Colorado Potato Beetle Vitality Against Pathogens After Exposure to Predators

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**Précis**

The Colorado potato beetle (CPB) is a key pest of potato crops in Washington. CPB larvae feed on plants aboveground, where they are attacked by predatory insects. Then, CPB pupate in the soil, where they are attacked by pathogens. CPB can be stressed when they are exposed to, but not killed by predators, and this stress might make them more susceptible to pathogens. We tested this hypothesis in field and laboratory experiments where CPB larvae were exposed to predators or a control and surviving pupae were exposed to pathogens. In the laboratory experiment CPB were exposed to predators by attaching a lady beetle or damsel bug to the end of a wooden dowel rod and forcing it into contact with CPB larvae. CPB not exposed to predators were contacted with a dowel rod alone. Following contact, we recorded the behavior of larvae to determine how they responded to each treatment. After larvae had completed development, we placed them in plastic deli cups containing soil with fungi and nematodes and recorded survival to the adult stage. This allowed us to determine if CPB previously exposed to predators had differing levels of mortality. In our field experiment we placed CPB larvae with potato plants in mesh cages and exposed them to both a combination of predator and density treatments. This exposed them to four different treatment combinations while they developed to their final instar stage. Resulting pupae were exposed to fungi and nematodes as in the laboratory experiment. Once again survival to adulthood was recorded. Our results revealed that predators decreased the CPB’s resistance to pathogens, suggesting predators and pathogens likely act in a complementary way to control CPB. CPB survival to adulthood after pathogen exposure was not significantly effected by the density treatment. If it is true that predators and pathogens can act in a way to suppress CPB pests, this experiment may add important knowledge to the field of
agriculture by providing insight into naturally controlling pests without using harmful insecticides.

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I. Introduction

Colorado potato beetles (CPB) (*Leptinotarsa decemlineata*) are an important pest of potato crops in Washington and other states (Koss et al. 2005). CPB exhibit a complex life cycle, where larvae feed on plant leaves above-ground before burying 3-6 cm in the soil to pupate (Hare 1990). Previous studies have shown that CPB larvae are attacked by a diverse community of predatory insects such as lady beetles (*Hippodamia convergens*) and damsel bugs (*Nabis alternatus*) on plant leaves (Hilbeck, et al 1996). In an attempt to escape from attack, CPB larvae deploy a suite of defensive behaviors when they are attacked by these predators (Ramirez and Snyder 2009; Lynch et al. 2013). These behaviors are potentially energetically costly to CPB, such that larvae exposed to predators above-ground may have a weakened immune system that renders them more vulnerable as pupae to pathogens and parasites that occur in the soil (Ramirez and Snyder 2009). Investigating whether predator exposure will lead to increased mortality of CPB pupae to pathogens has never been directly tested. Determining whether predators and pathogens act in a complementary way to control CPB may aid in developing effective natural pest control methods for this devastating pest.

II. Thesis Activity

We conducted laboratory and field experiments to determine whether CPB exposure to predators increased the beetles’ susceptibility to pathogens. If so, the two groups of natural enemies might be combined to manage this pest in potato cropping fields.
III. Methodology (Materials and Methods)

Collection:

Experiments occurred from April 2013 to August 2013. Field experiments were conducted on the R.B. Tukey Horticulture Orchard in Pullman, WA; laboratory and greenhouse experiments were also conducted in Pullman, WA. CPB were collected as eggs from potato plants in commercial fields throughout the Columbia Basin. The eggs were incubated in petri dishes at a temperature of 23° C, with 24h light, until hatching. Then the larvae were housed under the same conditions, with part of a potato plant and a moist cotton wick, until the beetles reach the fourth instar. The number of days it took for each larva to reach the next instar stage (2, 3 and 4) varied from 2-6 days on average.

Adult *H. convergens* and *N. alternatus* were collected by hand using plastic bug trappers or with D-Vac Vacuum samplers from alfalfa fields primarily in the Columbia Basin. Collections were staggered to ensure that recently collected predators we always available for use in experiments. Predators were housed in an incubator at a temperature of 5°c until they were used. This slowed predator feeding and movement, which helped reduce cannibalism and made it easier to capture and handle predators for use in our experiments.

Insecticide free Russet ranger Potatoes were collected from Washington State University’s Othello research unit (Othello, WA) and transplanted into potting containers in the greenhouse under 16hr light cycles. These plants would later be used to feed larvae in petri dishes or in mesh cages in the field experiment.
**Laboratory Experiment:**

In the laboratory (poking) experiment CPB larvae were exposed to either lady beetles (*H. convergens*), damsel bugs (*N. alternatus*), or a no-predator control treatment. Predator exposure was achieved by fixing the abdomen of a predator to the end of a wooden dowel rod with super glue; this method allowed the head and legs to move freely. The no-predator control treatment consisted of a bare dowel rod. In each experimental unit, CPB larvae were lightly touched by the dowel rod (whether or not it had a predator affixed). For the dowels with the predators, the predators’ head and upper body were touching the CPB. Contact lasted 30 seconds with a light touch elicited every second. All responses from the CPB were recorded following the exposure for 60 seconds. The responses observed included: walking away from the predator, rearing up on their hind legs, regurgitating onto their ventral surface, wiggling their bodies, and/or defecating. The responses were recorded in order to confirm whether exhibiting a response had any significant effect on the effectiveness of immune system and overall survival after exposure to pathogens.

Contact with the dowel was executed twice daily, once in the morning around 8-10am and once in the afternoon around 1-3 pm. Once the CPB completed all four stages of larval development and reached the end of their fourth instar larval stage, each CPB was placed in a 32oz plastic deli container that contained 700 grams of soil from Tukey Orchard. Larval stages usually lasted around 10-20 days. Also added to the soil about 30 minutes before placement of larvae was: distilled water to represent damp conditions that CPB pupae prefer, a nematode solution containing approximately 1000 of each nematode species per container (*Heterorhabditis*...
bacteriophera and Steinernema feltiae), and a solution of approximately 40 million spores of the fungus Beauveria bassiana, strain GHA. This combination of pathogens and fungi mimic the range seen in field conditions (Jabbour et al. 2011). The cups were checked daily to record any pupae that emerged as adults (usually taking 1-2 weeks). Survival rate of pupae to adulthood was then recorded. We allowed 5 weeks for the pupae to emerge before confirming that the adults were dead since this was more than double the time it takes for most teneral adults emerge from the soil (Hare 1990).

In this experiment, we had 60 replicates for each of the three treatments: control, damsel bug, lady beetle, causing there to be 180 experimental units total. Our experiment consisted of 3 blocks, each consisting of 60 replications. These blocks were conducted sequentially in the same location and under the same conditions between the months of June to August 2013.

Field Experiment:

We conducted a field experiment where the experimental units were BugDorm Insect Rearing Tents (fine mesh, 60x60x60 cm cages), set up in a bare-soil and insecticide-free field at Washington State University’s Tukey Orchard in Pullman, WA. We arranged these cages to mimic the true environment that CPB inhabit. Each cage enclosed two potted potato plants that were watered every other day. The experiment included two density levels of CPB (high, low) and two levels of predation pressure (predators absent, predators present). This resulted in four unique treatment combinations: One treatment consisting of a high density larvae with predator exposure. This consisted of 80 CPB larvae in each cage that were exposed to 4 nabids and 4 lady beetles. Another treatment consisted of a high-density larvae with no predator exposure. This consisted of 80 CPB in each cage, with no predators. Another treatment consisted of a low-density of larvae with predator exposure. This consisted of 40 CPB in each cage that were
exposed to 4 nabids and 4 lady beetles. The last treatment was the low-density larvae treatment with no predator control exposure. This consisted of 40 CPB in each cage and no predator exposure. There was a total of 3 blocks each containing 32 cages (with 8 cages for each treatment) occurring sequentially at the same location throughout the months of June and August 2013. Each block lasted about a month. These different blocks allowed for varying seasonal fluctuations to occur in order to better mimic real field conditions. The high-CPB-density treatment consisted of 80 CPB larvae while the low-density treatment consisted of 30 CPB larvae. CPB larvae were collected one day after they had hatched and then were placed on leaves to adjust for one day, and then placed evenly on the leaves of the potato plants in each field cage; that is, either 15 or 40 larvae were placed per plant depending on the CPB density treatment. These densities were chosen in attempt to mimic the range of larval clusters found in nature shortly after hatching. For the predator manipulation, the “predator absent” cages received no predators while the “predators present” cages received 4 H. convergens and 4 N. alternatus in each cage.

After the establishment of CPB density and predation-pressure manipulations, we waited for the larvae to reach their terminal, fourth larval instar (usually taking 10-20 days). Cages were checked bi-daily when watering to observe the CPB; once the CPB started to reach the third instar cages were checked daily to ensure capture of CPB at the appropriate stage. As all larvae reached late fourth instar they were counted and collected throughout the time period it took all surviving larvae to reach this stage (all of the larvae reached their fourth instar within a few days of each other). The surviving CPB reaching fourth instar were tallied in order to know the number that survived. After collection from the cages the beetles were put in the same soil/pathogen/fungi solution as in the laboratory experiment described above. However,
contrasting the poking experiment where only 1 larva was placed in each deli cup, for the field experiment 10 larvae randomly selected from the same cage were placed in each deli cup. If a cage did not have 10 larvae that survived to fourth instar then that treatment was thrown out to help eliminate unknown variables causing such a low number of larvae to survive in the first place. Placing 10 larvae in the deli cup instead of just 1 was meant to mimic field conditions where many larvae from the same plant typically pupate in the soil together. After the larvae were placed in the soil, we then waited 5 weeks while checking deli cups daily for emerging adults to determine the number of beetles successfully completing development.

**Data analysis:**

For the poking experiment all larvae that died before the first poke were excluded since these would not give us any insight into the effects of the treatments.

A two-way analysis of variance (ANOVA) statistical test was used to analyze the data since we compared the effects of multiple levels of two factors (predator and non-predator treatment) and since we had multiple observations at each level.

Response variables of interest were survival rate of first 1st larvae to fourth instar larvae, fourth instar larvae to adulthood, and the number of anti-predator responses.

**IV. Results**

**Laboratory Experiment:**

Larvae exposed to damsel bugs had significantly lower survival than larvae exposed to lady beetles or no predators (Fig. 1a, $\chi^2 = 9.83$, df = 2, $P = 0.0073$). However, survival though pathogen exposure as pupae was significantly lower for CPB that had been exposed to either
predator species as larvae compared with CPB that were never exposed to predators (Fig. 1b, $\chi^2 = 9.47, \text{df} = 2, P = 0.0088$)

Figure 1. (A) Larval survival to fourth instar with two predator treatments and a no-predator control. (B) Pupal survival to adulthood following exposure to pathogen and fungi for pupae that were exposed to one of two predators or a no-predator control in the larval stage.
Figure 1a suggests that nabic exposure lowered survival rates of the CPB larvae to the fourth instar, while exposure to lady beetles did not. Figure 1b suggests that exposure to either a nabic or lady beetle lowered survival rates of fourth instar larvae to adulthood, thus rendered beetles more susceptible to being killed by pathogens.
Fig. 2. The percentage of larvae that responded to each exposure to a (A) no-predator control (B) lady beetle, and (C) nabid. Values are shown for larvae of varying length of development.

Figure 2 represents how larvae responded significantly more often when exposed to a predator than when exposed to the no-predator control (Fig. 2, $F_{2,258} = 34.97, P < 0.0001$).

Although the response rate of larvae did not differ based on the total number of exposures
\( (F_{1,258} = 1.12, P = 0.29) \), there was a significant interaction between the number of days larvae were exposed and predator treatment \((F_{2,258} = 5.02, P = 0.0072)\). Larvae exposed to predators continued to respond consistently throughout their development, while the proportion of larvae responding to the no-predator control declined over time \((F_{2,258} = 5.02, P = 0.0072)\). This suggests that larvae exposed to the no-predator control modified their behavior over time and responded less as they developed.

**Field Experiment:**

There was a significant interaction between predator presence and larval density. The highest larval survival rate was observed when predators were absent and larvae were at low density \((F_{1,56} = 12.3, P = 0.0009)\). Larval survival was significantly lower when predators were present than when predators were absent \((F_{1,56} = 14.1, P = 0.004)\). Larval survival was also significantly lower in the high-density CPB treatment compared to the low-density CPB treatment \((F_{1,56} = 20.7, P < 0.0001)\). Although larval survival was significantly higher in block one compared with block 2 (but not block 3) \((F_{1,56} = 8.02, P = 0.0064)\), the effects of predators and larval density did not differ across blocks (all \(P > 0.45\)).

Survival of CPB after exposure to pathogens as pupae was significantly lower for CPB exposed to predators as larvae compared with CPB that were not \((F_{1,30} = 3.38, P = 0.0020)\). Ability of CPB to survive pathogen exposure was not affected by initial larval density, or the interaction between larval density and predator treatments (both \(P > 0.40\)). Furthermore, the effects of predators and initial larval density were consistent across blocks (all block x treatment interactions: \(P > 0.05\)).
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Figure 3. (A) The proportion of larvae surviving in cages with low (40 larvae) or high (80 larvae) initial larval densities and with two predator treatments (present/absent). (B) The proportion of CPB completing development from pupae to adult across these same treatments. In both panels, results are pooled across blocks, as density and predator effects were similar across blocks (P >0.12 for all analyses)

Figure 3a data indicate that beetle survival rate was high when predators were absent and beetles were at initially-low density, but reduced in the other three treatments.

Figure 3b data suggest that exposure to predators as larvae rendered beetles less likely to survive pathogen exposure, while larval density had no effect.
V. Discussion

In our poking experiment, survival in the pupal stage after exposure to pathogens in the soil was significantly lower for CPB that had been exposed to either predator species compared with CPB that were never exposed to predators. However, in the laboratory experiment only larvae survival rate was affected by nabid predators. One explanation for this is that only nabids are predators to CPB larvae, whereas lady beetles only prey on CPB eggs (Hilbeck and Kennedy 1996). There is a chance that the CPB larvae recognized this and fewer depleted a lethal amount of resources to ‘protect’ themselves from the lady beetle predators. It is also possible that there was metabolic or immunologic programming that was triggered by the nabids that further impaired their defense. In the long run, CPB survival to adulthood after exposure to pathogens was indirectly reduced by both types of predators, suggesting both nabids and lady beetles may have lowered their defense to pathogens.

Our laboratory experiment also looked into the response rate of CPB larvae to the three treatments in the laboratory experiment. Results showed that larvae responded significantly more often when exposed to a predator than when exposed to the no-predator control. Although, the total number of exposures did not dictate response rate of larvae, meaning the likelihood of CPB exhibiting an anti-predator behavior did not correlate with the total number of times a behavior was exhibited, nor did any particular response become more or less common over time. This might suggest that it was not the type of response that lowered CPB immunity, but the response itself or the stress that caused the response. At the same time, there was a significant interaction between the number of days larvae were exposed, predator treatment, and response rate. Larvae exposed to predators continued to respond consistently throughout their development, while the response rate of larvae responding to the no-predator
control declined over time (Fig. 2). This suggests that larvae exposed to the no-predator control modified their behavior over time and responded less as they developed. This may have been due to the control becoming less threatening as the CPB grew or possibly the cost of responding to the control treatment becoming greater than the risk of the treatment over time. However, when a predator was involved, the threat of that predator remained ‘costly’ throughout the CPB instars. It is also possible that the CPB consistently responded to predator exposure because this is a programmed response when threatened.

While both predator treatments produced consistent anti-predator responses throughout development, as well as increase in the number of responses, only CPB larvae exposed to nabids experienced significantly increased larval mortality. This may be due to the fact that larvae exposed to nabids on average responded more than the other two treatments (Figure 2). However, this does not explain why CPB exposed to lady beetles responded more than CPB exposed to the control, yet the CPB larval mortality rates were slightly higher for the control than for the lady beetle treatment, though this was not significant.

In our field experiment CPB exposed to both predators in cages had significantly lower survival rates to adulthood than larvae with no predator exposure. These results were independent of density. Thus, CPB survival to adulthood was indirectly lowered by predator exposure when pathogens were also present. When we take into account direct effects of predator exposure by looking at larval survival and density our results get a little more complex. As seen in figure 3a, at low densities larval survival was significantly lower when exposed to predators. However, density effected survival of larvae in the cages, essentially the high larval density treatment canceled out the effects of predators. Therefore, it is possible that intraspecific
competition for resources between CPB larvae has a greater effect on larval mortality than predation; that is, if the population is large enough and exceeds the carrying capacity.

Yet, as mentioned above, the survival rate of pupae was independent of density. Thus, intraspecific interactions between CPB exposures did not affect the survival rate to adulthood, thus non-lethal high-density exposure likely does not harm the CPB long-term. It is possible that the density effect was more temporary and only stressed them in the moment, without having a lasting effect later in life, like the predator treatment did. This supports the idea that the density effect on the larvae was due to the population exceeded carrying capacity (i.e. the maximum number of larvae the cage could support). This could be due to competition for resources, such as food and space. The effect of density and survivorship to adulthood may be something to further look into as well. For example, we may want to look into whether other non CPB species competing for either food or space also have a density effect on the limiting the number of CPB larvae. Also, looking into intraspecific and interspecific competition between predators may be important. Other studies have found that sometimes predators eat one another rather than the pests (Lynch et al. 2013). This would need to be taken into consideration when determining which predators or the density of predators to use when combating pests. Future experiments should also confirm the effects density has on CPB both short term and long term in order to better understand what factors effect CPB mortality and longevity.

Something else to consider is that in the field experiment both lady beetles and nabids were placed together for the predator treatment. While the field experiment showed that larvae survival was effected by predators in low density of CPB, the laboratory experiment showed that only damsel bugs significantly lowered survival of exposed larvae. Lady beetles and the no predator control had the same larvae survival rate. There is a chance that the nabids in the
predator treatment may have been the main source and reason for why the CPB had a significantly lower larvae survival. In the future we should have three different treatments (control, lady beetle, and nabid, just like in our lab experiment) to be able to separate the effects of lady beetles and nabids on CPB larvae survival. This would also allow us to see if our field results more closely relate to our laboratory results, in that nabids had the greatest effect on larvae survival. Further experiments testing different types of predator treatments may help us understand why this may have occurred. Also, this could help us determine the best combination of predators to use to suppress CPB in general and aid in us determining the most effective pest management treatment.

Previous experiments have shown that predators and pathogens individually lower survival rate of various agricultural pests by directly attacking them at different life stages (Lacey et al. 2001, Crowder, Aultman, Snyder 2011, Wilby et al. 2005). However, the work reported here represents one of the first experiments to show that predator and pathogen exposure does not just work individually, but works in harmony to further lower CPB survival rates, more than just the additive individual effects (but see Ramirez and Snyder 2009). Since past experiments have shown that pathogens, namely nematodes and fungi lower CPB survival, we assumed this would hold true for this experiment (Jabbour, Crowder, Aultman, Snyder 2011, Lacey et al. 2001, Ramirez and Snyder 2009). This is why we did not complicate our experiment with an extra control with no pathogens. However, in the future this could be added.

Future experiments should look into modulating predator and pathogen densities as well as the effects of combining predators versus the effects of predators working independently, such as just using nabids to suppress both larvae and pupae survival or using an array of different predators. Other experiments have shown that a combined effect of predators and high species
richness help suppress pests (Rigby and Jokela 2000, Dwyer and Yee 2004, Straub and Snyder 2006, Cardinale et al. 2003, Crowder et al. 2010). Also, the combined effects of pathogens and predators on shared prey/hosts have been shown to exceed that of either natural enemy alone for other species of pests (Rigby and Jokela). Thus, it is likely that using a larger assortment of predators and pathogens will only increase the suppressions of pests. Something to consider is that lady beetles eat CPB eggs, while nabids eat both CPB larvae and eggs. Using predators that attack different life stages of pests such as eggs and larvae, while still having a negative effect on pupae survival rate after pathogen exposure, may prove to be the best method of pest management.

We have robust evidence supporting our hypothesis that the combined effect of predators and pathogens complement each other and can exceed what any single predator or pathogen species can achieve on its own. This means that previous predator exposure likely stressed the surviving larvae, lowering their immune system, causing them to be more susceptible to pathogens in their pupae stage. Our experiments also showed that predator exposure (namely nabid exposure) not only increases mortality in the pupae stage, but also increases mortality directly to the pupae stage. Thus, nabid exposure decreases the number of larvae and the number of adults and may be an effective method to reducing CPB pests, especially when combined with pathogen treatments. Lady beetles are also effective at lowering survival rate to adulthood, and future experiments should examine the most effective combination of predators to raise CPB mortality overall.

This knowledge may be important in determining new ways of using natural enemies to limit damage of crops by CPB larvae that eat potato leaves and reduce yields. Initially reducing larvae will reduce the most crop damage, as larvae are the most destructive. Yet,
reducing pupae survival to adulthood will reduce the number of overall CPB produced in following generations. Methods to reduce both larval and pupal survival could be breeding and releasing additional predators (especially nabids) on croplands to help naturally reduce pests in addition to avoiding harmful insecticides. This could reduce the overall crop damage in the long run.

This experiment is a precedent for other experiments in the future. Ultimately we are looking for sustainable farming methods that do not incorporate insecticides, pesticides, or other harmful chemicals that could cause health and environmental harm. Future studies should investigate the most effective methods to using predators and pathogens to reduce succeeding populations of CPB. Also, finding out how many predators are needed to be effective at pest management, while still being cost effective will be important. The economic and environmental cost to implement safe and environmental pest management methods such as the one proposed should be compared to other pest management methods, for both organic and industrial farms.

Overall, both of our experiments seemed to show that larvae survival was lower when nabids were present. Our field experiment showed that the combination of both nabids and lady beetles had a significant effect on larvae survival, this effect was greater than the lab experiment possibly because of the combination of predators or the fact that larvae were exposed to predators throughout the entire day, whereas in the lab, they were just exposed twice a day for 30 seconds.

Also, before we use nematodes and fungi to manage pupae, we may want to conduct an experiment to determine whether these pathogens have any effects on the fitness of the plants being harvested. If they do not, then using a large amount of pathogens may be a good idea as one may be able to spray them on the ground just like one would spray insecticides. Although,
methods of placing the pathogens in the soil should be tested in order to determine the best ratio of nematode species to fungi species used, and the amount used.

On conventional potato farms that are sprayed often with insecticides, predator densities are low, and therefore there is less opportunity for predators to influence survival of CPB (directly or indirectly) as lower predator densities and diversity means less opportunity for predators to attack pests (Tscharntke et al. 2007, Tscharntke et al. 2005, Risch, Andow, Altieri 1983). Combatting pests with insecticides can cause ecological damage and safety hazards by the use of toxic chemicals (Lewis et al. 1997). This has caused a push towards safer, environmentally sound, economically viable, and more effective alternatives for pest management. Conventional farms are sprayed with insecticides and fertilizers, this high agrochemical input in crop fields is a primary causes for the rapid decrease of biodiversity in many of these sites (Benton et al. 2003, Bianchi, Booij, and Tscharntke 2006,). Biological control has been proposed because it uses natural enemies to reduce pest populations.

Recent studies related to ours suggesting that greater predator species richness often strengthens pest suppression. In turn, this increases the yield of economically important crops (Wilby 2002, Snyder and Ives 2003, Östman 2004, Naylor and Ehrlich 1997, Landis et al. 2000, Baggen, Gurr, Meats 1999). However, this is generally only true when the natural enemy species fill different feeding niches. Thus, conserving natural enemy diversity may have no effect on the strength of prey suppression when the enemy species is functionally redundant and share or compete for the same niche (Straub et al. 2008).
Predators and pathogens are two different classes of consumers that exhibit different ecological traits such as size, location, and resource acquisition strategy that allows for complementary effects on suppressing pests (Ramirez and Snyder 2009).

While recent experimental studies have examined the impact of predators, parasitoids, and pathogens on insect pests, many of these have only focused on one species at a time. There have been a few studies looking into increasing the richness of a population of natural enemies, or balancing and increasing predator and pathogen communities. Both can reduce the density of pests (Cardinale et al. 2003, Crowder et al. 2010). Thus, merging different enemy species together can suppress pest density more than predicted from the summed impact of each enemy alone. Other findings corroborate our own and suggest that these effects seen in predators carry over to pathogens as well (Jabbour et al. 2011).

Results from our study, and those of others performed in agroecosystems complement the broader debate over how biodiversity influence agricultural ecosystems. Since natural enemies also complement each other indirectly as we tested here, CPB may be regulated using natural biological control under organic farming systems that contain high densities of predators and pathogens. Thus, our experiments not only helped to understand the predator and pathogen connection to survival rate of the CPB, but it can help us make decisions in the future regarding the importance of conserving both predators and pathogens in farming systems.

Our poking experiment and field experiment results coincide and match our hypothesis that CPB exposed to predators in their larval stage are more susceptible to pathogens in their pupal stage, and ultimately have reduced survival to adulthood.

Our study was robust as we had large sample sizes and multiple experiments that helped control for factors including varying weather conditions and human errors. Also, we conducted
both a field and lab experiment in order to test a similar conclusion with different data and see if results were replicable in different settings with the same treatments, which they were. We also used two different predator treatments in the laboratory experiment for stronger results and to help determine which predators work best. In our field experiment we had the same predator treatment, but we also controlled for the effect density had on the CPB survival. For each experiment we collected all CPB eggs, predators, and potato plants used from the same locations in order to reduce lurking variables. However, in order to increase the robustness of our field experiments each separate trial had some variation either in location of where the predators, eggs, and potatoes were collected from, the month in which they were raised or born, and the weather in which the experiment was conducted. These variations represent natural fluctuations and disparities that would be seen in nature. This is a large reason we conducted a field experiment, to determine whether our results would be replicable in natural settings. In the future further studies could be done using larger scale models.

VI. Conclusion

Overall we found that natural predators and pathogens can be used as a natural pest control method to help combat Colorado Potato Beetles and possibly increase yield. This experiment is important to the larger field of entomology, pest management, and agriculture; it focuses on conservation biology as a tool to enhance survival of natural enemies to increase their efficiency in suppressing pests. In turn, by suppressing pests this can help increase yield for farmers, while at the same time not harming the environment with insecticides. Future experiments may employ looking at self-sustaining methods to uphold pest control with little maintenance. Conservation efforts like these may focus on moderating the use of insecticides and optimizing the use of natural biological forces to fight pests. This can help with reducing
the environmental footprint of farming, making agricultural pest management more effective both biologically and economically, and overall making agricultural production more sustainable for the future.

**VII. Acknowledgments**

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VIII. References Cited


Andow 1991; Kruess & Tscharntke 1994; Hawkins et al. 1999; Thies & Tscharntke 1999; Benton et al. 2003


**IX. Appendix**

Lady beetles (*Hippodamia convergens*) (pictured above)

http://www.flickr.com/photos/tedsla

Damsel bugs (*Nabis alternatus*) (pictured above)
CPB (*Leptinotarsa decemlineata*) larvae (pictured above)
http://farm4.static.flickr.com/3483/3260628063_afba7c0a6c.jpg

Adult CPB (*Leptinotarsa decemlineata*) (pictured above)
http://zoology.fns.uniba.sk/poznavacka/Insecta2.htm

2nd instar larvae being ‘poked’ by dowel (pictured above)
BugDorms at Tukey Orchard (pictured above)
Collecting predators at Othello using a D-Vac.

Petri Dishes with leaves, cotton wick, and larvae inside.