

BUTTERFLY ABUNDANCES, LARVAL PREDATION, AND EGG PARASITISM AS
DETERMINED BY PROXIMITY TO BUTTERFLY FARMS
IN FLORIDA AND COSTA RICA

By

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To my Mom, who has been a great role model and friend

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Abstract of Thesis Presented to the Graduate School
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Butterfly farming is an emerging and rapidly growing industry, both in developed and developing nations around the world. As an industry focused on breeding butterflies for sale, while providing a number of incentives to local communities such as jobs, revenue, education, and conservation, these farms are becoming popular enterprises and ecotourism destinations. However, little is known about the impacts of these farms from a biological and ecological viewpoint. This study aimed to collect data on 1) wild butterfly abundance, as indicated by oviposition rates, 2) larval predation, and 3) egg parasitism rates to determine any effect that proximity to the farm epicenter might have on each factor. Additionally, the experiment was duplicated in north central Florida, U.S.A and Guanacaste, Costa Rica, to test for any similarities or differences in the aforementioned factors and variables that may be attributable to location. It was found that butterflies tended to oviposit in greater numbers closer to the farm epicenter in north central Florida, suggesting that the farm could serve as an attractive force for gravid female butterflies. Although untested, components of the farm, such as enhanced host and nectar plant biomasses and high levels of conspecific density, could contribute to the higher butterfly abundance closer to the farm. The effect these components have on butterfly abundances and the

possibility of butterfly attraction to specific areas of the farm necessitates further research. Unexpectedly, there was no oviposition recorded during the entire Costa Rica trial. It is possible that visibility of the host plants and seasonal shifts in host plant preference by the gravid females severely affected oviposition in the tropical forest habitat. Larval predation was found to be more frequent in areas distal to the farm epicenters, both 100 meters from the farm as well as several kilometers. Lastly, in all trial sites, no egg parasitism was observed. These results suggest that while gravid female butterflies are more abundant closer to butterfly farms, there is less threat of predation to their offspring when ovipositing near the farm. The initial prediction that the farm may act as an ecological trap seems unlikely, as, in fact, larval survivorship is significantly higher near butterfly farms.

CHAPTER 1 INTRODUCTION

Butterfly Farming and Ecotourism

The breeding of butterflies for sale, known as butterfly farming, is increasingly regarded as a profitable microenterprise business. This is partly due to the fact that the overall use and the diversity of applications for these alluring lepidopterans are expanding. Today, butterflies are utilized in many ways, ranging from live butterfly releases at weddings to being displayed in live butterfly exhibits in countries around the world (Young 1986). In addition to this increasing popularity of these insects, techniques for farming, breeding, and rearing Lepidoptera are becoming more fine-tuned, therefore expanding output and ultimately escalating the success of the individual farmer. Organizations such as the International Butterfly Breeders Association (IBBA), founded in 1998, are contributing vast amounts of information to the overall scene. The IBBA coordinates workshops, manuals, and tutorials, allowing amateurs with little to no prior knowledge to initiate breeding operations and rapidly become proficient at farming butterflies. The resulting accessibility to butterfly farming through a virtually endless supply of knowledge and support, combined with a relatively high demand for these insects, make this industry increasingly more profitable and attractive. With some individual farms seeing annual revenues in the 6-digit range, it is easy to see it becomes desirable to enter this multi-million dollar industry.

At the same time, butterfly farming presents a novel situation in which to promote ecotourism. Defined as “responsible travel to natural areas in which to conserve the environment and promote the welfare of local people,” ecotourism is intrinsically associated with conservation (TIES 1998). Ecotourism is a powerful tool to promote conservation, both directly through investment in conservation programs as well as indirectly through education and

increased awareness, which are key components in many commercial butterfly farming operations.

These attractive qualities of butterfly farming render it very appealing to businessmen and naturalists alike. However, an additional sector is now participating: rural communities in developing nations. From a conservation perspective, this sector is very intriguing. As stated by Omenge (2002), butterfly farming operations in developing nations are contributing to local awareness and conservation of biodiversity, which is a very positive result. Although butterfly farms are typically regarded as positive influences in the environment on a macro-scale, one must not overlook the fact that they may *influence* their environment on a more local scale. Because of the rapidly increasing numbers of farms and the close association that these farms have with ecotourism, it is timely to assess some of the ecological processes, ecological impacts, and general influences that they exert on their surrounding ecosystems.

A neoteric study by Gordon and Ayiamba (2003) incorporated the monitoring of butterfly population abundances in the forest surrounding butterfly farms in Kenya in an effort to examine the impact such farms have on the relative butterfly abundances in the area. After establishing a highly successful butterfly farming operation aimed at deterring local communities from destroying the nearby forest, the Kipepeo project was born. Before the project began, however, butterfly abundance counts were taken. Then, after 4 years of farming operations (and after \$400,000 of farming revenue had come to the local community!), butterfly abundance counts were taken again (Gordon and Ayiamba 2003). In both instances, these counts were taken by researchers walking transects and counting sightings in each transect, which provided data on 60 species of butterflies. Of these 60 species, 23 were actively being reared at the farm at the time, while 37 were not. Results showed that butterfly abundances were significantly higher *after* the

farm had been in operation for the four-year period, but *relative* abundance rankings were not significantly different among the species surveyed (Gordon and Ayiemba 2003). In summary, butterfly populations increased proportionately during the first four years that the farm was in place. Gordon and Ayiemba concluded that this was not harmful to the natural butterfly populations since the original “balance” of common and rarer butterfly species in the local fauna remained intact (Gordon and Ayiemba 2003).

Parasitism and predation at butterfly farms have also been investigated in previous studies. Omenge (2002) reported that in small-scale butterfly farms in Kenya, most farmers experienced noticeable predation and parasitism from ants, spiders, wasps, and lizards (Omenge 2002). Not surprisingly, Omenge (2002) found that farmers with more sophisticated butterfly flight cages experienced fewer incidences of parasitism and predation than farmers with makeshift, more rudimentary cages. He reasoned that this was due to the disintegration of the screening on the poorly made and maintained flight cages, allowing parasites and predators to have direct access to their host or prey (Omenge 2002).

Objectives

To further the study of butterflies in relation to butterfly farms, the present project was initiated to investigate three major aspects of butterfly life history, all with respect to the butterflies’ proximity to the central operational area of the butterfly farm, which will be referred to hereafter as the farm epicenter. The first goal was to analyze butterfly abundance based on oviposition. Contrary to previous studies, such as Gordon and Ayiemba (2003), which used visual sightings of adults as indicators for abundances, my study used the deposition of eggs as an indicator for butterfly abundance. While egg censusing will not measure the presence of butterflies not laying eggs, such as males or non-gravid females, this method allows continuous data collection over 24-hour periods, as new egg counts were recorded regularly every 24 hours.

It was hypothesized that proximity to the farm would result in higher butterfly abundances so that the highest abundances would be observed right next to the farm epicenter.

The second research goal was to investigate larval predation rates based on proximity to the farm epicenter. It was hypothesized that larval predation rates would be highest at points closest to the farm.

The third and final goal was to study the rate of egg parasitism, again based on proximity to the farm epicenter. It was hypothesized that close proximity to the farm would yield higher rates of egg parasitism.

Application of this study's results could serve to improve the ecological health of the farm ecosystem, its surrounding area, and possibly result in the enhancement of overall productivity. Butterfly farming operations, both domestically here in the U.S. as well as in foreign countries, are often situated in relatively remote rural areas with significant amounts of surrounding undeveloped land. Relative to urban or suburban settings, these farms typically occupy a more pristine setting in which the surrounding ecosystem is healthier with higher levels of biodiversity. Although this is not *always* the case, as some farms are indeed proximal to urban areas, it is of great conservation concern to better understand the habits of all life stages of butterflies living within proximity to butterfly farms in order to ensure that these enterprises continue to contribute to biodiversity conservation and the principals of ecotourism.

Such a study is also beneficial to the butterfly farm as a way to increase understanding of how butterflies are parasitized, preyed upon and their oviposition habits. By quantifying parasitism rates, the farming operation may be able to adjust management practices in order to ensure higher productivity through lowering parasitism rates of the species actively being bred. This higher productivity, at a human social and community level, could result in more revenue

and job opportunities to the community involved. By measuring butterfly abundance rates, scientists are able to better understand the ecological dynamics and influences of a butterfly farm and its impact on the surrounding areas. Should irregular patterns of butterfly distribution and abundance occur (i.e., butterflies found to be not evenly dispersed throughout the farm property), it was the objective of this study to not only observe and record such patterns, but also to provide interpretation of the factors influencing such irregularity. More importantly, some investigators have suggested that the farm may act as an ecological trap, where the butterflies are being attracted to the farm epicenter under the auspices of reward for homing in on an environmental cue (Schlaepfer *et al.* 2002). Ecological traps will be discussed further in a later section starting on page 29. Finally, at a conservation level, understanding the reasons for patterns of parasitism, predation and butterfly abundance in areas surrounding the farm will allow for better management of the farm. Ultimately the hope would be that the results of this study might provide supportive evidence that will minimize any potential negative influence the farm may have on its environment, and result in a more stabilized, productive, and healthy ecosystem. This, in turn, will produce a healthier farm ecosystem and likely increase the sustainability and productivity of the butterfly farm and the surrounding human communities that may be economically dependent on the farm.

The remainder of this introduction will detail the major topics addressed in this study, including larval predation, egg parasitism, oviposition, dispersal, and ecological traps.

Larval Predation

The fitness of most organisms is closely related to the organisms' age and size at sexual maturity (Stearns 1992). Reaching an optimal combination of age and size as an adult is largely governed by how an organism performs in its juvenile stages, particularly concerning growth rate (Stearns 1992). Regarding juvenile survivorship, one can see the benefits of a rapid growth rate

(provided that there is sufficient food supply available). Theoretically, a faster growth rate results in less time spent in the vulnerable juvenile period, reducing the amount of time the larvae is exposed to threats, and increasing the probability that the immature organism will survive until reaching sexual maturity (Stearns 1992). However, Arendt (1997) suggests that oftentimes organisms grow at a slower rate than they are physiologically capable. If it is indeed true that a shorter juvenile period would lead to greater overall success of the animal (i.e., less time susceptible to predation, parasitism, and other threats), why wouldn't the animal attempt to maximize its growth rate? The answer is relatively simple. Growth rate and predation level are both functions of activity level in many organisms (Werner & Anholt 1993). Commonly, organisms that exhibit a maximum growth rate also require maximum nutrition and, therefore, devote more time to foraging activity, which puts the animal out in the open more often and increases its susceptibility to predation. Consequently, larval growth patterns are likely to be the result of making "strategic decisions" where the organism weighs the costs and benefits of various growth trajectories and chooses an optimal rate dependent upon its particular environment (Abrams *et al.* 1996, Nylin & Gotthard 1998, Gotthard 1999). In a laboratory experiment, Gotthard (2000) found that there is indeed a trade-off between butterfly larvae growth rate and predation risk, using the butterfly *Pararge aegeria* (Nymphalidae: Satyrinae). When comparing a "fast-growing larvae" and "slow-growing larvae", they found that the daily predation risk was 30 % higher in their "faster-growing larvae" treatment (Gotthard 2000). Therefore, we see that larval predation may be more of a problem for species that exhibit naturally high growth rates.

Butterfly larvae are at particular risk to predation due to their feeding habits. They are essentially feeding machines that devote their entire juvenile lives, as well as body plans, to

maximizing intake while also developing strategies to avoid or deter predation (Nylin & Gotthard 1998). Monarch butterflies (*Danaus plexippus*) employ a special tactic in deterring predation. By feeding on *Asclepias* species, they take advantage of the milky latex that is contained in a sealed system of vessels known as laticifers (Lucansky & Clough 1986). Monarch larvae are not only able to bypass this plant defense by effectively disabling the latex flow, but they also are able to sequester and concentrate the cardiac glycosides found in the latex (Dussourd 1990, Malcolm & Brower 1989). This facet of monarch biology has attracted quite a bit of attention and consequently has been researched extensively to produce indisputable evidence that the storage of these cardiac glycosides effectively deters vertebrate predators (Brower 1969, 1984; Brower & Fink 1985; Glendinning & Brower 1990). However, there are negative aspects to feeding on plants with latex and cardiac glycosides. In a study by Zalucki *et al.* (2001), results suggest that the amount of time spent avoiding excessive latex ingestion results in slower growth. Because younger and smaller larval stages are more susceptible to predation by ants, Hemiptera, Neuroptera, and other entomophages, additional time spent in a vulnerable stage reduces survival of the butterfly larvae (Zalucki *et al.* 2001). Another negative aspect of latex is the risk of accidental ingestion. While reports by Rothschild *et al.* (1977) and Dixon *et al.* (1978) indicate that fifth-instar larvae actively search for latex exuded from injured parts of the plant, Zalucki *et al.* (2001) report that ingestion of latex by first-instar larvae renders the larvae inactive or cataleptic. This cataleptic state could leave the organism vulnerable to predation. Although this prediction was not directly tested by them, Zalucki *et al.* (2001) observed that catalepsis and disorientation resulting from ingestion of latex by early-instar butterfly larvae caused larvae to fall off their host plants, increasing the likelihood of being predated upon by ants and other ground-dwelling predators. In line with these experiments,

many other studies of Lepidoptera have found that low survivorship of early instars is responsible for most of each generation's mortality, a truly grave realization (Cohen & Brower 1982, Zalucki & Kitching 1982, Dempster 1983, Kyi *et al.* 1991). For these reasons, third instar larvae were the subject for this part of the study. Third instars would not be as vulnerable to catalepsies, while also having less of a proclivity to sequester high levels of cardiac glycosides.

Despite these studies, there are very few published studies that estimate *natural* predation risks for butterfly larvae. However, one of these few studies found staggering results. Bernays (1997) made a comparison of predation risks while Lepidoptera larvae were feeding versus when they were resting. He found that predation levels while feeding increased three-fold for *Manduca sexta* and 100-fold for *Uresiphita reversalis*. There is a great need for more research to be done examining the predation risk for butterfly larvae in a natural environment.

Due to the extensive literature base, as well as their prevalence in butterfly farm cultures and in the Florida wild, Monarch butterflies (*Danaus plexippus*) were selected as the principal study organism for my experiment at north central Florida butterfly farms. Due to the predominant presence and abundance of the Common Morpho (*Morpho peleides*) at Costa Rica butterfly farms, this Morpho was selected as the primary study organism at Costa Rica butterfly farms.

Butterfly Parasitism

The relative importance of butterfly parasitism studies is being realized more and more as butterfly captive breeding and ecotourism programs continue to emerge and grow. While the majority of past studies have focused on the positive effects, or at least the theoretically positive effects, of parasites and parasitoids of Lepidoptera that are used in biocontrol, the research and findings on these situations are relevant and contribute to the overall understanding of lepidopteran parasitoid behavior. Nevertheless, there is a need for additional research to be done

on the possible negative effects of lepidopteran parasites, particularly those parasitoids affecting butterfly farming, captive breeding, and butterfly ecotourism programs.

When dealing with parasitoids that affect insects in the order Lepidoptera, butterfly farmers and biologists alike can identify several types of insects that *could* pose potential problems, such as Tachinid flies (Diptera: Tachinidae) and Braconid wasps (Hymenoptera: Braconidae), there is one key wasp group that overwhelmingly presents the greatest threat: species of the egg parasitoid, genus *Trichogramma* (Hymenoptera: Trichogrammatidae). Many species in this genus are important natural enemies and are used in inundative and inoculative biocontrol programs throughout the world (Li 1994, Smith 1996). While many species in the genus *Trichogramma* are generalists, the better-known species are specialists on parasitizing eggs of the members of the order Lepidoptera (Pinto & Stouthamer 1994). Because of their extremely small size, ranging from 0.2 to 1.5mm, these tiny wasps present a much greater problem than other parasitoids for the captive breeding of Lepidoptera (Pinto & Stouthamer 1994). This is because exclusionary tactics such as screens, netting, and cages need to be almost perfect (i.e., no gaps, tears, etc.) to ensure that these nearly microscopic insects do not have access to breeding centers and the Lepidoptera eggs within. While newer exclusionary technology allows for better parasite control, the costs of these more effective methods are often prohibitive to butterfly farmers in developing nations (Gordon & Ayiamba 2003). Because butterfly farming and ecotourism in developing nations are increasingly becoming vital sources of revenue and a means for biodiversity conservation, a better understanding of egg parasitoid behavior in these areas is critical.

Parasitoid Behavior

The behavior of *Trichogramma* parasitoids was studied as early as 1935 by G. Salt and J. Laing. Extensive experiments and reviews have been published subsequently, which have

contributed critical knowledge to our understanding of these organisms. Klomp and Teerink (1962) pooled data together from the aforementioned Salt and Laing (1935) study and other early experiments to divide *Trichogramma* egg parasitism behavior into four components. First, the wasp moves randomly on the substrate (i.e., host plant) and is visually attracted by small objects, which appear to rise above the surface. Eggs must be spotted from a distance of no more than 4mm, and thus walking is a key part of host searching behavior for these wasps (Wajnberg 1994). Plant structure plays a significantly large role in parasitism rates by *Trichogramma* wasps. Factors such as the plant size or surface area, abundance, and diversity of plant parts and connectivity between individual plants are all keys as to how effective *Trichogramma* wasps are at locating and then parasitizing their hosts (Andow & Prokrym 1990). Second, the wasp climbs up onto the egg and ritualistically walks all over the surface, making specific movements with its antennae known as “drumming.” Should the egg have already been visited by a different *Trichogramma* female, the wasp will reject the egg upon sensing the odor still present on the chorion of the egg. Otherwise, the parasitoid will proceed across the egg surface. Thirdly, the parasitoid will begin to drill on the surface of the object, apparently paying little attention to details of the surface such as texture and smell (provided it doesn’t smell like another female *Trichogramma*, at which time the parasitoid would arrest its examination and then move on to another egg) (Klomp & Teerink 1962). These wasps have been observed exhibiting the same behavior with small seeds and grains of sand, suggesting that texture and smell have little to do with whether the wasp accepts the egg as a viable host. However, the size of the object will be assessed, and if development of the wasp offspring is deemed impossible by the adult wasp, the wasp will immediately cease all further interaction with the egg and move on. Fourth, once the chorion is perforated by the wasp, she will deposit eggs. Should the host embryo inside the egg

be too far developed, or perhaps if it has already been parasitized, the wasp will abandon the egg and move on (Klomp & Teerink 1962).

These wasp parasites can be costly problems for Lepidoptera, a fact which has deemed them powerful bio-control agents on pest Lepidoptera in agriculture. Unfortunately, they also parasitize non-pest Lepidoptera that can thereby cause costly problems for butterfly farmers. As mentioned earlier, exclusionary measures are frequently used to protect butterfly stock from unacceptably high levels of parasitism. However, these measures are typically employed only in special labs and buildings for butterfly eggs and other immature stages. *Trichogramma* wasps still present problems in other areas of the butterfly farming operation, such as outdoor plant nurseries and gardens.

Parasitoids and the Farms

A common sight at butterfly farms is host and nectar plants growing in relatively vast numbers around the property. Some of these plants are found under the cover of screens in shadehouses, while others are out in the open to take advantage of sunlight as well as minimizing overhead costs to the farm. In both cases, these plants have the potential to attract parasitoids, creating a risk for the farm. As plants in the wild serve as resources to butterflies, so do plants that are found in the open areas at butterfly farms and breeding centers. The potential for high densities of butterfly eggs at these host plant nurseries would naturally attract parasitoids, especially *Trichogramma* wasps. Additionally, the close spacing of plants, common in these butterfly farm nurseries, makes it very easy for parasitoids to walk and jump to adjacent plants (Romeis *et al.* 2005). Research suggests that this augments parasitoid dispersal, which could make these areas prime habitats for *Trichogramma* wasps and other egg parasitoids (Surverkropp 1997). If this is indeed true, it means that butterfly farms could be supporting high populations of parasitoids in the same general area as where they harvest and store butterfly eggs. There is a

need for research that investigates the rate of egg parasitism within proximity to the butterfly farm to critically examine this problem.

Dispersal

Generalities and supportive documentation derived from previous dispersal studies have been difficult, mainly due to the amount of variation between the definitions of dispersal as well as the great number of differences in spatial scales used in such studies. Perhaps the two most common definitions for dispersal are (1) movement from the natal patch to the breeding patch, and (2) movement between breeding patches (Clobert *et al.* 2001). However, these definitions do not mention nor take into account the distances or even other reasons for dispersal, such as foraging, for example. To avoid complicated semantic issues, this study will adopt a broad definition of dispersal as stated by Bowler and Benton (2005), which defines dispersal as any movement between habitat patches, and habitat patches as areas of suitable habitat separated in space from other such areas, regardless of distance.

The consequences resulting from dispersal are great for both the individual actively dispersing as well as the populations involved (i.e., the population that the individual is immigrating to as well as the population that the individual is emigrating from). The act of simply moving from one patch to another may affect individual fitness, population dynamics, and genetics, as well as species distributions (Dunning *et al.* 1995, Hanski & Gilpin 1997, Hanski 1999, Clobert *et al.* 2001). The well-founded link between dispersal and population dynamics necessitates a better understanding of the patterns, causes and consequences of dispersal. This is critical to our overall understanding of population management and our ability to predict population responses to changes in the environment (Bowler & Benton 2005).

Factors Affecting Butterfly Dispersal

There are a number of different factors that influence a butterfly to either leave or remain in a patch of habitat. Some factors are individual characteristics of the organism such as age or sex (Kuussaari *et al.* 1996) while others are characteristics of the habitat, such as the presence of nectar sources and host plant density (Baker 1969, Shreeve 1995). The larval resource (host plants) and adult resource (nectar plants, sap flows, rotting fruit) are important ecological factors affecting movement in butterflies, as well as other holometabolous invertebrates (Brommer & Fred 1999). Similarly, the sex of the butterfly is correlated with the rate of dispersal, as is the insect's age. However, there is contention among experts as to the nature of the correlation between sex and age with rates of dispersal (Kuussaari *et al.* 1996, Lawrence 1987). Although these single factors may exhibit a certain degree of correlation with dispersal, one must question whether correlation proves causation. As seen in Bowler and Benton (2005), many of these single factors are associated with one unifying condition: conspecific density.

Conspecific density, which measures the density of organisms of the same species in a given area, is seen to have a great influence on butterfly movement and dispersal (Dethier & MacArthur 1964). While separate factors such as age, sex, and habitat resource availability may play their individual parts, conspecific density may be the most powerful, as it lumps many of the possible factors that govern dispersal rates into one central theme. Food availability, sex ratio, and parasite/parasitoid presence are all correlated to conspecific density (Bowler & Benton 2005). Therefore, understanding the effect that conspecific density has on dispersal is useful in understanding the entire system of dispersal. However, the correlation between conspecific density and butterfly dispersal is not as definitive as, say, the correlation between nectar source presence and butterfly movement into or out of a patch. With no surprise, there is a positive correlation between butterfly movement into the patch and the presence of high numbers of

nectar sources (Shreeve 1995). Nevertheless, understanding the effects that conspecific density has on dispersal is key in this study of butterfly abundances at butterfly farms, which have artificially high conspecific densities due to their butterfly stock and/or dense planting of nectar sources inside and outside the cages.

Conspecific density

Previous studies have observed that individuals disperse at a higher rate away from an area when the area exhibits a low conspecific density, suggesting a negatively density-dependent dispersal (Gilbert & Singer 1973; Kuussaari *et al.* 1996, 1998), while other observations find that individuals disperse at higher rates away from areas exhibiting high conspecific density, suggesting a positively density-dependent dispersal (Dethier & MacArthur 1964; Odendaal *et al.* 1989; Baguette *et al.* 1996, 1998). The next two sections will concentrate on the differences between these two concepts and common arguments in favor of one or the other.

Negatively density-dependent dispersal

Negatively density-dependent dispersal is a term used to describe a situation in which individuals disperse at a higher rate away from an area when the area exhibits a low conspecific density (Gilbert & Singer 1973; Kuussaari *et al.* 1996, 1998). According to Hanski *et al.* (1994) and Kuussaari *et al.* (1998), this could be the result of mate scarcity, as a butterfly inhabiting an area with low conspecific density would have a lesser chance of finding an appropriate mate and may choose to leave the patch in order to hopefully increase chance of finding a mate elsewhere. The concept of high dispersal rates away from areas of low conspecific density gave rise to the conspecific attraction hypothesis, which states that high density populations will result in higher immigration rates to and lower emigration rates away from the habitat in question (Ray *et al.*, 1991). There are two supporting arguments to this hypothesis. The first, given by Odendaal *et al.* (1989), is that butterflies are attracted to and ultimately remain in habitats with high

conspecific densities due to higher probabilities of locating suitable mates there. Specifically, males would likely be attracted to areas of high female density, (Hanski *et al.* 1994, Kuussaari 1998). The second argument was given by Gilbert and Singer (1973), who argued that a high density of butterflies in an area, especially of the same species, could be an indicator for suitable habitat. Therefore, an area with high conspecific density would trigger emigration from areas with low conspecific density as new adults would view the high density of same-species as a sign of suitable habitat, perhaps containing ample resources such as water, nectar, and host plants.

Positively density-dependent dispersal

On the other hand, there are also arguments supporting positively density-dependent dispersal, which is when individuals disperse at higher rates away from areas exhibiting high conspecific density (Dethier & MacArthur 1964; Odendaal *et al.* 1989; Baguette *et al.* 1996, 1998). These supporting arguments most often have to do with social interactions within the patch (Shapiro 1970; Odendaal *et al.* 1985, 1989; Baguette *et al.* 1996, 1998). For instance, in some species of butterflies, males will vigorously pursue females in order to mate with them. While this may be effective for males that are pursuing un-mated females, sometimes the females have already mated and are reluctant to mate again. This does not often dissuade the males, as studies have observed them harassing females to the point of the female leaving the patch (Odendaal *et al.* 1985). Should this happen frequently and repeatedly, a likely scenario in a high density population, the result would be a high dispersal rate away from the area by females. This, in turn, could trigger a similar dispersal pattern among males that may then leave the area too, in pursuit of females or in search of an area with more females (Baguette *et al.* 1998).

A high conspecific density in a particular patch may also directly affect habitat quality of the patch, which could lead to higher emigration rates should the patch's density surpass the

patch's carrying capacity (Dethier and MacArthur 1964). As the butterflies deplete the host and nectar plant resources in the habitat, a "migratory threshold" would be reached (Baker 1984). This threshold is a theoretical limit to the rate of migration so that as the threshold is exceeded, dispersal away from the patch would begin to surpass dispersal into the patch, continuing this way until returning to a stable density. This stable density would be below the carrying capacity and allow key resources of the habitat such as nectar and host plants to regenerate, allowing for more immigration in the future.

Dispersal and the Farms

The notion of conspecific density is of particular importance to this study of butterfly farms, which have artificially high conspecific densities. It is clear that farm enclosures are artificially stocked with butterflies, yielding this artificially high conspecific density. However, it has not been documented whether or not butterflies occur in high numbers around the exterior of the farm epicenter.

The enclosures at butterfly farms present a special circumstance when considering habitat patches and conspecific densities. They may be treated as separate habitats due to the walls excluding immigration and migration. At the same time, however, they may still serve as an attractive force (i.e., presence of host plants, nectar sources, pheromones and visual signals emanating from potential mates, etc.) to wild populations of butterflies, in which case they could be considered part of a larger patch that includes all areas of the butterfly farming operation. According to Hanski *et al.* (1994) and Kuussaari *et al.* (1998), butterflies may be attracted to high conspecific densities in search of an appropriate mate. Additionally, Gilbert and Singer (1973) suggested that high conspecific densities may also attract butterflies when acting as a cue for good habitat quality. Should these theories apply to butterfly farms as well, a great problem

arises. The butterfly enclosures could be serving as an ecological trap, in that they suggest appropriate habitat and substantial mate availability when in fact the opposite is the case.

Ecological Traps

In choosing an appropriate habitat, whether it is to breed, forage, lay eggs, or simply for refuge, organisms rely on cues exhibited by the environment as indicators. An organism's decision to choose a particular habitat is largely the result of evolution and an adaptation to rely on cues that previously have correlated with survival and reproductive success (Williams & Nichols 1984). With enough evolutionary time and a strong enough correlation, a sudden change in the environment could endanger the organism by altering the "time-tested" association between a particular cue (or set of cues) and a predictable outcome for the organism (Levins 1968).

Gates and Gysel (1978) were among the first to use the term "ecological trap" to describe the increasingly common occurrence of what was described in the previous paragraph. They observed a situation in which a bird's choice of nesting habitat led to nest failure due to recent anthropogenic change in the environment. This nest failure broke the connection between the normal cue and the habitat quality to which these birds were evolutionarily accustomed, and this resulted in a complete loss of brood by the nesting adults. Susceptibility to ecological traps is not restricted to birds, as these traps may victimize any organism whose ancestors recognized and acted upon a tight correlation between a cue and the present or future state of the environment (clearly an intelligent and historically successful behavior!).

Butterfly farms may present an example of how an ecological trap could work by displaying "false cues" for habitat quality. Ubiquitous at butterfly farms are specific host and nectar sources for the butterflies they breed. Similarly, the butterflies bred at farms are typically the same species that occur in the local area, due to climate, restrictions stated in government

permits, etc. Additionally, these plants are often kept outside in order to maximize sunlight and minimize overhead cost. Combining these three variables, there is a high chance that native butterfly populations in the area will find the farm suitable as an area in which to oviposit on the host plants, or to feed on the nectar sources.

Although this situation seems like it might be no more harmful than a butterfly garden, there are several key differences. Firstly, these plants are frequently moved, pruned, and even “de-egged” (eggs are removed just before using them in the butterfly enclosures to safeguard against the introduction of egg parasites in quarantined zones). These disturbances could cause the eggs to fall off or be removed from the plant. The second way that these nurseries differ from a butterfly garden is that the host plants are often very dense and clustered together in a homogenous pattern. This enables parasites to easily walk and jump to different plants (Romeis *et al.* 2005). Butterfly gardens are typically more heterogenous in the way the plants are arranged, serving as natural defenses to parasitoids. Lastly, predators such as vespid wasps, ants, lizards and birds are likely to exploit a predictable source of food in these nurseries, predated upon adults and larvae. It is clear that if the conditions are right, native butterfly populations could be in danger of falling into an ecological trap.

From an environmental standpoint, the presence of an ecological trap could be very detrimental, and the problem requires further investigation. However, it is not just an environmental problem; the farm could also be negatively impacted as well. In the case of both parasitoids and predators, high levels could pose problems for the farm’s butterfly stock. Predators and parasitoids may attempt to enter breeding and rearing centers, causing significant economic loss to the farm.

It was the goal of this study to conduct a survey of butterfly abundances in relation to their proximity to the butterfly farm. Should wild butterflies be significantly more abundant in areas closest to the farm, this may suggest that they are indeed being attracted to the farm. However, additional studies are necessary to either dismiss this as insignificant or to verify this attraction and distinguish this factor from the possibility that escaped larvae or adults are artificially inflating population numbers on the outskirts of the farm. In addition, larval predation and egg parasitism also need to be examined in this study as a function of proximity to the butterfly farm in an effort to document all facets of a possible ecological trap.

Oviposition

The evolutionary process of insect egg laying, or oviposition, is a topic that has received a significant amount of attention in past studies. As with other insects, oviposition in butterflies has been studied from many different angles. Those pertaining to variations in egg and clutch size, oviposition preference and oviposition habitat have particular application to this study.

Variation in Egg and Clutch Size

Egg size is a valuable measurement when studying insects, as it is closely related to fitness (Fox & Czesak 2000, Fischer *et al.* 2003). In an ultimate sense, it involves how mothers choose to allocate resources among their offspring. Almost always, as energy available for reproduction is held relatively constant, a larger egg size is correlated with a smaller clutch size, and vice versa (Smith & Fretwell 1974). However, it is not clearly known *how* a clutch is optimized by selection for either numbers, or for individual egg size. Although it may not be a panacean explanation for why any gravid female insect would choose greater egg size over greater clutch size, one study proposed that there is a correlation between temperature and egg size (Atkinson 1994). Atkinson (1994) coined the term *temperature-size rule*, which essentially states that as the temperature experienced by the mother is lowered, the size of her eggs become

larger, while the total *number* of eggs she lays is lessened. These initial beliefs have been confirmed in many recent studies (Azevedo *et al.* 1996; Yampolski & Scheiner 1996; Atkinson *et al.* 2001; Fischer *et al.* 2003, 2006a, 2006b; Steingenga & Fischer 2007) and could provide insight into butterfly emergence and dispersal patterns. This plasticity between egg size and clutch size is not an even trade-off, however, as total reproductive investment must be increased at higher temperatures (Avelar 1993; Ernsting & Isaaks 1997, 2000; Fischer *et al.* 2003). For example, lifetime fecundity was almost twice as high at 27° C than at 20° C in a study by Fischer *et al.* (2003). Although the mother's biological response of egg size and clutch size to temperature was gradual (two to three days of cold could alter egg laying behavior) in Fischer *et al.*'s study (2003), it was also found to be reversible after two to three days of warm weather. We can surmise from these studies that oviposition will be in greater numbers in warmer environments than in colder environments.

Oviposition Preference

Most butterflies are oligophagous. This habit of feeding on host plants from a very limited number of plant families, oftentimes only a single family, suggests that there may be basic similarities within plant families that signal or cue a butterfly to oviposit (Feeny 1991). The host plant may be attracting the butterfly by exuding a chemical, visual, or tactile cue. In any case, it is the job of the adult butterfly to choose the best host plant for its soon-to-be offspring. Larvae of many phytophagous and holometabolous insects are to a certain degree immobile and are not able to search for appropriate food over comparatively large distances (Rausher 1979a). Females that oviposit on plants with low larval survivorship or poor larval growth will likely leave fewer descendents than females that oviposit on the more suitable host

plants (Rausher 1979a). For this reason, it is imperative to the survival of the species that the female chooses an appropriate host plant correctly the vast majority of the time.

Habitat: Habitat is among the more important influences regarding host-plant selection among insects. A habitat that simply contains specific host plants is not enough for that habitat to be preferential to gravid female butterflies (Rausher 1979a). In fact, many ovipositing butterflies do not use all of the habitats in which the appropriate host plants grow (Shapiro & Carde 1970, Ehrlich *et al.* 1975). Egg laying patterns could be the result of patch size, connectivity, and landscape matrix as well (Rabasa *et al.* 2005). Plant-insect interactions within a site may also depend on processes occurring at larger spatial scales (Tscharntke & Brandl 2004). Nevertheless, patch size and landscape connectivity are postulated as key determinants of butterfly presence in fragmented landscapes, due to the fact that smaller patches experience higher rates of extinctions (James *et al.* 2003). A study by Rabasa *et al.* (2005) found that the probability of egg presences and oviposition for a rare European butterfly did not differ significantly between large and small patches.

Some previous studies suggest that oviposition search behavior continually evolves in order to ensure that females lay eggs in the habitats that are most well suited for the growth and development of the juvenile stages (Gilbert & Singer 1975, Wiklund 1977). As reported by Hovanitz and Chang (1963), food plant choice appears to be at least partly genetically determined in many insect species, with Lepidoptera being no exception. Therefore, one could expect that differences in the suitability of food plants to influence the evolution of genes or alleles controlling oviposition behavior (Rausher 1979a).

Forister (2004) tells us, however, that there is a positive correlation between female preference of optimal host plant and larval performance and this has been found and cited in

studies of various insect families (Mayhew 1997, Harris *et al.* 2001, Craig & Ohgushi 2002). Nevertheless, this presumption of a strongly positive correlation has been integrated in many biological models and taken as fact in many instances. Also termed the *naïve adaptationist* hypothesis, by Courtney and Kibota (1990), this is the most common hypothesis in regards to the selection of host plants by phytophagous insects. It states that natural selection effectively promotes female preference such that eggs are laid on the host plant that is the best host for her offspring (Levins & MacArthur 1969). Reiterating what Raucher (1979a) said, there are two ways that the plant can serve as best host: one way resulting in the highest survivorship rate among offspring, and the other being the relative size of the offspring without regard to overall survivorship rates (Rausher 1979a).

Oviposition is used in this study as an indicator for butterfly abundance. By standardizing the oviposition substrate (i.e., host plant), and environmental conditions such as seasonality and landscape connectivity, any fluctuations in the number of eggs deposited should indicate a fluctuation in butterfly abundance in the area.

CHAPTER 2 MATERIALS AND METHODS

Oviposition as Index to Butterfly Abundance

This experiment occurred in a total of three geographic locations, with all measurements and experiments set up identically in each location. The first two trials were conducted in north central Florida, at Shady Oaks Butterfly Farm and Greathouse Butterfly Farm. The third trial was conducted in northern Guanacaste Province, Costa Rica, at El Bosque Nuevo Butterfly Farm (Figure 2-1).

For each location in this study, the researcher designated an “epicenter,” as shown in Figure 2-2. The epicenter contained all butterfly host plants being grown at the farm (either for sale or for use in rearing and breeding), all butterfly cages, all rearing rooms, laboratories, and all gardens. The farm epicenter is essentially the operational part of the farm. If there was farm property not specifically used in the butterfly farming operation (such as access roads, parking lots, etc.), it was excluded from the epicenter. The epicenter circumference was then measured using a rolling-wheel electronic tape measurer.

A total of nine rows of potted (one-gallon size) butterfly host plants were placed, starting at the perimeter of the epicenter and radiating out to a distance of 100 meters for each row. Each 100-meter row was then divided further so that one host plant was placed at each 10-meter interval. Therefore, 11 host plants were placed along each row (Figure 2-2). In order to maintain the plants’ health, a water saucer was placed under each pot to maximize water retention. In order to distinguish the experimental plants from other nearby conspecifics, orange marking flags were used to identify the experimental plants.

Each host plant row was evenly spaced and angled from the two adjacent rows as accurately as possible so that no two rows were closer than other rows. Using the previous

measurement of circumference, the researcher was able to calculate equal distances and equal angles for the spacing between all rows.

A total of three species of host plants were used at each butterfly farm, with each intended to elicit oviposition from a specific species of butterfly. See Table 2-1 for the complete list of host plants and butterflies. Different species of host plants were used for a different set of butterflies at the Costa Rica butterfly farm (Table 2-1), but for both north central Florida locations the same plants were used. To recapitulate, one plant was placed at each 10m transect for each row and there were nine rows of host plants total. With three different host plants per farm, each host plant was replicated three times so that there were three rows for each plant. Each row contained 11 plants, from a distance of zero (adjacent to the epicenter perimeter) to a distance of 100 meters. Therefore, 99 individual plants were used for each farm.

Challenges in placing the plant rows presented themselves in the experiment. First, each farm was situated within 100 meters from a road. Therefore, several plant rows had to cross these roads at which points the researcher preserved the 10-meter spacing as much as possible. In the case of Costa Rica, dense jungle made 10-meter spacing difficult with two of the nine rows, and as a result the angle between them was slightly more acute than the other rows in this situation (Figure 2-3).

In the case of the north central Florida farms, the plants were placed on the edges of roads (Figure 2-4), which caused several transect intervals to be slightly longer than 10 meters. Another problem with having roads so close to the farm being studied was that several plants placed near the road were stolen. However, this happened only once, and the plants were able to be replaced on the following day, thus minimizing the loss of data.

Once the experiment was set up, data were then collected on the number of new eggs found on each plant per day (i.e., every 24 hours). These eggs were subsequently used in the next part of the experiment (see Egg Parasitism section – Page 37). All eggs were removed at the time of data collection each day. This was to prevent gravid females from passing up these plants when ovipositing in the following days, due to the possibility of females of that species having responded to past selection to relieve conspecific egg competition by visually inspecting host plants for the presence of previously laid eggs. Data collection was repeated on a daily basis (i.e., every 24 hours) over a 10-day period resulting in 30 replicate host plant at each transect number (distance from epicenter) for each farm location.

Egg Parasitism

The second part of the experiment was to test for egg parasitism at varying distances from the butterfly farm epicenter. Using eggs from the previous oviposition experiment, a total of 12 eggs was collected at random from each transect number and retained for use in this egg-parasitism study. Eggs were collected from the plants every 24 hours, at 8:00 A.M. (this samples eggs exposed to parasitism incidence from the preceding 24 hours). After collecting these eggs, they were placed individually in plastic containers covered with a fine mesh rubber-banded on top to allow air flow but to prevent any exit of the hatched larvae, or hatched parasite. The mesh also blocked entrance of subsequent searching parasites. Then, the eggs were observed on a daily basis until emergence of larvae (roughly four days), or parasitoid (Figure 2-5). Data were then collected on whether butterfly larvae or a parasite emerged.

Larval Predation

The final part of the experiment was to test predation effects on larvae of Monarch butterflies (*Danaus plexippus*) in north central Florida and Common Morpho butterflies (*Morpho peleides*) in Costa Rica. For each location, two host plant rows were placed radiating out from

the farm epicenter, much like the first experimental arrangement described earlier. However, since there were only two host plants per row, one was placed at the edge of the epicenter perimeter (distance = 0 meters) and the other was placed 100 meters away (distance = 100 meters) (Figure 2-6).

For each plant at the north central Florida location, five 3rd-instar Monarch butterfly larvae were directly placed on the plant and allowed to feed in a controlled laboratory environment for 24 hours (Figure 2-7). This step was to acclimate the larvae on the host plant and to make sure that they were not prone to wander off of the plant within the 24 hours that the trial was actually running. After this 24 hour acclimation period, the plants were taken from the laboratory and placed in their appropriate positions in the field. After another 24 hours, data were collected on the number of larvae remaining for each plant. This entire process was replicated twice for each north central Florida location.

For the Costa Rica location, two rows of host plants of the Common Morpho (*Morpho peleides*) were placed in an identical fashion as the plants at the north central Florida location (Figure 2-6). Because the host plants were trees and relatively large, they would not be able to fit into a controlled laboratory room, as the Monarch larvae were. Therefore, an alternate method was used. With the potted host plants already in the field, five 3rd instar Common Morpho larvae were placed directly on the plant and then enclosed in a “predator screen” (Figure 2-8). This screen was a fine mesh bag tied at both ends (using a secure overhand reef knot), both preventing the larvae from wandering as well as excluding parasites and predators from attacking the larvae while they were being acclimated to the host plant (Figure 2-9). These screens are used in the very same way by the butterfly farm and have provided successful results throughout

the years, according to the farm manager. After 24 hours, the predator screens were removed. Then 24 hours later, data were collected on the number of larvae remaining on each plant.

In addition, a control variable was used for this experiment at all geographic locations. At a separate location away from the Butterfly farm, in similar habitat, a “false epicenter” was marked with marking flags. This epicenter was the same size and dimensions as its experimental counterpart, but did not have any butterfly farming operations taking place within its borders (Figure 2-10). Two rows of host plants were set up in an identical fashion as described for the experimental variable. The researcher oriented the rows so that both experimental and control variables were along the same compass direction. Acclimation and data collection procedures were identical to the procedure used for the experimental variable.



Figure 2-1. Entrances to the three butterfly farms used throughout the experiment. Clockwise from upper left, entrances to El Bosque Nuevo, Shady Oaks, and Greathouse butterfly farms

Table 2-1. List of butterfly and host plants used

Butterfly Species for Costa Rica	Host plant Species for Costa Rica
<i>Heliconius sapho</i>	<i>Passiflora auriculata</i>
<i>Caligo memnon</i>	<i>Heliconia sp.</i>
<i>Morpho peleides</i>	<i>Leguminosa cristobal</i>
Butterfly Species for Florida	Host plant Species for Florida
<i>Danaus plexippus</i>	<i>Asclepias curassivica</i>
<i>Junonia coena</i>	<i>Plantago lanceolata</i>
<i>Papilio polyxenes</i>	<i>Petroselinum crispum</i>

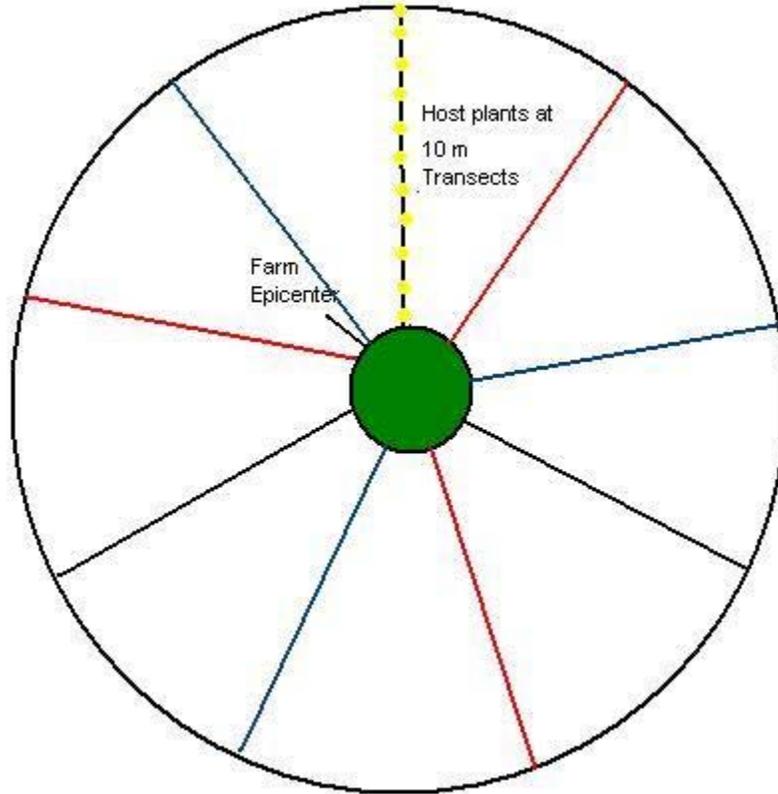


Figure 2-2. Diagram of farm epicenter (green circle) with radiation rows of hostplants at 10 meter transects (yellow dots). Transects repeated for each row, but shown here for only one row. Three different host plants were used, corresponding with the three differently colored rows in this diagram



Figure 2-3. Dense jungle causing one row of host plants to be shifted slightly



Figure 2-4. Several plants needed to be placed close to roads in the Florida experiments



Figure 2-5. Screened vials and stereoscope used to observe the emergence of parasites or larvae

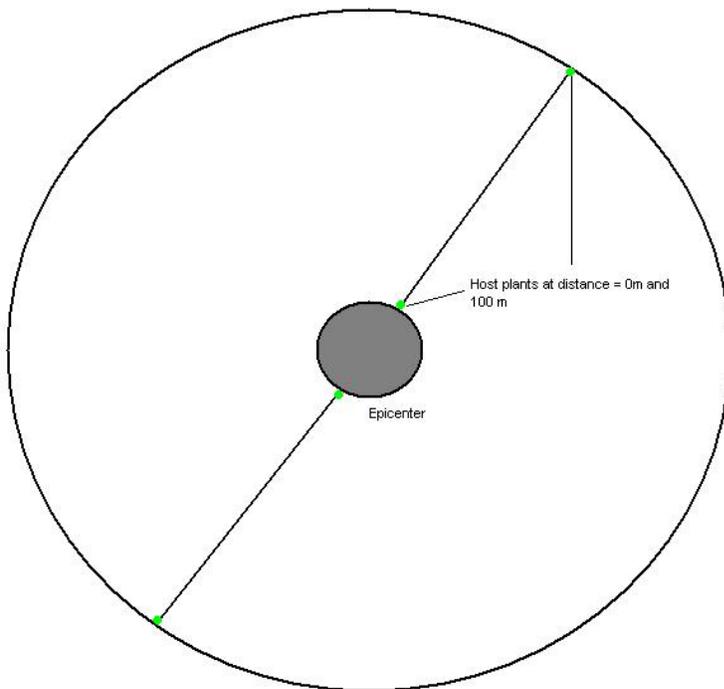


Figure 2-6. Diagram of larval predation experiment



Figure 2-7. Controlled environment used to acclimate Monarch larvae to their host plant



Figure 2-8. *Morpho peleides* host plant *Leguminosa cristobal* before and after predator screen application



Figure 2-9. Close-up view of predator screen



Figure 2-10. Control plot used in north central Florida experiments

CHAPTER 3 RESULTS

Main Experiment – Butterfly Oviposition in North Central Florida

After a period of 10 days of data collection at two north central Florida butterfly farms, 60 observations (30 for each farm) were taken on oviposition for each of 11 transects. At these two farms, sufficient data were collected from Monarch butterfly oviposition, but neither farm yielded sufficient oviposition data for the Eastern Black Swallowtail or the Common Buckeye. Although Buckeyes and Swallowtails were frequently seen at both farms by the researcher, no Buckeye eggs and only 2 Swallowtail eggs were observed during the entire 10-day period on all transects. Therefore, statistical models and summary statistics are meaningless for these two butterflies. Monarch oviposition, however, was indeed commonplace, and therefore is the main subject for this oviposition experiment in north central Florida.

Mean egg number per day was highest for those transects closest to the farm epicenter. Then, egg number proceeded to decline and level out at a lower mean for transects more distal to the farm epicenter (Figure 3-1). Figure 3-1 provides a summary of mean egg numbers per day at each transect for monarch oviposition at the north central Florida farms. Summary statistics are provided in Table 3-1. From these data, it is evident that the highest egg numbers are found closer to the farm, with transects 1, 2, 3, and 4 yielding the highest egg numbers (3.033, 2.933, 2.933, and 2.700 eggs/day respectively). Starting with transect 5, which exhibited an average of 1.683 eggs/day, egg numbers then dropped off by approximately 1.5 eggs per day. Transects 5 through 7 exhibit a downward sloping trend as egg numbers proceeded to decline until rising again at transect 8. However, transect 9 exhibited the lowest egg number of all, at 1.067 eggs/day. Then, a slight increase was exhibited with transect 10 seeing 1.4 eggs/day and transect 11 seeing 1.633 eggs/day. This up and down pattern between 5 and 11 is only slight when

compared to the drop from 1 to 5. From these data, there appears to be a noticeable difference in egg numbers between transects in regards to distance from the farm epicenter.

Looking at the data summarized in Table 3-1, we see that the highest standard deviations correspond with the highest mean egg numbers. These high standard deviations wreak havoc on statistical tests, lessening any significance that the data may inherently possess. Consequently, we cannot say that there is a significant difference in egg averages between transect 1 and transect 11, much less between other more closely-spaced transects. However, when we look at the 3rd quartile data (Table 3-1), there is a clear increase in frequency of higher egg numbers with transects more proximal to the epicenter, specifically transects 1 through 4. Additionally, median values are significantly higher with transects 1 through 3. Perhaps due to the sample size, we cannot prove significant differences between any of the transects using summary statistics alone. The next section will detail more complicated statistical analyses and findings.

In order to further analyze butterfly oviposition, the researcher used the model of repeated measures over time, treating the experiment as a randomized block design with day, location and transect as the factors. This allows the researcher to put the fixed effects of location, day and transect into blocks in order to measure how influential each fixed effect is to the variance in egg number. The raw data were then transformed (i.e., egg numbers) using a log+1 transformation in SAS to standardize the residuals in order to meet the assumptions of the model. The transformed data were then analyzed in SAS using a *proc mixed procedure*, which generated the significance statistics found in Table 3-2.

Variations in egg number can be attributed to 4 different effects: location, transect, day, and the interaction between location and day. There were no reported interaction effects between location and transect, nor day and transect. Table 3-2 shows a list of the effects and

their corresponding F-values and P-values, all at *preset alpha* = 0.100. Transect effect was found to have a p-value of 0.2049, which is slightly larger than the *pre-set alpha* level. However, this is still a relatively low value. In conjunction with the summary statistics described previously, there is enough evidence to suspect that the transect number (i.e., distance from the epicenter) has an effect on butterfly oviposition.

Due to the strong effect that location and day had on egg number, it merited further examination of the data based on individual locations and individual days. See Figure 3-2 and Figure 3-3 for separate graphs for average egg number per day at each location in north central Florida. From the data shown in Figure 3-2 and Figure 3-3, we can see a definite difference between locations. Shady Oaks has a more pronounced negative correlation between egg number and transect, while for Greathouse, egg number drops sharply around transect 5 and 6, but then rises from 6 to 11 (Figure 3-2 and Figure 3-3).

As mentioned before, there is an interaction effect between location and day. Figure 3-4 shows the egg number broken down by individual days as well as the individual farm. This interaction means that the combination of day and location yields more variance in egg number than simply the sum of their individual main effects. We see that Shady Oaks yielded overall fewer numbers of eggs in a vacillating fashion, while Greathouse exhibited a bell-shaped pattern, with most oviposition occurring on day 5 and 6, with a sharp drop off on day 7 through 10.

The day effect was quite pronounced as well. Most eggs were deposited during the middle of the experiment, with days 4, 5 and 6 seeing the most eggs at 201, 189 and 252 eggs respectively (Figure 3-5). Then, on day 7, a sharp decline in oviposition was observed, which then continued until the end of the experiment.

Finally, the location effect is shown below in Figure 3-6. There was over a 3-fold increase in total oviposition from Shady Oaks to Greathouse, with 288 and 997 eggs deposited, respectively, over the 10-day period.

Main Experiment: Butterfly Oviposition in Costa Rica

Oviposition in the Costa Rica trial was very surprising, as absolutely no eggs were oviposited on any of the hostplants during the entire 10-day duration of the experiment. Although this was an unexpected result, it does raise some interesting questions, which will be addressed in the Discussion chapter.

Larval Experiment: Larval Survivorship as Determined by Distance from Farm Epicenters in North Central Florida

The larval predation experiment suggests that there is indeed an effect that the butterfly farm has on its environment, but perhaps not in the same way as was originally predicted.

After running the experiment at the farm and running a control in a field of similar habitat approximately 5 miles away from the farm, several comparisons were made. First, total larval survivorship (i.e., not accounting for distance from the epicenter) at the experimental plot was compared to a control plot. Higher levels of survivorship among larvae at the experimental plot were found when compared to the control plots (Figure 3-7). Survivorship at the experimental plot was 41%, while survivorship at the control plot was 14% (Table 3-3). Using a T-test at $\alpha = 0.005$, the researcher was able to conclude that there is a significant difference between the experimental and control plots, in terms of larval survivorship. Second, it was determined that there was also a significantly higher level of survivorship for larvae situated closer (i.e., located at transect station 1, which was adjacent to the perimeter of the farm epicenter) to the farm epicenter, when compared to larvae situated farther (i.e., located at transect station 11, which was 100 meters away from the perimeter of the farm epicenter) from the farm epicenter

(T-test at $\alpha = 0.005$). An average of 58% larval survivorship on plants in the near location is significantly greater than the 25% larval survivorship at the far location (Figure 3-8). Lastly, larval survivorship between near and far locations in the control plot was found not to differ significantly (Table 3-5).

Larval Experiment: Larval Survivorship as Determined by Distance from Farm Epicenters in Costa Rica

Identical to the north central Florida farms experiment, a control plot was run in conjunction with the experimental farm plot. After collecting the data, several comparisons were made. First, using a T-test at $\alpha = 0.01$ (larger alpha due to smaller sample size), it was determined that there was no significant difference in total survivorship between control and experimental plots (i.e., not accounting for distance from the epicenter). Percent survivorship for the experimental plot was 83% versus 63% for the control plot (Table 3-3). The second comparison was between near and far locations for larval survivorship in the experimental plot. With near and far points exhibiting 90% and 75% larval survivorship, respectively, a T-test at $\alpha = 0.01$ concluded that there was no significant difference between the two distances (Table 3-4). However, a T-test at $\alpha = 0.01$ *did* find a significant difference in larval survivorship when comparing near and far treatments in the control plot (Figure 3-9). Percent survivorship in this control plot was found to be 40% for the near plot and 85% for the far plot (Table 3-5).

Egg Parasitism

The egg parasitism study in north central Florida generated significant data. However, the findings were very unexpected. By collecting a total of 12 eggs per transect, without regard to day, none of the collected eggs yielded any evidence of parasitism. One hundred percent of eggs collected from all transects hatched to produce live Monarch larvae. Due to the limited

number of eggs deposited at transects 9, 10, and 11 at Shady Oaks, the full 12 replications were not able to be conducted. Rather, the sample size was 6, 9, and 6, respectively.

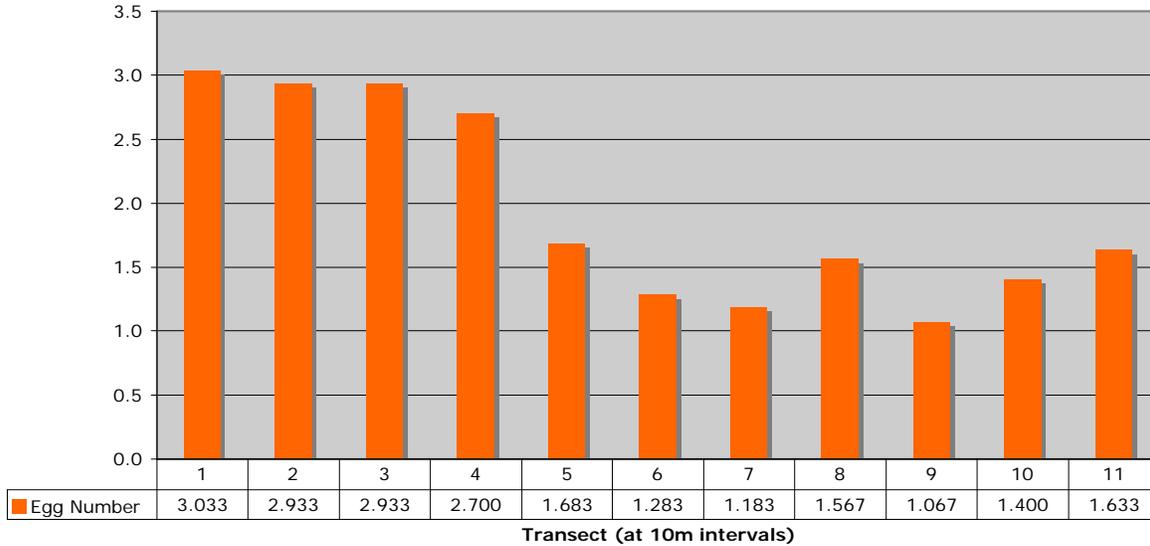


Figure 3-1. Summary graph of average egg number for each transect

Table 3-1. Summary statistics for North central Florida oviposition experiment

Transect	Sample size	Mean egg number	St Dev	Qmax	Q3	Median	Q1	Min
1	60	3.033	3.835	15	6	1	0	0
2	60	2.933	3.901	14	4.5	1	0	0
3	60	2.933	4.137	14	4	1	0	0
4	60	2.700	4.777	21	3	0	0	0
5	60	1.683	2.861	13	2	0	0	0
6	60	1.283	2.387	10	1	0	0	0
7	60	1.183	1.987	9	2	0	0	0
8	60	1.567	3.061	12	2	0	0	0
9	60	1.067	1.812	7	2	0	0	0
10	60	1.400	2.688	11	2	0	0	0
11	60	1.633	3.650	17	1.5	0	0	0

Table 3-2. Significance values obtained from the SAS proc mixed procedure ($\alpha=0.1$)

Effect	F-Value	P-Value
Location	32.18	<.0001
Transect	1.42	0.2049
Day	13.64	<.0001
Location*Day	24.25	<.0001

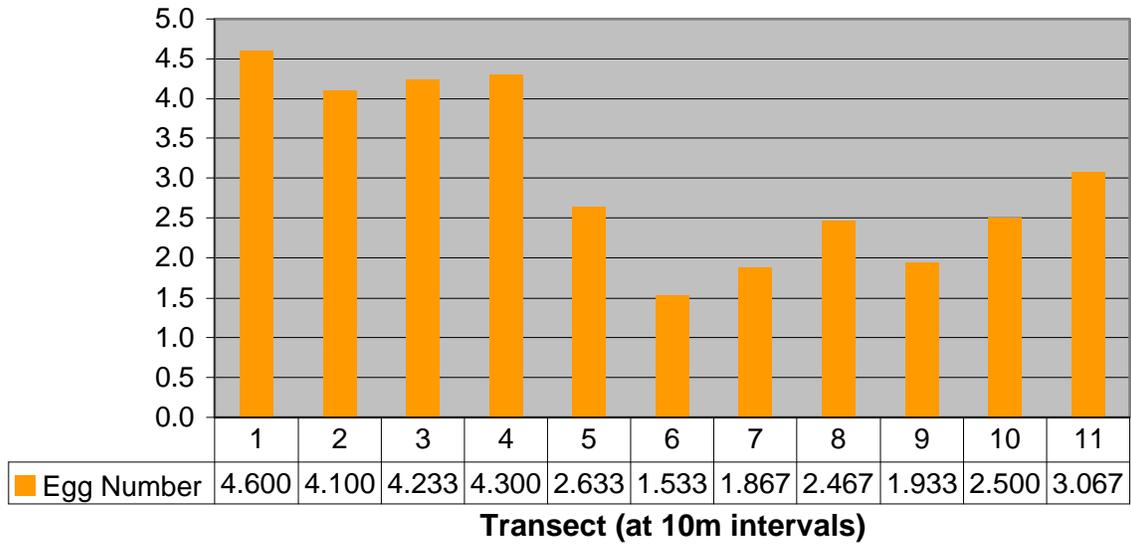


Figure 3-2. Mean egg number at each transect for Greathouse Butterfly Farm

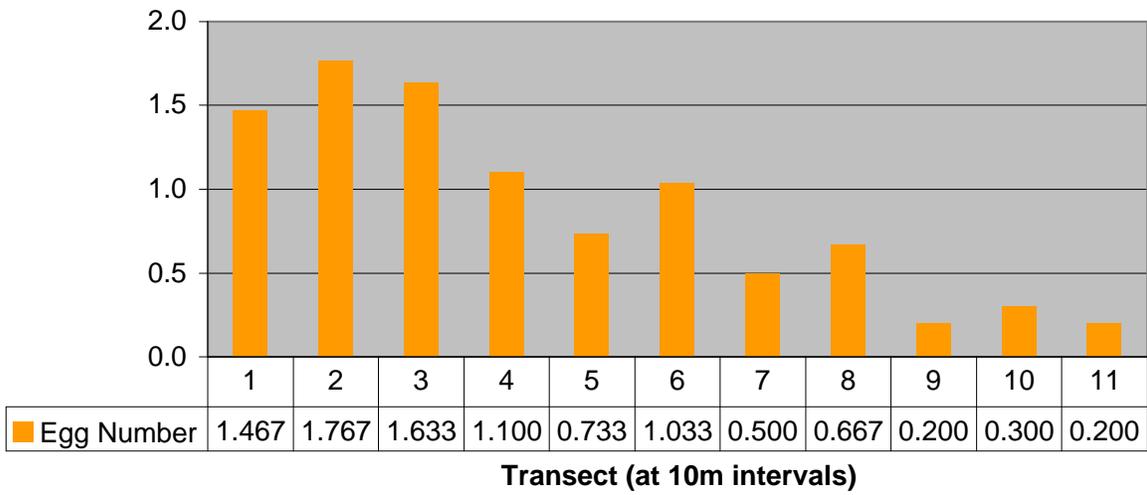


Figure 3-3. Mean egg number at each transect for Shady Oaks Butterfly Farm

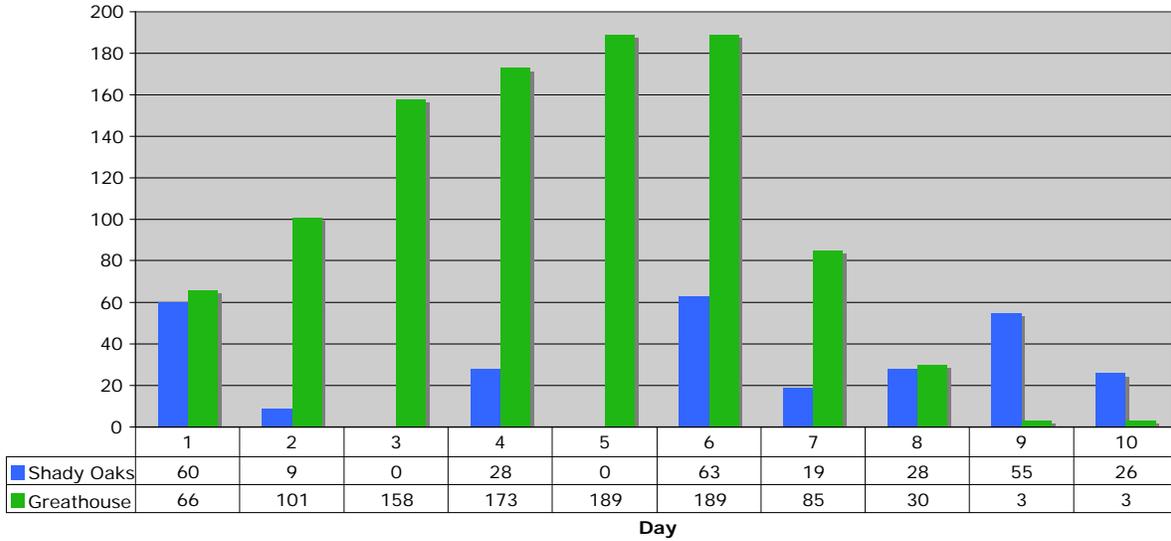


Figure 3-4. Location and day affected egg number more than just the sum of their individual roles

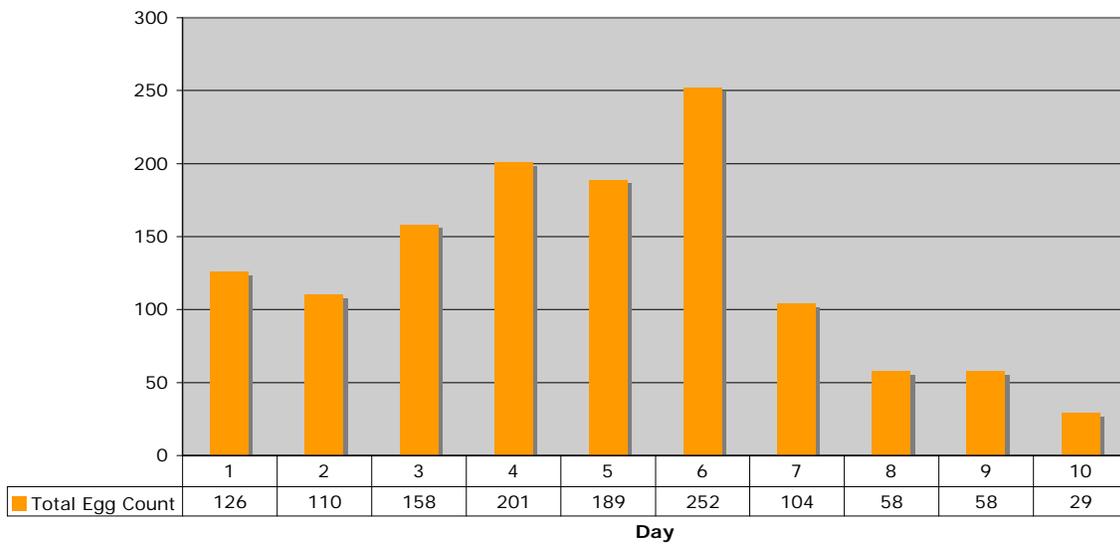


Figure 3-5. The day effect on egg number was relatively bell-shaped, with a peak in egg counts for days 4 through 6 after initial placement of the potted plants

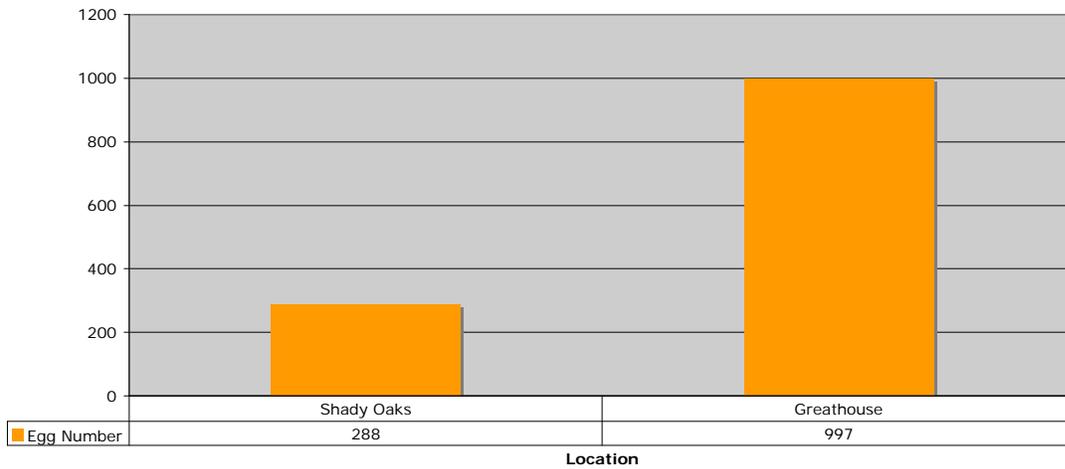


Figure 3-6. Location had a pronounced effect on egg number, with almost 3 times as many eggs at Greathouse when compared to Shady Oaks

Table 3-3. Mean % survivorship among larvae at experimental and control plots for all geographic locations

Farm	Experimental	Control
Shady Oaks	43	18
Greathouse	40	10
Florida combined	41	14
El Bosque	83	63

Table 3-4. Mean % survivorship among larvae at far and near distances in the experimental plot for all geographic locations

Farm	Far	Near
Shady Oaks	25	60
Greathouse	25	55
Florida combined	25	58
El Bosque	75	90

Table 3-5. Mean % survivorship among larvae at far and near distances in the control plot for all geographic locations

Farm	Far	Near
Shady Oaks	35	0
Greathouse	0	20
Florida combined	18	10
El Bosque	85	40

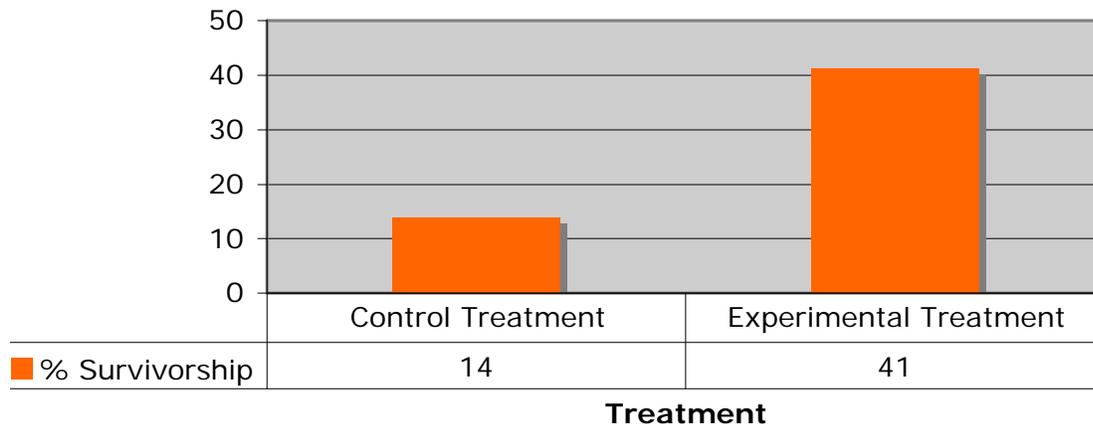


Figure 3-7. Mean % survivorship among larvae at experimental and control plots for north central Florida farms

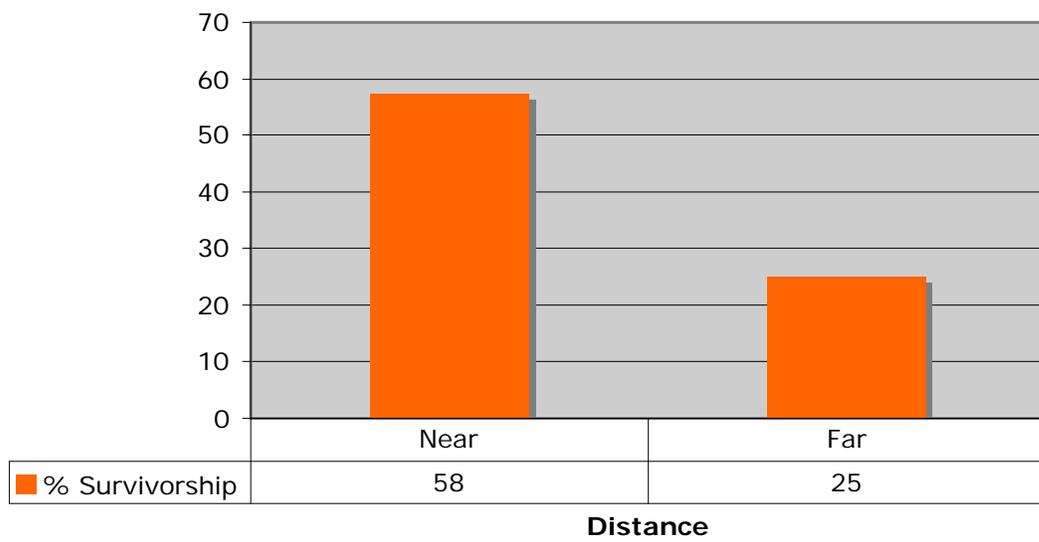


Figure 3-8. Mean % survivorship among larvae at far and near distances in the experimental plot for north central Florida farms

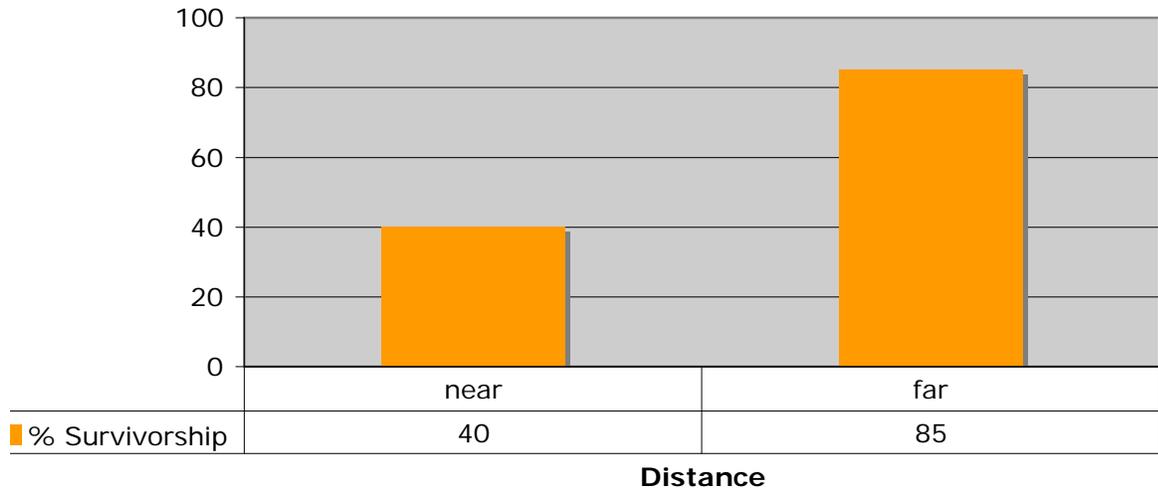


Figure 3-9. Mean % survivorship among larvae at far and near distances in the control plot for the northern Costa Rica farm

CHAPTER 5 DISCUSSION

Throughout the world today, butterfly farming is becoming increasingly common as a source of revenue or commerce for communities in both developed and developing nations. It has been reported that this industry can generate six-digit annual revenues in even the most rural of communities in developing nations (Gordon & Ayiemba 2003). While specializing in butterfly rearing and breeding, many of such farms also provide educational opportunities for the surrounding communities, which serve to educate the public on farming practices as well as butterfly and habitat conservation. By providing economic incentives to the local community and actively engaging in nature education and conservation, butterfly farming is rapidly becoming a part of ecotourism and conservation efforts across the globe.

With the emergence of butterfly farming operations, so too emerges a need to better understand factors affecting butterfly natural history around the farm. It was the objective of this study to better understand the impacts of farms on butterfly abundances, using oviposition as an indicator, butterfly larvae predation rates, and butterfly egg parasitism, all with regard to proximity to butterfly farms.

Butterfly Abundance and Oviposition at North Central Florida Farms

Monarch butterfly oviposition was, on average, in greater numbers at transect stations 1 through 4 when compared to transect stations 5 through 11. Although there is *not* a smooth downward sloping trend in egg number as one goes from transect stations 1 to 11, we can say that Monarch butterflies are much more likely to be found ovipositing on plants within 40 meters of the butterfly farm epicenter. When examining transect stations 1 through 4, specifically, we observed a completely negative correlation between egg number and transect station number as one goes from transect station 1 to transect station 4, which was hypothesized to occur

throughout the entire range of transect stations 1 through 11. These data ultimately suggest that butterflies are more abundant in the areas directly adjacent to the butterfly farm epicenter, and are correspondingly less abundant farther from the farm epicenter. This idea will be discussed more later, but for now, it is necessary to stress the fact that there was a marked difference between transect stations 1 through 4, and transect stations 5 through 11.

The difference between 1 through 4 and 5 through 11 is likely due to the researcher's placement of the farm epicenter perimeter. This imaginary perimeter line was placed in order to designate the perimeter of the actual working part of the farm. It was designed to incorporate all the hypothesized attractive forces, such as host plants, nectar plants, rearing cages, butterfly gardens, and laboratories that could potentially influence oviposition preference by containing resources used by female butterflies. Because there were multiple areas with host and nectar plants throughout each of the farm epicenters, some of the less attractive and less resource-loaded components of the farm epicenters, such as closed laboratories and more shielded plant nurseries, may have acted as a buffer between the perimeter and the more attractive rearing cages and host and nectar plant nurseries. If wild populations of butterflies are being attracted principally by these interiorly located plants and/or the associated butterflies, they may fly directly to these areas and subsequently limit future flights to a set radius around these host and nectar plants, while searching for alternatives depending on their oviposition preference. Perhaps the 30 meter transect station (at station #4) approached the outer limit to their searching behavior, rendering transects 5 through 11 less obvious. According to Renwick & Chew (1994), learning plays a vital role in the recognition of suitable sites for oviposition. Thus, it could be possible for butterflies to have learned the location of the best host plants within the farm epicenter prior to the start of this experiment, having already assigned them oviposition priority.

Previous research suggests that when female butterflies are confronted with an array of potential hosts, they exhibit a hierarchy in preferences, laying most eggs on the preferred plant, and fewer on the next, and so on (Courtney *et al.* 1989, Thompson 1988).

A second scenario possibly explaining the difference between station groups 1-4 and 5-11 could be that egg number data (i.e., oviposition) may have come from butterflies from within the farm rather than wild butterflies. These “within farm” butterflies could be ones that have escaped from the enclosures, or possibly hatched from eggs that remained undetected by farm workers as the host plants are moved around the farm. Oviposition from escapee and other farm-raised butterflies may be more contained to the interior of the farm epicenter, depending on location of the particular rearing cage from which the butterfly escaped or the host plant from which the butterfly emerged. Previous studies have found that visual conspicuousness of host plants is related to increased oviposition (Courtney 1982). Therefore, plants located at closer transects may be more conspicuous to the escapee and farm-raised butterflies, and would likely exhibit higher levels of oviposition. Although this may apply to the steady decline in egg number from transect stations 1 through 7, it does little to explain the drastic drop from transect station 4 to transect station 5.

The conspecific attraction hypothesis states that high-density populations yield higher immigration rates to, and lower emigration rates away from, the habitat in question (Ray *et al.* 1991). Should these escapee butterflies be content with the available resources of the farm, many may reside inside the farm epicenter. The likelihood of wild butterflies being present in the outdoor nurseries and butterfly gardens, in addition to these escapee butterflies, could serve as motivation for butterflies to remain in the area, according to the conspecific attraction hypothesis.

In this set of experiments examining butterfly abundance and oviposition around north central Florida butterfly farms, there were three main effects: day, location, and transect. The following sections will discuss possible explanations for these results in detail.

Main Effect 1: Day

The effect that the number of days host plant exposure to gravid females in the vicinity had on egg number throughout the experiment was very significant (p-value < .0001). With days four, five, and six exhibiting the most oviposition, it is plain to see that butterfly abundances were highest during the middle of the experiment, forming a bell curve. The most probable explanation for this initial spike is that the first few days were required for the butterflies to locate, accept, and learn the location of the host plants, which resulted in high oviposition levels on the subsequent days. Then, once the host plants became “heavily” used and environmental conditions stressed the plants, they became less suitable to gravid females, resulting in less frequent oviposition. Butterflies rely on specific sensory cues in searching for appropriate host plants, with visual perception playing a major role (Prokopy & Owens 1983, Singer 1993). Using visual perception, butterflies overwhelmingly use the shape and color of the host plant as a search tool, with the color and shape of *leaves* particularly important (Stanton 1984). According to Courtney and Forsberg (1988), landing frequencies by gravid female butterflies have been seen to depend on the relative abundance of the host plant in the area. With a wealth of competing host plants in the general vicinity, all providing similar visual cues, it is possible that some gravid females did not detect the experimental host plants immediately. As these experimental host plants were detected by more and more gravid females, the oviposition rate increased until reaching a peak -- in the middle of the experiment.

Once the plants elicited the maximum oviposition rate during the temporal median of the experiment, oviposition began to decline sharply. It is possible that during the days that these

plants were in the field, they became degraded due to high levels of oviposition and stressful environmental conditions such as intense sunlight, high temperatures, hard rainfall or wind dehydration of leaves. A study by Ilse (1937) was the first to report that females in most families of Lepidoptera engage in a “drumming” action on the surface of the leaf on which they intend to oviposit. Later described as a “scrapping” action by Fox (1966), Fox suggested that the spines on the foretarsi abraded the leaf surface in order to release essential oils from the leaf, which were likely used in host plant selection and acceptance. However, this supposed degradation of the leaf surface is not limited to just *female* lepidopterans. Some male danaid butterflies have been reported to scratch withered leaves in order to attain pyrrolizidine alkaloids from the plant (Boppre 1983). Two studies, one by Schurr & Holdaway (1970) and the other by Renwick and Radke (1982), suggest that injured and stressed plants may actually stimulate the release of volatiles from host plants, which then act as oviposition deterrents to several species of Lepidoptera. Should butterflies be instigating their host plant to release an oviposition deterrent caused by plant damage and stress, we would expect high levels of oviposition to be followed by a sharp decline, as seen in this experiment.

Environmental conditions may have also taken their toll on the plants, possibly rendering them relatively unsuitable for oviposition. This experiment was conducted in August, which is typically a very hot month in Florida, and it is not uncommon to see temperatures above 100° Fahrenheit. Temperature readings during this experiment were consistent with historical records, and temperatures in the high 90’s were frequently observed. Summer storms are also common, bringing strong wind gusts as well as flooding rain. Although these plants were cared for daily, many were in direct sunlight for the majority of daylight hours and also exposed to the daily pounding rain. It was evident that as time went on, some plants were showing signs of

degradation. As stated previously, color of the host plant plays a major role in whether or not a female butterfly is successful in finding an acceptable host (Prokopy & Owens 1983). As stated by Myers (1985) and Wolfson (1980), color may depend on plant chemistry, which is directly related to the physiological condition of the plant. It is possible that as the experiment progressed, the physiological condition of the plants gradually rendered them less and less visible, attractive, and suitable to gravid female butterflies.

Conspecific egg loads have been found to be oviposition deterrents for many species of butterflies, including those in the genus *Danaus* (Rausher 1979b, Rothschild & Schoonhoven 1977). For this very reason, eggs were removed daily in an effort to minimize oviposition deterrence. However, some species of Lepidoptera have been known to chemically detect the presence of conspecifics (Renwick & Radke 1980). It is possible that residual chemicals from the egg remained on the leaf surface, which may have acted as an oviposition deterrent.

Despite these possible explanations for a bell-shaped curve in oviposition for day and the very significant p-value, we must not overlook the fact that this bell-shaped curve was observed solely at Greathouse. Because Greathouse yielded significantly more data than Shady Oaks, the analysis of the combined data overemphasized the results from Greathouse. However, there was clearly a pronounced day effect at Shady Oaks as well, with fluctuations up and down throughout the 10-day trial. Analysis of the day main effect suggests that more trials need to be conducted if we are to confidently say that a bell-shaped pattern is indeed occurring.

Main Effect 2: Geographic Location

The effect that geographic location had on egg number was also very significant (p-value < 0.0001). When the data are analyzed by location, we see that the Greathouse trial yielded approximately three times more eggs overall than the Shady Oaks trial. Similarly, almost three

times more eggs were found, on average, for each transect. For certain days, almost 10 times more eggs were found at Greathouse versus Shady Oaks.

One possible reason for this difference is the farm epicenter size, which was observed by the researcher to be directly proportional to the number of nurseries, butterfly rearing houses, and other parts of the farm involved in butterfly production. The farm epicenter for Shady Oaks was nearly 7,000 square meters, while that of Greathouse was just over 12,600 square meters. With twice the total area, occupied by nectar plants, host plants, and butterfly enclosures at Greathouse, we see that there may be a positive correlation between the size of the butterfly farm and rates of butterfly abundances around the farm. The greater size and number of butterfly enclosures could increase the potential for butterflies escaping on the principle that more enclosures create more risk of escape.

A second difference between the two farms was the landscape surrounding the farm epicenter, and there were two major differences. First is the amount of forested land surrounding the farm. The type of forested land was similar at both farms, consisting of mostly pine and oak trees, with some undergrowth, all growing in sandy, well-drained soil. Shady Oaks was bordered by far more forested areas with all but two rows traversing part of the surrounding forest. Although the majority of host plants were placed in the grassy areas that occupied most of the experimental plot, some plants were placed in the forested land, rendering them much less visible to gravid female butterflies (Figure 4-1). Conversely, Greathouse butterfly farm was situated in a way that only one row of hostplants traversed forested land. The vast majority of plants were situated in well-kept grass, allowing the visual cues to be better perceived by searching butterflies (Figure 4-2). The second landscape characteristic that may have led to greater oviposition rates at Greathouse was the presence of a residence within close proximity to the

butterfly farm epicenter (Figure 4-3). At this residence was a butterfly garden. Filled with abundant nectar sources and covering an area of approximately 200 square meters, this garden was a butterfly attractant, as observed by the researcher. Although only one row of host plants traversed this area, high rates of oviposition was observed for those transects proximal to the garden in this particular row. Although this residence and garden were very close to the farm epicenter, they could not be counted as part of the epicenter because it was not involved in the operations at the butterfly farm.

One more thing to note about the Greathouse trial was the presence of a large monarch butterfly enclosure, filled with hundreds of milkweed plants and adult and larval Monarch butterflies located at the very edge of the farm epicenter (Figure 4-4). It just so happened that one of the Monarch butterfly host plant rows used in this experiment was placed directly adjacent to this enclosure, as dictated by the randomization scheme designed prior to the start of the experiment. Not coincidentally, the first three host plants radiating out from the epicenter here also exhibited the highest number of Monarch eggs throughout the entire experiment, with some days seeing upwards of 20 eggs on each plant. High oviposition levels here are possibly the result of high plant visibility, and may have been attracting gravid females. When the females arrive to oviposit on what they feel will be suitable habitat, they are excluded from the enclosure and choose to oviposit elsewhere. With several of the experimental host plants located in close range, they become a probable choice for the gravid females. Although these plants did have relatively high levels of oviposition, these transect stations were not outliers in terms of *overly* high levels of oviposition when compared to the other data from this study. Oviposition rates were affected by the number of days the plants at these transects were exposed to gravid

females in the vicinity just as other plants were that were not located near this Monarch butterfly enclosure.

Main Effect 3: Transect Station

The effect of transect station on oviposition was not as statistically significant as either day or location, but still received a relatively low p-value (p-value = 0.2049) and interesting summary statistics.

From the data, we found that there is a positive correlation between high oviposition levels and proximity to butterfly farm epicenters. With oviposition also serving as an indicator for butterfly abundance, the question must be posed as to why butterflies would tend to be more abundant within 40, perhaps 30, meters from the perimeter of the farm epicenter?

As stated by Rausher (1979a), females that oviposit on plants yielding low larval survivorship or poor larval growth will likely leave fewer descendents than females that oviposit on the more appropriate host plants. For this reason, the choice that a female butterfly makes on where to oviposit is critical. Although exceptions have been found, it is widely accepted that oviposition search behavior has evolved to ensure that females lay eggs in the habitats that are most suitable for the growth and development of the juvenile stages (Gilbert & Singer 1975, Wiklund 1977). Therefore, gravid females may prefer the plants closer to the farm epicenter because these plants are perceived by the female to be more suitable for development of their offspring. Evidently, as the butterflies evaluated the balance between positive and negative signals from the plant and external stimuli, such as presence of eggs and other insects, the plants closer to the farm epicenter are more suitable. Although unproven here, it is possible that the farm may be acting as a magnet, both attracting butterflies from wild populations, as well as retaining any farm-raised or escapee butterflies from within the farm itself.

Each of the farms tested in this experiment report that they have thousands of adult butterflies present at any one time, as well as thousands of host and nectar plants within the borders of the farm epicenter. With high levels of butterflies present for breeding at the farm, as well as the probability of host and nectar plants serving to attract even more butterflies, we can say that there are high conspecific densities among butterflies being reared at the farm. In turn, these high conspecific densities could themselves serve as attractants, according to the conspecific attraction hypothesis, further increasing levels of conspecific densities (Ray *et al.*, 1991). According to Odendaal *et al.* (1989), butterflies are commonly attracted to areas of high conspecific densities due to the increased probability of finding a suitable mate. Additionally, Gilbert and Singer (1973) suggested that a high density of butterflies in an area, especially of the same species, could be used by a butterfly as an indicator for suitable habitat. The conditions at the farm in which high densities of butterflies are continually present is a possible force of attraction that may cause high levels of oviposition closer to the farm epicenter. With the epicenter providing host plants, nectar plants, and possibly mating opportunities for butterflies, it is not surprising that there was less oviposition on those plants further away from the farm. This study, however, did not examine butterfly abundance *within* the farm epicenter. There is a need for further research to be done regarding the populations of butterflies actually inside the butterfly farm epicenter. Although the results from this study indicate that the farm *may* serve as an attractive force, the data can only suggest that butterflies are likely to be more abundant closer to the farm epicenter. Further studies are needed to ascertain whether or not higher butterfly abundance is caused by attraction, and if so, to what degree.

Larval Predation in Florida and Costa Rica

A very unexpected result of this study was that the presence of the butterfly farm, in all geographic locations tested, provided for higher levels of larval survivorship. This is true when

comparing the experimental plot with the control plot, as well as when comparing the near variable with the far variable at the experimental plot.

Higher levels of predation on larvae that are situated further from the butterfly farm could be the result of either a higher presence of predators or higher rates of larval detection by predators. It could also be possible that there are multiple populations of predators surrounding the farm, with separate populations preying upon the larvae at different distances from the farm epicenter. Perhaps the predator populations situated closer to the farm predate mainly on larvae in and around the farm's gardens, nurseries, etc., and were satiated without needing to predate upon the larvae presented to them in this experiment. In addition, it is possible the predator population away from the farm, not typically having easy prey such as the larvae that were placed in this experiment, took advantage of the opportunity to predate upon these easy prey items. It could have also been possible that different species of predators were at the different distances from the farm. Perhaps high predation rates at the control plot were due to one species, while predation at the farm plot was from another species. The control plot in Florida, although similar habitat to the farm plot, was unmowed, brushy and in very sandy soil. *Solenopsis* ants were observed here and could have been the main predator involved. Similarly, predation at the farm could have differed depending on the species of potential predator as well. Although *Solenopsis* ants were also found here, they could have been more contained due to the mowing regime (approximately once a week), rendering them a subordinate predator.

Lastly, relatively high levels of human activity could have affected predation levels. It is possible that the near constant presence of ecotourists and farm workers around the farm grounds was enough to impose a "threat" to avian and lizard predators (Figure 4-5). By trying to avoid humans, potential predators would be indirectly deterred away from the farm. Should this be

true, it is a unique example of the benefits of integrating ecotourism (especially the high energy and noisy student groups) into a butterfly farming operation.

Although statistical T-tests indicate that Costa Rica did not follow the same pattern of significant differences for north central Florida, the raw data clearly follow the same pattern as north central Florida. For this reason, the researcher does not feel that there is any notable difference between the Costa Rica and north central Florida trials. In order to test for significant differences between the localities, additional studies are needed.

There are numerous possibilities for why an overall decline in predation was observed as one gets closer to the farm epicenter, but we cannot ignore the plain fact that there were statistically significant differences allowing us to make this conclusion in the first place. There is a great need for further research to be done on predation rates in regards to proximity to butterfly farms. In particular, future studies should concentrate on causes for these unexpected results.

Egg Parasitism in Florida and Costa Rica

Egg parasitism was found to be non-existent among monarch butterfly eggs during the 10-day experiment at all three geographic locations in Florida and Costa Rica. Although this bodes well for the prognosis of overall parasitism rates around these particular butterfly farms, we cannot make any general conclusions from this experiment. These results certainly do not mean that parasites and parasitoids are absent at butterfly farms, and they likely do not mean that they are necessarily absent from the farms used in this experiment. What *is* likely is that the experiment was not designed in a way to “attract” parasites and parasitoids to the butterfly eggs.

The first problem is that the butterfly eggs may not have been available to the parasitoids for long enough. In a study by Fatouros *et al.* (2005), wasps were found to be more attracted to leaves with eggs deposited for 48 hr, rather than eggs for 24 hr. Fatouros *et al.* (2005) also found

that eggs deposited for 72 hr elicited and even more significantly stronger response and suggested that substances associated with egg deposition could have diffused into the leaf tissue or the leaf's wax layer, triggering a chemical response by the leaf. This chemical, in turn, would at very least arrest, and possibly attract parasitoids. In my experiment, eggs were left on the plant for no more than 24 hr, which may not have been enough time for synergistic effects between the plant and the egg to attract parasitoids. The reason that eggs were removed daily was to prevent oviposition competition in the oviposition study. Research has suggested that gravid females may pass up suitable host plants due to the presence of conspecific eggs (Rausher 1979b, Rothschild 1977).

The second problem is that the plants were spaced, from a parasitoid's perspective, relatively far apart from each other. This prevented parasitoids from dispersing by means of walking and jumping, which is a vital means of locomotion for butterfly parasitoids (Pak *et al.* 1985). While nurseries inside the farm epicenter provided for high connectivity between host plants, the 10-meter spacing used in the experiment provided relatively no connectivity. Gingras and Boivin (2002) stated that connectivity between host plants and host plant parts best explains variability in parasitism rates.

Due to these reasons, it is likely that parasitoid presence is concentrated to areas within the farm epicenter. Areas in which eggs are left on host plants for greater than 48 hrs, as well as high levels of plant connectivity and homogeneity, are likely to present ideal habitats for populations of egg parasitoids, thus, there was little need for them to disperse to the outer areas of the farm (Lukianchus & Smith 1997). It would be beneficial to conduct further studies from within the farm epicenter and select specific habitats and specific groups of plants rather than the entire farm, as was done in this experiment.

Butterfly Abundance and Oviposition at the Northern Costa Rica Farm

The results from the butterfly abundance study in Costa Rica were unexpected and very surprising. With the number of eggs deposited in the north central Florida trials ranging from several hundred to almost one thousand during the 10-day experiment, finding absolutely zero eggs during the Costa Rica trial is perhaps as interesting as if there were hundreds of eggs found. There are several factors that may contribute to this overall lack of oviposition.

One explanation has to do with the extreme competition among host plants in the tropical environment. As stated before, butterflies make tremendous use of visual stimuli to locate host plants, particularly the color and shape of the host plants' leaves (Stanton 1984). Furthermore, relative abundance of host plants in an area has been shown to affect landing frequencies by gravid female butterflies (Courtney & Forsberg 1988). Consequently, the tremendous diversity of plants in the rainforest, some as host plants for these species, others with similarly shaped and colored leaves, could have acted as competition towards the potted plants placed out in this experiment. With the experiment lasting 10 days, perhaps this was too short of a duration for butterflies to recognize and learn the location of such host plants in a sea of both nearly identical and intricately complex stimuli.

Seasonality may have played a part in the visual homogeneity of the landscape as well. This experiment was conducted during the rainy season in Costa Rica and, thus, there was little to no flowering exhibited by the plants. According to Janzen (1967), tropical plants in Guanacaste, Costa Rica, flower and fruit in the dry season due to the fact that investing into non-vegetative activity is least detrimental to their vegetative competitive ability when rains are less frequent. The presence of flowers may have increased oviposition by providing another visual cue for the butterflies, which may have yielded oviposition on the host plant.

The host plants used in this experiment were the same species used by the farm for breeding their own butterfly stock. However, these host plants could have been the wrong choice, nonetheless. Landing frequencies by gravid female butterflies, and ultimately oviposition rates, have been seen to fluctuate with the seasonal changes in host plant abundances (Rausher 1979a). It is possible that while farm-raised butterflies will oviposit on these host plants year-round, wild butterflies may be seasonally adapted to searching for different species during the time this experiment was conducted. This could also apply to the north central Florida experiment, providing explanation for why there was little to no oviposition by both Eastern Black Swallowtails and Common Buckeyes.

The layout of the Costa Rica farm posed a particular problem for measuring oviposition of *Caligo memnon*. Near the farm epicenter, but outside of the operational part of the farm, there was a huge stand of *Heliconia* plants. These were the same species used at the farm to rear their own *Caligos* and consequently, the same species used in this experiment (Figure 4-6). With hundreds of *Heliconia* plants very near two of the experimental *Heliconia* rows, the hostplant competition here for visiting female *Caligo* was especially high.

Throughout the beginning phases of the experiment, there was a very high abundance of *Anartia fatima* butterflies around the farm. As the experiment progressed, and day after day no eggs were found at any of the host plant transects stations, several host plants for *Anartia fatima* were placed by the researcher around certain nectar sources and other areas that they frequented. Thinking that perhaps the wrong indicator species was chosen to test for abundance (although *Morpho peleides* sightings were common), the researcher began to monitor these *Anartia* host plants in addition to the other experimental plants. Despite being within an arm's reach of

literally hundreds of *Anartia* over the course of five days, not a single egg was found on the host plants.

After the experiment was complete, the researcher began to compare the north central Florida test versus the Costa Rica test in order to reason why there was such a drastic difference in oviposition levels. There was indeed one glaring difference between the trials. In both north central Florida and Costa Rica, orange marking flags were used to delineate transects and mark the experimental host plants. It could have been possible that Monarch females saw the orange marking flag and were attracted to it as being a possible mate, subsequently ovipositing on the plant, or returning at a later time to oviposit. At this point, shiny blue plastic material was used to mimic butterfly presence in the same way the orange flags were presumed to do. After the blue plastic was tied to the host plants, the 10-day experiment was repeated (Figure 4-7). Again, there was no oviposition. Although the orange marking flags *could* have served as an attractant in the north central Florida trials, there is no proof.

This brings us to the question of why Monarch butterflies were the only species that oviposited during the north central Florida trials? Although Swallowtails and Buckeyes were seen regularly in and around the farms, there was virtually no oviposition (there were two eggs seen of the Swallowtail during the entire experiment). There are two main factors believed to be the cause for this lack of oviposition. The first is a relative lack of visual conspicuousness. Courtney (1982) suggested that the level of visual conspicuousness (i.e., how obvious a plant is compared to its surroundings) directly affects oviposition in butterflies. Specifically, as visual conspicuousness increases, so does oviposition. Based on the size, color, and leaf shape of the two host plants used for the Swallowtail and Buckeye (parsley and plantain, respectively), they likely exhibited a relatively low level of visual conspicuousness in relation to their surroundings.

These plants were low to the ground, had leaves only 10 cm above the grass line, grew almost prostrate in the field, and did not have any flowers or other color patterns to distinguish them from the surrounding grasses. These qualities are nearly the exact opposite of the milkweed used for the Monarch butterflies. The milkweeds grew vertically, exhibited a height of about ½ meter, commonly had flowering parts, and the leaves were well above the grass line.

A second possible reason for the lack of oviposition is an improper choice of host plants, despite the fact that these were the host plants actively used for rearing of the respective butterfly species at both farms. Rausher (1979a) suggests that landing frequencies among butterflies may fluctuate and change seasonally, depending on host abundance and quality. It is possible that there was a temporary or seasonal host plant shift prior to the start of the experiment. In addition, Courtney and Forsberg (1988) suggest that landing frequencies may depend on the relative abundance of a preferred host plant. Should the numbers or quality of these host plants in the wild have declined recently, or perhaps quantities of another host plant surged prior to the experiment, landing frequencies, and consequently oviposition, could have been negatively affected for these butterflies.



Figure 4-1. Although most host plants were situated in fields like this at Shady Oaks Butterfly Farm, some plants were in more forested areas as seen in the background



Figure 4-2. More open layout of the area surrounding Greathouse farm. In the distance, there is a residence with a butterfly garden – a probable attractive factor



Figure 4-3. A residence in the area surrounding Greathouse farm, complete with butterfly garden



Figure 4-4. Large screen enclosure housing hundreds of milkweed plants



Figure 4-5. Student groups participating in ecotourism may be perceived as threatening by larger butterfly predators, such as birds and lizards



Figure 4-6. *Heliconia* plants around the exterior of the farm epicenter could have acted as competition for oviposition in the area



Figure 4-7. Blue plastic material used in hopes of stimulating oviposition by *Morpho peleides*

CHAPTER 6 CONCLUSIONS

The results from this experiment show that there is indeed an effect that the typical butterfly farm has on wild butterfly oviposition, and in turn, wild butterfly abundances. The tendency for butterflies to be in greater abundances closer to the farm could be the result of a combination of limited dispersal among butterflies from within the farm, whether escaped or hatched in the nurseries, as well as wild butterflies being attracted to the farm epicenter and the butterfly resources it contains. It is possible that the farm is emitting specific cues indicating habitat quality as well as the presence of viable mates, although untested in this experiment. This experiment cannot prove or disprove that these cues are real, nor if there is indeed a force attracting wild butterflies. However, we can say that there are greater butterfly abundances closer to the farm, whether from wild populations or butterflies from within the farm. It would be a very interesting and helpful follow-up study to examine these cues and the attractive stimuli these farms may elicit with regards to wild butterflies.

By testing for larval predation around the farm, we see that the farm poses no clear threat of abnormally high levels of predation closer to the farm. In fact, the opposite is the case. There appears to be abnormally low levels of predation as one gets closer to the farm. This could be due to discrepancies in predator populations and searching behavior. It could also be the result of heightened levels of human activity, in the form of farm workers and ecotourists, which may be perceived as a threat by potential predators.

By testing for egg parasitism, the data suggest that proximity to the farm does not elicit higher levels of parasitism when compared to more distal areas. However, zero parasitism compared with zero parasitism does not provide convincing enough statistics to authoritatively claim no difference.

An initial hypothesis of the researcher was that butterfly farms may be acting as ecological traps, presenting adult butterflies with seemingly suitable resources and habitat, only to have their eggs parasitized and larvae predated upon at higher than normal rates. Although it was evident that butterflies were in higher numbers surrounding the farm, larval predation was less of a threat at the farm and especially low quite near the farm epicenter. Therefore, this study suggests that butterfly farms may, in fact, not serve as ecological traps and in a way could have the opposite effect by acting as a source for metapopulations in fragmented landscapes. Although this is a relatively unsubstantiated assumption, it could provide the framework for an intriguing follow-up study.

Being a preliminary study of butterfly abundance, larval predation, and egg parasitism at butterfly farms and ecotourism areas, this set of experiments yielded interesting and helpful results. Many possible explanations for trends in these data involve butterfly activity from within the epicenter as well as possible attraction *to* the epicenter. Further studies should concentrate on the specific areas in question, such as nurseries, outdoor gardens, and breeding and rearing centers in order to conclusively evaluate specific attractive qualities they may possess.

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BIOGRAPHICAL SKETCH

Court Whelan was born in July of 1983, in Clearwater, Florida. His love for nature and a passion for living things began almost immediately and progressed through his participation in nature programs, from summer camps to school field trips. His formal training in biology and entomology began during the second semester of his undergraduate career at the University of Florida (Spring 2002), upon taking a basic entomology course taught by Dr. Don Hall of the department of Entomology and Nematology. After meeting with Dr. Hall to discuss his affinity for entomology and the programs and advanced classes that were offered at UF, Court declared his major as entomology immediately and has never looked back since. During his junior year of undergraduate work (2004), he was offered a student internship with an ecotourism tour operator to co-lead a group of 18 high school students on a 2-week field trip to the rainforests and coral reefs of Belize. With the responsibility to instruct the students on tropical entomology and ecology as well as co-lead the group throughout the trip, a strong passion for ecotourism emerged simultaneously with a passion for tropical entomology and ecology. Following the trip, his passion for ecotourism and entomology only grew stronger, as he immediately began working with Dr. Thomas Emmel, Dr. Jaret Daniels and Dr. John Capinera on developing a new graduate program entitled “ecotourism entomology.” While continuing his undergraduate career, he began working with a local tour operator, which specialized in tropical ecotourism, in arranging and leading ecotourism trips. After receiving his Bachelor of Science degree in the Spring of 2005, Court received an Alumni Fellowship from the University of Florida and was admitted to graduate school in the Department of Entomology and Nematology. He received his M.S. degree in 2008 and continues to arrange and lead ecotourism trips that are focused on tropical biology, ecology and entomology while pursuing his Ph.D. in the newly formed discipline of ecotourism entomology.