Developing a Standard Method for Rearing of *Chrysoperla comanche* For Biological Control

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Abstract

Biological controls, which involve introducing natural enemies for pest management, offer a promising alternative to chemical pesticides, which are known for their detrimental human and environmental impacts. While biological controls provide a sustainable and cost effective option, their efficacy depends on thorough research and effective implementation. My research aimed to develop a method to rear the Comanche Green Lacewing, *Chrysoperla comanche*, a commonly used genus in biological control, and a commonly found species in Colorado. In order to develop a rearing method, I tested 5 different food sources for the larval diet: Mediterranean Flour Moth (*Ephestia kuehniella*) eggs, a mix of *Ephestia* eggs and pea aphids (*Acyrthosiphon pisum*), hornworm (*Manduca sexta*) paste, mealworms (*Tenebrio molitor*), and other lacewing larvae (*Chrysoperla carnea*). The results of my study showed that a diet consisting of a mix of *Ephestia* eggs and pea aphids (*Acyrthosiphon pisum*) resulted in the highest percentage of lacewings surviving through their larval stage and emerging as adults. By finding the most efficient rearing protocol, I generated knowledge and understanding that could help to support an economically feasible quantity of lacewings to agricultural settings. This will enhance the accessibility of lacewings as an Integrated Pest Management tool, promoting sustainable agriculture, reducing pesticide use, and mitigating associated health and environmental risks, locally in Boulder and in agricultural settings across North America.

Introduction

Agriculture has always faced challenges of water management, soil quality, plant quality and yield as well as facing crop losses to pests and pathogens. A pest is a species of either plant or animal (including insects) that damages or competes with a commodity of economic value, crops, animals (humans), wetlands/rivers, landscapes, or structures (Ordish, 1977). Whereas humans have found a variety of ways to control the amount of damage to crops from pests, the perfect pest control method remains elusive. Theoretically, a perfect pest control method would target only pests, avoid harming other organisms, break down safely, prevent resistance, be cost effective, however no pesticide as of 2022 meets all these requirements (Gossett, 2024). While modern farming practices employ synthetic pesticides almost exclusively due to the ease of use and economic feasibility, biological control remains among the most targeted, effective, and ancient means of managing pests (van Lenteren, 2012).

Consequently, many different environmental issues have arisen, and humans continue to strive to find methods of pest control that come closer to meeting the goals of a perfect pest control method. In recent decades, it has become clear that widely used synthetic pesticides are responsible for a wide range of negative consequences, including to human health and environmental issues (Gossett, 2024; Tudi et al., 2021). For this reason, it is important to pursue less harmful methods of pest control. Therefore, I aimed to develop a method for rearing one biological control species, the Comanche Green Lacewing, *Chrysoperla comanche*.

Section 1: Background on Synthetic Pesticides: The Good and the Bad

Why we need some form of pest control

Pesticides, to an extent, are indispensable to modern agricultural methods. Synthetic chemical pesticides are able to prevent human sickness and death, increase crop yield, and increase profit for farmers by quickly managing a wide range of pests. About a third of agricultural products depend on the use of synthetic pesticides; without these, there would be an estimated 78% loss of fruit production, a 54% loss of vegetable production, and a 32% loss of cereal production (Tudi et al., 2021). Even with the currently used pesticides, insects and other pests reduce the world's potential food supply by more than 50 percent (Gossett, 2024). Nevertheless, synthetic chemical pesticides are a substantial contributor to the human population increase over the last century from 1.5 billion in 1900 to 6.1 billion in 2000 (Tudi et al., 2021; Carvalho, 2017). Whereas several factors, such as modern medicine and improved technology contributed to this increase, synthetic pesticides have been a critical factor by lessening crop losses (Tudi et al., 2021; Aktar et al., 2009). It has been estimated that every dollar invested in pesticides results in a yield increase ranging from three to five dollars for farmers (Popp et al., 2013). Additionally, pesticides have been used in a variety of ways to address public health concerns. For example, during the time Dichlorodiphenyltrichloroethane (DDT) was in use, pesticides were seen as instrumental in averting approximately seven million premature human deaths caused by insect-transmitted diseases, such as bubonic plague, typhus, and malaria (Gossett, 2024). This has been done specifically by reducing disease vectors, like mosquitoes, ticks, rats, and mice in places like houses, workplaces, and others. Insecticides are considered the most practical way to control and prevent diseases, preventing about 5,000 deaths globally every day (Tudi et al., 2021; Ross, 2005).

Human and Environmental Health Consequences of Synthetic Pesticides

Although there is an urgent need for pest control, it is imperative to find a form of pest control that is not based on synthetic chemical pesticides that have dire health consequences to both human and environmental health (Gossett, 2024; Tudi et al., 2021). Even as data overall become more accessible and transparent through the internet, there are still significant challenges in having widely accessible, accurate, and thorough records of pesticide risk assessments (Lewis et al., 2016), leading to many unknowns about how pesticides truly affect the world around us.

It has been estimated that while three billion kg of pesticides are used globally every year, only 1% of the total pesticides are effectively used for their intended purpose (Bernardes et al., 2015). Methods of pesticide spraying vary in their efficiency and can contribute to air pollution which exposes the general public to these chemicals (Aktar et al., 2009). Pesticide residues undergo volatilization, dispersion, and long-distance transportation, engaging in a process of environmental recycling between the air and terrestrial surroundings (Benka-Coker et al., 2020; Doan et al., 2021). Additionally, when spraying pesticides, approximately 2% to 25% are lost due to drift volatilization (Pan, 2020). The remaining excessive quantities of pesticides

infiltrate non-target plants and ecosystems, polluting the environment and causing adverse effects on human health (Tudi et al., 2021). Because volatilization causes liquids or solids to commingle with the air and become integrated with the atmosphere, assessing the air pollution caused by pesticides becomes challenging due to the complex and interconnected nature of these environmental processes (Tudi et al., 2021).

Pesticides are suspected of causing long-term health problems like cancer, by acting as carcinogens and/or endocrine disruptors (Gossett, 2024; Environmental Protection Agency, 2024). These effects can even be transmitted to the next generation through epigenetic changes (e.g. there is a 50% increased risk of leukemia, lymphoma and brain cancer in children linked to paternal exposure to pesticides (Ali et al., 2021; Vinson et al., 2011)). Additionally, under some circumstances, cancer risk and mental health problems can increase by 25–30% after exposure to pesticides (Ali et al., 2021; Abd-Alrahman et al., 2014). Many agricultural workers, especially in developing countries lacking strict guidelines for pesticide handling, have lost their health and even their lives due to direct exposure to these chemicals (Gossett, 2024). Additionally, employees in pesticide factories constitute a high-risk group, with a considerable number experiencing poisoning as a result of occupational contact with these chemicals (Gossett, 2024). Every year, 385 million people, or 44% the world's farming population, are exposed to unintentional acute pesticide poisoning, with roughly 11,000 fatalities (Moebus and Boedeker, 2021; Centre for Pesticide Suicide Prevention 2024)

When pesticides are employed, only a fraction of the applied substances serves a protective function, while a significant portion of the pesticides reach the soil, leading to soil pollution (Qin et al., 2014). Runoff, the movement of pesticides in water over sloping surfaces, is influenced by factors such as slope, soil composition, rainfall, and irrigation (Geng et al., 2017). Pesticide mobility in water results in contamination of both surface water and groundwater, posing serious global issues in freshwater and coastal ecosystems. Reports worldwide highlight pesticide contamination in water, impacting local drinking water quality and affecting other species through the food chain. The soil's capacity to filter and degrade pesticides is crucial, but persistent residues can threaten aquatic and terrestrial ecosystems, making soil the principal reservoir of environmental pesticides with implications for water and the food chain (Shakeri et al., 2015).

Insect resistance to synthetic pesticides

The development of pest resistance due to overexposure to a chemical poses a challenge with continued use of chemical pesticides. Over a relatively short evolutionary span of 65 years, starting with the initial report of pesticide resistance by Melander in 1914, pesticide resistance grew exponentially. By the 1990s, roughly 200 insect species had a genetic resistance to DDT (Gossett, 2024), and more than 440 species of insects and mites have documented resistance to one or more pesticides (Tabashnik and Roush, 1990). Despite efforts to replace DDT with newer insecticides, many insects developed resistance to these alternatives, leaving the pest problem largely unabated (Gossett, 2024). The resistance in disease vector species, notably

malaria-transmitting mosquitoes, had posed a significant threat to public health globally. In an attempt to fight pesticide resistance, pesticides are typically increased in concentration or in frequency, also known as the pesticide treadmill. However, this response typically replaces a selective pesticide with a more general one, which can prevent the later use of biological controls (Roush 1990).

Section 2: Background on Biological Control: An Alternative to Pesticides

Consumer demand for safer, healthier, and pesticide free food (Nitzko, 2024; Leppla et al., 2018) requires the development of pest control methods that increasingly approach the ideals of a perfect pest control method. Biological controls, or the control of a pest by the introduction of a natural enemy, typically a parasitoid, pathogen, or predator (often arthropod), make a contribution to this demand. Biological controls can also result in synergistic effects when paired with other Integrated Pest Management techniques (Barzman et al., 2015), such as cultural control (i.e., habitat improvements for natural enemies), mechanical or physical control methods, and the use of genetically modified organisms. Studies indicate that genetically modified crops engineered with insecticidal proteins not only avoid harming natural enemies, but also promote greater insect biodiversity, contributing to sustainable development goals (Romeis et al., 2019; Lu et al., 2012).

There are three broadly recognized approaches to biological controls: conservation, introductory or classical, and augmentative (Naranjo et al., 2019). Conservation biological control involves modifying the environment to enhance the habitat and effectiveness of resident natural enemies in controlling pests. This is typically done through designing agricultural environments to promote the presence and activity of these natural enemies (Barbosa, 1998; Landis et al., 2000). Introductory, or classical, control targets exotic pest species by introducing natural enemies from the pest's native region to achieve long-term pest management (DeBach, 1964). Finally, augmentative biological control involves introducing native or exotic natural enemies either once (inoculation) or repeatedly (inundation), to reduce pest populations. This is the form of biological control that is commonly used in integrated pest management and has a commercial industry built around it (Naranjo et al., 2019).

Little potential for resistance

The risks of pest species developing a resistance are low or non-existent in biological control, while they are high in chemical control (van Lenteren, 2012). Biological control agents also possess higher target specificity than pesticides when properly implemented, minimizing harm to non-target organisms and avoiding accumulation of harmful residues in the environment and food chain. It has previously been shown that decreased use of pesticides in Europe and North America in tandem with biological control methods in greenhouse vegetable production has helped slow the pesticide treadmill (Merino-Pachero, 2007; Pilkington et al., 2010).

Mass production of biological controls has had a substantial history, spanning roughly 120 years, proving to be a successful strategy for pest control (van Lenteren, 2012). More than

7,000 introductions involving almost 2,700 species of exotic arthropod agents for control of arthropod pests in 196 countries or islands during the past 120 years rarely have resulted in substantial negative environmental effects (Cock et al., 2010a, 2010b; van Lenteren, 2012).

Little Potential To Become An Invasive Species

Due to this highly specialized predator-prey relationship and screening of host-specific agents, it is unlikely that the current use of natural predators as a biological control strategy would risk becoming invasive. Like many pest management strategies, biological controls involve intervention in natural ecosystem dynamics, which inherently carry uncertainties. Therefore, conducting a risk assessment is essential prior to any intervention (Mason, 2021). Insect control agents against insect pests were not extensively screened before they were introduced before 1988 (Waage and Greathead, 1988); as a result, many of the instances in which insect biological controls became invasive occurred before screening was required. One of the most infamous occurrences of an insect biological control becoming invasive is the case of the cactus moth, *Cactoblastis cactorum*, employed in Australia to control non-native prickly-pear cacti (*Opuntia*). This was initially deemed a success case; however the spread of the moth to Florida in 1989 raised concerns due to the threat to native *Opuntia* species in North and Central America. The moth's spread from Australia to other areas endangered the culturally significant cactus *O. ficus-indica* (Zimmermann et al., 2000). An important distinction is that *Cactoblastis* was introduced in the 1920s (Zimmermann et al., 2000), which was before the necessary screening measures for insect biological controls were established.

Although efforts to establish natural predators as biological controls created an invasive species in the past, these instances were due to a lack of critical analysis of what would happen and were not properly screened. Databases have since been established and refined in the past decades to analyze the safety and stability of insect predators as a biological control. These databases address the relation between success and the taxonomic group to which the natural enemy and pest belong, the stability of the pest's habitat, and the continuity of its populations (Waage and Greathead, 1988).

Efficient and cost effective

The substantial monetary benefits derived from natural pest control further emphasize the economic and ecological advantages of biological control in agroecosystems. Much of the overall costs of pesticide use are not reflected in the immediate cost to the agricultural producer, due to the indirect nature of the detrimental effects on the environment and human health. Additionally, agricultural producers who release mobile beneficial organisms end up benefiting other producers nearby when the organisms migrate to their field (Baker, 1988). It is estimated that if inclusion of the real environmental cost was reflected in the cost to the agricultural producer, pesticides would be at least three times more expensive than biological controls (Pimentel et al., 1980; Pimentel, 2009; van Lenteren, 2012).

Section 3: Background on The Benefit of Using Lacewings as Biological Controls

In my work as a Student Farming Assistant at the University of Colorado Boulder's Village Center Greenhouse, Common Green Lacewings (*Chrysoperla carnea*) were used as part of the integrated pest management system, alongside parasitoid wasps and synthetic pesticides. As a production greenhouse, this facility supplied all of the lettuce and greens to the Village Center Dining Hall at the university. Having a natural pest control option allowed for produce that contained less synthetic chemicals in the food served to students. These lacewings had to be purchased from a middle man, and then shipped overnight from Europe. This resulted in large shipping and overhead costs that our budget no longer allowed for, and also represents a substantial carbon footprint. The need for a way to grow sufficient quantities of produce for the students using a cost effective and locally sourced biological control agent spurred the idea for my thesis.

Initially, it was difficult to determine whether *Chrysoperla carnea* was native to Colorado, or what lacewing species in general were native to Colorado. Online sources simply said they were "commonly found" or used common names rather than scientific names. Because I wanted to use a species that wouldn't cause an imbalance in the ecosystem, I asked the University of Colorado Natural History Museum's entomology curator, Virginia Scott, to help me investigate what species were considered to be native to Colorado. After looking through the collections, we determined that *Chrysoperla carnea* is actually not commonly found in Colorado. For my research, I used the Comanche Green Lacewing (*Chrysoperla Comanche*), as it is in the same genus but commonly found in Colorado, and I was able to catch them outside on the University of Colorado Boulder campus to start my colony.

In general, green lacewings in the genus *Chrysoperla* are frequently employed as biological control agents due to their predatory larvae (Canard, 2010). Figure 1 illustrates how these larvae use their elongate sickle-shaped jaws to tightly grasp their prey and suck out the nutrients (Canard, 2010). These mouthparts allow the lacewing larvae to effectively prey on common agricultural pests such as aphids, thrips, white flies, spider mites, small caterpillars, some insect eggs, and others (Varenhorst, 2023). These mouthparts also allow the larvae to kill prey far larger than they are, increasing their efficacy. Importantly, lacewings do not pose a threat to humans, as they neither bite nor sting (Newton, 2008), and

Figure 1. Small lacewing larva (right) eating large aphid (left).

they exhibit lower dispersal rates (Bessin, 2022) compared to other biological control agents, such as ladybugs.

Despite the proven effectiveness of lacewings as biological control agents, there are notable limitations to their widespread use. Procuring lacewings often requires sourcing them from companies that ship the insects from countries like Denmark or the Netherlands via overnight delivery, which incurs substantial costs (Brown, 2024). Furthermore, during shipment, these organisms may be exposed to abnormal circadian rhythms, overcrowding, reduced oxygen levels, condensation, and extreme temperatures, all of which can negatively affect their efficacy or even lead to mortality (Leppla et al., 2017). Compounding these challenges, the companies that have developed successful methods for rearing lacewings often treat rearing methods as proprietary (Buehrer, 2024), limiting public access to reliable information. I experienced this when I met with the rearing specialist from a biological control consulting agency, who was kind but evasive about specifics of their rearing process. Consequently, the few openly available resources on rearing green lacewings are often outdated, or use ineffective food sources.

There is little already being done policy-wise to enforce the use of sustainable pest control (van Lenteren, 2012), and general policy change is often slow to materialize, so making an already efficient biological control option more affordable could drive widespread adoption without the need for regulatory reform. This market-driven approach may be more effective than waiting for policy initiatives to mandate the use of sustainable pest management practices, as current policies are limited in their enforcement of such methods.

For my research, I initially aimed to develop a standard rearing procedure for *Chrysoperla carnea* (Common Green Lacewing). However, after learning that this species is not established in Colorado, I shifted to developing the procedure for *Chrysoperla comanche* (Comanche Green Lacewing). This enhances both the financial and logistical access to lacewings as a biological control method, making them a more viable alternative for local farmers. By offering a sustainable pest management solution that is locally produced and cost-effective, one can reduce the need for expensive overnight, overseas shipments, minimize shipping-related mortality and physiological changes in the insects, and lower transportation emissions. Moreover, this approach would provide a species that is already native, or at least commonly found, in Colorado and North America, making it a more ecologically appropriate pest control solution.

Materials and Methods

Materials

Item	Name of product/Brand	Cost	Picture of item
Insect mesh	No See Um Netting	Can be procured on Amazon for $20 for 54" wide x 5 yard	
Multicell container	27 cell multipurpose stackable organizer (boxb)	$9 (each)	
Insect rearing cups	Sure Fresh Mini Storage Containers with Lids, 10-ct. Packs	$1.25 for 10 cups	 (Image from Dollar Tree website)

Insectislip	PTFE Plus - PTFE Escape Prevention Coating	$34.99 (set of 3)	
Anti-static spray	Static-Guard	$5.22	
Curved forceps	sparkfun	$4.50	

Ephestia kuehniella eggs	Koppert Entofood 10g Ephestia *kuehniella*, 50g *Artemia spp*. (product number: 09700)	$61.44	
Mealworms (*Tenebrio molitor*)	Procured from Petco / Imagitarium	$8.00 (100 count)	 (Image from Petco website)
Aphids (Pea aphids- *Acyrthosiphon pisum* Cabbage aphids- *Brevicoryne brassicae*)	NA	Free, pea aphids sent by a Cornell lab, cabbage aphids acquired locally	
Hornworms (*Manduca sexta*)	Procured from Scales ‘N Tails	$15.99 per container (by weight, but typically 25-35 individuals)	 (Image from Scales ‘N Tails website)

Methods

Because standardized methodology is lacking for lacewing rearing, the goal of this study was to determine what methods of rearing are most effective and sustainable to then build a standard protocol. My metric for success is the ability to complete the full lifecycle for lacewings in a laboratory setting. Survivorship and developmental time in each life stage was measured to assess efficacy.

My study began June 12, 2024. While the colony is still alive, the data in this study are up to September 19, 2024. The original lacewing stock used in this study were *Chrysoperla carnea* larvae and eggs donated by Koppert Biological Control Consulting Agency, headquartered at 1502 Old US-23, Howell, MI 48843. However, most died during my initial protocol development. I then caught thirteen adult lacewings on June 26th, 2024, by Boulder Creek (40.01018ºN, 105.25273ºW), then three adult lacewings on July 8, 2024, on the CU Boulder campus, close to Varsity Lake (40.01001°N, 105.27312° W), which were identified as the Comanche Green Lacewing (Figure 2). I maintained this wild caught stock in a mesh cage, in which they then laid eggs. I was able to create a protocol based using these lacewings.

Figure 2. Chrysoperla comanche under a microscope.

To start the process of hatching the eggs, I used curved forceps to individually pluck each egg from its silk strand. I noticed during this process that it was easy to accidentally crush the egg or to pull it off the stalk while trying to grab it, so I recommend doing this with a slight diagonal downward motion. I then placed the eggs on a previously sanitized insect rearing cup container lid sprayed with Static-Guard and allowed to dry. Static-Guard is essential for the eggs and the larvae since they are so small that they can easily be killed by static. Then, I prepared the multicelled organization tray in which I placed the eggs. I sprayed the container with Static-Guard, allowed it to dry, and placed cut up paper towels at the bottom of each cell. The paper towel provides a place for the newly hatched larvae to hide in order to reduce stress. I also tried using rice hulls instead of paper towels at the bottom of the cell, however it was very difficult to find the newly hatched larvae unless they were crawling on the sides or top of the cell.

Then, using a small paintbrush, I placed one egg into each cell of the container. I then placed insect mesh on top of the cells before placing the lid on top of it. I then placed insect mesh on top of the cells before placing the lid on top of it as it became apparent after several rounds of these protocols that the larvae are capable of traveling between cells to prey upon their siblings. I tried using insecti-slip and found it inadequate to prevent transit between cells. In some instances, I put one egg in an individual cup (same kind I used for the larvae) but there are

usually many eggs at a time and it is quite resource and time intensive to have one egg per cup. I taped the lid down to the container, since the mesh between the container and lid creates a bit of space that could potentially allow the lacewing larvae to escape, so I close that gap by tightly taping the lid to the container. I labeled the container with the date and how many eggs are in it, so that I could later record how long it took for the larvae to hatch. I placed the container(s) in the incubator at 28ºC (82ºF). Our incubator did not control humidity, so I put a small bowl of water in the incubator. In general, the humidity should be maintained at about 30-50% (Rincon Vitova Insectaries). Placing the eggs in the incubator was not strictly necessary, since many eggs left in the mesh cages with the adults did hatch. However, incubation may lead to a more consistent and potentially quicker hatching rate.

Once the larvae hatched, I started the process of setting up their enclosure by preparing the cups they would be kept in. I did this before taking the larvae out of the container since they move quickly and can be lost easily. I used a small resealable cup that I bought from the Dollar Tree for each larva's enclosure, as they can be easily washed, reused and sealed. Once I bought the cups, I drilled a small hole in the lid of each, and then hot-glued insect mesh over the hole, doing so carefully to prevent the glue from covering the hole. This allowed airflow into the cup without allowing the larvae to escape. I set up the cups by sanitizing them by spraying 70% ethanol in the cup and wiping it away with a towel. I then lightly sprayed it with Static-Guard and allowed it to dry. Finally, I placed a small piece of paper towel at the bottom, cut to the size of the bottom of the cup. I initially also lightly coated the inside of the lid with vegetable oil to prevent the larvae from climbing on the lid, however this resulted in many of the larvae getting stuck to it and dying, so I stopped doing this after the first round of larvae.

Figure 3. Insect rearing cup set up (without food source).

Figure 4. Air tight sealed container with labeled insect-rearing cups containing larvae inside.

Once the cups were prepared, I removed the tape from the container, and removed the mesh to expose each cell individually to prevent any larvae from escaping. I used a small paintbrush to transfer each larva into their own individual cup. I then labeled the cups with the date hatched, the food source, and assigned a number to that individual to be able to track it throughout its life cycle. Depending on where I was in my study, the rearing cups may have been placed together in an air sealed container with multiple wet paper towels at the bottom of it. I didn't try this method until after initially trying several rounds of treatments including the *Ephestia* eggs, mealworms, and the lacewing larvae, and finding that humidity was an important factor to keeping the larvae alive. There should only be enough paper towels to cover the bottom, and no more, since too much moisture inside the air sealed container can cause molding and even enough condensation within each cup to drown the larvae.

I tried five different food sources for the larvae: Mediterranean flour moth eggs, a mix of *Ephestia* eggs and aphids, hornworm paste, lacewing larvae, and mealworms. I chose to use Ephestia eggs as a food source as this was recommended by the consultants at Koppert. The *Ephestia* eggs I received from Koppert were a mix of 10g *Ephestia kuehniella* eggs and 50g *Artemia* spp. eggs, however, the larvae never touched the Artemia eggs. To feed the larvae the *Ephestia* eggs, I lightly coated the tip of a soft bristled paintbrush with the eggs, then lightly tapped it on the paper towel in the cup. I replaced the eggs every other day to prevent mold growing. I found the easiest way to do this is by using the soft bristled paintbrush to pick up the larvae and place it on the lid, then use the paintbrush to sweep the remaining eggs into the trash. I also thoroughly cleaned each cup once a week, more if mold grew, by placing the larva into a different cup or small test tube, rinsing the original cup with soap and warm water, spraying with 70% ethanol, drying it, lightly spraying it with Static-Guard and allowing that to air-dry. I then replaced the paper towel at the bottom of the cup, tapped more eggs into the cup with the paintbrush, and replaced the larva back into the cup.

Figure 5. Amount of Ephestia egg mix fed to larvae shown on paintbrush and in cup.

I chose to feed the larvae aphids since the larvae eat aphids when outside. The aphids I fed the larvae were initially a pea aphid (*Acyrthosiphon pisum*) colony sent to me from a lab located at Cornell University. However, keeping this colony alive was unsustainable, since it required fava bean plants that would be replaced once or twice a week. Since my accessibility to aphids was not consistent, I supplemented the diet for these larvae with the *Ephestia* eggs. Later

on, I replaced the pea aphid colony with a cabbage aphid (Brevicoryne brassicae) colony, supplied by a local garden. In order to keep the pea aphid colony alive, I had to plant five fava bean plants twice a week- every Monday and Thursday, I then placed the plants in a mesh cage on a plastic tray, which I would fill with an inch of water weekly. The plants were also replaced twice a week, as aphids are incredibly sensitive when kept in captivity and need healthy, young plants to stay alive. To feed the cabbage aphid colony alive, I kept them in a terrarium rather than a mesh cage to increase and maintain humidity. I put a Red Russian kale plant in this terrarium, and in order to make this an easier process, I place two new kale leaves in the terrarium every day and remove the old leaves.

I chose to feed the larvae hornworm paste, as the larvae eat small hornworms when in an outside setting (Groves, 2020), but the hornworms I initially bought were too big for the larvae to eat. To feed the larvae hornworm paste, I froze a colony of Tobacco hornworms (*Manduca sexta*) procured from Scales N' Tails, a reptile store in Boulder, CO. I then used a mortar and pestle to mash the frozen worms into a paste. I separated the mixture into small dots into a petri dish, which I placed back into the freezer. Once the paste in dot form was frozen, I used forceps to transfer a paste dot onto the paper towel in the larva cup. I made sure to replace the paper towel and paste dot daily to prevent molding. I also thoroughly cleaned each cup once a week, or more often if mold grew. I cleaned the cup by placing the larva into a different cup or small test tube, rinsing the original cup with soap and warm water, spraying with 70% ethanol, drying it, lightly spraying it with Static-Guard and allowing that to air-dry. I then replaced the paper towel at the bottom of the cup, placed another paste dot on the paper towel, and replaced the larva back into the cup. The paste dots were stored in the refrigerator.

Figure 6. Mortar and pestle used to prepare *Manduca sexta* as diet for lacewing larvae, insect-rearing cup set up for hornworm paste.

I chose to feed the larvae other larvae because cannibalism is already common for lacewing larvae (Rojgt, 2009), so I was curious if this could result in the ability to survive as larvae and become adults. To feed the larvae other lacewing larvae, I used a soft-bristled paintbrush to place 2 first instar larvae into the cup. I replaced the larvae carcasses daily to prevent mold. I cleaned the cup weekly, or more often if mold grew, in the same way as described for the *Ephestia* and hornworm paste food source method. Some larvae that were sent

by Koppert were kept together in a large container filled with buckwheat hulls. This was because they sent 10,000 larvae, which was not possible to create individual containers for.

I chose to feed the larvae mealworms (*Tenebrio molitor*) because previous literature mentions that this is a successful option (Loru et al., 2014; Weaver, 2018) To feed the larvae mealworms , one small mealworm was placed into the cup onto the paper towel. Once the lacewing larvae reached the third, final, instar, I placed a small piece of corrugated cardboard into the cup. I was able to tell that a larva was in its final instar by its size, as it is visibly significantly larger than any other instar. I was able to see that larvae can pupate without the cardboard, sometimes choosing to pupate on the paper towel or on the cup lid, however they more often chose to pupate inside the corrugated cardboard. Because the larvae will most likely choose to pupate inside the cardboard, I made sure to clean the cup and replace the cardboard at least twice a week when a larva reached its final instar to prevent mold that could potentially spread to the pupa.

Once the larvae did pupate, I removed the cardboard with the pupa inside of it and threw away the contents of the cup. I cleaned the cup and the lid with soap, warm water, and sprayed with ethanol and dried with a paper towel. I then placed a clean piece of paper towel at the bottom of the cup, then replaced the cardboard with the pupa in it back in the cup. If the larva pupated on the paper towel, I cut off the excess paper towel around it and placed it on top of the new paper towel. If the larva pupated on the lid, I carefully wiped the surrounding area of the lid with ethanol, avoiding the pupa. I added a marking on the cup's label that it has pupated along with the date of pupation. I then placed the cup back into the air sealed container, as it is very important to control humidity in the pupal stage. I checked daily for any mold, wiping off any with ethanol on a paper towel. I checked daily for emergence of the adult stage.

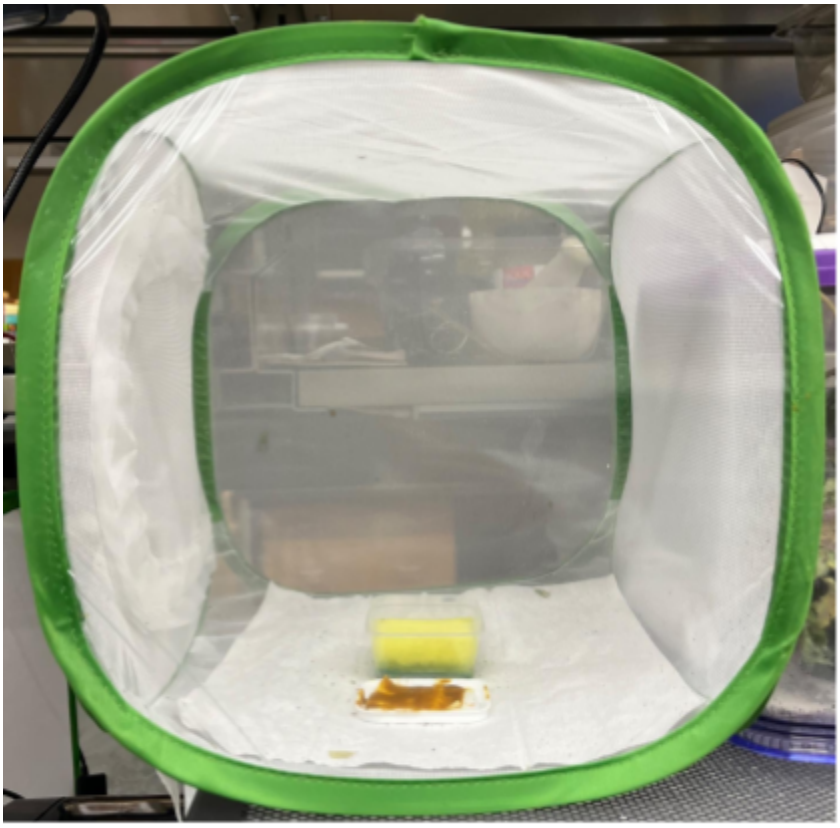


Figure 7. Mesh cage with food and water source inside.

Once the adult emerged, I documented the date of emergence and sex (females have a larger, rounder, abdomen). I took note of this to decide where to place the adult. For example, if a mesh cage already had more males than females, it would be beneficial to place a newly hatched female into that cage, while it was beneficial to place a male into a cage with more females to balance out the ratio. I carefully opened

the cup into the mesh cage to allow the adult in.

The overall mesh cage set-up changed as the season transitioned from summer to fall. In the summer, the mesh cage was set on the lab table. The bottom of the cage was lined with a paper towel, which was replaced weekly to prevent the frass from molding. I initially placed a wet sponge on top of the cage and covered it with a larvae cup, but it dried out very quickly, so I changed this part of my protocol to have the larvae cup partially filled with water inside the mesh cage with the sponge inside of it to allow the adults to have somewhere to stand while they drink and not drown. I initially placed the food mixture (described below) directly onto a larvae cup lid, however many lacewings died due to getting stuck and drowning in the mixture. To combat this, I placed a piece of paper towel onto the larvae cup lid and smeared the food mixture onto the paper towel; no adults have died since I started this method.

The food recipe consists of:

- 2/3 cup warm water
- 1/4 cup brewer's yeast
- 2 teaspoons honey
- ½ cup bee pollen
- 1 tablespoon vegetable oil (this was not originally part of the food recipe, but I noticed the food source dried out very quickly, so this prevents that from happening)

Lacewings overwinter as adults or as pupae, which matched my observations once summer ended, as my adults stopped laying eggs. To avoid overwintering behaviors in the fall, I placed a reptile heat mat to the side of the cage, as well as placing the mesh cages inside a tank with plastic lining to control humidity. The bottom of the tank is lined with wet towels to control humidity, which I rehydrated daily by using a spray bottle to spray warm water. I also lined the bottom of the mesh cages with paper towels, which are also replaced weekly to prevent the frass from molding. I placed a larvae cup partially filled with water inside the mesh cage with the sponge inside of it, and I placed a piece of paper towel onto the larvae cup lid and smeared the food mixture onto the paper towel. I also incorporated an aster plant into one of the cages. In the cage I had the aster plant in, I used a paintbrush to transfer as many pea aphids as would fit on the tip in hopes that it would further stimulate oviposition. However, the adults did not lay eggs due to the presence of pea aphids.

For both the summer and winter set-ups, I replaced food and water daily, and sanitized the sponge weekly by spraying it with ethanol and rinsing thoroughly.

Results

Final Protocol:

Eggs:

1. Using forceps, gently pluck each egg from its silk stalk and place them on a previously sanitized insect rearing cup lid sprayed with Static-Guard. Once gripping the stalk with the forceps, use a downward then sideways motion in order to prevent accidentally cutting the egg off the stalk.
    a. Alternatively, if there are many eggs condensed next to each other, they can be snipped off their stalk using fine scissors with an insect rearing cup lid below.
    b. Place the mesh cage on top of a piece of black paper to see if any eggs were lost. If so, they can be picked up using a small, soft bristle paintbrush.
2. Then, prepare the multicelled organization tray to put the eggs into.
    a. Spray the container with 70% ethanol to sanitize.
    b. After drying, lightly spray the container with Static-Guard spray. This prevents the eggs and future hatched larvae from dying from the static due to their small size.
    c. Allow the spray to dry.
    d. After the spray is dry, place small pieces of paper towels that fit into each individual cell.
3. Using a small paintbrush, place one egg into each cell of the container to prevent cannibalism.
4. If the container you are using has any space between the cells and the lid, place insect mesh on top of the cells before placing the lid on top of it to prevent larvae from crossing from cell to cell.
    a. Insectislip will not work for this.
5. Tape the lid down to the container
    a. The mesh between the container and lid creates a bit of space that could potentially allow the lacewing larvae to get out so it is important to close that gap by tightly taping the lid to the container.
    b. It is helpful to partially fold down a small part of the tape at each end to make it easier to pull it off later.
6. Label container by using a small piece of masking tape with the date and egg abundance.
7. Place the container(s) in the incubator at 28°C (82°F)
    a. If the incubator does not control humidity, place a bowl of water in the incubator.
    b. If the incubator can control humidity, set it to 30-50% (Rincon-Vitova Insectaries)
    c. Eggs should hatch within 3 to 4 days.
    d. This step is not necessarily a must, as eggs can still hatch at room temperature, but it is not guaranteed.
    e. If there is no access to an incubator, it may help to keep the eggs in an air sealed container with wet paper towels at the bottom, preferably on a heating pad.

Larvae:

1. Prepare the cup:
    a. Sanitize the cup by spraying 70% ethanol and wiping it away with a towel.
    b. Spray the cup with Static-Guard (lightly) and allow it to dry.
    c. Place a small piece of paper towel that is cut to the size of the bottom of the cup.
2. Once the larvae hatch, remove the tape, and remove the mesh to expose each cell individually, one at a time, to prevent any cannibalisation while transfering.
3. Use a small paintbrush to transfer each larvae into their own individual lidded cup.
    a. The cup's lid should have a small hole drilled in it for airflow, with a small piece of insect mesh hot glued over it. Ensure that the hot glue does not cover the hole.
4. Label the cups:
    a. Date hatched or transferred into the cup.
    b. Food source if you are testing new options.
    c. Number the cup for the purpose of observation.
5. Place the individual cups together in an air sealed container with just enough wet paper towels to fully line the bottom. This helps to control humidity and prevent desiccation.
    a. Do not put too many wet paper towels at the bottom, and make sure to pour out any excess water, as too much water can condense inside the cups and potentially drown the pupa.
6. Feeding:
    a. Using a small paintbrush, place 2-4 aphids in the cup.
        i. Size of aphids depends on the size of the larvae. For example, the first instar should be given younger, smaller aphids, while older larvae can be given adult aphids. The larvae can eat aphids slightly larger than them, but adult aphids shouldn't be given to newly hatched larvae.
    b. *Ephestia* eggs can be used as well but often do not all get eaten and can quickly mold
        i. If using *Ephestia* eggs, use a small paintbrush to scoop a pea sized amount of the eggs onto the paper towel.
    c. Food should be replaced daily.
    d. Make sure to remove aphid carcasses daily before replacing to prevent mold.
7. Once larvae reach 4th instar, put a small piece of corrugated cardboard into the cup. The larvae can pupate without it, but often choose to pupate inside it if given the choice.
8. Cleaning: The cup should be cleaned twice a week (every other day if using *Ephestia* eggs), more often if you can see mold or other substances that shouldn't be there.
    a. Using a paintbrush, place the larva in a different cup or test tube (just something to contain it that has a lid while cleaning the cup).
    b. Throw away the contents of the cup.
    c. Rinse the cup with warm water.
    d. Wipe cup and lid down using 70% ethanol, dry.

e. Lightly spray static-guard, allow to dry.
f. Replace paper towel, cardboard, and food source.
g. Put larva back into the cup.
h. Wipe down the transfer cup with 70% ethanol before cleaning the next cup.

Pupae:

1. Larvae will most likely choose to pupate inside cardboard, which is why it is important to clean the cup and cardboard at least twice a week, otherwise the cardboard will likely grow mold.
    a. If the pupa is in the cardboard, it is difficult to transfer the pupa from the cardboard without damaging it, but it is possible. Preferably make sure it doesn't mold in the first place.
    b. If the cardboard does mold, carefully pull it apart, use a hard-bristled paintbrush to pick up the pupa, lightly pull loosen the corrugation of the new cardboard piece, and gently nudge the pupa inside the cardboard using the paintbrush.
2. Once the larva pupates, it will most likely be inside the corrugated cardboard. Remove the cardboard and place it to the side. Throw away the contents of the cup.
3. Clean the cup and the lid with ethanol and allow to dry.
4. Place a clean piece of paper towel at the bottom of the cup.
5. Place the cardboard with the pupa in it back in the cup and place the lid back on.
    a. If the larva pupates on the paper towel, cut off the excess paper towel around it and place it on top of the new paper towel.
    b. If the larva pupates on the lid, still (very gently and carefully) wipe the surrounding area of the lid with ethanol, avoiding the pupa and the area around it. Allow it to dry before placing the lid back on.
6. Add a marking on the label that it has pupated along with the date of pupation.
7. Place the cup back into the air sealed container- it is very important to control humidity in the pupal stage.
8. Check daily for any emergence of mold. If there is any, wipe it off using ethanol.
9. Check daily for the emergence of an adult.

Adults:

1. Once the adult emerges, document the date of emergence and gender (females will have a larger, rounder abdomen), this will help understand how different variables that change affect the hatching rate, and documenting gender will help decide where to place them (for example if a mesh cage has more males than females, it would be beneficial to place a newly hatched female into that cage).
2. Carefully open the cup into a mesh cage to release the adult.
3. Overall mesh cage set-up:
    a. During the summer:

i. Mesh cage can be set on a counter-top.
ii. Line the bottom with a paper towel (replaced weekly).
iii. Place a larva cup partially filled with water with a small sponge in it (for the purpose of drinking water), rinse and replace daily.
iv. Smear the food mixture on a paper towel on the rearing cup lid.
    1. Clean the lid daily with warm water and ethanol.
    2. Replace paper towel with food daily.

b. During the fall/winter:
i. Place the mesh cage inside a tank with a plastic sheet between the tank and the lid to control humidity. Poke small holes in the plastic sheet to allow for some air circulation.
ii. Line the bottom of the tank damp towels to control humidity.
iii. Line the bottom of the cage with a paper towel (replace weekly, or more often if mold begins to form).
iv. Fill a larvae cup partially filled with water with a small sponge in it (for the purpose of drinking water), rinse and replace daily.
v. Smear the food mixture on a paper towel on the rearing cup lid.
    1. Clean lid daily with warm water and ethanol
    2. Replace paper towel with food daily
vi. Place heat mat on the side of tank

4. Food recipe:
    a. 2/3 cup warm water
    b. 1/4 cup brewer's yeast
    c. 2 teaspoons honey
    d. ½ cup bee pollen
    e. 1 tablespoon vegetable oil (as I noticed food source dried out very quickly, this prevents that from happening)
5. Sanitize sponge weekly using soap and warm water, then, ethanol, and thoroughly rinsing.

Statistical Analysis

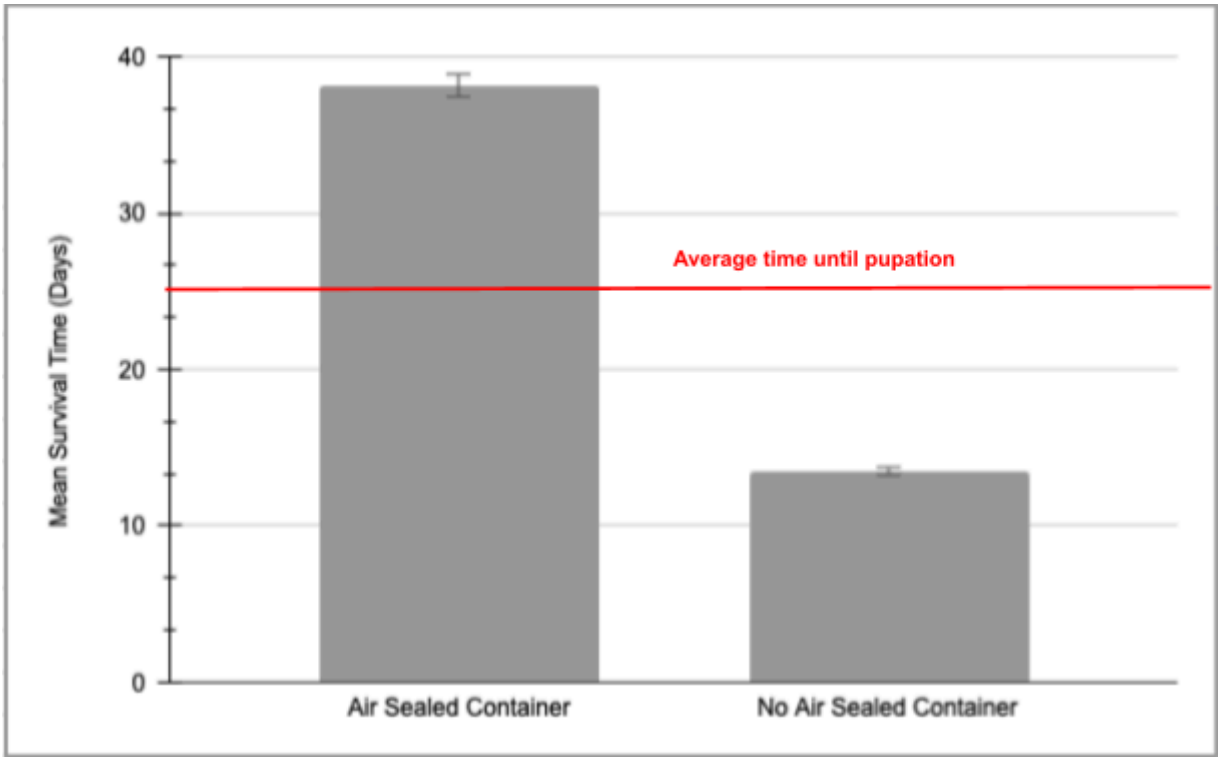
I used a Cox-Proportional Hazards Model to compare survivorship of *Ephestia* eggs to the other food treatments, and to show the relative significance of my results. This model shows how likely an individual is to die under a different treatment. A smaller coefficient shows that a treatment is less likely to result in mortality and a larger coefficient shows that a treatment is more likely to result in mortality. For the purpose of the model, I chose one treatment to compare the rest of the treatments to. I chose *Ephestia* eggs to compare the other treatments to due to its relatively large sample size.

Quantification of Outcomes

Survivorship

I reared 428 larvae according to the above procedure, with adjustments to both storage system and diet, measuring survival, time to pupation, and time to adulthood. It is important to note that out of the 428 larvae, 118 of those larvae did not have a distinct hatch date either because they were found outside, found in the mesh cage, or were sent as larvae by Koppert. The survival experiment ended after 45 days, the pupation experiment ended after 47 days, and the emergence experiment ended after 62 days.

Figure 8: Survivorship (± Standard Error) With and Without Air-Sealed Food Storage Container.



This bar graph shows the mean survival time of larvae (in days) for the *Ephestia* egg treatment in both air-sealed and non-air-sealed food storage containers, with standard error bars included. The red line represents the average time until pupation. Larvae in the air-sealed container demonstrated a significantly longer mean survival time compared to those in the non-air-sealed container. This data is based solely on the *Ephestia* egg treatment, which had the largest sample size.

In the absence of humidity, larvae across all treatments experienced high mortality. To maintain high humidity, I kept the rearing-cups in an air-sealed container with the bottom lined with wet paper towels. The air-sealed container was able to contain a lot of moisture which increased the humidity content, preventing desiccation and increased survival time (Fig. 8). The air sealed container method was able to increase survival time for the larvae by about 25 days, where the air sealed container method survived 40 days (± 1.68) on average, while not using the air sealed container method survived about 15 days (± 0.696) on average. Clearly, the larvae held

in the air sealed container survived longer on average than larvae not in the air sealed container ($p < 2.2 \times 10^{-16}$, Two-sample t-test).

I compared larval survival numbers (Table 1; Table 2; Figure 9) and the amount of time observed to reach significant life stages of pupation (Table 3; Figure 10) and emergence (Table 4; Table 5; Figure 11) among the different treatments. Diet treatments began as soon as I transferred each larva into its own cup after hatching. In all experimental treatments, I observed high larval mortality within the first 15 days.

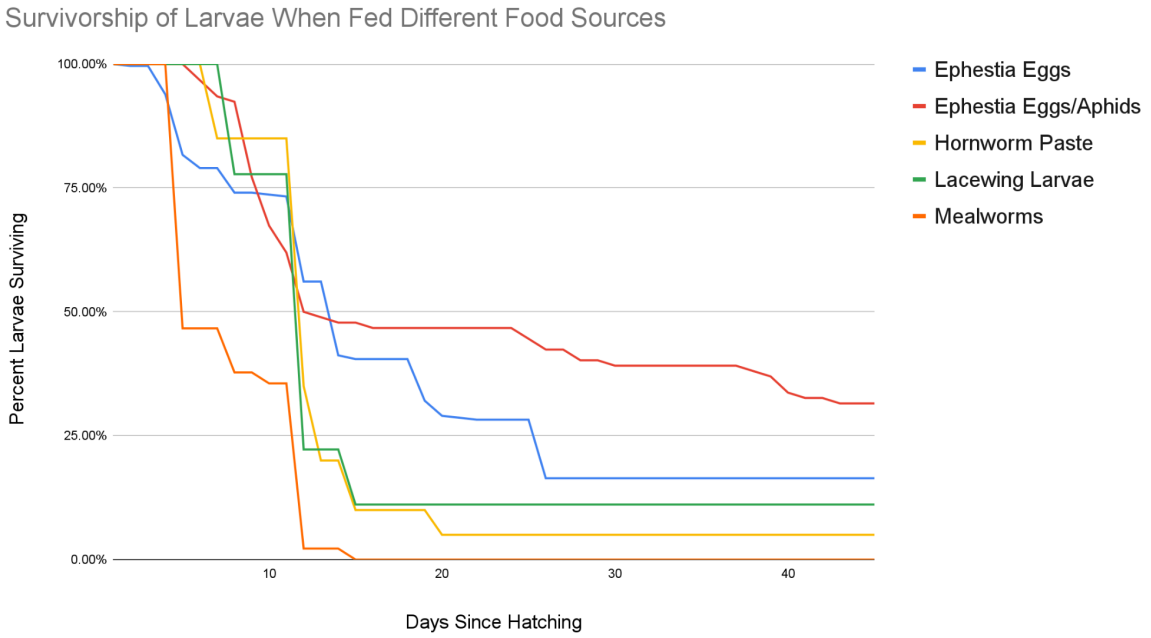
Of the 428 larvae that were used in this study, 44 survived to adulthood. However, it is important to indicate that food source plays a key role in emergence. For each specific treatment of food sources used, initial observations were of the numbers of larvae which survived to pupation. A mix of aphids and *Ephestia* eggs resulted in the highest number of larvae pupating and emerging into adults (Table 1; Table 2; Figure 9).

Table 1: Number And Percent Of Larvae Survived Per Treatment

Treatment	Sample Size of Larvae Started With	# Larvae Survived To pupation	% Larvae Survived To Pupation
Ephestia eggs	262	43	16.4%
Ephestia eggs/Aphids	92	29	31.5%
Hornworm Paste	20	1	5%
Lacewing Larvae	9	1	11.1%
Mealworms	45	0	0%

This table displays the survival outcomes for larvae under various treatment conditions. The treatments include *Ephestia* eggs, *Ephestia* eggs combined with aphids, hornworm paste, lacewing larvae, and mealworms. For each treatment, the sample size, number of larvae that survived to pupation, and the corresponding percentage survival rate are shown. The highest survival rate was observed in the EP/APH treatment (31.5%), while the MW treatment resulted in no survival.

Figure 9:



This survival curve illustrates the percentage of lacewing larvae surviving over time when fed different food sources: *Ephestia* eggs, *Ephestia* eggs with aphids, hornworm paste, lacewing larvae, and mealworms. The y-axis represents the percent of larvae surviving, while the x-axis shows the number of days since hatching. Larvae fed *Ephestia* eggs with aphids demonstrated the highest initial survival rate, while those fed mealworms showed the lowest survival, with all larvae in this group dying within about 11 days.

Table 2: Statistics for larvae survived per treatment

Treatment	Coefficient	P-value
Ephestia Eggs/Aphids	-0.3018	0.0378 *
Hornworm Paste	0.4929	0.0413 *
Lacewing Larvae	0.5661	0.0975
Mealworms	1.4176	4.48×10^{-16} *

* = statistically significant

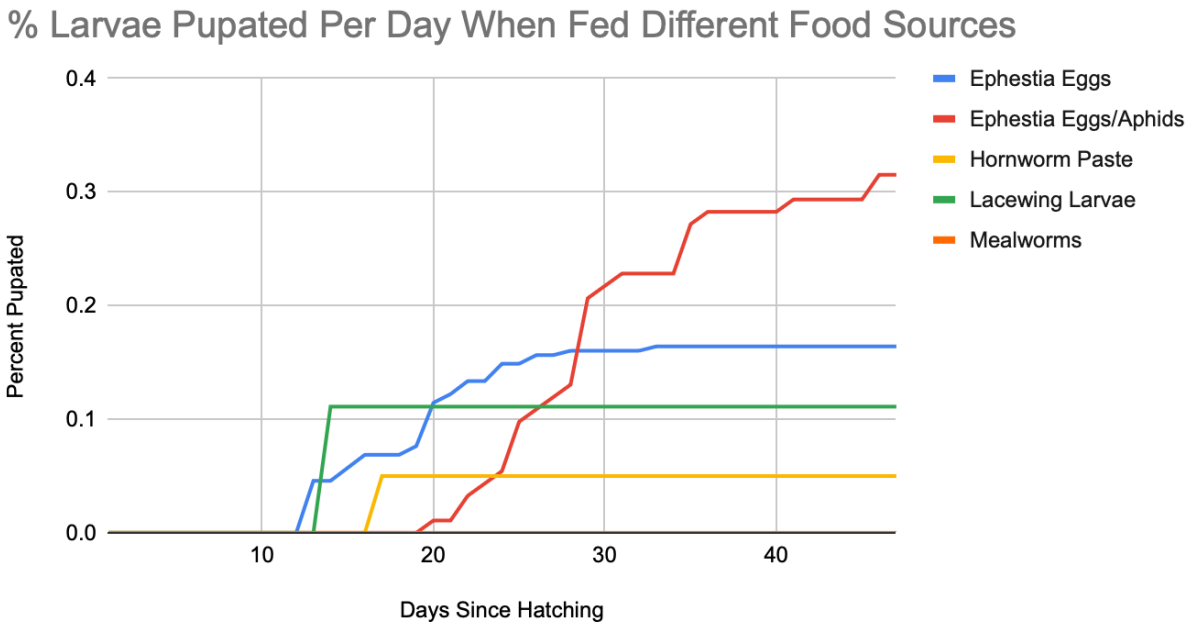
The larval survival data was analyzed using the Cox-Proportional Hazards Model.

Statistical analysis of survivorship with different food sources compared to *Ephestia* eggs shows that the mix of *Ephestia* eggs and aphids, hornworm paste, and mealworm diet showed a statistically significant change in survivorship (Table 2). Specifically, the mix of *Ephestia* eggs and aphids increased survival, while hornworm paste and mealworm decreased survival.

Lacewing Development and Diet

My data indicate that successful pupation relies on the type of larval diet. Figure 10 shows the percent of larvae that pupated for each food source after a certain number of days. A horizontal line indicates that all surviving larvae pupated. These data were also analyzed using a Cox-proportional hazards model, except instead of time to death, I used time to pupation.

Figure 10:



This graph illustrates the percentage of lacewings that pupated over time when fed different food sources: *Ephestia* eggs, *Ephestia* eggs with aphids, hornworm paste, lacewing larvae, and mealworms. The y-axis represents the percent of larvae pupating, while the x-axis shows the number of days since hatching. Larvae fed *Ephestia* eggs with aphids demonstrated the highest pupation rate, while those fed mealworms showed no pupation, with the data points of this group remaining on the x-axis.

Table 3: Statistics For Larvae Pupated Per Treatment

Treatment	Coefficient	P-value
Ephestia Eggs/Aphids	0.7843	0.0028 *
Hornworm Paste	-1.005	0.3220
Lacewing Larvae	-0.1518	0.8812
Mealworms	-18.12	0.9957

* = statistically significant

The pupation data was analyzed using the Cox-Proportional Hazards Model, however instead of time until death, time to pupation was analyzed

My data show that a food source consisting of *Ephestia* eggs and aphids is significantly superior to *Ephestia* eggs alone for the larvae to successfully emerge from pupation into adults. It is also important to note that the one lacewing larva from the lacewing larvae food source treatment that did pupate and emerge, died a few hours after emerging, so although it was able to make it to adulthood, it cannot conclusively be said that it is a successful food source. Although Figure 11 may show that lacewing larvae as a food source compare well to the other treatments, the survival of one lacewing is not a statistically significant sample size to make a definitive conclusion. These data were also analyzed using a proportional hazards model, except instead of time to death, I used time to adult (Table 5).

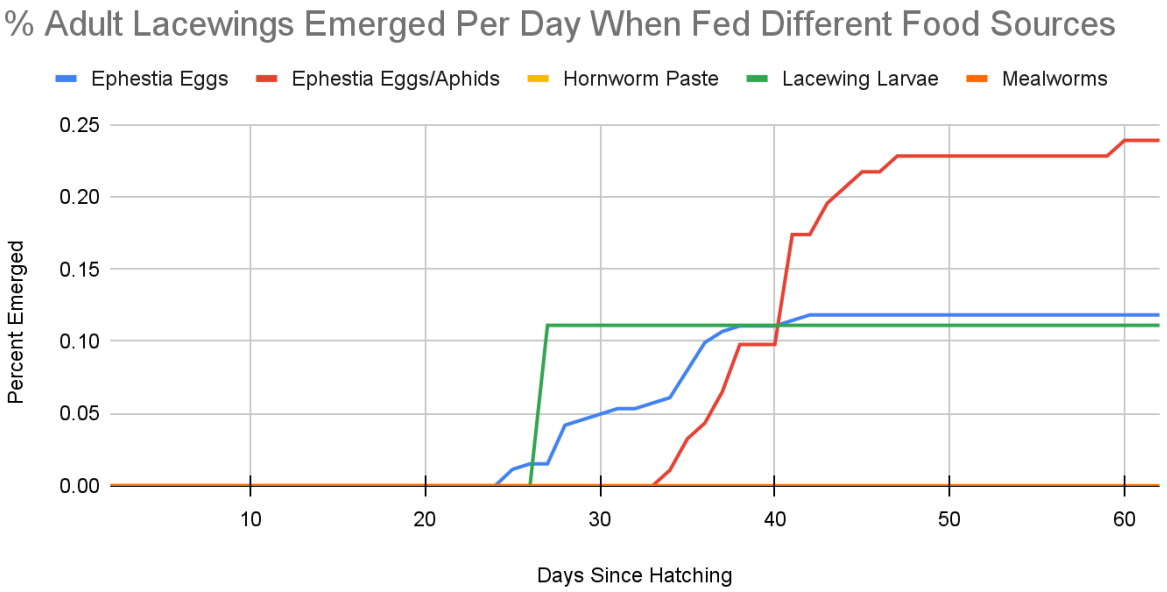
Additionally, each larva was observed through emergence to adulthood for each food source used (Table 4).

Table 4: Number and Percent of Adult Lacewing Emerged Per Treatment

Treatment	Sample Size of Larvae Started With	# Larvae Survived to pupation	# Emerged As Adults	% Emerged As Adults
Ephestia eggs	262	43	31	11.8%
Ephestia Eggs/Aphids	92	29	22	23.9%
Hornworm Paste	20	1	0	0%
Lacewing Larvae	9	1	1	11.1%
Mealworms	45	0	0	0%

The number and percent of adult lacewings emerging as adults were compared among treatments.

Figure 11:



This graph illustrates the percentage of lacewings that emerged over time when fed different food sources: *Ephestia* eggs, *Ephestia* eggs with aphids, hornworm paste, lacewing larvae, and mealworms. The y-axis represents the percent of adult lacewings emerging, while the x-axis shows the number of days since hatching. Larvae fed *Ephestia* eggs with aphids demonstrated the highest emergence rate, while those fed mealworms showed no emergence, with the data points of this group remaining on the x-axis.

Table 5: Statistics For Lacewings Reaching Adulthood Per Treatment

Treatment	Coefficient	P-value
Ephestia eggs/Aphids	0.7069	0.0222 *
Hornworm Paste	-18.15	0.9976
Lacewing Larvae	0.1774	0.8619
Mealworms	-18.15	0.9964

* = statistically significant

The adult emergence data was analyzed using the Cox-Proportional Hazards Model, however instead of time until death, time to emergence as an adult was analyzed.

Discussion, Study Limitations, and Future Research:

My study focused on developing and optimizing protocols for the rearing of lacewing larvae. Throughout the process, changes were made iteratively until the method was adequate for studying the effect of food sources, as shown in the results. The rearing cups containing mealworms and lacewing larvae as a food source treatment were not kept in the air tight container, and if they were, my data suggest their lifespans would have been longer. Conversely, I tried the hornworm paste method before and after I tried the airtight container method, and the paste quickly molded.

Additionally, if I were to do this study over again, I would use the most improved protocol for all treatments and would have the same sample size for all the treatments. This should be considered a preliminary study that I based my next experiment on. Although this may have provided too many variables and too many things changing at a time, it was necessary to change in order to keep the lacewings alive, as this was my main goal.

The effect of larval survival with different food sources became clear throughout the study. Aphids proved to be a successful food source when used in tandem with the *Ephestia* eggs, however I believe it would have worked by itself as well. Currently, I am experimenting with using aphids as a food source without adding *Ephestia*, and it is proving to be successful in keeping the larvae alive and getting them to pupation. Because my initial two aphid colonies died, I procured cabbage aphids, *Brevicoryne brassicae*, from a local garden, and have been using these as the aphid food source. Using the aphid food source proved to be a good method as I could procure them for free and keep them alive easily after finding the correct method to do so, however it isn't necessarily a stable food source. When in a lab setting, aphids are already very stressed and need precise conditions to prevent substantial mortality. Humidity needs to stay high and consistent, and because they can quickly tell if the quality of their food source is decreasing, new plants or leaves need to be replaced in the enclosure frequently. This can be done; however, the *Ephestia* eggs proved to be a more stable food source because the bottle can be stored in the fridge for months at a time and without significant spoilage, requiring significantly less time and resources than rearing another colony of insects. However, the *Ephestia* eggs cost $61.44 to procure, and although it lasts for months to feed the larvae, may not necessarily be obtainable for someone trying to replicate this protocol on a limited budget.

When I gave the lacewing larvae the mealworms as a food source, ostensibly the integument of the mealworm was too thick for the larvae's jaws to pierce. When the larvae prey on an organism, they pierce it with their mandibles and suck the contents of the insect through their jaws. If the exoskeleton of the prey is too thick, the larvae cannot get the nutritional contents of it. Lacewing larvae are specialists in feeding on soft-bodied (minimally sclerotized) insects like aphids, caterpillars, and other lacewing larvae. The hornworm paste may not have worked as a food source because it either dried too quickly before I started using the air sealed container method, or molded after a day when kept in the air sealed container. If I were to replicate this part of the experiment, I would ensure to replace the hornworm paste deposit every day.

A few papers mention other food sources that may be used to rear lacewings. "Techniques for Rearing Lacewings" by Butler (1971) and "Rearing of Adult Green Lacewing, *Chrysoperla carnea* (Stephens) on Different Artificial Diets in the Laboratory" by Balouch et al. (2016) both mention using Angoumois grain moth, *Sitotroga cerealalla*, as a food source. They most likely chose this food source because, similar to *Ephestia* eggs, the grain moth eggs can be refrigerated for long periods of time, serving as a constant and secure food source. They are both moth eggs, and it is common for lacewing larvae to eat lepidopteran eggs. However, Butler's paper mentioned that "aphids that may be common on roses, ivy, or other plants and in the school yard at certain times of the year can be used as food; or pea aphids, *Acyrthosiphon pisum*, may be collected from alfalfa fields or from sweet peas and transferred to dishes containing Chinese pea pods". However, aphids are very host-specific, and do not like to change diets once they have been set on one (Mahr, University of Wisconsin-Madison Horticulture). Therefore, the procuring of aphids from alfalfa fields or from sweet peas followed by transfer to a different food source most likely would result in death rather quickly.

"An innovative, low cost, small-scale rearing method for green lacewings" by Loru et al. (2014), as well as "Small Scale Rearing of Lacewings, Predatory Mites, and Entomopathogenic Nematodes" by Weaver (2018) mention using mealworms (*Tenebrio molitor*) as a food source. Another study describes the advantages of using mealworms that require little space, are highly productive, and produce little waste (Ramos-Elorduy et al., 2002). Although I did not kill the mealworms as suggested in the paper, I had them previously refrigerated to slow them down significantly, and the lacewing larvae had a 100% mortality rate.

Additionally, the USDA published information referenced a study conducted by Cohen in 1997 entitled "Mass-Reared Insects Get Fast Food" that used ground beef and beef liver, cooked hen's eggs, and antimicrobial agents (like potassium sorbate or streptomycin) as a diet for lacewing larvae. Although this is an accessible diet, it seems time intensive, and counterintuitive to purchase beef to feed to an insect that I'm trying to use to reduce overall pollution.

I am currently continuing the process of rearing my lacewings, trying different food treatments like turtle food and just aphids (*Brevicoryne brassicae*) rather than a mix of aphids and *Ephestia* eggs. In the future I would like to manipulate variables like time until pupation, time until hatching from pupation, time until eggs laid, number of eggs laid, and hatching time. Many of these can be manipulated through heat and humidity, which could be manipulated using the lab's new incubator, which controls humidity as well as temperature.

One thing I'm curious about would be how to keep the lacewings in their larval stage for longer. Since their larval stage is when they act as pest control agents, it could be advantageous to keep them in that stage for long periods of time. This could be manipulated through the levels of the juvenile hormone and the ecdysone hormone in their body (Adams 2009), and would require more research into what exactly controls the levels of these hormones. Because both of these hormones regulate molting into the next life stage (Adams 2009), manipulating the levels of these hormones could result in an extended larval stage. Additionally, I would try putting multiple larvae together on an aphid-infested plant and see how many larvae can be placed on a

plant to control the aphids effectively while preventing cannibalism. I also want to understand how a larval diet may impact the average number of eggs laid as an adult.

If I were to use this method of biocontrol in a greenhouse, I would need to be able to provide for the general input rate recommendation, i.e., how many larvae are needed per area unit of space, typically in square feet. The general input rate recommendations for the genus *Chrysoperla* is 1-2 larvae per square foot, as mentioned to me by a Koppert consultant. However, if the pest infestation is severe, up to 20 larvae may be recommended. However, the specifics of the general input rate recommendation depends on crop type, whether it is inside or outside, and temperature and humidity. The greenhouse I worked in had an area of about 2,700 square feet, meaning that 2,700-5,400 larvae would be needed in total (1-2 larvae x 2,700 square feet). This would obviously require scaling up my process in order to supply even just one greenhouse. At the beginning of my initial study, I only started out with 428 larvae, therefore this process would thus need to be scaled 6-12x the current scale, if I kept my protocol the same.

My supply of larvae would be derived from my adult lacewings that lay eggs, which I could either keep in the fridge for a couple weeks, or would hatch in my controlled environment. In the future, if a greenhouse or farm decided to buy lacewings to use as a biocontrol, the abundance and life stage would depend on the severity of the pest infestation. If an immediate treatment were needed for a pest problem for a more severe infestation, I would provide larvae or eggs. If a larger area needed to be treated, or a farmer wants to create a standing population within the area, then I would provide the adults (Arbico Organics).

My adult lacewings laid about 40 eggs on average per day among roughly 20 individuals, averaging to about 2 eggs per adult lacewing per day (however this number did increase when I started keeping the cages in the humidity control tank with the heat pad on it). In order to supply 2,700-5,400 larvae needed for my greenhouse, I would need to find a way to increase the average eggs laid per day, or I would need 193-386 adult lacewings to provide that number of eggs in a week (2 eggs per day per adult lacewing, 7 days in a week).

Although supplying lacewings for a large outdoor agricultural setting is beyond the scale and scope of my study, it is an important goal for me to reach one day much further down the line. Rearing lacewings can require large amounts of space, and although I was able to make the most of my space by stacking the air tight larva containers on top of of another, it is important to strive to have the most efficient protocol rather than the most amount of lacewings (although ideally both would be nice). Having an efficient protocol and most ideal environment for the larvae and adults would leave me with a higher survival rate and higher oviposition rate. This would then result in lower time and resources invested in the rearing process, ideally resulting in the lowest cost possible to farmers when it is time to sell.

If a commercial source is used, most major suppliers require customers to contact them for guidance on how to manage their pest problems. It is necessary to accurately define key variables, such as the pest species and their abundance, and current use of chemical pesticides (Brown, 2024). This information is required to determine the species of natural enemies needed, the timing, release rates, and associated cost, and to decide if biological control is feasible

(Brown, 2024). The cost of biological control products varies widely and is not usually advertised because suppliers work with customers to design and price successful pest management programs (Leppla et al., 2017).

If I were able to meet the demand necessary to provide adequate biological control for a large outdoor agricultural setting, there would still be significant barriers to widespread acceptance. Besides cost, the current largest barrier to adoption of biological control is the lack of education surrounding biological controls among farmers (van Lenteren, 2012; Baker et al., 2019). This often results in reluctance to use, as some can't understand why they would introduce more insects to address an insect-based problem. Farmers tend to believe that registered pesticides are safe for the environment as well as for themselves to use, so there is no incentive for them to change (van Lenteren, 2012). Many farmers believe that crops cannot be grown without pesticides. This is true for many modern crops, which have been developed under heavy pesticide use to prioritize high yields or appearance. Others believe that farming should be approached with a strictly organic technique. However, making such a drastic and immediate shift can result in catastrophic results (Seufert et al., 2012). Sri Lanka's drastic shift to strictly organic farming in April of 2022 caused a 20% drop in rice production, forcing $450 million in imports and a 50% rise in domestic prices (Nordhaus, 2022). This proved to be so catastrophic that the government offered $200 million to farmers as compensation and $149 million in price subsidies to farmers who incurred losses. Better alternatives than the extremes of pesticides, tested for their economic viability, are desperately needed before farmers can be expected to adopt new practices. Changing the mindset from relying on the current pesticide treadmill will require significant creativity and effort (van Lenteren, 2012), which is what I aim to accomplish with the goal of eventually making a biocontrol option cheaper than synthetic chemical pesticides. Additionally, complex application techniques can prove to be a barrier to implementation among farmers (Baker et al., 2020). However, lacewing eggs and larvae have a simple application technique that requires placing a card containing the eggs on or near the infested plant, or placing a small cup of the larvae in grain hulls by the infested plant.

Conclusion

The findings of my study contribute to moving the scientific and agricultural community a step further by making green lacewings more feasible as a biological control for local greenhouse managers, gardeners, and eventually commercial agriculture. Because my study focuses on rearing lacewings specifically found in Boulder, CO, where aphids can be found locally as a food source, it is a feasible method for local biological pest control. If I am able to get my work published in the future, other researchers could build on my findings to increase the scale of my work and manipulate variables that I didn't have time to address, like the number of eggs laid, time until hatching from pupation, etc. The process I developed does not require a large budget; most materials are easily accessible and access to a lab is not required for replication. A disadvantage is that culturing lacewing larvae is a time-consuming process which requires a lot of manipulation. With further research, this protocol could potentially become an

ideal pest control method, by only targeting pests, avoiding harm to other organisms, involving no toxic chemicals, and being cost effective.

This study's implications extend well beyond Colorado, potentially influencing the broader field of biological pest control in agriculture. By establishing a protocol for rearing the Comanche Green Lacewing, this research could catalyze further studies aimed at refining biological control methods for other green lacewings. As lacewings are already present in many agricultural ecosystems across North America, expanding research on breeding them could encourage growers, from local gardeners to large-scale farmers, to adopt more sustainable pest management practices. Additionally, this work could inspire research into other native predators, strengthening a movement toward pest control strategies that promote biodiversity, protect natural predators, and reduce the consequences of using synthetic chemical pesticides. As more data is gathered, the use of lacewings and similar natural enemies might gain broader acceptance, fostering a shift in agricultural systems towards more eco-friendly, effective pesticides.

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