Abstract

By Matthew Sorge

Do Cool Temperatures Impact the Parasitism Rate of *Psyttalia humilis*?

A biological control is needed to combat the olive fruit fly, because it is threatening California's $90 million olive industry. *Psyttalia humilis*, a parasitoid wasp, is being released by scientists to control the insect, but there have been problems creating permanent populations possibly because of cool winter temperatures. My project will determine if the cool winter temperatures in coastal San Diego County affect the ability of *P. humilis* to parasitize the olive fruit fly larvae inside of the olive fruit. Dr. Marshall Johnson of U. C. Riverside's Entomology Department advised me on how to conduct the experiments and provided me with 160 olives infested with fruit fly larvae and 40 mated female *P. humilis*. I prepared 20 identical test units. There were 4 treatments: Infested olives without *P. humilis* held at room temp; infested olives with *P. humilis* held at room temp; infested olives without *P. humilis* held at cool outside temp; infested olives with *P. humilis* held at cool outside temp. Each treatment was replicated 5 times. The high and low temperature was recorded daily. After seven days, all the containers were placed inside and the parasitoids were removed. The results show that cool temperatures did impact *P. humilis*' ability to parasitize the fruit fly larvae, but overall *P. humilis* was able to reproduce at numbers similar to the parasitoids in warmer temperatures. Therefore, this experiment could be useful in predicting if creating permanent populations of *P. humilis* is possible in Southern California. It is recommended that another year of winter experiments be done.

Acknowledgements

I would like to thank Marshall Johnson, PhD., because without his assistance this project would never have been possible. In addition, I would like to thank Kent Daane, PhD. and Xingeng Wang, PhD. for supplying great advice, the parasitoids, and olives. I would also like to thank Bill Schnetz for explaining what it is like to be an olive grower that has experienced an olive fruit fly invasion. Finally, I would like to thank Mrs. Culley and my parents for all of the driving and encouragement.

Dr. Johnson and me at U.C. Riverside. Photo by Jill Sorge

Introduction

I became interested in parasitoid wasps when my older sister did a science project on eucalyptus trees and the red gum lerp psyllid that was killing them throughout San Diego County. A parasitoid wasp was released to control the psyllid, and it saved all of the eucalyptus trees from dying. If the trees had died, my town would have burned down during 2007 firestorm. After that, I had great respect for the tiny parasitoid wasp, and that is why I decided to do my science project on *P. humilis*.

In my research on different types of parasitoids, I discovered that California's olives were being attacked by the olive fruit fly, and it was severely impacting the olive industry. Since I really like California black olives on pizza and all of my sandwiches, and because I think parasitoid wasps are very cool, I decided to help California's olive industry by doing experiments on *P. humilis*.

After doing more research, I discovered that two major universities had recently released *P. humilis* in some areas of California, but they were unsure if the insect could survive or reproduce during the cool temperatures of fall and winter months. After contacting the University of California, Riverside, I met Marshall Johnson, PhD. who arranged to have Xingeng Wang, PhD. send me sample wasps and olives infested with the fruit fly, so I could conduct experiments on *P. humilis*' ability to reproduce in cool weather.

Review of the Literature

The olive tree has existed for thousands of years along with insects that infest its fruit. One of these insects is the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), and it is the most destructive insect to the olive fruit and the livelihood of olive growers (Daane 1). The olive fruit fly is now a threat to California's $90 million olive industry. The insect is new to California, and scientists have been trying to find a way to control it. Although some natural enemies (e.g., ants, tiny wasps, etc.) of the fruit fly exist in California, none are effective enough to keep the olive fruit fly at non-damaging levels. Pesticides have not worked well in coastal areas where the pest's numbers reach very high levels. In addition, some pesticides can harm the environment and kill insects that are beneficial to the environment. Therefore, scientists in the United States have been searching the world for other insect natural enemies (i.e., predators and parasitoids) that could be introduced to control the olive fruit fly. All of their research and travels have led to the importing of many different parasitoid wasps that could potentially regulate the fly's population.

The history of the olive goes back 8,000 years, and was originally grown in Syria and Asia Minor (Lindsay). The olive tree is older than written language, and some olive trees are believed to be over a thousand years old. It grows very slowly and must be carefully nurtured. When it was discovered that the fruit could be pressed to release savory oil, it became one of the first trees to be cultivated by man (Carter 137).

The olive tree was first introduced to North America by the Spanish Jesuits when they brought olive cuttings from Spain to the missions in Mexico. From there, the missionaries who traveled north into California took olive trees with them and established orchards to produce oil. The first trees in the United States were planted in California at the Mission San Diego de Alcala in about 1769 (Carter 138). From 1769 to 1823, olive farming was popular at the 21 missions spread from San Diego and Sonoma, California. Fermin Francisco de Lasuen wrote in the Biennial Report of 1803, "In some missions they have already begun to harvest olives; and at San Diego they have already made some very good olive oil" (Carter 138). Years later, a woman named Mrs. Freda Ehmann built a factory in Orville, California, where the modern day olive industry was born (Lindsay).

Recent studies in the United States showed that Americans consume 50 million gallons of olive oil each year or seven percent of the world's production (Beck). The United States only produces 300,000 gallons of olive oil each year, but the demand for olives has increased by 88 percent in the last few years. In addition, gourmet chefs and consumers are very interested in buying local, fresh olive oil (Vossen 1). With the demand for olives and olive oil increasing, California's olives are more important than they have ever been. The entomologists Timothy Collier and Robert Van Steenwyk explained this in a research article about California's olive production, "In 2001, California growers produced 99% of the commercial olives grown in the United States, 134,000 tons of olives on 36,000 acres for a total value of $90 million."

About 13 years ago, the olive fruit fly was accidentally introduced into Mexico and California, and was first found in a survey fly trap in Los Angeles in 1998 (Collier). This insect originated in the Mediterranean region where infestations of olive fruit have been common from biblical times. The insect is also found in North and South Africa where wild olive trees are found. In the Mediterranean area, the insect has been the most destructive olive fruit pest for the last 2,000 years (Collier). Because it has no natural enemies in California that can keep the population from rapidly expanding, it quickly spread across the state (Hoelmer 1006). The University of California's Center for Invasive Species said that the olive fruit fly has caused the loss of up to 80% of oil value and 100% of table olives (Johnson). This is because the larvae cause the fruit to drop early and impacts the quality of the olives and olive oil. Even more devastating is that just a few larvae can cause the entire crop of table olives to be rejected. If the olive trees in California are left untreated the olive fruit fly could infest over 90% of the olive fruit (Collier).

Bill Schnetz of Schnetz Landscaping in Escondido, California, is an olive grower, and he explained in a personal interview that he decided to start growing olive trees because avocado farming required a lot of water and cost too much money. When his olive trees finally began to produce fruit he was very excited because it was a bumper crop. Sadly, he lost 100 percent of the olives due to the olive fruit fly. Since then, Schnetz has become very interested in using a biological control to save him time and money in his olive growing business.

Before 2003, olive growers in California had three ways to combat the olive fruit fly: pesticides, fly traps, or a mineral clay mixture (particle film). Pesticides can be expensive, are very harmful to the environment, and kill helpful insects. Fly traps are time consuming, ugly, and not always effective. The clay mixture is sprayed on the tree as a repellant and is highly effective in preventing flies from infesting the olive fruit, but growers do not like the white powdery residue left of their trees (Devarenne 2). This pest is a severe danger to California's olive industry (Hoelmer 1006). Although olive growers do have different ways to try to save their crops, it is a very difficult battle because of the way the olive fruit fly reproduces.

The adult female fly can lay 50 to 400 eggs and usually one in each olive. These hatch into larvae (i.e., maggots) that are extremely tiny. The larvae make a tunnel through the fruit as they feed and to escape. Tiny microorganisms (bacteria) that feed on the olive fly-damaged fruit make the fruit rot and their by-products increase the acid level of olive oil. The adult fly is 4 to 5 millimeters with a reddish brown skin and red eyes. The olive fruit fly has three to five generations of offspring per year. The number of these flies decreases greatly over the late fall and winter months. In mid-spring (April – May) the first generation of adults appears. The highest number of infested olive fruit appears during the fall when the olive begins to soften and turn color (Vossen 1).

Next, the second generation of offspring appears in mid-summer. These eggs hatch in two to three days, and the larvae develop in about 20 days. The adult fly can live from 2 to 8 months depending on the temperature and the amount of food they have.-The female adults lay their eggs in the larger olives. Olive fruit flies survive best in cooler coastal climates, but are also found in hotter places such as Greece, Italy, Spain, Mexico, and the Central Valley of California. These flies can live in places that range from 20 to 40º C. During a rainy winter, the number of adult flies caught in traps decreases, because the adult flies are inactive and the larvae do not develop below 10 ºC (Wang 1). As the numbers of fruit flies increase, the damage to fruit increases, and the olive industry struggles to produce enough olives. Therefore, it is important to find a natural enemy to combat this destructive bug (Vossen 2).

Insects that are natural enemies may be divided into predators and parasitoids. An insect parasitoid is similar to a medical parasite (e.g., tape worm, leech) because it develops and survives at the expense of its host, but a medical parasite does not always kill its host, and an insect parasitoid almost always kills its host (Bonet). Insect parasitoids may be found in several insect orders, but the most are from the order Hymenoptera (wasps and bees). There are over 600,000 species of parasitoids, which includes many species of wasps. Although the parasitoid wasps have stingers, they are so tiny (less than a quarter inch) that most people have never seen them (Science Daily). According to John Werren, Professor of Biology, University of Rochester, "Parasitoid wasps are like 'smart bombs' that seek out and kill only specific kinds of insects.

Therefore, if we can harness their full potential, they would be vastly preferable to chemical pesticides, which broadly kill or poison many organisms in the environment, including us." Werren went on to say that without parasitoid wasps and other natural enemies, our planet would be over-run with pest insects (Science Daily).

Parasitoids are beneficial because they feed on developing larvae (McEvoy 1). The parasitoid wasp *Psyttalia humilis* (Hymenoptera: Braconidae) lays its egg inside the larva of an olive fruit fly within an olive. The parasitoid larva will feed off of the fruit fly larva to survive and develop. It takes about 22 days for *P. humilis* to grow from an egg into an adult, and the temperature needs to be about 25º C degrees. At temperatures below 14 ºC or above 32 ºC, it is difficult for the parasitoid to develop or survive (Wang 1). If the adults are provided with honey and water, they will live from 36 to 78 days. If the adults have no food or water they live less than five days (Daane 5). Some of the insect victims of parasitoids have defense reactions. An organism such as a caterpillar will show an external defense reaction by wiggling around to throw off the parasitoid. An organism with an internal defense reaction will show cellular reactions to predators (Fisher). Parasitoids do more than just kill harmful pests. By using parasitoids as a biological control, it is much better than using pesticides, because poison harms the natural predators of the pests (Vossen 2).

In 2003, scientists from the University of California, Berkeley, and the University of California, Riverside, began looking all over the world for parasitoids to combat the olive fruit fly. They explored the Republic of South Africa, India, Namibia, China, Kenya, and other countries (Daane 2). Once the researchers found parasitoids that could work in California, they obtained government-issued permits to import them to the United States. Once they received the permits, the imported parasitoids were shipped to the University of California, Berkeley, where they were kept in quarantine to prevent an unwanted release into the environment. The quarantine is needed to determine if the parasitoids will attack other insect species and not just the olive fruit fly. Scientists discovered that *Psyttalia lounsburyi* and the *P. humilis* only attacked and reproduced on the olive fruit fly and no other insect species tested. They were also a good fit for California's climate (Daane 3). *Psyttalia humilis* was originally found in South Africa, and was first imported to Hawaii in 1913 to combat the Mediterranean fruit fly (medfly). The parasitoid was so successful that the medfly was nearly eliminated. *P. humilis* was established there until it mysteriously vanished sometime before 1949 (Wharton).

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture gave the scientists approval to release *P. lounsburyi* and *P. humilis*, and researchers are now releasing the two parasitoids (Daane 6). So far, their studies have shown that *P. lounsburyi* was found in the field, but does not seem to survive the winter. Meanwhile, *P. humilis* is easier to raise in the laboratory, but researchers are not sure if it will do well in the wild (Daane 6). In addition, more research needs to be done to determine if it is possible for the wasp to survive the winter in California. According to one study by Victoria Yokoyama, "*P. humilis* has shown some promise for olive fruit fly suppression… although successful year-round establishment has not been achieved" (Yokoyama 90).

In conclusion, the olive tree and mankind have lived together for thousands of years, and mankind has benefited from the olive tree and its fruit. In California, the olive industry is an important business, but with the threat of the olive fruit fly, it is important to find an effective biological control. The parasitoid *P. humilis* could be an excellent way to combat the olive fruit fly, but more research needs to be done to determine if it can survive the cool winter and establish a substantial population. Fortunately, scientists like Drs. Kent Daane (UC Berkeley), Xingeng Wang (UC Berkeley & Riverside), and Marshall W. Johnson (UC Riverside) are continuing to study *P. humilis* and other parasitoids as a solution to the olive fruit fly crises in California.

Statement of Purpose

The purpose of this project is to determine if *Psyttalia humilis* is able to search for and parasitize olive fruit fly larvae in cool temperatures. It is important to find a biological control to combat the olive fruit fly because it is a severe threat to California's $90 million olive industry. Although some natural enemies, (ants, tiny wasps, etc.) of the fruit fly exist in California, none are effective enough to keep the olive fruit fly at non-damaging levels. Even though there are other ways to combat the insect, (pesticides, fly traps, etc.) they are very expensive, time consuming, and can harm the environment. Consequently, scientists are releasing *Psyttalia humilis*, a small parasitoid wasp, to control the olive fruit fly in California.

The problem is that most cold-blooded insects are dramatically affected by cool weather, and most activity stops at temperatures below 16° C. In order for *P. humilis* to become permanently established in California, scientists must discover if it can reproduce in cool winter climates. Therefore, any information on how well *P. humilis* can search for and parasitize the olive fruit fly during California's winter months is useful to predict if it is possible to establish permanent populations of this parasitoid in the state.

If my project is successful it will assist scientists in confirming that coastal San Diego County's cool temperatures are not too cold for permanent populations of *P. humilis* to become established. This will help San Diego County's local olive growers. With these very useful insects assisting olive growers, it will insure that they can continue to grow delicious California black olives for sandwiches and pizzas and olive oil can continue to be produced in the state.

Hypothesis

Based on my research, this experiment will determine if the cool winter temperatures in San Diego County affect the ability of *Psyttalia humilis* to parasitize the olive fruit fly larvae inside of the olive fruit.

Materials

1. Twenty 12x13x18 cm plastic containers
2. Twenty 18 cm circles fine organza fabric for the tops of the containers
3. Twenty rubber bands to hold the mesh covers onto the tops of the containers
4. Twenty 13x13 cm metal grids (hardware cloth) to go inside each plastic container to keep the olives off of the floor of the container
5. Wire cutting scissors
6. Gloves for hand protection from sharp wires
7. Twenty squares of paper towels to line the bottom of the containers
8. 200 olives infested with olive fruit fly larvae (provided by U.C. Riverside's Entomology Department)
9. Fifty one-week-old mated *P. humilis* females larvae (provided by U.C. Riverside's Entomology Department)
10. Twenty balls of cotton
11. Cotton swabs
12. Water
13. Honey
14. Indoor/outdoor thermometer
15. Log book

Controls:

- Five containers were kept inside the house with four mated female wasps that were the same age and eight olive fruit fly infested olives with larvae at the same stage of development per container
- Five containers were kept inside the house with only eight olive fruit fly infested olives per container and no wasps
- Five containers were kept outside the house with mated female *P. humilis* and eight olive fruit fly infested olives per container
- Five containers were kept outside the house only eight olive fruit fly infested olives per container and no wasps

Controlled Variables:

- All of the containers were the same

- All of the containers had the same number of olives
- All of the containers had olives infested with the olive fruit fly larvae
- All of the olives were exposed to the wasps at about the same time
- All of the outside containers were exposed to cool weather for the same length of time

Independent Variables:

- The inside verses outside weather

Dependent Variables:

- Number of olive fly larvae that emerges
- Number of adult fruit flies produced
- Number of adult wasps produced

Procedure

1. UC Berkeley sent 0 olives infested with the olive fruit fly and 40 mated female *P. humilis* parasitoid wasps.
2. 20 rectangle plastic containers were purchased from Smart and Final and the lids were removed.
3. A piece of paper towel was put in the bottom of each container.
4. Twenty 13x13cm wire mesh hardware cloth was cut and put at the bottom of each container. It was raised about 2 cm by bending each corner so it is above the bottom of the container and over paper towel. See Figures 1 and 2.

Photos by Matthew Sorge

Figure 1 Figure 2

5. Eight olives infested with the olive fruit fly larvae were randomly selected from a brown paper bag and placed into each of the 20 containers on top of the wire mesh.
6. 20 pieces of fine organza screening was cut into circles and put over the top of each container, and it was secured with a rubber band.
7. Ten of the containers were labeled with the letter I for inside. Five of the containers were labeled with the letters A-E, and five were numbered 1-5.
8. Ten of the containers were labeled with the letter O for outside. Five of the containers were labeled with the letters A-E, and five were numbered 1-5. See Figure 2.
9. Four wasps were released into each of the inside containers labeled I/A-E, and the container was recovered and secured with a rubber band.
10. Four wasps were released into each of the outside containers labeled O/A-E, and the container was recovered and secured with a rubber band.
11. The ten containers labeled O/A-E and O/1-5 were placed outside of the house in an area sheltered from wind, rain, and direct sunshine.

12. The ten containers labeled I/A-E and I/1-5 were placed inside the house in an area where they would not be disturbed by the family or pets and were kept out of the direct sunshine.
13. A wet cotton ball was placed on top of all 20 containers to provide water for the insects that would emerge. See Figure 2.
14. A tiny bit of honey was placed on a cotton swab and dabbed on the fabric covering the ten containers with live wasps to provide food. It is important not to overuse the honey or the insects will become trapped in it and die.
15. Each day all of the containers were examined, the high and low temperatures were recorded, and the wasps were checked to see if they were still alive.
16. Every few days the wasps were given food and water.
17. The outside containers were left outside for a total of eight days, and then they were brought inside and placed next to the inside containers.
18. The wasps were removed from all of the containers.
19. All of the containers were checked every day to count how many maggots had emerged from the olives, and how many fruit flies or wasps would emerge from the maggots. All of these were counted every day.

Conclusion

My hypothesis, "The cool winter temperatures in San Diego County affect the ability of *Psyttalia humilis* to parasitize the olive fruit fly larvae inside of the olive fruit" is correct. More adult fruit flies did develop from the cool temperature larvae exposed to *P. humilis*, but *P. humilis* was still able to stop a large number of the larvae from developing into adult fruit flies. In addition, the cool temperatures slowed the development of *P. humilis* by ten days. Although the cool temperature treatment suggested that *P. humilis'* mobility is affected when parasitizing fly larvae, the most important fact is that the cool temperature treatment also produced adult *P. humilis* parasitoids. This means there is promise in using *Psyttalia humilis* as a biological control, and there is the possibility of establishing permanent populations in Southern California.

Recommendations

It is recommended that experiments be done over a few winters at cooler temperatures to determine how cold is too cold for *P. humilis* to successfully parasitize the olive fruit fly and reproduce. This project shows there is hope for establishing permanent populations of the wasp in Southern California, but until more cool temperature experiments are successfully completed it is too soon to begin using *Psyttalia humilis* as a biological control.

Bibliography

Beck, Caroline J. "Olive Fly Control." The Olive Oil Source. The Olive Oil Source. 27 Oct. 2010. 20 Oct. 2011.

Bonet, A. "Parasitoid Wasps, Natural Enemy of Insects." Topical Biology and Conservation Management. Encyclopedia of Life Support Systems: 2002. 11 Nov. 2011.

Carter, Nancy Carol. "San Diego Olives: Origin of a California Industry." The Journal of San Diego History. The San Diego Historical Society. Summer 2008: 137-138.

Collier, Timothy and Robert Van Steenwyk. "Prospects for Integrated Control of Olive Fruit Fly are Promising." California Agriculture. Division of Agriculture and Natural Resources, University of California. Jan.-Mar. 2003: 28. 19 Oct. 2011.

Daane, Kent, Marshall Johnson, Charles Pickett, Karen Sime, Xin-Gene Wany, Hannah Nade, John Andrews Jr., and Kim Hoelmer. "Biological Controls Investigated to Aid Management of Olive Fruit Fly in California." California Agriculture. Division of Agriculture and Natural Resources, University of California. Jan.-Mar. 2011: 2-6. 21 Oct. 2011.

Devarenne, Alexandra and Paul Vossen. "Controlling Olive Fruit Fly at Home." University of California Cooperative Extension. University of California, Davis. 1 Sept. 2006: 1-2. 11 Nov. 2011

Fisher, N., R. Ubaidillah, P. Reina, and J. La Salle. "Liriomyza Parasitoids in South East Asia." Csiro Australia. Csiro Publishing. 2005. 19 Oct. 2011.

Hoelmer, Kim A., Alan Kirk, Charles Pickett, Kent Daane, and Marshall W. Johnson. "Prospects for improving biological control of olive fruit fly, Bactrocera oleae (Diptera: Tephritidae), with introduced parasitoids (Hymenoptera)." Biocontrol Science and Technology: 1005-1025.

Johnson, Marshall W. "Olive Fruit Fly." Center for Invasive Research. University of California, Riverside. 2009. 11 Nov. 2011.

Lindsay Olives. "The History of the Olive." Lindsay Olives. Bell-Carter Foods, Inc. 2011. 23 Oct. 2011.

McEvoy, Peter B. "Parasitoids." Urban Entomology. Oregon State University. 2005. 21 Oct. 2011.

"Parasitic Wasp." AgriLife Extension. Texas A & M University. 1999. 12 Dec. 2011.

Schnetz, Bill. Schnetz Landscaping. Personal Interview. 04 Nov. 2011.

ScienceDaily. “Parasitic Wasps' Genomes Provides New Insights Into Pest Control, Genetics.” ScienceDaily. 14 Jan. 2010. 13 Nov. 2011.

Vossen, Paul, Lucia Varela, and Alexandra Devarenne. “Olive Fruit Fly.” University of California Cooperative Extension. University of California. April 2004: 1-2. 17 Oct. 2011.

Wang, Xin-geng, Karmit Levy, Youngsoo Son, Marshall W. Johnson, Kent M. Daane. “Comparison of the thermal performance between a population of the olive fruit 3 fly and its co-adapted parasitoids.” Biological Control. Elsevier Inc. 13 Dec. 2011: 1-8.

Wharton, R.A. and M.J. Yoder. “Parasitoids of Fruit-Infesting Tephritidae.” paroffit.org, paroffit.org, n.d. 11 Nov. 2011.

Yokoyama, Victoria Y., Carlos Cáceres, L.P.S. Kuenen, , Xin-Geng Wang , Pedro A. Rendón Marshall W. Johnson, Kent M. Daane. “Field performance and fitness of an olive fruit fly parasitoid, Psyttalia humilis (Hymenoptera: Braconidae), mass reared on irradiated medfly.” Biological Control. Elsevier Inc. 22 Apr. 2010: 90–99. 7 Nov. 2011.