues are measured in mean micrograms per gram (ppm) of active ingredient per fruit.

	Insecticide							
	phosmet		carbaryl		zeta-cypermethrin		imidacloprid	
Rainfall (mm)	surface	subsurface	surface	subsurface	surface	subsurface	surface	subsurface
0	3.69	0.41	1.15	3.48	0.28	0.17	0.08	0.00
12.7	0.99	0.23	0.74	1.46	0.09	0.49	0.04	0.00
25.4	0.67	0.38	0.51	2.34	0.11	0.43	0.04	0.00
50.8	0.26	0.17	1.20	1.08	0.04	0.11	0.05	0.00

Carbaryl residues on blueberry leaf surfaces did not show a pattern of loss as rainfall increased (Table 3.3). Carbaryl residues in the subsurface of blueberry leaves decreased by approximately 90% when comparing 0 mm of rain to 50.8 mm of rain; most of the decrease was found after 12.7 mm of rain (Table 3.3). On the surface of blueberry fruit, no pattern of wash off was found for carbaryl residues (Table 3.4). In the subsurface of blueberry fruit, carbaryl residues decreased by approximately 70% when comparing 0 mm of rain to 50.8 mm of rain (Table 3.4).

Zeta-cypermethrin residues on blueberry leaf surfaces did not show a pattern of loss as rainfall increased (Table 3.3). Zeta-cypermethrin residues in the subsurface of blueberry leaves showed an approximately 70% when comparing 0 mm of rain to 50.8 mm of rain, although there a loss of residue was only found after 50.8 mm of rain (Table 3.3). Zeta-cypermethrin residues on the surface of blueberry fruit did not show a pattern of wash off, although there was an approximately 85% decrease when comparing 0 mm of rain to 50.8 mm of rain (Table 3.4). A pattern of wash off was not observed for zeta-cypermethrin in the subsurface of the fruit (Table 3.4).

Imidacloprid residues on blueberry leaf surfaces showed approximately 35% decrease when comparing 0 mm of rain to 50.8 mm of rain (Table 3.3). There was an approximately 75% decrease in the imidacloprid residues when comparing 0 mm of rain to 50.8 mm of rain (Table 3.3). Imidacloprid residues on blueberry fruit surface decreased by approximately 40% when comparing 0 mm of rain to 50.8 mm, although that decrease was seen after the 12.7 mm of rain (Table 3.4). Imidacloprid residues were not detected in the subsurface of blueberry fruit (Table 3.4).

Discussion

This study increases the understanding of the rainfastness of insecticides used to control Japanese beetles as well as the inherent toxicity and field residual activity of the compounds.

Based on the analysis done on the beetle condition data from bioassay chambers when no rain was simulated, it is difficult to determine which insecticide was the most inherently toxic (when no rainfall was simulated). All insecticides performed significantly better than the untreated control, but not significantly different than each other (Figure 3.1, Figure 3.2). Therefore, the LT_{50} calculations were used to determine that zeta-cypermethrin was the most inherently toxic (Table 3.2).

All compounds showed a general trend of lower toxicity as rainfall increased, with the LT_{50} values providing a measure of toxicity. However, zeta-cypermethrin showed the smallest decrease in toxicity from rainfall and was the most toxic insecticide after 50.8 mm of rain had been simulated, although not significantly more toxic than imidacloprid (Table 3.2).

A physical attribute of pesticides which 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)

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

K_{ow} varies from approximately 10^{-3} to 10^{7} and is usually expressed as $\log(K_{\text{ow}}) = P_{\text{ow}}$. 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).

Phosmet has a P_{ow} of 2.83 (Chiou et al. 1977) meaning that phosmet has a relatively low solubility in water and is lipophilic. This would suggest that phosmet will adhere to the plant epicuticle, but not penetrate readily through the cuticle. In this study, it was found that the biggest decrease in phosmet residues occurred on the surface (Table 3.3, Table 3.4).

Carbaryl has a P_{ow} of 2.34 (Noble 1993) which means that carbaryl has a relatively low solubility in water and is lipophilic. This might confer some wash off resistance to carbaryl. With carbaryl, most of the residue was lost after 12.7 mm of rain was simulated and mostly subsurface residue was lost (Table 3.3, Table 3.4). The maintenance of surface residues is likely a result of partitioning of subsurface residues as surface residues are removed by rainfall.

Imidacloprid has a relatively low P_{ow} of 0.57 and is systemic (Elbert et al. 1991). This compound is highly soluble in water and is the reason for its systemic nature. This can be seen by the relatively high proportion of the insecticide in the subsurface of the leaves (Table 3.3). Imidacloprid was not detected in the subsurface of the fruit which could indicate that imidacloprid is unable to penetrate into the fruit, which has also been found in other studies (Wise et al. 2007).

Zeta-cypermethrin has a P_{ow} of 4.47 (Noble 1993). This indicates zeta-cypermethrin is relatively lipophilic and has relatively low water solubility. It is likely these properties of zeta-cypermethtin are the reason for the insecticide's relative rainfastness. This is supported by the residue analysis portion of the study; there was not a general pattern of decrease in the residues of zeta-cypermethrin (Table 3.3, Table 3.4). These properties of zeta-cypermethrin may also contribute to the insecticide's effectiveness as the compound will be able to quickly penetrate the insect cuticle.

The results of this study will help blueberry growers 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.

CHAPTER 4:LEACHING OF INSECTICIDES USED IN BLUEBERRY PRODUCTION AND THEIR TOXICITY TO REDWORMS

Abstract

Soil columns were collected from a blueberry field, and insecticide solutions were allowed to flow through these columns. The insecticides were applied at two different rates: the concentration at which the insecticides wash off blueberries under rainfall conditions and the labeled field rate at which they are sprayed. The soil columns were divided into thirds; top, middle and bottom. Soil bioassays using *Eisenia foetida* Savigny were set up to determine the toxicity of the insecticides at a top, middle and bottom layer of the soil column. The concentration at which insecticides wash off of blueberries was not toxic to the *E. foetida*. The mass of *E. foetida* was also measured after the end of the bioassay experiment. Under field rate leaching conditions, carbaryl showed the highest level of toxicity in the middle layer of soil suggesting that it has the highest risk to organisms from leaching. Insecticide residues were quantified in the layers of soil for each insecticide to support the mortality data. This study will help growers make informed decisions about insecticide use, which can help minimize contamination of the environment.

Introduction

In order for crop production to sustain a growing population and remain profitable, commercial growers have intensified agriculture significantly. This has been done by increasing the land area used for agriculture, using higher yielding varieties, and increased use of fertilizers and pesticides (Matson et al. 1997). Increased use of pesticides is of concern because only about 1% of pesticides may reach the target organism with the rest reaching non target organisms or end up in other areas of the environment (PSAC 1965, Pimentel and Edwards 1982). At the same time, lack of effective control against pests such as insect pests can cause enormous damage to the crops. Often, economic factors such as consumer preferences and industry standards create standards for food products that are difficult to achieve without the use of conventional insecticide sprays. The specialty crop industry generally has a zero tolerance standard for insect contamination at pack-out. This creates additional challenges for growers of crops which have pests present during harvest such as cherry, peach, plum and blueberry (*Vaccinium* spp.). The pressure on growers for clean fruit at the time of harvest is enormous and insecticides are typically used for that purpose.

Older insecticides present much greater risks of contamination because of their stability in the environment. Older organochlorines such as DDT and dieldrin are far more persistent in the environment than current conventional insecticides such as organophosphates such as parathion and carbamates such as carbaryl (Eichelberger and Lichtenberg 1971). Modern insecticides are likely to degrade over days and weeks, whereas older compounds may persist in the environment long enough to affect years into the future. However, constant use of these insecticides can cause contamination in close proximity to their use. It is believed that degradation via microbial activity, photolysis, and hydrolysis are the main sources of insecticide degradation (Eichelberger and Lichtenberg 1971, Zepp and Cline 1977, Gupta et al. 2008). Some insecticides have physical properties which allow them to bind to particles of soil and remain relatively immobile others are more susceptible to leaching. Insecticides pose the greatest risk if they do not readily degrade in soil and do not bind to the soil. These insecticides may eventually reach ground water or other larger bodies of water.

Blueberries are an economically important crop for Michigan ("USDA-NASS Quick Stats (Chem)" 2010). The average monthly rainfall during the blueberry growing season in Michigan ranges from approximately 50 mm to 100 mm ("Michigan Automated Weather Network" 2011). The application of insecticides in combination with the high likelihood of rain presents both a production challenge for growers and a contamination problem when insecticides are carried by rain water and leach into soils. Growers may also discard the leftover rinsate from spray tanks which results in point source deposits of insecticides.

It is well known that insecticide applications can have adverse effects on beneficial organisms in agriculture and current Integrated Pest Management strategies plan for the use of insecticides which minimizes damage to beneficial insects (Elzen 2001, Michaud and Grant 2003, Michaud and McKenzie 2004). Within the soil, ecosystems can be affected by insecticide application as well (Gyldenkærne and Jørgensen 2000). Studies have been performed aimed at assessing the economic and environmental cost of pesticide application, including the leaching of pesticides (Römbke et al. 1996, Leach and Mumford 2008).

There has been considerable work done on how various pesticides leach through various soils (Sakata et al. 1986, Zhou et al. 1997, Gupta et al. 2002, 2008, Wauchope et al. 2004). These studies have focused on how a single, insecticide behaves in detail, but not a variety of insecticides and none in soil appropriate for growing blueberries. There are few studies which look at how different insecticides leach through the same soil, in this case Michigan soils appropriate for growing blueberries.

The objective of this study was designed to look at how insecticides commonly used in blueberry production leach through soil appropriate for growing blueberries. This was done by leaching 4 different insecticides from different chemical classes through soil columns taken from a blueberry field. The insecticides were leached at two different rates: a rate which simulated wash-off by rain, and a rate which would simulate drift, discard or spill of the insecticide. Red worms, *Eisenia feoetida* Savigny, were used in bioassays in the soil to assess the bioavailability of the insecticides in the soil.

Methods and Materials

Worms

E. feoetida adults were purchased from Carolina Biological Supply Company, Burlington, NC in July 2010. Upon arrival, the worms were placed in the culturing container previously set up. The culturing container consisted of a 35.6 x 45.7 x 20.3 cm plastic bin with a layer of damp shredded newspaper in the bottom and full of potting soil. Oats were mixed in to the soil to provide the *E. feoetida* with food until they were needed for the bioassay experiments. Over the top of the culturing container, a damp towel was placed to keep the container moist. Water was lightly sprayed in the chamber once a week to maintain the dampness of the soil. Healthy adult *E. feoetida* were used in bioassay experiments.

Soil Columns and Treatment Applications

Soil columns were collected from the blueberry field at the Michigan State University Trevor Nichols Research Center in Fennville, MI (+42° 36' 12.59", -86° 9' 13.86"). The soil columns consisted of a PVC pipe approximately 50 cm in length and 7.62 cm in diameter. The PVC pipes were hammered into the ground at the field site using a sledgehammer. Once the pipes had been hammered to a depth of 25 cm, they were removed from the ground with the soil column still in the PVC pipe. The soil was characterized by the Michigan State University Soil and Plant Nutrient Laboratory as sandy loam and had the following composition: % Sand = 74.7, % Silt = 13.5, % Clay = 11.8, TKN (Total Kjeldahl Nitrogen) = 0.09%, pH = 3.8, % OM (Organic Matter) = 2.8, and CEC (Cation Exchange Capacity) = 8.2 me/100g. The bottoms of the soil columns were placed in bins of water to allow the water to percolate up through the soil for 24 h and then allowed to drain for 24 h before the insecticide treatment to ensure uniform water level in all of the soil columns.

Two different sets of insecticide application of the soil columns took place. The rates of the first application were calculated by using the observed wash off rate of the insecticides from previous research (Hulbert, unpublished data) (Table 4.1). The second rate of insecticide application was the rate at which the insecticides are labeled for use in the field.

Table 4.1 Formulated compounds, field rates and concentrations used for bioassay experiments and residue analysis.

Formulated name	Chemical Class	Active ingredient	ppm[1]	ppm[2]	Company
Imidan® 70 WP	Organophosphate	phosmet	33.350	3355	Gowan Company, Yuma, AZ
Sevin XLR® 4L	Carbamate	carbaryl	39.843	9586	Bayer CropScience, Pittsburgh, PA
Mustang Max® 0.8 EC	Pyrethroid	zeta-cypermethrin	1.168	5	FMC Corp., Princeton, NJ
Provado® 1.6 SC	Neonicotinoid	imidacloprid	1.237	19	Bayer CropScience, Pittsburgh, PA

[1] Insecticide rates calculated from previous wash-off studies

[2] Insecticide rates labeled for field use

This study was designed to test how insecticides leach through soils after they have been washed off by rain, so the volume of rain that was equivalent to 25.4 mm (1 inch) of rain over an area equal to the area of the opening of the soil column (45.604 cm^2) was calculated based on figures from the U.S. Geological Survey website ("Rain and precipitation, USGS Water Science for Schools" 2010). It was calculated that 117.20 mL of water is equivalent to 25.4 mm of rain over a surface area of 45.604 cm^2. This volume of distilled water was allowed to flow through the soil columns with the concentrations of insecticides listed in Table 4.1. Untreated control soil columns had 117.20 ml of distilled water poured into them. Funnels with spray bottle nozzles attached were used to drip the insecticide solutions onto the soil columns to approximate the natural rate of rainfall. After the insecticide application, the soil columns were allowed to sit in a cool, dark area overnight until they were prepared for the next part of the experiment.

After 24 h, the soil column was cut into 3 equal segments laterally, each 8.3 cm in length. Then these discs were cut in half longitudinally so that half of the soil at a particular depth could be used for bioassays and the other half could be used for residue analysis. For each insecticide and the untreated control there were 5 soil columns at both of the insecticide concentrations. All 5 columns from each treatment were used to provide soil for 5 replicates for the bioassay experiment and 3 columns from each treatment were used to provide soil for the residue analysis portion of the study.

Bioassays

Bioassays were used to determine the toxicity of the four insecticides against *E. feoetida* at the 3 different soil depths. Half of the soil column at a particular depth (~190 cm^3) was placed into a glass 1 pint canning jar. The lid was placed on upside down so that a seal was not created. Each of these containers was considered an experimental unit in the bioassays.

Ten randomly selected healthy adult *E. feoetida* were placed in the bioassay chambers which were held in the laboratory at 21°C and a photoperiod of 16:8 (L:D) h. There were five replicates for each treatment at each treatment and depth combination. The number of *E. feoetida* alive and dead was recorded after 0, 24, 168 and 336 h (0, 1, 7, 14 d). The bioassays were evaluated by dumping the contents onto a clean sheet of paper and a probe was used to find the worms in the soil. The worms were probed to determine whether they were living. After the final bioassay evaluation at 336 h the total mass of *E. feoetida* in the bioassays were measured. The mean mass of the worms was compared among insecticide treatment at the same depth and also across depth within an insecticide treatment using analysis of variance (ANOVA). Mean separation was done using Tukey's honestly significant difference (HSD) test. The mortality data was analyzed by logistic regression to determine the time at which half of the organisms were in the immobile condition (LT_{50}) (Robertson et al. 2007). Logistic regression analyses were performed at every insecticide treatment and depth combination. These analyses were conducted in R version 2.12.1 (R Development Core Team 2010) using the MASS library (Venables, W. N. & Ripley, B. D. 2002), the lmtest library (Achim Zeileis, Torsten Hothorn, 2002), and the doBy library (Søren Højsgaard, Ulrich Halekoh with contributions from Ulrich Halekoh, Jim Robison-Cox, Kevin Wright and Alessandro A. Leidi, 2011).

Insecticide Residue Analysis

Ten g of the treated soil samples designated for residue analysis were placed in 150 ml of high-performance liquid chromatography (HPLC)-grade acetonitrile (EMD Chemicals, Inc., Gibbstown, NJ) and sonicated for 10-15 s. The acetonitrile was decanted through 5 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water. The sample was dried via rotary evaporation and brought up in acetonitrile for HPLC analysis. The remaining soil samples were ground in 50 ml of HPLC grade dichloromethane (Burdick&Jackson, Muskegon, MI). The extracts were passed through 5 g of anhydrous sodium sulfate. The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45-µm Acrodisc 13-mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for insecticide residue with a Waters 2690 Separator Module HPLC equipped with a Waters 2487 Dual Wavelength Absorbance Detector (Waters, Milford, MA) set at 270 nm, and a C18 reversed phase column (150 by 4.6mm bore, 5 µm particle size, Restek, Bellefonte, PA)(Bayer 1998). Themobile phase was water/ acetonitrile (80:20) at 55°C. The HPLC level of quantification was 0.457 µg/g (ppm) of active ingredient, and level of detection was 0.138 ppm.

Results

Bioassays

a) Wash-off simulated rates: There were no observable toxic effects on the worms when the wash-off rate of insecticides were leached through the soil columns.

b) Field-rate simulated study: The time at which half of the *E. feoetida* were killed (LT_{50}) by the insecticides was calculated as a measure of toxicity for each insecticide at all soil depths. An overlap test of the 95% confidence intervals was used to determine significant differences in insecticide toxicity. In the top layer of soil, there was evidence that imidacloprid had a significantly lower LT_{50} value than phosmet which had a significantly lower LT_{50} value than both zeta-cypermethrin and the untreated control (Table 4.2). In the middle layer of soil, there was evidence that carbaryl had a significantly lower LT_{50} value than zeta-cypermethrin (Table 4.2). In the bottom layer of soil there was no evidence any of the treatments had significantly different LT_{50} values (Table 4.2).

The top layer of soil treated with phosmet had a significantly lower LT_{50} value than the bottom layer of soil treated with phosmet (Table 4.2). Layers of soil treated with zeta-cypermethrin had a similar LT_{50} values (Table 4.2). The top layer of soil treated with imidacloprid had a significantly lower LT_{50} value than the bottom layer (Table 4.2). The middle layer of soil treated with carbaryl had a significantly lower LT_{50} value than the bottom layer (Table 4.2).

Table 4.2 LT_{50} values for insecticides leached through soil at the different soil depths for *E. feoetida* adults.

Insecticide	Depth	n	Slope (+ SE)	LT_{50} (h)	95% CI	Chi square
UTC	Top	50	-0.009 (+ 0.002)	367	(297.34, 436.15)	< 0.0001
	Middle	50	-0.004 (+ 0.003)	980	(-119.18, 2078.41)	0.1608
	Bottom	50	-0.005 (+ 0.002)	727	(239.41, 1214.25)	0.0345
Phosmet	Top	50	-0.009 (+ 0.001)	135	(104.07, 164.98)	< 0.0001
	Middle	50	-0.003 (+ 0.002)	1107	(-292.29, 2506.57)	0.1895
	Bottom	50	-0.005 (+ 0.002)	744	(225.95, 1262.48)	0.0379
Zeta-Cypermethrin	Top	50	-0.006 (+ 0.002)	455	(319.67, 589.81)	< 0.0001
	Middle	50	-0.004 (+ 0.002)	778	(184.29, 1371.04)	0.0473
	Bottom	50	-0.006 (+ 0.002)	542	(321.06, 762.74)	0.0017
Imidacloprid	Top	50	-0.030 (+ 0.004)	78	(65.85, 90.60)	< 0.0001
	Middle	50	-0.001 (+ 0.002)	1423	(-1336.01, 4182.62)	0.3662
	Bottom	50	-0.004 (+ 0.002)	798	(172.24, 1423.73)	0.0562
Carbaryl	Top	50	-0.324 (+ 62.600)	67	(-1810.81, 1944.89)	0.9959
	Middle	50	-0.007 (+ 0.001)	134	(98.62, 169.35)	< 0.0001
	Bottom	50	-0.003 (+ 0.001)	602	(211.16, 992.90)	0.0247

The mass of *E. feoetida* in each bioassay at the end of the bioassay experiment was measured as another measure of toxicity. In the UTC soil columns, the results of the overall ANOVA across depth was $F = 4.19$; df = 2, 12; $P = 0.04$, but none of the individual comparisons using Tukey's HSD were significant at $\alpha = 0.05$. Within bioassays with soil treated with phosmet, there was a significant difference between the masses of *E. feoetida* across soil depths ($F = 9.77$; df = 2, 12; $P = 0.003$) (Figure 4.1). Within bioassays treated with zeta-cypermethrin there was no evidence of a significant difference between the masses of *E. feoetida* across soil depth ($F = 1.47$; df = 2, 12; $P = 0.26$) (Figure 4.1). Within bioassays treated with imidaclopid there was a significant difference between the masses of *E. feoetida* across soil depth ($F = 26.57$; df = 2, 12; $P < 0.001$) (Figure 4.1). Within bioassays treated with carbaryl there was a significant difference between the masses of *E. Foetida* across depth ($F = 25.77$; df = 2, 12; $P < 0.001$) (Figure 4.1).

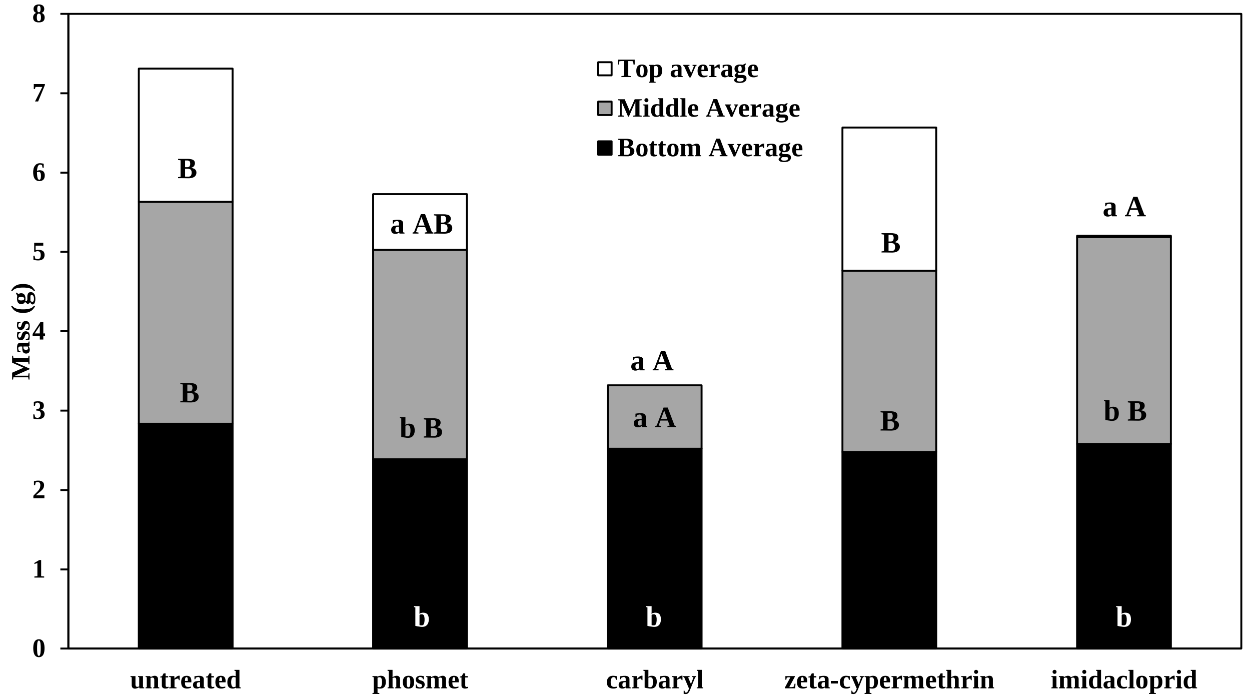

Figure 4.1 Average mass of *E. feoetida* in the bioassay chambers for all insecticide at each depth. Lower case letters show significant differences across depth within a single insecticide treatment. Upper case letters show significant differences across treatments in the same depth. Mean separation was done using Tukey's HSD ($P < 0.05$).

Insecticide Residue Analysis

All insecticides were recovered in progressively lower concentrations as depth increased (Table 4.3, Table 4.4). Much higher concentrations were recovered in soil that was treated with the labeled spray rate than the wash-off rate. In soil treated with phosmet at the washoff rate, residue detection was approximately 90% less in the bottom layer than in the top layer (Table 4.3). In soil treated with phosmet at the labeled spray rate, residue detection was approximately 99% less in the middle layer than in the top layer (Table 4.4). In soil treated with zeta-cypermethrn at the wash-off rate, detection only occurred in the top layer of soil (Table 4.3). In soil treated with zeta-cypermethrin at the labeled spray rate, detected residues in the middle layer were approximately 98% less than in the top layer and detected residues were approximately

90% less in the bottom layer than in the middle layer (Table 4.4). In soil treated with imidacloprid at the wash-off rate, residues detected in the middle and bottom layers were approximately 93% less than in the top layer (Table 4.3). In soil treated with imidacloprid at the labeled spray rate, residues detected in the bottom layer were approximately 99% less than in the top layer (Table 4.4). In soil treated with carbaryl at the wash-off rate, detected residues were approximately 13% lower in the middle layer than the top layer and 99% lower in the bottom layer than the middle layer (Table 4.3). In soil treated with carbaryl at the labeled spray rate, residues detected in the middle layer were approximately 98% less than in the top layer and approximately 92% lower in the bottom layer than in the middle layer (Table 4.4).

Table 4.3 Average insecticide residues recovered from blueberry soil across soil depth after the wash-off rate of insecticides was applied to the soil. Residues are measured in micrograms per gram (ppm) of active ingredient per soil sample.

Depth	Phosmet	Zeta-Cypermethrin	Imidacloprid	Carbaryl
Top	1.632	0.8816	0.07904	1.29
Middle	0	0	0.00606	1.117
Bottom	0.117	0	0.00542	0.0107

Table 4.4 Average insecticide residues recovered from blueberry soil across soil depth after the labeled spray rate of insecticides was applied to the soil. Residues are measured in micrograms per gram (ppm) of active ingredient per soil sample.

Depth	Phosmet	Zeta-Cypermethrin	Imidacloprid	Carbaryl
Top	47.5562	9.67	18.14	769
Middle	0.216	0.2252	0.1386	14.672
Bottom	0	0.0238	0.19372	1.1722

Discussion

We found that when insecticides were leached through soil at concentrations likely to occur after a rainfall event there were no toxic effects seen on the worms. This has important implications for insecticide contamination that is likely to occur after rainfall. Namely, that there is little risk of contamination and leaching for these insecticides after rain. The primary risk associated with rainfall is the loss of efficacy of these insecticides for the grower. When the insecticides were leached at concentrations that simulated a spill, there was evidence that only the insecticide carbaryl leached. Imidacloprid and imidan were toxic in only the top layer of soil and no toxic effects in the middle or bottom layer.

This study provides evidence that newer insecticides pose a lower risk of soil contamination, especially in the context of insecticides used in the last 60 years, and trends in insecticide use show a trend towards decreasing risk of contamination by insecticides. When carbaryl was tested against other older insecticides such as DDT, dieldrin and lindane it was found that carbaryl had lower adsorption than the other insecticides (Sharom et al. 1980). Carbaryl showed a higher degree of leaching than the other insecticides tested in our experiment, which are more recent inventions.

An important reason for some of the patterns of toxicity and leaching seen in this study is likely the rates at which these insecticides were applied to the soil columns. carbaryl, which showed the highest degree of leaching as evidenced by toxicity and residues recovered, was applied at a rate a few times higher than phosmet, hundreds of times higher than that of imidacloprid and thousands of times higher than that of zeta-cypermethrin (Table 4.1). Identical concentrations of insecticides would have been used had the purpose of this study been to determine, based strictly on the physical properties of the compounds, which insecticide was most susceptible to leaching. The purpose, however, was to determine what is more likely to occur in the field given normal blueberry production practices. For this reason, insecticides were applied at rates which would be likely to reach soils in terms of their absolute concentration and their relative concentration to other insecticides.

We found that there were similar levels of toxicity in the top layers of soil treated with carbaryl and imidacloprid, however in the middle layers carbaryl had much larger toxic effects (Table 4.2, Figure 4.1). This shows that in practice, carbaryl would have a larger leaching capacity, making carbaryl a greater risk than imidacloprid.

Water solubility and octanol-water partitioning coefficient K_{ow} are properties which significantly correlate with the mobility of insecticides in soils and K_{ow} has been found to be a better predictor than water solubility (Somasundaram et al. 1991). The octanol-water partitioning coefficient is defined as the ratio of a chemical's concentration in an octanol solution ([Ci]octanol) over its concentration in aqueous solution ([Ci]water) (Leo et al. 1971)

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

K_{ow} varies from approximately 10^{-3} to 10^{7} and is usually expressed as $\log(K_{ow}) = P_{ow}$. Compounds with lower P_{ow} are polar and have high solubility in water. Compounds with higher P_{ow} are non-polar and have low water solubility and are lipophilic (Ragnarsdottir 2000).

Phosmet has a P_{ow} of 2.83 (Chiou et al. 1977) meaning that phosmet has relatively low solubility in water and is lipophilic. Phosmet is known to degrade relatively rapidly in soil and the hydrolysis of phosmet in water is buffered at neutral and alkaline pH (Menn et al. 1965). It has also been found that phosmet may degrade faster in soils with a higher organic content (Suter et al. 2002). These factors likely influenced the degradation over the course of our experiment which took place over the course of a couple weeks.

Carbaryl has a P_{ow} of 2.34 (Noble 1993) which means that carbaryl has a relatively low solubility in water and is lipohilic. Carbaryl showed the largest effects of leaching in this study which may be attributed in part to the rate at which it was applied. Considerable work has been done to determine how carbaryl degrades in soil and water and its environmental fate (Kazano et al. 1972, Wolfe et al. 1978). Carbaryl can bond moderately with soil and may leach into groundwater. The degradation of carbaryl has been characterized in a sandy loam soil similar to the soil used in our experiment and it was found that hydrolysis was the main pathway of degradation (Kazano et al. 1972).

Zeta-cypermethrin has a P_{ow} of 4.47 (Noble 1993) indicating zeta-cypermethrin is relatively lipophilic. The insecticide is highly toxic to insects which is why a relatively low rate is applied per acre. The leaching potential of cypermethrin has been described as “limited” because of its low water solubility and adsorption to soil (Sakata et al. 1986). Mixtures of pyrethroids, including zeta-cypermethrin are used with neonicotinoids to control termites in soil because the mixture has high toxicity to termites at low application rates and has “good soil mobility for a very effective continuous chemical barrier” (Ballard et al. 2008).

Imidacloprid is a systemic insecticide with P_{ow} of 0.57 (Elbert et al. 1991). Under high levels of simulated rainfall, imidacloprid has been found to have a high potential for leaching (Gupta et al. 2002), but other studies have found that under non-monsoon conditions, imidacloprid is relatively immobile (Hellpointer 1998). Different formulations of the insecticide are also shown to reduce the risk of leaching and contamination of imidacloprid (Fernández-Pérez et al. 1998). As stated above, imidacloprid, when mixed with a pyrethroid, has been used and studied for its control of termites in soils (Baskaran et al. 1999, Ballard et al. 2008).

The question of how insecticides leach through soils is complex and the answer is dependent on a number of factors, such as soil moisture, temperature, insecticide formulation, soil pH and composition among others. With this study, we hope to advance the knowledge of the mobility of insecticides used in blueberry production and how it affects soil organisms. We hope that growers can make informed decisions about their insecticide use which will help minimize contamination in the environment. Specifically, blueberry growers should probably use newer insecticides such as zeta-cypermethrin or imidacloprid because they caused lower toxicity to the worms and did not leach through the soil even at high concentrations.

CHAPTER 5:CONCLUSION

This thesis contained three separate experiments designed to answer related questions. Two of the experiments were designed to answer very applied questions of how does rainfall affect the performance of insecticides used to control an early season pest of blueberries, cranberry fruitworm (*Acrobasis vaccinii* Riley), and a late season blueberry pest, Japanese beetle (*Popillia japonica* Newman). I felt it was a natural progression to investigate the question of what happens to the insecticides if and when they wash off the blueberry bushes. This is a complicated question and was simplified to a somewhat more manageable scope.

In the cranberry fruitworm experiment, none of the insecticides investigated seemed to be affected by rainfall and the insecticides, with the strongest toxic effects were spinetoram and methoxyfenozide. The residue data did reveal some interesting residue profiles of the insecticides as rainfall was simulated, but I have been reserved in drawing conclusions from this data because the low number of replicates in this part of the experiment. In the Japanese beetle experiment, the bioassays revealed that zeta-cypermethrin and imidacloprid were highly resistant to loss of efficacy because of rainfall, and were at least as toxic as phosmet and carbaryl under zero-rainfall conditions. This is an important result because it shows that these newer reduced risk insecticides are at least as toxic to Japanese beetles as older more conventional insecticides which are sprayed at much higher rates, and these newer insecticides are more resistant to rainfall than the older ones.

Newer insecticides, in particular pyrethroids, are less persistent in the environment, degrading readily by photolysis and other mechanisms. This is a common trend among insecticides throughout history. The organophosphates and carbamates, which are today considered older and more “conventional”, are much less persistent in the environment than their predecessors such as the organochlorines. Obviously, this trend has advantages from an environmental conservation perspective, and disadvantages from a crop protection perspective. A highly unstable molecule would pose little risk as an environmental contaminant, but may also not have much utility, after its initial application, as an insecticide in the field. This represents a balancing act with no easy answer. Although, as more selective insecticides are developed and other IPM practices become more widely used, the dependence on broad-spectrum insecticides will probably be lessened.

As previously stated, the insecticide leaching experiment was designed to be the next logical step for investigation after insecticide wash off was investigated. Even investigating how much insecticide actually reaches the top of the soil after a rainfall is an enormously complicated question which I am not equipped to tackle on my own. Because of this, relatively simple methods were used to calculate the concentration of insecticide to leach through soil columns. The wash-off rate concentrations leached through the soil approximated the residues lost in the Japanese beetle experiment (Chapter 3). The wash-off rate concentration was not toxic to our test organism, redworms (*Eisenia feoetida*), so a higher rate meant to simulate an insecticide spill or dumping was also used. Because the concentrations at which newer insecticides are applied are relatively low, it could be argued that the amount of insecticide which reaches the soil and subsequently leaches through would have little measureable effect. This was the case with zeta-cypermethrin, but imidacloprid did show toxic effects to the worms in the study even though it was applied at a relatively low concentration. This is important because it shows that even newer reduced risk insecticides still may have the potential to be harmful to soil ecosystems.

I believe that insecticides are a powerful tool requiring due responsibility when yielded. Part of that responsibility comes from understanding how they can be effective under compromising circumstances (the Japanese beetle and cranberry fruitworm experiments) and another part of that responsibility comes from understanding the risks associated with their use (the leaching experiment). By understanding how to maximize efficiency and the risks of insecticide use, responsible practices can be achieved.

Pesticides in general have profoundly altered human existence and the entire planet. With them, humans are able to grow more food quicker and more efficiently than ever before to feed an ever increasing population. With current population trends, it is likely that the dependence on pesticides will only increase. Because of irresponsible human use of pesticides, ecosystems have been destabilized, species lost and many fellow human beings inadvertently poisoned. We clearly have a responsibility from a selfish anthropocentric perspective and as stewards of the earth to responsibly use pesticides.

Understanding how to maximize pesticide efficiency while minimizing risks is a very lofty goal which requires innumerable hours of investigation especially as new pesticides are always being added to our tool-kit. Through incremental studies, such as the ones in this thesis, a greater understanding can be achieved.

Looking forward, there are several directions this research could contimue. One example is an experiment on the rainfastness of insecticides to control cherry fruitworm which is another serious blueberry pest. Also, looking at the effect of adjuvants on the rainfastness and leaching of insecticides would be a valuable direction to go. Investigating how insecticides dissipate in the field and in soil over time has been investigated for several insecticides, but would be worth studying for the insecticides in this thesis and in different types of soil. Finally, the development of a guide for growers to help them better plan their insecticide applications and react appropriately to rainfall would be a benefit to IPM.

APPENDICES

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2011-04

Title of thesis or dissertation (or other research projects):

Rainfastness and leaching of insecticides used in blueberry production

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Daniel Lloyd Hulbert

Date 19 August 2010

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Adults	Museum where deposited
Acrobasis vaccinii Riley	Michigan Allegan Co. Douglas blueberry field 26-July-2011 D. Hulbert coll.	20							MSU
Popillia japonica Newman	Michigan Allegan Co Fennville – TNRC Floral and pheromone trap 26 July 2011 D. Hulbert coll.					10	10		MSU
Eisenia feoetida Savigny	Ex. Ordered from Carolina Biological Supply Company Ordered 6 August 2010 D. Hulbert coll.							20	MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Daniel Lloyd Hulbert

Date 19 August 2011

Voucher No. 2011-04

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

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