Testing the Toxicity of Organic and IPM Compatible Insecticides on *Anagyrus pseudococci*, a Parasitoid of the Vine Mealybug, *Planococcus ficus*, Over Multiple Generations

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ABSTRACT

This study seeks to understand the effects of three chemicals on the reproductive fitness of the parasitoid *Anagyrus pseudococci* used to control the vine mealybug, *Planococcus ficus*. JMS Styet Oil and PyGanic are chemicals compatible with an organic pest management system, while Movento is compatible with an Integrated Pest Management (IPM) management system. Fecundity was used as a measure of the effect of the chemicals over three generations to give a holistic view of the effects of the pesticides. This study system will mimic a sprayed vineyard in California where these chemicals are sprayed to control the vine mealybug and where *A. pseudococci* are introduced as biological control agents. Both organic pesticides-JMS Stylet Oil and PyGanic-had 100 percent mortality while the Movento treatment had no significant difference in offspring emergence from the control treatment. This indicates the need for further research into the effects of organic pesticides that have little data on their effects on specific species of biological control agents. These results are important for vineyard managers as incompatible pest management approaches have economic implications, making the labeling of pesticides very important and in need of improvement.

KEYWORDS

Integrated Pest Management, alternative pest management, multigenerational study, biological control, fecundity, toxicity bioassay

INTRODUCTION

With the recent increase in research into the effects of pesticides on human health (Maroni et al. 2000), a number of pesticides are being pulled from the market due to adverse effects on human health (Zalom et al. 2005). This has contributed to the a shift towards alternative pesticides and away from certain formerly common pesticides. Worries about the effects of pesticides on human health have propelled the movement towards organic production as well as towards Integrated Pest Management (IPM) (Kogan 1998). IPM is a movement to use alternative pest control methods to the conventional method of applying toxic pesticides regardless of the externalities against a target pest and possibly non-target insects (Baker et al. 2002). IPM involves combining methods of control and restrictions on what chemicals can be sprayed based on the various elements of the IPM system. Some examples of IPM approaches to pest control include biological control, developing pest resistant crops, and crop rotation (Kogan 1998). IPM differs from an organic management system, which the United States Department of Agriculture defines as "seek[ing] to reduce or completely avoid the use of synthetic compounds" (Browne et al. 2000). In California these movements have been popular due to the importance of agriculture to the state's economy. It is also a center of many pest outbreaks, which pose a threat to California's economic health.

In recent years, the invasive scale insect *Planococcus ficus*-commonly called the vine mealybug-has become a major threat to the California wine industry (Daane and Weber et al. 2004). California is a major producer of wine grapes, table grapes, raisins, and other grape products, making the viability of these crops of great economic importance. *P. ficus* is a major threat to crops as it is a known vector for grape leafroll disease (GLD), which is incredibly deadly for grapevines (Cabaleiro et al. 1997, Daane and Weber et al. 2004). Vine mealybugs also feed on the sap of grapevines and secrete honeydew, which leads to the growth of sooty mold, which in turn reduces the viability of crops (Guthman 1999).

In order to maintain high yields of grapes for a wide variety of industries that are important to California's economy, the vine mealybug and GLD must be prevented from spreading. *Anagyrus pseudococci* is a parasitoid commonly used as a biological control agent of the vine mealybug (Daane, Weber et al. 2004) in both organic and IPM vineyards. *A. pseudococci* is a successful biological control agent due its host specificity (Beddington 1978) to

P. ficus. In organic California vineyards infested with mealybugs JMS Stylet Oil-which suffocates pests upon contact-and PyGanic-a botanical that acts as a contact pesticide-are allowed according to California Department of Food and Agriculture (CDFA) regulations. Spirotetramat trademarked as Movento® (a systemic pesticide which is integrated into the vascular system of the plant to poison insects that feed on the sprayed plant) is recognized by the USDA as compatible with IPM systems (Brück 2009). Each of these chemicals has a different mode of action. Modes of action are useful for assessing how different chemicals in IPM systems interact with *A. pseudococci* in order to determine if it would be economical and effective to introduce the parasitoids if the target vines are also sprayed with these chemicals.

Although there is extensive literature on the toxicity of pesticides on parasitoids at high doses (Wilkinson et al. 1975), there are few studies documenting multigenerational effects of pesticides on common biological control agents due to the costs and time associated with conducting multigenerational studies. A limited number of toxicity bioassays have been done on *A. pseudococci* (Brück et al. 2009), yet none outline the possible effects beyond contact toxicity. Longevity (length of time an organism is alive) and fecundity (ability to successfully produce offspring) are two commonly used measures of chemical toxicity, yet they are normally only assessed for one period of reproduction or for one generation. This study will follow three generations of *A. pseudococci* (including the generation exposed) and uses fecundity as measures of toxicity of the pesticides Movento®, JMS Stylet Oil, and PyGanic in order to see if their presence leads to reduction in the effectiveness of pest management regiments. This may be a step towards understanding long term effects of different pesticides on biological control agents used in both organic and IPM systems. Of the chemicals used to assess toxicity, I believe that Movento will be the most toxic of the pesticides in part due to having a systemic mode of action (Halm et al. 2006). This is in part due to there being relatively little research on the systemic mode of action. I think that JMS Stylet Oil will be the least toxic pesticide due to the lack of restriction on the sale of mineral oils such as this one, implying regulatory agencies do not deem oils to have many negative externalities to monitor. This study hopes to represent a step towards understanding long term effects of pesticides that are becoming more popular and have an increased likelihood of contact with biological control agents used in both organic and IPM systems.

METHODS

Conditions for experiment

All *A. pseudococci* used in the experiment were raised on *P. ficus* from a colony grown on butternut squash in the Daane Laboratory. *A. pseudococci* used for the experiment were provided with an optimal reproductive environment. The experiment was conducted in a room with a 24-hour exposure to florescent light and at a constant temperature of 18 °C. This temperature was maintained as at 18 °C, *A. pseudococci* are the most reproductively active (Daane, Malakar-Kuenen et al. 2004) and longevity of the parasitoid increases between 18°C and 30°C (Tingle et al. 1989).

All parasitoids used had emerged from mummies from the laboratory colony within 24 hours prior to exposure. Throughout the experiment and prior to exposure to *A. pseudococci,* the mealybugs for the experiment were raised on sprouted potatoes to provide a steady food source to keep them viable as hosts. The potatoes were USDA certified organic, and purchased from the San Francisco farmers market and then washed in 1 percent bleach in the laboratory for sterilization. During the experiment, the potatoes with mealybug hosts from the colony to be used for the experiment were kept in Fluon treated cups to reduce the number of mealybugs that could leave the cups, further facilitating maximum parasitism rates by *A. pseudococci*. Mealybug hosts were replaced four days after exposure to allow for time to parasitize. Following this four-day period, potatoes were rotated every two days to maintain a steady availability of hosts.

Exposure method

Exposure to treatment chemicals was via feeding on honey and preening, a behavior that has been shown to occur more often when insects are exposed to neurotoxic pesticides (Haynes 1988). I painted the treatment (each of which will be at the field rate dose) on the insides of cages with mesh for ventilation. A honey mixture with water (2:1 ratio of water to honey) was added when the containers were still wet to allow mixing of the treatments and the honey mixture that the parasitoids would ingest when feeding.

The treatments were either of the field rate doses (FRD) of Movento, PyGanic, JMS Stylet Oil, or distilled water (Table 1). Exposure to treatments was through the actions of feeding specifically on honey, resulting in the greatest number of offspring (Islam et al. 1997). *A. pseudococci* was indirectly exposed via preening during the experiment, as tis led to further ingestion of the treatments. Preening is the behavior of cleaning via rubbing legs against mouthpieces. I sprayed *A. pseudococci* in a Potter Spray Tower to ensure ingestion of the chemical treatments while preening as the entire parasitoid's body was covered in the treatment following spraying. These treatments remained on the cages and were allowed to naturally break down or be ingested by the generation of parasitoids exposed.

Table 1. Information on pesticides used in study. Pesticide names, what management system they are compatible with, and the amount of pesticide sprayed is shown below.

Pesticide Name	Classification	Mode of action	Field Rate Dose (FRD)
Movento	Integrated Pest Management compatible	Systemic	62.6 mL / 50 mL of distilled water
JMS Stylet Oil	USDA Organic	A contact pesticide, suffocant	1 mL / 50 mL distilled water
PyGanic	USDA Organic	Botanical, contact pesticide	780 µl / 50 mL of distilled water

Similarity to natural environment

This synthetic environment replicates a freshly sprayed vineyard in which parasitoids would be exposed to the field rate dose of the treatment (Guthman 1999). Spraying was conducted only once for Movento as in the field it would only be sprayed once per growing season. PyGanic and JMS Stylet Oil are sprayed typically around twice per growing season. These two chemicals were sprayed on the initial generation and the second generation following exposure. The purpose of replicating the field as closely as possible is to make results as applicable as possible and to monitor possible effects of IPM compatible or organic chemicals on the fecundity of parasitoids.

Data collection

After spraying, fecundity was monitored every two days for up to ten days, as this is the period during which *A. pseudococci* is the most reproductively active. This will be done by introducing thirty or more second or third instar mealybugs as these stages are preferred for parasitism by *A. pseudococci* (Islam et al. 1997). With each potato rotation, 30 more mealybugs of these instars were introduced. In the following 20 days during which offspring will emerge, the cups that contained the potatoes with emerged offspring were frozen. The exception was for the *A. pseudococci* to emerge as the offspring that emerged were monitored, recorded and killed until a male and female pair per potatoes emerged within 48 hours to be used as the parents of the next generation. These offspring were then counted and the sex of the offspring was determined.

Statistical analysis

In order to analyze the fecundity data, I first determined the normality of the data using boxplots, histograms, and the Shapiro-Wilk test and confirmed that the data was normal and can be analyzed in R. The data was determined to be normal enough to use robust statistical tests such as the Wilcoxon rank sum test, Pearson chi square test, and Welch two sample two sample t-test (McDonald 2009).

My analysis consisted of using Wilcoxon rank-sum tests to compare each generation for treatments versus controls to determine if there is a significant difference in the control and Movento data. This is due to the independence of my samples although they are drawn from the same colony population and each male and female pair was chosen randomly. In order to compare the entire number of offspring for the Movento and control groups, I used the Welch two sample t-test to test if there was a statistically significant difference in the total mean of offspring for all three generations between the Movento and control groups. This was used due to the unequal variances that were probably present due to the data analyzed being biological data. I also used the Pearson chi-squared test with Yates' continuity corrections to test the independence of the sex ratios of parasitoids exposed to treatments versus the sex ratio of those parasitoids exposed to the control. This was done in order to determine if the chemicals have had an effect on the ratio of males to females compared to the control data. This makes the assumption that each pair of parasitoids has the capability of producing any number of offspring, within biological limits. The continuity correction was used as the chi-squared test used data from a contingency table, which introduces error as the chi-square test assumes a binomial distribution, which is not exactly true for this data set. The test is commonly used with the Yates' continuity correction for comparing sex ratios in toxicity studies (Burnett et al. 1998).

RESULTS

Results for JMS Stylet Oil and PyGanic

Overall, JMS Stylet Oil and PyGanic both led to statistically significant mortality rates for *A. pseudococci.* 100 percent of replicates died following spraying, while 100 percent of the controls survived. However, the replicates treated with Movento survived and reproduced for three generations. The Movento data can be analyzed as both the control and treated replicates survived the treatment, yielding data that can be analyzed further.

Determining normality

The Shapiro-Wilk normality test confirmed the normality of the data for the Movento treated group, control, and for the data collected for each generation. The Shapiro-Wilk normality test was used, and all the data was normal, except for the Movento data. The histograms and box plots made of the different data groups supported this conclusion. The Movento treated data collected for the first generation, which yielded a p-value of 0.005, indicating this data is most likely not normal. However, both chi-squared and t-tests are fairly robust so all categories of data were treated as normal (McDonald 1989).

Determining difference in offspring emergence for number of offspring

There appears to be no statistically significant difference between the total number of Movento and the total number of control offspring emergence. There appears to be fewer offspring from the Movento group, but also more variation (Figure 1).

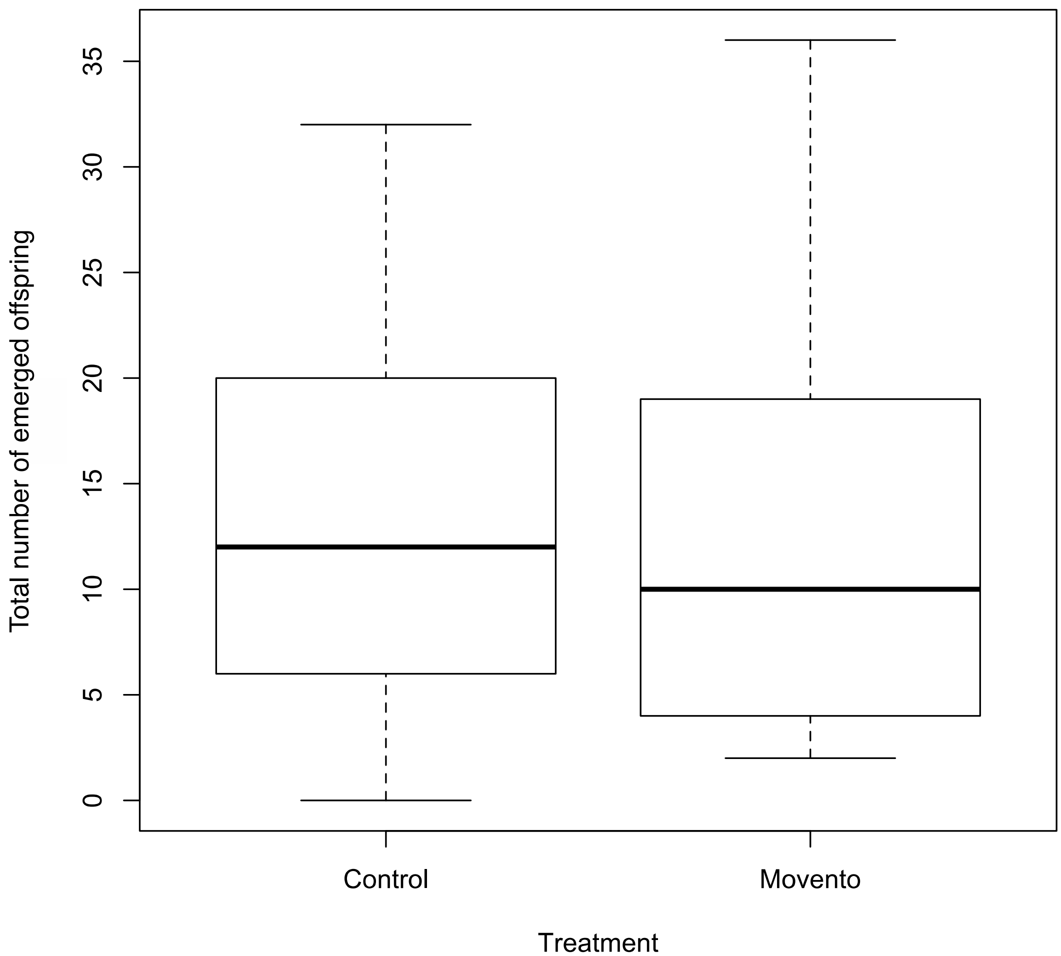

Figure 1. Total emerged offspring and treatments. The number of emerged offspring over three generations were used to determine the effect of Movento on *A. pseudococci*. The average for overall offspring emergence from the Movento group was lower, although this group also had more variance than the control.

When the Welch two sample t-test was run for the data (assuming independent error) the p-value was 0.77, well above the 0.05 statistically significant threshold. This supports the null hypothesis that the true difference in the means of the control and Movento data are negligible.

Determining difference in sex ratio

To assess if there is a real difference in the ratio of males to females for the Movento data, the Pearson's chi-squared test with Yates' continuity correction was used. The test yielded a p-value of 0.41, which is above the statistically significant threshold. This supports the null hypothesis, which states that the difference in the ratio of males to females for the Movento and control treatments is not significant.

Determining the difference of offspring across generations

To analyze the difference in offspring emergence for generations one, two, and three I used Wilcoxon rank sum tests with continuity corrections, which yielded p values of 0.13, 0.26, and 0.24 for each respective generation. All of the p-values are above the 0.05 statistical significance threshold. This supports the conclusion that the difference between offspring emergence among the three generations is not significant. It seems that there are fewer offspring that emerged from the Movento group than control, although this can perhaps be attributed to error (Figure 2) as the Wilcoxin rank sum test results support this not difference not being statistically significant.

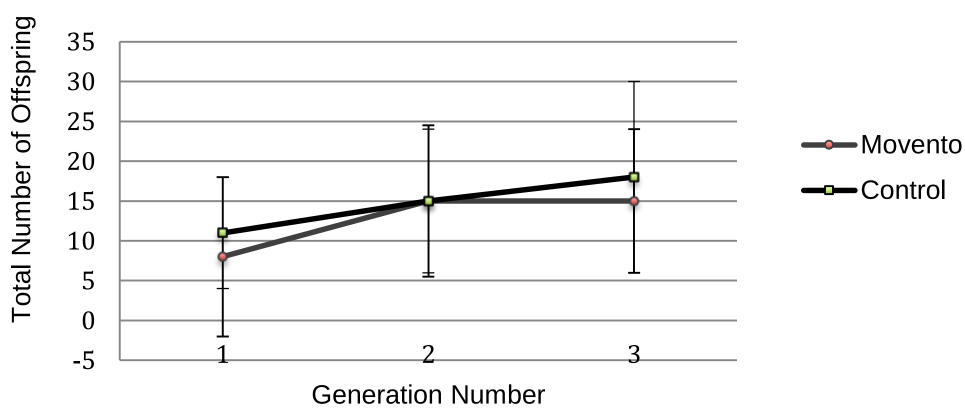

Figure 2. Emerged offspring for each treatment over three generations. The number of emerged offspring over each of the three generations was used to determine the effect of the pesticide Movento on *A. pseudococci* versus the control treatment. There appears to be fewer Movento offspring for generations one and three, although this may be attributed to error.

Summary of results from statistical tests

Due to the lack of statistically significant data, it appears that there is no real difference in the number of offspring yielded from the Movento and control treatments. The results from the Pearson's chi-squared test, Welch two-sample t-test, and Wilcoxon rank sum tests with continuity corrections were all not statistically significant. For all these tests, the null hypothesis could not be proven probably incorrect making it difficult to make any assertions about the results of these tests.

DISCUSSION

The toxicity of exposures to organic and IPM compatible pesticides had mixed effects on the parasitoid *A. pseudococci.* The chemicals designated as organic resulted in 100% mortality of the treatment group, while the IPM designated pesticide had mixed results. Statistical tests did not yield significant results with p-values below the threshold of alpha-0.05; this lends support to the null hypothesis for the Movento treatment. Before conducting the experiment, I anticipated that the number of offspring for the Movento group would be far lower than for the Pyganic and Stylet Oil groups. I also anticipated fewer offspring for the first generation than the second and third generations due to spraying disturbing the parasitoids during a period when they could be reproducing. Although there was statistically significant mortality for the PyGanic and Stylet Oil groups, this does not necessarily mean that these treatments are always toxic to *A. pseudococci* as I had only 10 replicates for each treatment. However, these results do seem to provide evidence that Movento is safer than both PyGanic and JMS Stylet Oil for use with *A. pseudococci* in vineyards in California as previous literature suggests (Brück et al. 2009).

Implications for organic pesticides

The two tested organic pesticides were toxic to *A. pseudococci*. This has important implications for California vineyards, the study system that encompasses *A. pseudococci*. The results of this study support the need to further investigate the toxicity of USDA labeled organic pesticides. Vineyard managers who believe that a labeled organic pesticide may be compatible with biological control agents should determine if the insecticides they use are toxic to the specific biological control species they have chosen to introduce. Many organic pesticides have not been investigated fully as to their effects on specific species, with most labels indicating their general compatibility with beneficial arthropods. This was the case with both JMS Stylet Oil and PyGanic. Beneficial arthropods are a broad category and research on the effects of chemical families on specific species is needed to truly understand the effects of pesticides, whether registered as USDA organic or IPM certified. The labeling of organic and IPM pesticides is important from an economic standpoint as agribusinesses increasingly appropriates the organic market in California (Guthman 2004). This research is also cost effective as generally insects destroy about 13% of crops in the United States annually and for every dollar invested in pesticide control there is a return of about $4 in protected crops (Pimentel 1997). With a public push towards alternative pest management systems due to concerns about human exposure to pesticides, this economically important industry has had to change.

There has been a well-documented shift away from chemicals that have been identified as risk pesticides to a greater number of new pesticides at greater concentrations that have unknown effects on human health (Epstein et al. 2003, Wilson et al. 2001). Pyrethroids such as PyGanic have been rapidly replacing organophosphates between the 1990s and 2000 but need further research to identify their effects (Epstein et al. 2003). The terms ‘organic’ and ‘natural’ have been associated with and many-including policy makers-have come to believe natural chemicals are safe (Richardson et al. 2005). However, a chemical that is natural is not necessarily safe for consumption by humans or arthropods as the dose makes the poison of any substance (Richardson et al. 2005). Similar to organic production, studies on the effects of IPM certified pesticides needs to be conducted to determine the effect of IPM certified chemicals on specific species of beneficial arthropods such as *A. pseudococci*.

Implications for IPM certified pesticides

As Movento had no apparent effect on *A. pseudococci* offspring, it appears to be safer than the two organics that caused 100 percent mortality. Although no effect on the offspring has been found from this or other studies, this does not prove Movento has no effect on beneficial insects, rather an effect may not been found yet. In risk assessment, “nondetects” are not equivalent to "not present" meaning a chemical is not necessarily safe if an effect is not seen in a study (Faustaman 2001). IPM pesticides and practices are less toxic than conventional agricultural practices and pesticides, but IPM systems allow a greater number of pesticides which can be found on IPM produce than organic produce in the United States which may have synergistic effects as little is understood about the interactions between the chemicals that compose pesticides (Baker et al. 2002). More studies must be done for a holistic understanding of the effects of pesticides used as part of an IPM system as there may be effects of pesticides past the general time frame used for toxicity bioassays (Briggs et al. 2005). There are also very few studies on synergistic effects of chemicals on arthropods as well as on human health. In the case of human health, some studies have been conducted that support the claim that ingesting multiple pesticides can have a greater effect than that from separate exposures, even if the exposure is a low dose of several pesticides (Ramamoorthy et al. 1997). Although there may be effects on human health from the IPM approach, the regulation of pesticides and limits set by involving biological control agents does have benefits.

IPM approaches have led to a number of long-term benefits of control, which contributed to the USDA establishing a goal of implementing IPM practices on 75% of the nation's acreage by 2000 (Stephenson 2001). This goal was questioned as early as 2001 by the General Accounting Office due to the lack of leadership and understanding between farmers and the government as to what qualified as IPM leading to questionable practices being used by IPM farmers (Stephenson 2001). It is therefore questionable if this 75% federal goal was ever truly met. However, there are not just environmental benefits of implementing IPM and most likely using fewer environmentally persistent pesticides, but economic benefits that generally derive from averted environmental risks (Mullen et al. 1997). With increased wealth and education, farmers decreased pesticide use or adopted IPM approaches even in countries not under the pressure that the USDA placed on reaching its 75% goal (Schoell et al. 2008). Although some IPM certified chemicals may be toxic for biological control agents, they generally are chosen for IPM systems as they deteriorate quickly, therefore becoming less persistent in the environment (Stephenson 2001).

Limitations

Due to a small number of replicates, the implications for this study are limited. In a study with more than 10 replicates, more substantive conclusions could be drawn about the mortality of all the JMS Stylet Oil and PyGanic treatment groups. The high mortality rate of *A. pseudococci* may be attributable to a factor beyond the toxicity of the organic pesticides sprayed. Sample size and a high amount of experimental error may have heavily contributed to the results of this study. Also, the pesticide may affect the longevity *of A. pseudococci* and the results may not be seen in this study due to the study not being conducted for the full life span of *A. pseudococci*. In the field, the results may have been dramatically different than in the laboratory as there is more variation in vineyards than in a controlled setting. The mode of exposure for *A. pseudococci* may also vary in the field as well as some parasitoids may enter a sprayed vineyard environment following spraying, not just during as the study assumed. Due to the many limitations of this study, there are many directions for future studies that could be pursued.

Future directions

A study to test the effects of Movento on the longevity of the parasitoid *A. pseudococci* and on exposure through sprayed mealybug hosts affect offspring may make the results of this study clearer. Movento may not have been directly toxic as the chemical may have effects on the parasitoids only if they parasitize a host exposed to a non-lethal dose of a treatment. A study on the effects of the pesticide mode of action would determine how systemic pesticides might vary from contact pesticides on *A. pseudococci.* As the mode of exposure is different, it may affect the parasitoid differently than if the exposure was through host exposure to a non-lethal dose. The study only followed *A. pseudococci* through its most active reproductive period; a study analyzing the effects of different treatments on the longevity of the parasitoid may yield interesting results. As this study was conducted in a laboratory setting, a study in the field comparing the number of biological control agents found in fields sprayed with different treatments could have interesting results due to the differences between laboratory and field conditions. With more studies conducted on possible aspects of the experiment that could have produced confounding results, the results of this study may have clearer implications for the field of biological control.

Broader implications

Despite the results of this study not being statistically significant, the trend the data shows supports what has been seen in the literature. Generally, it appears that there needs to be more scientific studies to determine the effects certain organic pesticides as they are substituting old 'risk' pesticides and have unknown results on environmental and human health. Major agencies such as the USDA are encouraging increased testing and regulation of the effects of new chemicals replacing these old 'risk' pesticides as a greater number of chemicals are now being used (Stephenson 2001). Studies on the effects of pesticides need to be conducted over a longer span of time as current studies are usually conducted for less than a generation for a beneficial arthropod, which does not give a clear picture of the effects of a pesticide. Overall, this study follows the general body of literature that supports the need for increased testing of pesticides to determine how they may impact specific species within a study system that uses IPM as well as human health.

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