Mortality and Predation of Olive Fly (*Bactrocera oleae*) Pupae on the Soil in a Davis, California Olive Orchard

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Abstract The olive fly (*Bactrocera oleae*), which plagues most olive-growing countries of the world, arrived in California in 1998. The larvae infest olive fruits, and even small populations can ruin entire crops. Many mechanisms of control have been developed for it, including chemical spraying and biological methods (parasitoid wasps). One avenue that has been largely overlooked is control of olive fly populations at the pupal stage. Most olive flies pupate on the ground underneath the trees, where they are potentially vulnerable to many elements. This study examined the effects of predation, climate, and soil-borne organisms (such as fungi, nematodes bacteria and other pathogens) on pupal mortality. To determine the contribution of these various factors to pupal mortality, I utilized a combination of observation and release-recapture of *B. oleae* pupae exposed to different conditions on the surface of the soil in an olive orchard in California's Central Valley. By comparing recovery and emergence rates of pupae in four treatments of increasing exclusion, I determined that predation (up to 67% mortality) and climate (up to 83% mortality) both contribute significantly to pupal mortality, while soil-borne organisms do not. The main predators of *B. oleae* pupae were ants. This study has potential implications on the future of biological control in olive orchards, including suggestions for insecticide and/or herbicide application strategies to encourage generalist ground predator populations.

Introduction

Olive fly (*Bactrocera oleae*) is an invasive and destructive tephritid fruit fly species that plagues most of the world's olive producing countries. Olive flies are native to the African continent (Bartlett et al. 1978), but can also be found in Greece, Spain, France, Portugal, Israel, Turkey, India and Pakistan (Rice et al. 2003). They were not found in the United States until 1998, when they were discovered in an orchard in Los Angeles County (Collier and Van Steenwyk 2003). Since that time, olive flies have spread to nearly every olive-growing county in California and now pose a serious threat to California's $90 million olive industry (Collier and Van Steenwyk 2003).

After mating, adult female olive flies deposit a single egg into the pulp of an olive, beneath the surface of the skin. They generally lay as few eggs (often only one) as possible in each olive (CDFA 2003), presumably to reduce competition between offspring. Each oviposition leaves behind a visible "sting" mark on the surface of the olive.

Under the olive's skin, the eggs hatch and develop into first instar larvae. They grow larger as second instars and continue consuming the pulp of the olive, moving closer to the olive's pit. When they develop into third instars, they move to the surface of the olive. Eventually, the larvae emerge from the olive, drop to the ground and pupate in the litter and soil beneath the tree. Most, but not all larvae pupate in the ground. Some larvae in younger, firmer olives pupate inside the olives on the trees. After approximately twelve days (depending on temperature), the adult olive fly emerges. The entire duration of development, from egg to adult, is approximately 24 days at 25° C (Sime et al. 2006), but varies from season to season. In the winter development takes much longer than in the summer, and because there are few (if any) olives on the trees in the winter, most olive flies overwinter as pupae on or in the soil and litter (Bartlett et al. 1978).

Olive flies are an especially severe problem for table olive producers. The presence of olive fly larvae leads to the premature dropping of olives, and the discovery of even a few larvae in an orchard can lead to the rejection of an entire crop (Collier and Van Steenwyk 2003). The problem is less critical for olive oil production, but the larvae do cause increased olive oil acidity, thereby reducing overall oil quality (Torres-Vila et al. 2003; Collier and Van Steenwyk 2003). In untreated areas, olive flies are capable of infesting one hundred percent of olives (Hoelmer et al. 2003).

Insecticide treatments for olive fly are common, including dimethoate and fenthion (Collier and Van Steenwyk 2003), but some farmers and consumers prefer to avoid potentially harmful chemicals. Aside from health concerns, these chemicals are expensive. It is also important to consider that insecticides are not specific, so they may eliminate beneficial insects in addition to the targeted pest. Moreover, most olive trees in landscaping (in suburban housing developments, for example) and in abandoned orchards will likely not be treated by insecticides. These areas can therefore serve as reservoirs of olive flies for reinvasion, rendering insecticide application ineffective for any nearby olive growers in the long run. Alternative methods of control are therefore being sought out. Other potential means include a bacterium called *Bacillus thuringiensis* (Dimitriadis and Domouhtsidou 1996), and classical biological control involving parasitoid wasps. Several parasitoid wasps have been studied as biocontrol agents and are being considered for introduction into California (Sime et al. 2006). None of them are native to California, though they are all specific to tephritid fruit fly species. Most of the parasitoid species that are currently being studied target the egg and larval stages of olive fly development. They sense the movement of the developing fly from the surface of the olive and pierce through the olive's skin, into the larva's body, and lay an egg.

To date, little is known about the mortality and predation of the pupal stage. Because the pupae occur on (and under) the soil's surface they are more exposed to predators and pathogens than the larvae which are protected by the fruit, and it is likely that substantial mortality occurs in the pupal stage. A handful of studies have been conducted on pupal mortality of other tephritid fruit fly species. Many of them find ants and other arthropods to be important predators. One such study found fire ant, spider and beetle predation on Mexican fruit fly (*Anastrepha ludens*) pupae on the soil (Thomas 1995). In one particular instance in this study, fire ants alone were responsible for destroying 94 percent of the released fruit fly larvae and pupae (Thomas 1995). A similar study on a fruit fly in Brazil (*A. oblique*) discovered ants preying on fly larvae, pupae and young adults. Control by ants and other predators of fruit flies was significant (Bressan-Nascimento 2001). Other important mortality factors in this study included parasitism, disease, and fungal infection. Argentine ants were also found to prey on Mediterranean fruit fly pupae in Hawaii (Wong et al. 1984). In that experiment, mortality of pupae (38.8%) was much higher than larval mortality, which averaged only 3.1%. (Wong et al. 1984).

Previous work has also been done on olive fly pupae in Europe. Bateman's (1976) findings in southern France suggested that ants were the principal predators, and that predation was happening more actively in the winter than in summer. A study on olive fly in Crete (Bigler et al. 1986) saw both birds and arthropods (predominantly ants) playing a significant role in the predation of pupae and larvae (inside olives and out). Cavalloro and Delrio also found that predation of olive fly pupae in the ground was an important factor in controlling populations in Italy (1975).

Nothing is known of pupal mortality of the olive fly in California. The study I conducted involved field and laboratory observation as well as the release and recapture of olive fly pupae in a Central Valley orchard. My objectives were to identify potential predators of olive fly pupae and to monitor and compare the mortality of pupae exposed to different combinations of mortality factors. Based on the responses (recovery and emergence) I determined which factors contribute significantly to pupal mortality. I hypothesized that predation, soil-borne organisms, and climate all significantly affect olive fly pupal mortality.

Methods

For my study I used the USDA's experimental olive orchard at Wolfskill in Winters, California. The project took place during the summer and fall of 2005. The site was not treated with insecticides, so there was no concern for pupal mortality due to chemical exposure during my experiment.

To distinguish mortality caused by the three sets of factors (predation, exposure to soil-borne organisms, and climate), I established four treatments, including a control. In order to minimize uncontrolled variables, I attempted to keep as many elements of the treatments as similar as possible. All four treatments utilized identical sturdy, 48-ounce plastic (Gladware) containers. In total I used eighty of these containers; twenty per treatment. Each container held twenty-five olive fly pupae. The first twenty containers were designed for the exposed treatment pupae. These containers had their tops and bottoms removed, so the pupae were directly exposed to all of the elements in the field (soil, sky, predators, etc.). The sides of these containers were pushed into the earth (partially buried) so that only the top rim of each container was visible at the surface of the soil. This precaution was necessary in order to guarantee that no predators were excluded by the height of the plastic container. The pupae were carefully placed onto the soil

within the perimeter of the container, and were lightly covered with loose soil and leaf litter (if any was present in the area), in order to mimic the surrounding area. Larvae on the ground are more likely to pupate under this litter than on top of it. At least thirty minutes after placing the last exposed pupae in each container, I returned and observed the area in order to record any predators and predator-pupae interactions for five minutes. A minimum thirty minute rest period was necessary for the area to recover from any disturbance caused by my presence, so that observations of natural predator activity would be more accurate.

Unlike the "exposed" containers, the containers for the predator-exclusion treatment were whole. They did not have their bottoms cut off. Instead, they were filled with predator-free dirt, partially buried, and topped with paint-strainer mesh that was glued around the perimeter of the containers' lids. The mesh was breathable in order to minimize climatic differences between these containers and the exposed containers. This design was intended to exclude predators, including birds and arthropods that forage in and on the soil, such as ants and ground beetles. But since the pupae were exposed to the soil, they were vulnerable to nematodes, pathogens, fungi and bacteria in the soil. Again, pupae were placed on the surface of the soil within the container and covered with leaf litter in order to mimic the surrounding area. The leaf litter was checked for predators before adding it to the containers.

The total-exclusion treatment containers also had intact bottoms and the same mesh tops used for the predator exclusion containers. The pupae inside these containers were protected from everything (predators and soil-borne organisms) except climate. They therefore served as a test of how the local climate affects pupal mortality. There was no dirt or leaf litter present in these containers; only the pupae. I had originally planned to partially bury these containers, just like the predator exclusion containers, in order to minimize differences between treatments. But due to the extremely hard nature of the soil and the fact that the site was usually windy, I was concerned that the lack of weight inside the containers would make them easy to blow away. So instead of burying, I placed these containers on top of the soil and anchored them with metal stakes.

The containers used for the lab control pupae were identical to those used for both the predator exclusion and total exclusion treatments, except these were kept in an insectary at room temperature (25 ± 2 °C). The purpose of having a set of lab control pupae was to measure the un-manipulated emergence rate, so that I could test the relative contributions to mortality of the

factors in the field. All of the containers (except for the lab control containers) were placed under twenty randomly-assigned trees in the orchard. I chose the trees from thirteen rows in a twenty-six row orchard. There were eleven trees per row, though several trees were missing, presumably pulled out because of age or disease. When the randomly selected trees were missing from the orchard, the nearest available tree was used. Each selected tree had an exposed, predator exclusion and total exclusion container beneath it. I blocked the treatments like this so that I could account for variability within the orchard. Potential differences within the orchard could have included temperature, moisture, exposure to sunlight, exposure to wind, and concentrations of predators and/or nematodes, pathogens and bacteria in the soil. All of the containers were placed beneath the trees in order to mimic where pupae would naturally fall from the olives.

After four days in the field, I returned to collect the pupae. At this point many of the exposed pupae were gone, due presumably to the predation of birds and arthropods. However, some were lost to human error, since pupae are tiny and difficult to find. This human error can be accounted for based on the predator exclusion treatment collection/recovery rates, since these pupae were also in the dirt but were not exposed to predators. Each group of pupae was put into its own Petri dish, brought back to the insectary, and allowed to emerge. Once brought back to the lab, the pupae that had been in the field were kept in the same environment as the lab control pupae. After allowing sufficient time for the pupae to emerge (about two weeks after the first pupae began to emerge), I counted the emerged adults. A small number of flies developed fully but died as a result of being stuck in their pupal casings. I counted these as emerged.

I measured the effects of the possible mortality factors with the recovery and emergence rates of the pupae. Pupae not recovered in the exposed treatment were assumed to have been consumed and/or carried away by bird and arthropod predators. If pathogens or other soil-borne elements affected the pupae in the field, I would have obtained fewer adult flies from the predator exclusion pupae than either the total exclusion or the laboratory control pupae. In addition, differences found in emergences rates between the predator exclusion and exposed pupae recovered from the field give me further clues about differences in predation. For example, some predators may bite or suck the pupae but leave them more or less intact and in place (instead of removing them completely), and their effects may not have been apparent until I attempted to rear the pupae. Differences found between the emergence rates of the total

exclusion and lab control pupae may lead to the conclusion that climate conditions in the field have an effect on pupal mortality. This information is summarized in Table 1.

Table 1: Possible mortality contributors for each treatment

	Treatment			
	Exposed	**Predator Exclusion**	**Total Exclusion**	**Laboratory Control**
Surface predators	X			
Nematodes, bacteria, fungi, and pathogens in the soil	X	X		
Climate	X	X	X	
Genetic defects, etc.	X	X	X	X

All pupae used in the experiment were insectary-raised. The methods used are summarized in Sime et al. (2006). The pupae came from olives that were infested in the lab, and the timing for infestation was strategically planned so that I had pupae close together in development. They were all recently pupated so that no flies emerged during field exposure.

The entire experiment was repeated three times throughout the season, in June, August, and October 2005. Differences in climate, flora, and fauna throughout the season may have lead to differences in the overall mortality rates, and the relative contributions of predators as opposed to soil-borne organisms may have differed. The June, August and October studies all took place at the same orchard, but the specific locations of the containers differed. The trees were randomly chosen each time.

To get an idea of the potential predators existing in the orchard, I set out 10 pitfall traps before each experimental trial. They were white plastic, quart-sized containers that I buried to the rim and filled with soapy water. I left them for four days, and then strained the contents with cheesecloth and placed all arthropods into ethanol-filled tubes (one tube per pitfall trap). Due to the nature of cheesecloth and the often muddy conditions of the pitfall traps' contents, some very small arthropods may have gone undetected. In addition, windy conditions during October caused many of the pitfall traps to fill with leaves, potentially allowing for the escape of some

potential predators. The contents of the pitfall traps were later examined in the laboratory under a microscope at 8X magnification, and were identified by family.

Prior to analysis I re-counted the emergences for 10 random pupae groups per month in order to ensure that no additional flies (possibly in diapause) had emerged. No additional flies were found.

Results

The recovery and emergence data were transformed in order to stabilize variance via (arcsin(?*p*)), where *p* is the proportion of pupae recovered or the proportion of recovered pupae from which adults emerged. Problematic replicates (those June predator exclusion containers that became contaminated by predators, and those total exclusion containers that blew away into direct sunlight) were excluded from analyses. ANOVA was performed on the transformed data, with treatment means separated using Tukeys HSD test (SYSTAT 2000). There was a significant treatment impact on the percentage recovered in trials conducted in June ($F = 58.51$, $df = 3,76$, $P < 0.0001$), August ($F = 104.4$, $df = 3,76$, $P < 0.0001$), and October ($F = 46.65$, $df = 3,76$, $P < 0.0001$) (Fig. 1). It is important to note that the exposed treatment was the only intended treatment of interest regarding pupal recovery. All other treatments should have, in theory, had 100 percent recovery rates. This wasn't always the case, due to difficulty in finding pupae and occasions of miscounting. In all three months there was no significant difference between the lab control and the total exclusion recovery of pupae, but the predator exclusion and exposed treatments were both significantly lower. In June, the predator exclusion treatment was about 70 percent lower than the total exclusion and lab control recovery rates, while it was only about 10 percent lower in August and October. The recovery should have been much higher for this treatment in June, however a design flaw allowed predators into the predator exclusion treatment containers for this month. The predator exclusion and exposed treatments differed significantly from each other in August and October, but not in June (also presumably because of the design flaw). The difference between the predator exclusion and the total exclusion/lab control recovery rates is apparently due to human error in collecting for August and October, since these predator exclusion treatment containers were predator-free. For this reason, it must be assumed that some of the pupae gone after the 96 hour period in the exposed treatment were not necessarily preyed upon. However, we can presume that loss rate (from pupae collection) is

similar for the predator exclusion and exposed treatments. If this is the case, then we can calculate a more accurate predation rate as:

Actual predation in Exposed treatment = loss in Exposed treatment - loss in Predator Exclusion treatment

For example, using August's recovery rates: 74 % (loss in exposed treatment) - 7% (loss in predator exclusion collection) = approximately 67% pupae lost to predation. Unfortunately, this calculation is only applicable for August and October (June design flaw).

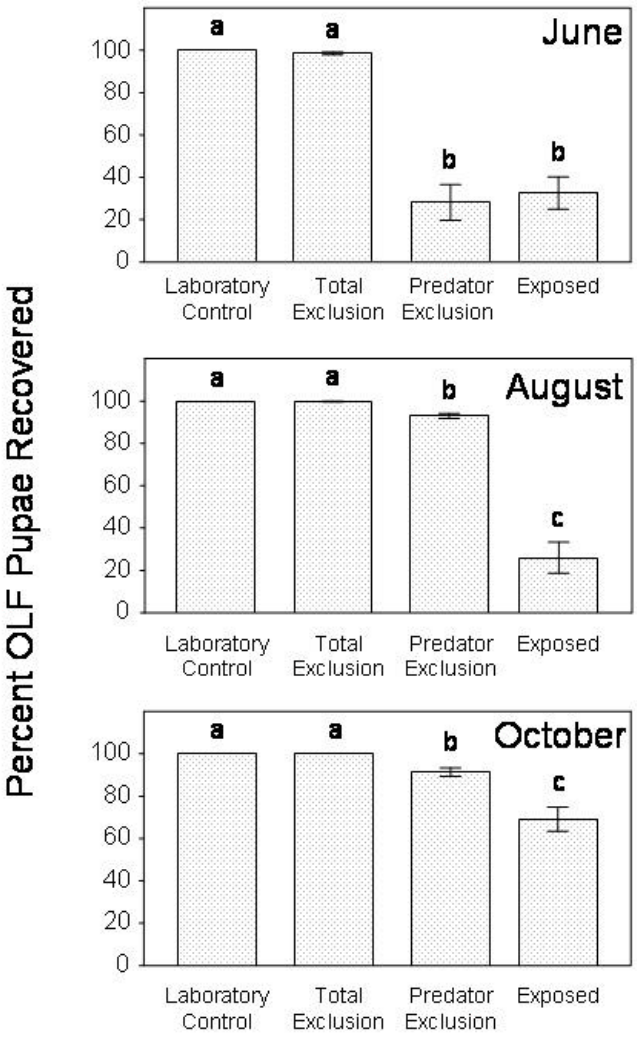

Figure 1. Mean percentage olive fruit fly pupae recovered after a 96 hr period in the laboratory or in the field under total exclusion, predator exclusion or exposed conditions. Error bars represent +/- standard error of the mean. In some cases standard errors were zero because of 100 percent recovery. Treatments with the same letter above the bars within each graph were not significantly different (Tukey's HSD test, $P < 0.05$). Graphed by Kent Daane.

In addition, there was significant treatment impact on the percentage of adult fly emergence from recovered pupae in trials conducted in June ($F = 5.911$, df = 3,55, $P = 0.0014$) and August ($F = 14.66$, df = 3,65, $P < 0.0001$), though there was no treatment impact for emergence in the October trial ($F = 2.137$, df = 3,69, $P = 0.1033$) (Fig. 2). The June design flaw did not appear to have any effect on emergence rates for the predator exclusion treatment, and overall June emergences turned out as expected. Lab control pupae had the highest emergence rate, followed by the total exclusion treatment, then the predator exclusion treatment, and finally the exposed treatment. This same pattern did not form so neatly in August or October. In August (the warmest month) the lab control and total exclusion treatment emergence rates differed significantly, though this was not the case June or October. The emergences for total exclusion-treated pupae were much lower in August (about 20 %), than in June (about 55 %) or October (about 70 %). In October none of the four treatments differed significantly from each other (they varied only between about 65 and 80 % emergence). In June the exposed treatment and predator exclusion treatment emergence rates did not differ significantly. In August, none of the three field treatments differed significantly from each other, though the total exclusion rate (again, 20 %), was much lower than either the predator exclusion or exposed treatment rates (approximately 40 % and 30 %, respectively). The total exclusion emergence levels in August were much lower than the levels in June and October, possibly because of the exceedingly warm weather. The emergence levels for the field treatments in October were all relatively high when compared to those in June and August.

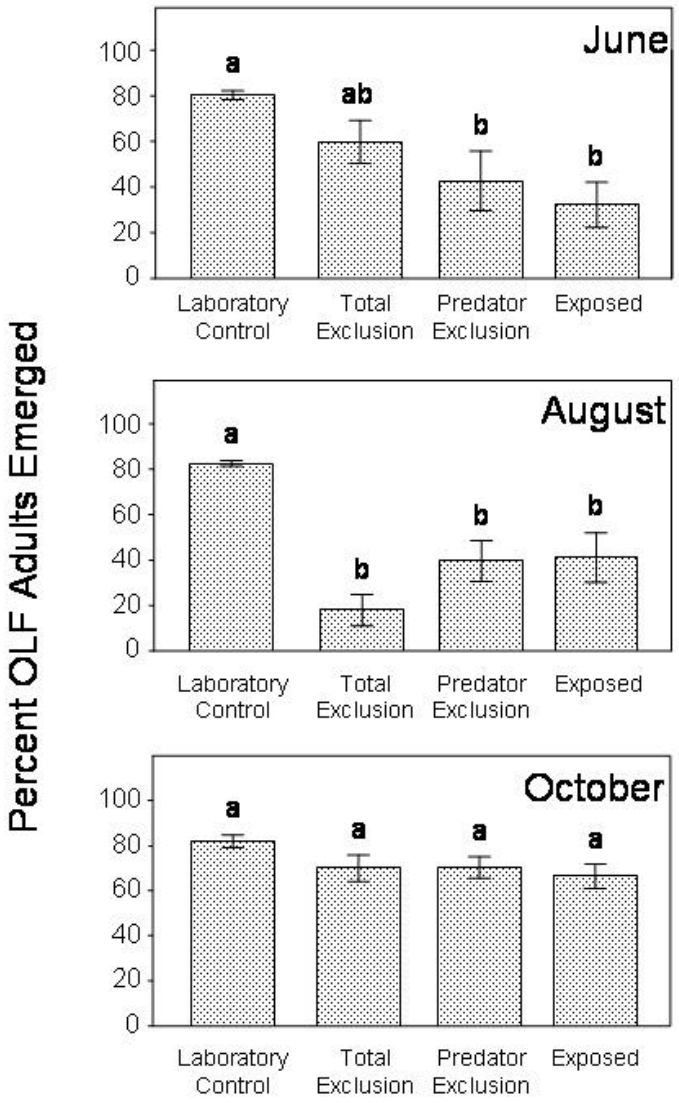

Figure 2. Mean percentage olive fruit fly adult emergence from recovered pupae after a 96 hr period either in the laboratory or in the field (under total exclusion, predator exclusion or fully exposed conditions). Error bars represent +/- standard error of the mean. Treatments with the same letter above the bars within each graph were not significantly different (Tukey's HSD test, $P < 0.05$). Graphed by Kent Daane.

During the five-minute observation periods, ants (*Tetramorium caespitum* and *Formica aerate*) were the most numerous predators spotted for all three months (Table 2). Ants were also responsible for all of the interactions between predators and olive flies observed (eight in June and two in August). These interactions include ants that were found touching, attempting to carry, or carrying the pupae. Mites, beetles and spiders accounted for the majority of the other observations. Identification of these species was limited because the potential predators observed were not collected. With regard to the pitfall traps, ants accounted for the majority of

likely predators captured, followed by ground beetles and earwigs (Table 3). It must be noted that the technique for collecting the pitfall traps' contents could have caused me to find an incorrectly low number of tiny potential predators such as mites. Also, October was exceptionally windy and caused most of the pitfall traps to fill with leaves, thereby allowing some catches to escape. Overall though, it was the case in both the observations and pitfall traps that potential predators observed/caught varied between June, August and October.

Table 2. Summary of potential predators observed. Ant species identified by Philip Ward (University of California, Davis).

Summary of Activity	***May***	***August***	***October***
Tetramorium caespitum (small ants)	44	35	0
Formica aerate (large ants)	23	33	16
Lacewing larvae	1	0	0
Unidentifiable arthropods	0	2	0
Spiders	5	5	1
Mites	0	0	41
Beetles	9	2	0

Table 3. Potential predators of olive fly larvae/pupae caught in pitfall traps. Most predators were classified to family. Families where all (or most) species are carnivorous and likely to prey upon olive fly larvae are listed as "Likely Predators." Families where only some species are carnivorous or are carnivorous but unlikely to eat olive fly larvae are listed as "Possible Predators." Other catches and families caught only once were omitted.

Likely predators			
	May	***August***	***October***
Formicidae (Ants)	185	67	144
Carabidae (Ground beetles)	5	11	0
Forficulidae (Earwigs)	5	0	4
Chrysopidae (Lacewings)	2	0	0
Staphylinidae (Rove beetles)	1	1	0
Possible predators			
Araneae (Spiders)	26	15	13
Isopoda (Pill bugs)	0	18	4
Apocrita (Wasps)	3	4	2
Elateridae (Click beetles)	2	6	0
Blattidae (Cockroaches)	0	2	4
Acari (Mites)	0	6	0

Discussion

This experiment showed that there is great potential for alternative methods of controlling olive flies at the pupal stage of development. In particular, it was found that generalist predators and warm weather could both be valuable tools. Based on the recovery rates of the exposed treatment pupae, we can determine that predation (predominantly ants) is a major source of mortality for olive fly pupae on the soil's surface (up to 67% mortality). Climate and other abiotic factors *can* also affect pupal mortality, as was evidenced by emergence rates in June and August (especially August, which had an 83% mortality rate due to climate and is often the

hottest month in this area). This was not the case in October, when the weather was milder. The only hypothesis that was not supported was the one relating to exposure to soil-borne agents (bacteria, nematodes, fungi, other pathogens). Contrary to expectation, total exclusion pupae did not suffer significantly lower mortality than either the predator exclusion or exposed treatment emergence pupae. In fact, none of the three field treatments differed significantly from each other in emergence rates in any month. This suggests one of two possibilities: 1. There is no effect of bacteria, nematodes fungi, and/or other pathogens (in this orchard) on pupal development and mortality; or 2. Any bacteria, fungi, or other pathogens in the soil are also present in the air (or in the case of nematodes, they could have been transported by the wind), and therefore would have affected total exclusion pupae as well.

Looking at the literature, my findings generally agree with those of my predecessors. Bigler et al. (1986) completed a study somewhat similar to mine in western Crete, and though the methods were considerably different, the results were similar. This experiment focused on both arthropods and birds as predators of olive fly pupae. It found that birds were responsible for 70.2% of pupal predation, with the other 29.8% attributed to arthropods (mainly ants, with some carabids and others). These findings suggest that perhaps some of the predation on pupae in my exposed treatment could have been attributed to birds. However, there were no indications of bird predation such as feathers or bird droppings near the containers, and there were no observations of birds near the exposed treatment containers during the five-minute observations (arguably, this would have been unlikely anyway because most birds avoid human activity).

Delrio and Cavalloro (1977), who did a similar experiment on olive fly pupae in Italy, also reported ants, carabids, and birds as important predators of olive fly pupae, as well as centipedes. They also found that temperature was an important mortality factor for pupae and adults. In addition to Delrio and Cavalloro, Bateman's (1976) studies on olive flies shed some light on olive fly pupal mortality. He focused on the effects of both abiotic factors and predation, much like my project. Bateman's methods were different, as he buried the pupae at various depths under the soil. He found that predation was more prevalent in winter than in the warmer months. These observations were made at a depth of 5 cm, and Bateman suggests that predators go deeper than 5 cm during the summer. Ants were found to be the primary predators, though Bateman also observed mites in the pupal samples.

For growers, the most useful and applicable results of my study are the pupal mortality caused by predation, and the sensitivity to sun and warm temperatures. Generalist predators like ants and ground beetles have great potential as controllers of olive fly populations at the pupal stage. Boosting these populations can be done fairly easily. To begin with, growers can either stop insecticide spraying, or alter the spray timing and/or technique. Insecticides kill ants and ground beetles in addition to the targeted pests, but there may be certain times of the year or day when beneficial generalist mortality can be minimized. For example, ants seem to be more active outside when the weather is warmer. Perhaps spraying during cooler weather would minimize unwanted ant casualties.

Ants have been found to be beneficial in other agricultural settings, as well. Perfecto (1991) found that ants contributed significantly to controlling fall armyworm (*Spodoptera frugiperda*) and corn leafhopper (*Dalbulus maidus*) populations in irrigated maize in Nicaragua. Plots with high ant populations suffered from significantly less pest damage. Daane and Dlott (1998) also found ants to be useful in controlling peach twig borer populations in peach orchards. It seems that this could be the case for olive fly pupae, however it is important to note that ants would not be beneficial in every olive orchard. In some instances, ants have been found to tend black scales (Barzman and Daane 2001). Ants protect the scales from their natural enemies and populations grow as a result. Since black scales can also be pests in olive orchards (Moursi and Hegazi 1983), it would not be advisable for olive growers to boost ant populations in groves that have black scale problems.

Carabids also have potential to assist in controlling olive fly populations at the pupal stage, and these populations can be fairly easily boosted, according to O'Neal et al. (2003). This study showed that certain ground covers are better than others for supporting carabids (*Harpalus pensylvanicus* in this case). In this experiment, carabids were more abundant in clover and ryegrass ground cover treatments than in buckwheat or herbicide-treated bare ground in highbush blueberry fields. A seemingly contradictory result came of Miñarro and Dapena's (2003) ground beetle study in an apple orchard. This one found that ground beetle populations were most diverse and rich in tilled and herbicide-treated plots when compared to plots covered with plastic mulch, pine bark mulch, soil, or straw mulch. The herbicide used in this study was glyphosate, applied twice in the season. O'Neal et al. (2003) also used glyphosate for its bare-earth

treatment, although it did not state how much or how often (and perhaps this omission is key to the difference in result).

The apparent sensitivity to warm temperatures and direct sunlight may also be of use to growers. As previously mentioned, August in particular had a very low emergence rate in the total exclusion treatment, contrary to what was expected. These pupae did not have the cooling benefits of soil or litter, as they sat undisturbed in their containers beneath the trees. Their high mortality suggests that it may be advantageous for growers to clear the litter from the ground in the hottest part of the summer. It would remove some pupae's protection and expose them to the sun. Arguably, this may just encourage more larvae to burrow under the soil to pupate. There is also some concern about how clearing the litter could affect generalist predator populations. In any case, this would be something worth studying in the future.

There were a number of factors that could have contributed to error in this experiment. To begin with, the initial design for the predator exclusion treatment (used in June) was faulty and gave predators access to the pupae inside the containers. As previously mentioned, this was reflected in the low recovery rate for this treatment in June. There were also suspicions about the design of the total exclusion treatment containers (for all three trials). Unlike the other two field treatments, these containers were not buried. They sat on top of the soil and were anchored by thin metal stakes. These containers may have been warmer as a result, since the soil could have provided cooling benefits for the two treatments with buried containers. Also, the total exclusion pupae may have even been exposed to direct sunlight, as they were not covered by leaf litter like the other two treatments' pupae. This may explain the relatively low emergence rate for the total exclusion treatment pupae in August (the warmest month of the three). In theory, these pupae should have had higher emergence rates than those of the other two field treatments, since they were not exposed to predators or soil-borne threats. So in hindsight, it would have been better to bury the total exclusion containers. It would have minimized this potential variability between treatments. On the positive side of things, it was learned that pupae are very sensitive to warmer temperatures and exposure to direct sunlight, and as mentioned before this may be of use to growers.

There was also potential for mistake in the filtering method for the pitfall traps. After four days in the field, leaves and dirt had often blown into the water in the pitfall traps. I used forceps to remove the potential predators from the water and mud. In retrospect it is possible that small

predators such as mites may have remained hidden in the mud, and my numbers for these species (such as mites) may be incorrectly low.

As always, there are several opportunities for future research projects. For example, it may be the case that predation of pupae at the surface is higher in the winter than in the summer, as Bateman (1976) found in southern France. If this is also the case in California, it may be especially important for olive growers to boost generalist predator populations during this period of the year. Also, as previously mentioned, it might be worth studying the advantages and disadvantages of clearing the litter in an olive orchard; would it increase mortality of pupae on the surface or would it simply hurt generalist predator populations and encourage larvae to pupate underground? Lastly, Cavalloro and Delrio (1975) and Bigler et al. (1986) found birds to be extremely important sources of predation of olive fly pupae. If birds are also found to be important predators in California, it would be in the interest of olive growers to boost populations of bird species (perhaps with artificial nests) that do not consume olives, and that (ideally) aren't problematic for any crops adjacent to the olive orchards or for pollinator populations such as bees.

The findings in this study are promising. There are many possible avenues for the development of sustainable agricultural practices in olive groves, and I have touched on but one possibility. There is also great potential in the realm of specialist parasitoid wasps, bacterial agents, and safer chemical dispersion techniques. Whatever the method, I look forward to healthier ecosystems surrounding our agricultural fields and orchards.

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