Selected Pesticides' Effects on the Convergent Lady Beetle (*Hippodamia convergens*)

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ABSTRACT

A novel wave of pesticides has been introduced as "reduced risk" towards the human population. However, the application of pesticides will affect nontarget organisms and beneficial insects such as the Convergent Lady Beetle (*Hippodamia convergens*). This research examined how the direct application of seven of this new class of pesticides, Altacor, Cyazypyr, Manzate, Rimon, Delegate, Kumulus, and Warrior, will affect *H. convergens* pupae emergence rates in a laboratory setting. Seven different pesticides were applied topically onto first day pupae and emergence rates were recorded. The synthetic pyrethroid, Warrior, was found to decrease emergence rates the most. Rimon also caused a statistically significant amount of mortality, whereas Diamides, fungicides, and the fungal metabolite appeared to have little to no effect on pupae emergence rates. The knowledge of how these pesticides will affect *H. convergens* population will help provide an understanding for pesticide application in the field.

KEYWORDS

Hippodamia convergens pupae, beneficial insects, reduced-risk, insecticides, bioassay

INTRODUCTION

Crop pests have severe environmental and economic ramifications, incurring costs of several billion dollars per year in damages alone in the United States (Pimental 2002). To combat these pests, the U.S. spends $10 billion dollars annually on roughly 500 million kg of pesticides (Pimental 2005). However, the application of pesticides can inadvertently create new pest problems. These pests, termed secondary pests, are normally kept under control by natural predators and beneficial arthropods (Elzen 1998). The use of pesticides can destroy these natural predators and beneficial insect populations, allowing secondary pests to multiply out of control (Elzen 1998). Biological conservation of beneficial insects is therefore an important component of successful pest management.

One such beneficial natural predator is *Hippodamia convergens* (Coleoptera: Coccinellidae), commonly known as the lady beetle. *H. convergens* is a widely used arthropod in biocontrol to reduce aphid populations, a common pest in many crop fields such as soybean, cotton, and wheat (Obrycki 2009). Not only do aphids consume crops, they can transmit hazardous diseases to the crops (Conway 2010). If untreated, aphids are capable of reducing a crop's yield by up to 50% (Alt 2012). A release of 80-120 *H. convergens* larvae and adult each in Egyptian squash fields has been shown to reduce aphid populations of up to 79.27% (El-Din 2013). Research in Florida citrus orchards also showed that *H. convergens* could subsist on its three major Homopteran citrus pests (Qureshi and Stansly 2011). However, as the growing season progresses, crop plants grow larger fruiting structures and pest populations naturally increases, making crops more vulnerable to attacks (Frisbie 1989). Pesticides thus often become necessary to maintain crop profitability. Determining the relative toxicity of various pesticides to *H. convergens* is important in selecting pesticides that will maximize their survivorship.

Seven novel EPA-classified "reduced risk" pesticides are DuPont's Altacor, Cyzaypyr, and Manzate, Makhteshim-Agan's Rimon, Dow Agrosciences' Delegate, BASF's Kumulus, and Syngenta's Warrior. Altacor and Cyazypyr are diamide pesticides that act on ryanodine receptors in insects and forces calcium channels to remain open, causing uncontrolled calcium release and preventing feeding, leading to death (Jeanguenat 2013). Rimon is an insect growth regulator that interferes with the molting process of immature insects by preventing chitin formation. This means that adult insects are unaffected because their chitin is already fully formed, and these pesticides must be either absorbed by eggs or ingested by larvae. Delegate is a fungal metabolite which uses the compound spinetoram to interfere with nicotinic and GABA receptors, preventing proper neurotransmitter release to cause death. Kumulus and Kocide-Manzate are fungicides containing sulphur and zinc ions that disrupt fungal cell function. Warrior is a pyrethroid pesticide that uses lambda-cyhalothrin to prevent repolarization of sodium channels, leaving organisms paralyzed. Although pyrethroid lambda-cyhalothrin pesticides are tested to be ineffective against aphids (Tillman 2000), they are extremely toxic towards beneficial insects (Tillman 1995). These seven new pesticides are developed to reduce human health risks, benefitting scientists, field laborers, and pesticide applicators. However, little is known about their effect on pest predators. Because *H. convergens* often pupate on top of leaves or other stable surfaces, they become directly exposed to pesticide spray. If these pesticides can kill *H. convergens* pupae, lady beetle populations will decrease dramatically. Because there is a lack in knowledge in how these seven pesticides will affect emergence rates of *H. convergens* pupae, it is important to investigate this issue.

This research seeks to examine how direct application of pesticides will affect *H. convergens* pupae emergence rates in a laboratory setting. Which class of pesticides: diamides, insect growth regulator (IGR), fungal metabolite, fungicides, and pyrethroid, will have the highest pupae mortality rate? More specifically, which of the seven pesticides will affect survivorship of pupae rates the most? I hypothesize that Warrior, the pyrethroid pesticide, will cause the highest mortality rate among *H. convergens* pupae, because pyrethroids are synthetic compounds with a history of high toxicity. On the other hand, the diamide pesticides are hypothesized to have a lower mortality rate because its mode of action may not affect insects undergoing metamorphosis. Determining the emergence rates of each group will reveal which pesticide will be the most toxic to *H. convergens*. This information will help determine which pesticide will be the best choice for field pest control while conserving *H. convergens* populations.

METHODS

Study System

This experimental study took place at the Natural Resource Laboratory at UC Berkeley. *H. convergens* were raised in lab from a year old colony in separate individual containers under fluorescent light with a 16 hr day and 8 hr night cycle at a temperature of 73° F and a humidity of 70%. Subjects were fed ample pea aphids, *Acyrthosiphon pisum* (Hemiptera: Aphididae) daily until pupation. Aphids were raised in a greenhouse on fava bean plants. *H. convergens* underwent experimental treatment within 24 hours of pupation.

Pesticides

There were a total of seven pesticides classified among five different classes listed below. In order to simulate field usage, I measured out each pesticide in their respective 100% field dosage.

Table 1. Selected Pesticides and basic information

Pesticide	Manufacturer	Class	Active Ingredient	Mode of Action	Field Dosage (solvent/$H_2$0)
Altacor	DuPont	Diamide	35% Chlorantraniliprole	Acts on insect ryanodine receptors	0.337g /1L
Cyazypyr	DuPont	Diamide	95% Cyazypyr	Acts on insect ryanodine receptors	0.8ml /0.5L
Delegate	Dow Agrosciences	Fungal metabolite	25% Spinetoram	Prevents neurotransmitter release by disrupting nicotinic and GABA receptors	0.524g/1L
Kocide-Manzate	DuPont	Fungicide	46.1% Copper Hydroxide	Disrupts fungal cell function	0.861201g/50ml
Kumulus	BASF	Fungicide	80% Sulfur	Disrupts fungal cell function	0.36g /75ml

Rimon	Chemtura	Insect growth regulator	9.3% Novaluron	Disrupts cuticle formation and deposition during molting	0.1952ml/50ml
Warrior	Syngenta	Pyrethroid	11.4% Lambda-cyhalothrin	Prevents sodium channel repolarization	1ml/4.14173L

Data Collection

To determine how each pesticide will affect percent emergence of *H. convergens* pupae, I placed 30 pupae per experimental and control group for each pesticide. I coated experimental pupae directly using a pipette with 200 microliters of its pesticide at 100% field dosage. I then drained out excess solvent from each container. I treated control groups in the same manner as experimental groups with water in place of pesticide. Each group remained in a fume hood for 24 hours post-treatment to dry out and prevent the possibility of drowning. I placed subjects back in an incubator set at 73° F. I checked each group daily, recorded percent emergence and identified gender of emerged adults.

Data Analysis

The percent survivals of each group from their pesticide are displayed in a table. A chi-squared test was conducted on each pesticide to determine if there is an association between pesticide use and pupae mortality (R commander).

RESULTS

Data

At the 100% field dosage test, I found that Warrior had by far the lowest survival rate of all the pesticides with 0% of the group emerging (Table 2). Kumulus, Kocide-Manzate, and Altacor all had 100% survival rates. Cyazypyr had a 90% survival rate. Rimon and Delegate had a survival rate of 83.33 and 90% respectively, but a large majority of these adults had developmental issues and died shortly after emerging.

By class, none of the *H. convergens* pupae treated with synthetic pyrethroid emerged. The fungal metabolite and insect growth regulator had the second lowest survival rates with 90% and 83.33% respectively, and these emerging adults showed fatal developmental issues. The diamides had the second highest survival rates with a total average emergence of 95%, and the adults that emerged were completely healthy. The fungicides had the highest survival rates, with every pupae emerging developmentally healthy.

Table 2. Survival rates of *H. convergens* exposed to selected pesticides

Pesticide	Class	Control % Emergence	Treatment % Emergence
Altacor	Diamide	96.67	100
Cyazypyr	Diamide	100	90
Delegate	Fungal Metabolite	100	90
Kocide-Manzate	Fungicide	96.67	100
Kumulus	Fungicide	96.67	100
Rimon	Insect Growth Regulator	100	83.33
Warrior	Synthetic Pyrethroid	90	0

Data Analysis

I ran a chi-squared test for each pesticide. My null hypothesis is that there is no association between the pesticide and death. A standard p-value below 0.05 was statistically significant and meant the null hypothesis was rejected. The calculated odds ratio determines how many times more likely a pupa would die when treated with the pesticide than water. Statistically significant results are shown below.

Table 3. Rimon

	Emerged	Died	Total
Treatment	25	5	30
Control	30	0	30
Total	55	5	60

p-value: 0.01952

(1, N=60, $p<0.05$)

odds ratio=0.0017

Table 4. Warrior

	Emerged	Died	Total
Treatment	0	30	30
Control	27	3	30
Total	27	33	60

p-value = 2.444e-12

(1, N=60, $p<0.05$)

odds ratio=3.7e-5

DISCUSSION

My results indicated that my hypotheses were largely correct; however, it also brought about some unexpected findings. I found that Warrior, the synthetic pyrethroid, and Rimon, the insect growth regulator, caused a statistically significant amount of mortality. On the other hand, diamides, fungicides, and the fungal metabolite had little to no effect on pupae emergence.

In accordance with my hypothesis, Warrior had the lowest survival rate among all pesticides tested. Warrior's active ingredient is lambda-cyhalothrin, a synthetic pyrethroid chemical developed to be more potent than its natural counterpart, pyrethrin. Warrior works as a neurotoxin by preventing sodium channel repolarization, causing paralysis and death. Warrior's statistically significant 100% mortality rate in my experiment suggests the lethality of synthetic pyrethroids. Past research has shown treatments of lambda-cyhalothrin, its active ingredient, to have lethal effects on other insects such as *Deraeocoris brevis* (Hemiptera: Miridae), the mired bug (Amarasekare and Shearer 2013). Another study on pyrethroid insecticides found that similarly found pyrethroids were toxic towards terrestrial and aquatic insects (Siegfried 1993). Although these studies tested on different insects, they both came to the same conclusion that pyrethroid compounds were significantly toxic towards insects.

Rimon showed a statistically significant albeit much higher survival rate. As an insect growth regulator, it specialized in preventing chitin formation, thus targeting immature insects (Krysan and Dunley 1993). As a result, even though 83.33% of Rimon tested pupae emerged, all had developmental issues and died shortly due to their abnormal exoskeleton formation, giving them a 0% survival rate. Thus, if post-emergence survival was a measured parameter, Rimon can be considered as toxic to *H. convergens* as Warrior is.

The diamides, Altacor and Cyazypyr, the fungal metabolite, Delegate, and the fungicides, Kocide-Manzate and Kumulus, all had either 0% or statistically insignificant pupae mortality rates. The diamides function by acting on ryanodine receptors in muscles to force calcium channels open to cause uncontrolled calcium release and prevent feeding. However, this may not have affected pupae because there is no feeding while pupating, hence the 95% average emergence rate. Delegate, the fungal metabolite, interferes with nicotinic and GABA receptors to prevent proper neurotransmitter release, affecting muscle control. My results showed a 90% emergence rates for Delegate, so it is possible that pupating insects have no use for muscle control, and as a result, are not affected by fungal metabolite pesticides. However, because post-treatment survival and reproduction of the new adults were not measured parameters, it is ultimately unclear if there are any other effects diamides might have on *H. convergens*. However, a previous study testing the effect of spinosad, a different version of the spinetoram compound in Delegate, on *H. convergens* adults revealed a similarly high survival rate of 100% (Elzen 1998). This could indicate that *H. convergens* is particularly resistance to this class of pesticide, but it is important to note that study tested on adults, which are biologically different from pupae. Furthermore, Elzen's study involved different methods of pesticide exposure; it treated its subjects through spray chambers and residual treatment. The last class, fungicides, all had 100% emergence rates. Because these pesticides use sulfur and copper hydroxide ions to inhibit fungal cell function, they may have no effect on insect cell function. Supporting these implications are previous papers that showed sulfur and copper hydroxide fungicides causing no mortality among tested insects (Amarasekare and Shearer 2013). These could have major implications because studies have shown that coccinellid beetles in California are susceptible to fungal pathogens such as *Erynia neoaphidis* (Zygomycetes: Entomopthorales), which can lead to rise in aphids (Gutierrez and Ponti 2013).

Limitations and Future Directions

There are some limitations of this study that may impede scientific inference. My research question on the effects of pesticides on pupae emergence rates should extend towards post-emergence survival. My results with Rimon showed that only measuring emergence rate as a parameter may not fully disclose the toxicity of some pesticides. It's also important to note that my study is a laboratory experiment and may not be indicative of nature's process. *H. convergens* may pupate under cover which may reduce their exposure to pesticide spray and reduce its effect. My study involves a topical treatment of pesticide, just one of many possible methods of pesticide exposure. Furthermore, *H. convergens* in field may not be as fed as all my subjects were, which could amplify the effects of pesticides. Future research should focus on the other stages of *H. convergens* and other means of pesticide application. *H. convergens* larvae and adults may react differently to a pesticide's mode of action due to changes in body composition and mode of exposure, such as residual or ingestive.

Conclusion

In consideration of my results and the scope of my research, I conclude that Warrior and Rimon are most lethal towards *H. convergens* pupae, whereas Altacor, Cyazypyr, Delegate, Kocide-Manzate, and Kumulus have the least effect on pupae emergence. Although I cannot draw complete conclusions on which pesticide would be the best choice for maintaining *H. convergens* populations, my findings do suggest that synthetic pyrethroids should not be applied to crop fields where conservation of *H. convergens* is necessary. Thus, I believe that my research has been successful in bridging part of our gap in knowledge on these seven novel pesticide's effect on *H. convergens* pupae.

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APPENDIX A: Pesticide Data Analysis

Table 5. Altacor

	Emerged	Died	Total
Treatment	30	0	30
Control	29	1	30
Total	59	1	60

p-value = 0.3132

(1, N=60, p>0.05)

odds ratio:103

Table 6. Cyazypyr

	Emerged	Died	Total
Treatment	27	3	30
Control	30	0	30
Total	57	3	60

p-value =0.07556

(1, N=60, p>0.05)

odds ratio =0.003

Table 7. Delegate

	Emerged	Died	Total
Treatment	27	3	30
Control	30	0	30
Total	57	3	60

p-value =0.07556

(1, N=60, p>0.05)

odds ratio =0.003

Table 8. Kocide-Manzate

	Emerged	Died	Total
Treatment	30	0	30
Control	29	1	30
Total	59	1	60

p-value = 0.3132

(1, N=60, p>0.05)

odds ratio =103

Table 9. Kumulus

	Emerged	Died	Total
Treatment	30	0	30
Control	29	1	30
Total	59	1	60

p-value = 0.3132

(1, N=60, p>0.05)

odds ratio =103