Project report submitted to the Organic Farming Research Foundation:

Project Title:

Investigations into the Ecology of Microtheca ochroloma ***and control methods for organic farmers***

FINAL PROJECT REPORT

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Project Summary

The purpose of this project was to describe and explain aspects of dormancy and host finding behavior of the yellowmargined leaf beetle (YMLB), *Microtheca ochroloma*. We were interested in three main YMLB behaviors. Firstly, we were interested in describing their dormancy during the summer. Although aestival behavior has been reported (Oliver and Chapin 1983), is not clear whether their dormancy is quiescence or true aestivation (see Danks 1987). Once beetles became active again, we wanted to know how beetles found and colonized crop plants in an agricultural field post dormancy. In an already infested field, we wanted to test whether intercropping could protect host plants from damage by *M. ochroloma*. Anecdotal evidence from organic farmers suggested that host plants might be more susceptible to YMLB attack once they had been initially damaged, e.g. from the farmer harvesting part of the host plant, so that was an additional area of interest. Finally, our intention in answering these questions was to provide an ecological basis for control methods of *M. ochroloma* that were suitable for organic farmers.

In this case study, we found that beetles appear to be quiescent rather than aestivating. They recolonized this farm from within the field, rather than returning from an alternative dormancy habitat. Intercropping host plants did not provide additional protection from YMLB attack. *M. ochroloma* does not respond to mechanically damaged host plants in lab experiments.

Introduction

Beginning around 2000, organic farmers in Florida began reporting severe problems with beetle outbreaks in high value *Brassica rapa* crops, including mizuna, mibuna, napa cabbage as well as in *Brassica juncea* (mustards) and *Nasturtium officinale* (watercress). Very little has been published on *M. ochroloma* and no field studies had been done. As a result of YMLB damage to brassica crops, many farmers reduced their acreage of these valuable crops or stopped growing them altogether.

Objectives

The original objectives of the project were as follows:

1. To determine if beetles remain active into summer if crucifers are present under field conditions.
2. To determine if initial infestation of *M. ochroloma* arise from within the field from the soil subsurface or whether beetle enter the field from field edges after oversummering.
3. To determine whether intercropping crucifers protects them from YMLB by hiding them among non-host plants.
4. To determine if cutting crucifers makes them easier for the beetles to find, therefore increasing infestation.

After further reviewing the literature and consulting with the participating farmer, my thesis committee and other entomologists, I determined that I would have to revise the initial experiments proposed for objectives one and four. I continued to sporadically sample what was left of the host vegetation in the field over the summer, instead of planting more host plants. I found adults in the field and by the time the host plants were ready to be transplanted for the objective two experiment, I found eggs and first instars on the transplants that had been in the greenhouse, indicating that there was an active population during the supposed aestival period. Also, the beetles that I collected over the summer and placed in environmental chambers resumed feeding and oviposition immediately. This response to favorable environmental and resource conditions indicates that they were in quiescence rather than undergoing a true diapause. In March 2005, I began field cage trials to test this working hypothesis.

Olfactometer bioassays are the standard methodology for testing insect responses to host plant volatiles, so the experiments of objective four were carried out (and continue to be carried out) using those protocols. Additionally, we felt that the low number of beetles and high numbers of other crucifer-feeding insects that were attracted overall in the spring 2003 would prevent this experiment from providing useful and meaningful results.

The materials and methods, results and discussion for the rest of this project report focus on the experiments for objectives two and three, which were carried out on the organic farm as described in the original project proposal.

Materials and Methods

Edge Versus Interior Experiment

I carried out both experiments on Rosie's Organic Farm, a certified organic farm located in southwest Gainesville, Florida. I chose this farm because it is a well-established certified organic farm that had experienced outbreaks of *M. ochroloma* and the farmer was willing to participate in the research. The farm consists of two non-contiguous fields, one of eight acres (3.2 ha) and the other of seven acres (2.8 ha). This experiment involved only the eight-acre (3.2-ha) field. The soils are Entisols, mineral, sandy soils with no obvious soil profile.

The field has been certified organic and managed organically since 1993. A typical season begins at the end of the summer when the cover crop (a combination of cowpea and millet) is mowed and disked. Well-rotted chicken manure, the only fertility amendment, is applied to the fields at the rate of 2 tons/acre (1814 kg/ha). Vegetables and flowers are then seeded directly or transplanted from one of two greenhouses on the farm.

I seeded mizuna, *Brassica rapa*, var. Kyona (untreated seed, from Johnny's Seeds, Albion, ME) into four 72-cell transplant trays on 3 August 2001 and maintained the plants in a greenhouse until transplant. I used Scotts Metro-Mix, specially blended without starter fertilizer or wetting agents as the transplant media. No fertilizer was added to the transplants initially, which halted the seedlings' growth, and delayed transplanting by several weeks. I thinned the cells three weeks after planting, leaving only the largest transplant in each cell. A single application of composted chicken manure was added one month after seeding (during the first week of September).

I had initially planned to use the entire field for this experiment, and surveyed it accordingly the last week of September. Because of the long growing season and diversified nature of the farm, beds are prepared, planted, and harvested on an as-needed basis. During the growing season at the farm, a single bed may be rotated through three different crops. The result is that the eight-acre area is heterogeneous; some areas are still in their summer cover crop while others are mowed and disked and ready to be planted and some have already been planted. After considering these factors, I decided to adjust the experimental design. The heterogeneity of the research site led me to divide the field and choose a single homogeneous area for this experiment. The mizuna host plants were transplanted to into the experimental area on 2 October 2001.

I chose an experimental area in the northeast part of the field. The area consisted of 18 rows on 6 ft. (1.83 m.) centers, running north-south, approximately 250 ft. (76.2 m.) long (Figure 1). The rows had been planted in green beans and cucumbers during the first week of September 2001. The north border was the edge of the field, bordered by successional vegetation typical of this area of north Florida, including trees and shrubs and forbs. The south border was the irrigation lines and walkway dividing the north and south sections of the field. The east edge was disked and subsequently planted in mixed crucifers that were not sampled as part of the experiment (labeled in Figure 1 non-experimental host vegetation). The west edge was two rows of turnips that had been planted around the middle of July 2001. In August 2001, I surveyed the turnip rows and found several adult *M. ochroloma*, but no eggs or larvae and no evident plant damage.

Beginning with the second (easternmost) row of turnips, I measured out experimental plots of 3 ft. (0.914 m) long at either end (woods and interior) of every third row. In each experimental plot, I planted 12 host plants in a three by four block. I assigned interior plots the letters A through F and edge plots the letters G through L (Figure 1). I recorded the number of beetles (adults, larvae and eggs) on each host plant in each plot at least twice a week beginning on 9 October 2001 and ending on 31 October 2001. By the end of October, many of the host plants had been completely eaten by yellowmargined leaf beetles and I was no longer able to sample.

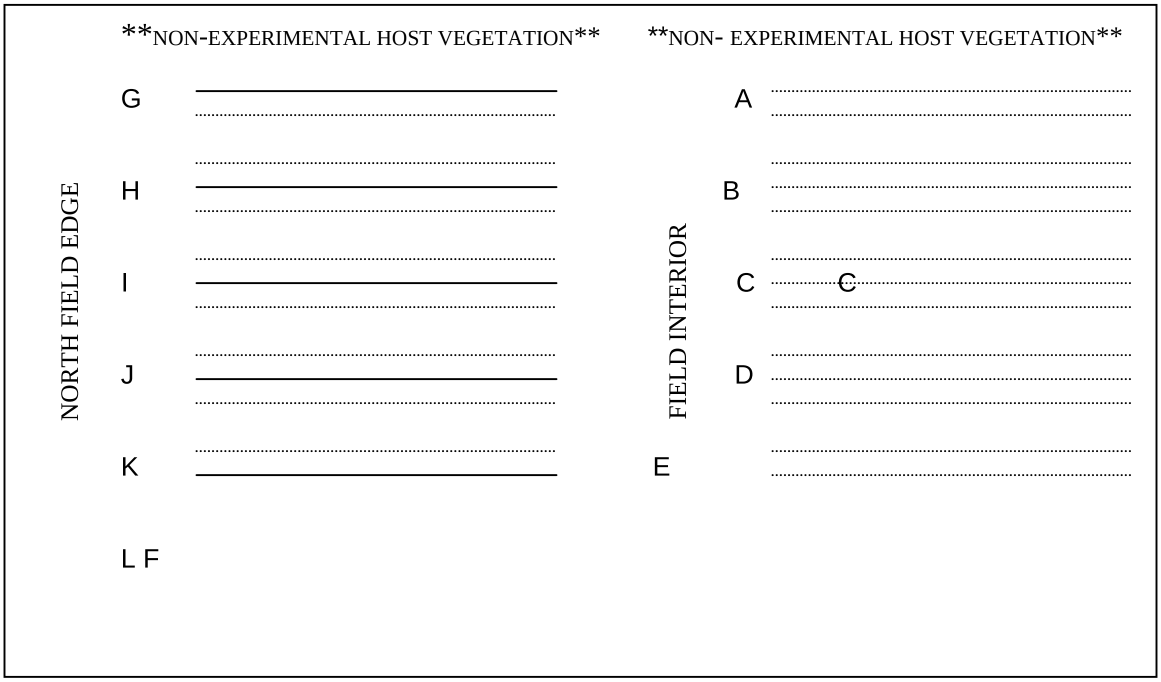

Figure 1. Field layout for edge versus interior experiment. The letters A-L represent experimental plots composed of 12 mizuna plants each. The solid lines are farm rows containing non-host vegetation (beans, cucumbers, etc.) that are bounded on north and south ends by the experimental plots. The dashed lines represent rows that were not bounded on either end with experimental plots.

On two occasions (25 October and 29 October), I marked adult beetles using a water-based paint and small paintbrush. I assigned each of the interior plots a color and marked all the adults accordingly. The first time, I marked the beetles as I counted them. The second time I collected the beetles in a container and then marked and counted them. I then held the marked beetles in the container for 10 minutes, by which time the paint appeared dry. I then released the beetles back into the middle of their respective plots. On the two subsequent sampling dates (27 October and 31 October), I recorded the number and mark of all the beetles found in the experimental plots.

I used non-parametric methods to analyze the data. Graphing the untransformed data, I found that the mean and variance were highly correlated. Neither square root nor log transformations succeeded in diminishing this relationship to the point where the parametric measures would have been appropriate. I used the Wilcoxon rank sum test (Hollander and Wolfe 1999) to determine if there were differences between the numbers of beetles on the field edges versus in the field interior. I used the Kruskal-Wallis multiple comparison test (Hollander and Wolfe 1999) to determine if there were differences among the plots which were nearby, far away or a medium distance from the non-experimental host plots. I used Wilcoxon comparisons *post-hoc* to test for differences between the three pairs of treatments (near-far, near-mid, and far-mid) (Hollander and Wolfe 1999).

I had three problems during this experiment. When I transplanted the host plants, I noticed that there was evidence of beetle activity in one of the transplant trays. There were several adults, a clutch of eggs and one newly hatched larva distributed among three of the plants in this tray. I removed and killed the beetles, since I did not want to introduce beetles to the field. Because the host plants were transplanted at a small size and the beetles are very evident, I don't believe there were any more beetles on previously transplanted trays and that this was an isolated incident.

A second problem was that each plot did not have a uniform host plant biomass, nor did I correct my calculations to take this into account. The differences were not systematic, i.e. all the plants on the interior were smaller than the ones on the edge, but nonetheless present.

Finally, my attempts to mark the yellowmargined leaf beetles failed. I did not recapture a single marked beetle in subsequent sampling. I believe that the paint either rubbed or washed off, or that I did not mark enough beetles relative to the total number of beetles in the field to recapture them.

Intercropping Experiment

This experiment took place in a different part of the same field at Rosie's Organic Farm. I seeded three 72-cell transplant trays of mizuna, *Brassica rapa*, var. Kyona (untreated seed, from Johnny's Seeds, Albion ME) and six trays of oak leaf lettuce, *Lactuca sativa* var. Berenice (untreated seed, Johnny's Seeds, Albion, ME) during the second week of January 2002. Plants were maintained as previously described. On 15 March, I transplanted the seedlings to the field. The experimental design was a complete randomized block, consisting of four treatments replicated four times. Each block consisted of 32 plants. The treatments were three densities of non-host plant (oak leaf lettuce) to host plants --15:1, 7:1, and 3:1-- plus a control plot that contained only mizuna. The resulting blocks had 30 non-host plants plus two host plants, 28 non-host plants plus four host plants, 24 non-host plants plus eight host plants and 32 host plants. The sixteen blocks were randomized between two adjacent rows. In each row, I left a border space of 6 ft. (1.82 m) between each treatment plot. The location of the host plants within the treatment block was selected using a random number table. There were no other host plants within 33 ft. (10 meters) of the experimental plots. Twice a week, from 16 March to 16 April, I recorded the number of beetles per host plant and the position of the host plant.

I encountered several problems during this experiment. No adult yellowmargined leaf beetles were present until the first week of April. I did not find any larvae or eggs during the entire course of the experiment. I stopped sampling after 16 April because the host plants were so badly damaged by other insects that I could not continue the experiment. The other factor was that the nutsedge and pigweed that had been controlled through hand weeding early in the season became completely out of control and took over several of the experimental plots.

Again I used non-parametric methods for data analysis because of the correlation between mean and variance. I used the Jockheere-Terpstra test for ordered alternatives (Hollander and Wolfe 1999) to determine if there were differences among the treatments. I used the multiple-comparison Hayter-Stone test (based on Wilcoxon ranks) *post hoc* to determine which plots were different.

Results

Edge Versus Interior Experiment

There were significantly more adult yellowmargined leaf beetles in the interior plots than in those plots bordering the field edges ($W = 52$, $P = 0.04$). The edge plots (G-L) had a mean ± SE of 8.17 ± 3.67 adult beetles while the interior plots (A-F) had 54.5 ± 20.42 beetles (Figure 2). There were no differences between edge versus interior location for either larvae or eggs ($P = 0.45$ and $P = 0.42$, respectively), although the plots where eggs were laid reflected a pattern similar to that of the adult population (Figure 3). The distribution of the yellowmargined leaf beetle larvae did not reflect the bimodal distribution of the adults and eggs.

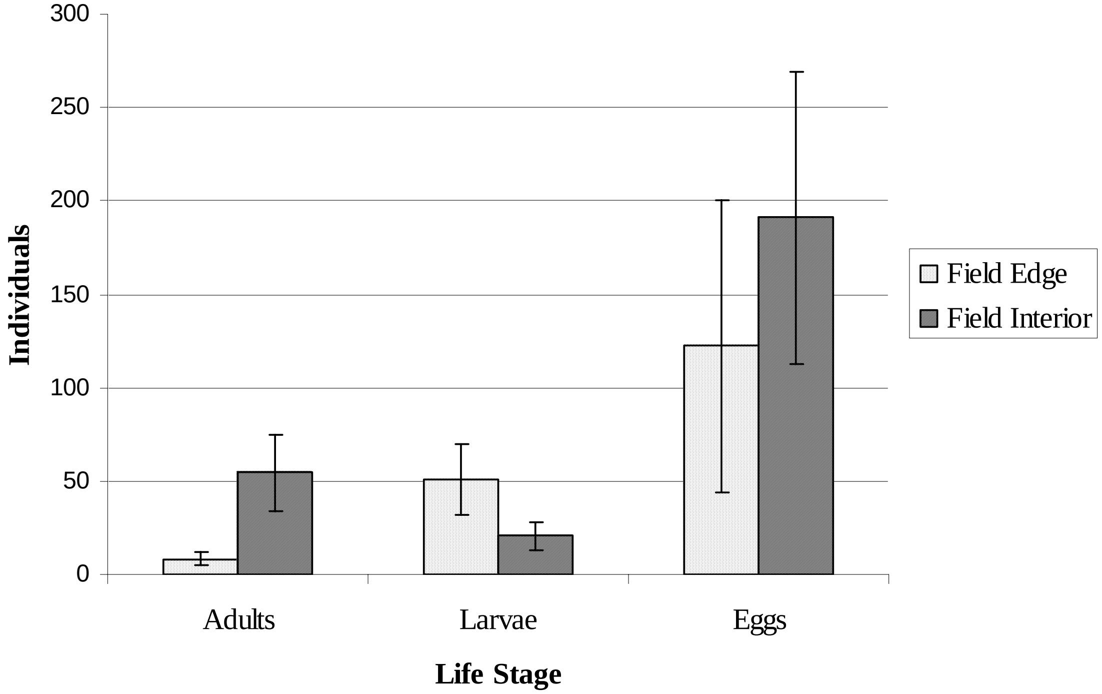

Figure 2. Mean number of yellowmargined leaf beetles in edge versus interior plots according to life stage, Gainesville, FL 2001.

Beetle populations varied widely between plots (Figure 3). Within the six experimental plots that comprised each of the variables "edge" and "interior", the adults, larvae and eggs were highly aggregated into one or two plots. For example, I counted a total of 142 adult beetles in interior plot A, but no beetles in plot B. Similarly, there were two eggs in interior plot B, but 467 in interior plot A. I found the most larvae in the edge plot 'I', which had 101 larvae, but there were no larvae in edge plot L.

The relationship between number of adults and number of offspring was inconsistent throughout the experimental plots. Plots with high larval populations and high egg counts did were not always the plots with high adult populations in previous weeks (Figure 4). Plot A had the most adult beetles, as well as the most beetle eggs but only the fourth highest larval count. For example, plots D, 'I', and J, had relatively few numbers of adult yellowmargined leaf beetles, but the second, third and fourth highest egg counts later that week. Plots C, D, and E all had moderate and comparable numbers of adults on 24 October, but subsequent location of eggs varied as did their larval populations (Figure 4).

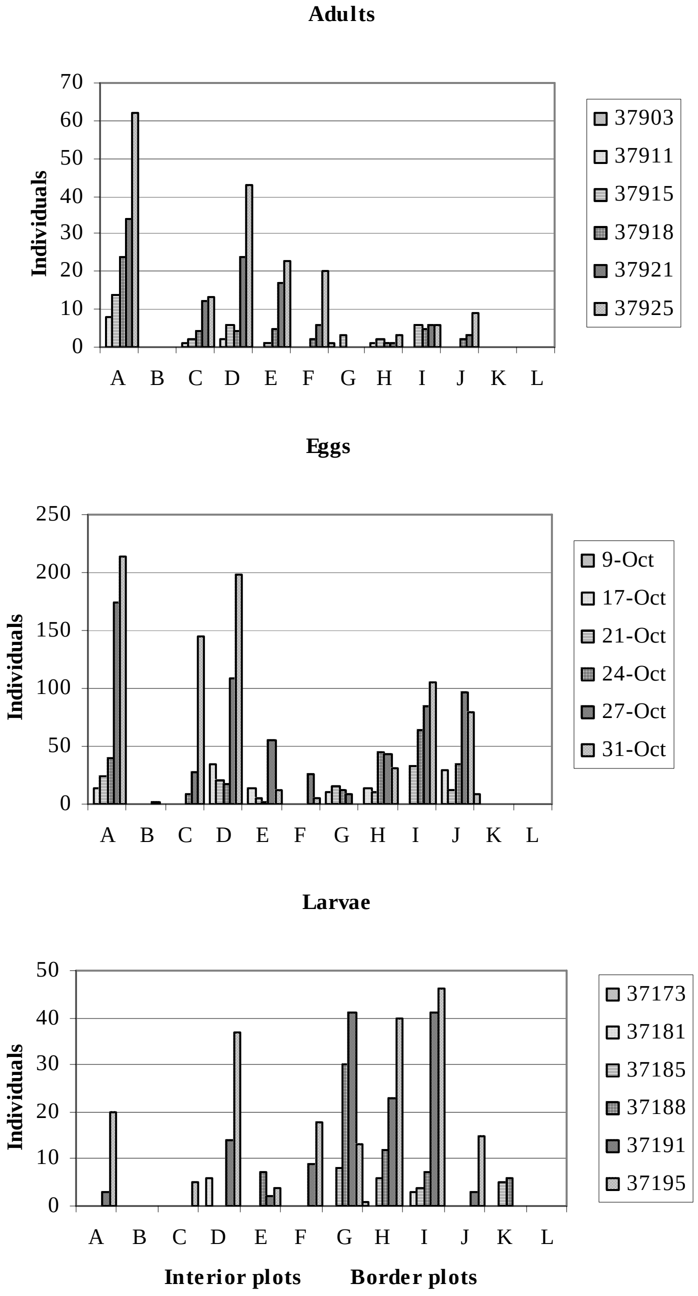

Figure 3. Total number of yellowmargined leaf beetles collected from host plants, Gainesville, FL 2001. Plots A-F are the interior plots; G-L are edge plots. Note the different y-axis scales.

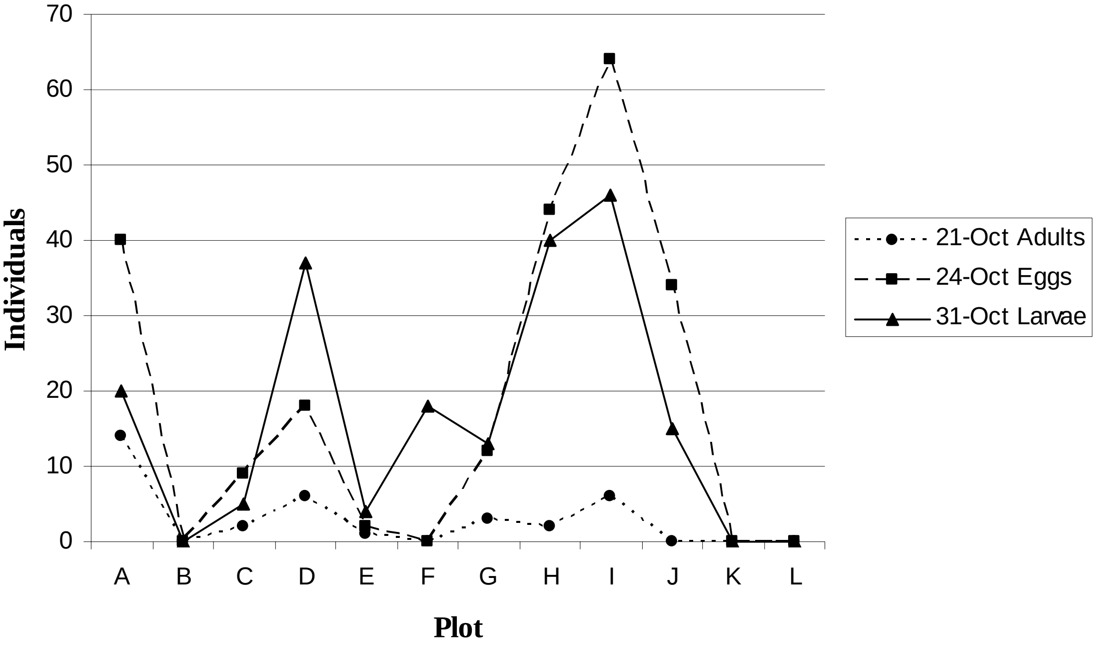

Figure 4. Yellowmargined leaf beetle adult (AD), egg (EG) and larval (LA) distributions during a one-week sampling period, Gainesville, FL 2001.

When I categorized the plots as nearby, medium distance or far away from other crucifer plantings, there was no difference in the number of adult ($X^2 = 1.91$, $P = 0.43$) or larval ($X^2 = 0.94$, $P = 0.66$) yellowmargined leaf beetles in the experimental plots. Three out of four of the plots categorized as near (plots F, G, and L) ranked towards the bottom of all the plots for number of adult beetles and eggs. Plot A had the highest number of adults overall, with 142. Plots A, F, and L ranked towards the bottom for number of larvae, but Plot G had the second highest number of larvae, with 82.

The quantity of eggs in a plot did correspond to its proximity to other crucifer plantings ($X^2 = 4.41$, $P = 0.11$). Multiple comparisons using Wilcoxon ranks revealed that the plots farthest away had more eggs than mid-distance plots ($W = 3.266$, $P = 0.0739$), but that there was no difference between the number of eggs in the nearest plot and the farthest, or between the nearest and the mid-distance plot. The plots farthest from other crucifer plantings had a mean ± SE of 274.75 ± 41.15 eggs, while the medium distance plots had 60.25 ± 33.51 eggs and the nearest plots had 135.75 ± 110.82 (Figure 5).

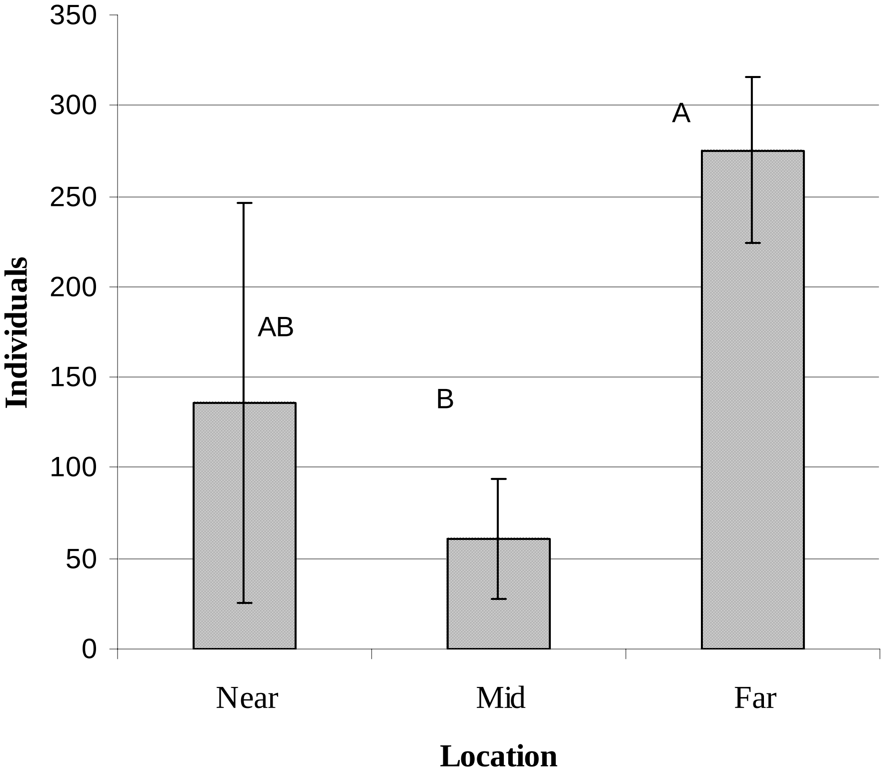

Figure 5. Mean number of yellowmargined leaf beetle eggs collected from experimental plots based on the proximity of the plots to non-experimental host plants, Gainesville, FL 2001.

Intercropping Experiment

I used the Jonckheere-Terpstra test for ordered alternatives to establish that there was an increasing treatment effect (*i.e.* plots with fewer host plants had fewer beetles) ($J = 79$, $P < 0.0056$) (Figure 6). Multiple comparisons based on Wilcoxon ranks did not establish which plots were different[1]. When plant density was taken into account, there were no treatment differences among the intercrop plots ($J = 48.5$, $P = 0.481$). There were no more adult yellowmargined leaf beetles per plant in the monocropped (control) plots than in any of the intercropped (treatment) plots, nor were there differences among yellowmargined leaf beetle populations per plant resulting from the various densities of intercropped plants in the treatment plots. I did not find any larvae or eggs in the control or treatment plots during the course of this experiment. However, there was a trend towards higher number of adult yellowmargined leaf beetles as the experiment progressed, indicating a seasonality effect (Figure 7).

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[1] The critical value for the Hayter-Stone test at $= 0.10$ is $W = 2.873$. The highest statistic for these comparisons was for the monocrop to 1:15 treatment, which was $W = 1.4$.

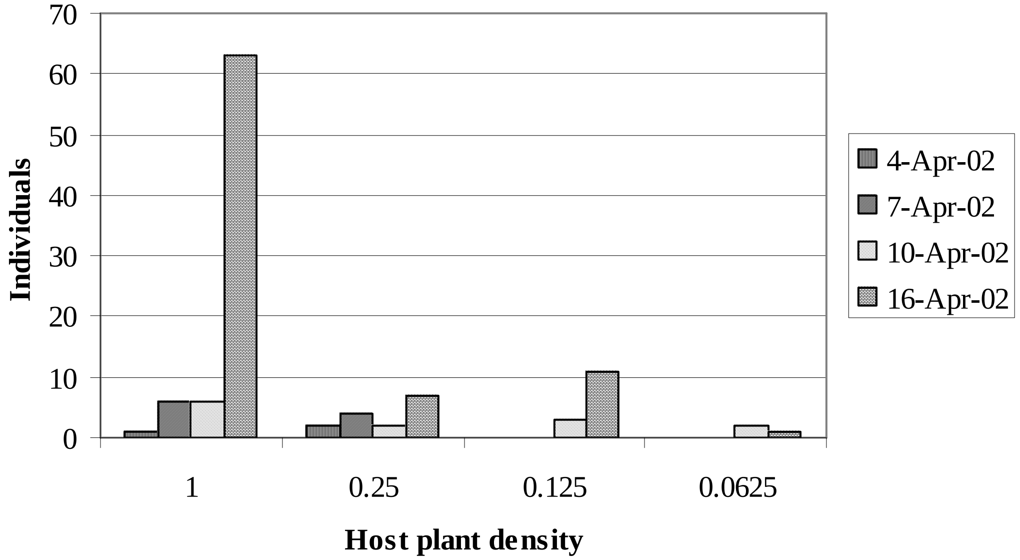
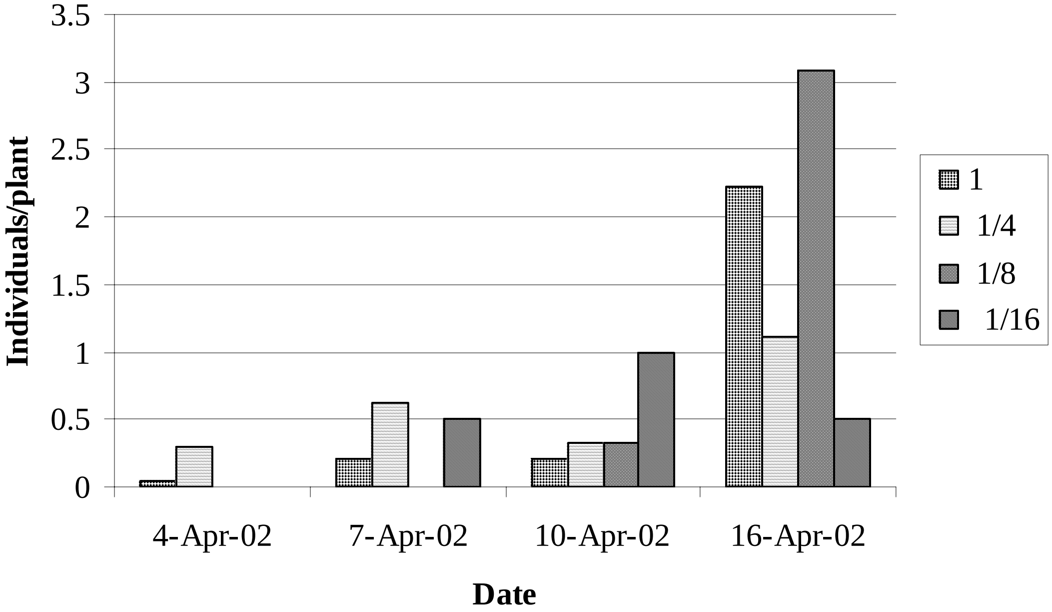


Figure 6. Mean number of yellowmargined leaf beetles collected on intercropped host plants by date and host plant density[2], Gainesville, FL 2002



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[2] Host plant density is the ratio of non-host plants to host plants. The least dense plots of host plants (0.0625) had two host plants and 30 non-host plants (a ratio of 1:15 non-host plants to host plants). The other treatment ratios are 1:7 (0.125), 1:3 (0.25) and a monocrop (control) plot containing all host plants, represented by 1.

Figure 7. Mean number of yellowmargined leaf beetles found on host plants, Gainesville, FL 2002. Legend refers to the fraction of plants in a block that are host plants.

Discussion

Adult *M. ochroloma* colonized the interior plots before the field edge plots and remained in those interior plots longer, resulting in more damage to host plants in the interior plot. This indicates that this field may have had a local, non-migratory population of YMLB that oversummered within the field and quickly recolonized host plants in the autumn. The pattern of ovipositioning in the field indicates that adult females did not remain strictly in these interior plots. Larvae were recorded in plots where few eggs had been found, indicating that they also moved between plots. Insect distribution within a field results from insects' movement towards host plants or mates, or to avoid competition, predation, parasitism or inhospitable microclimates. It is not yet clear how these factors influence abundance and distribution of *M. ochroloma*.

The proximity of non-experimental host vegetation to the experimental site may have influenced the resulting population dynamics of *M. ochroloma*. YMLB have expressed host plant preferences in lab studies (Ameen and Story 1997a), but none of the plants offered by Ameen and Story 1997 were included in the edge versus interior experiment. I did observe that once adult YMLB populations reached high levels in the experimental plots, the beetles would emigrate from this depleted food resource. In effect, they moved away from their own herbivory. This phenomenon has been observed in other leaf beetles (Chrysomelidae) as well (see Lewis 1994 and Morris et al. 1996).

While the intercropping experiment did not provide any protection from YMLB damage in this experiment, an alternative intercropping and/or trap cropping design might prove more effective. In this intercropping experiment, simply decreasing the density of suitable host plants in an area was not a viable control technique. Intercropping strategies that include repellant plants, plants with more varied plant architecture, or plants that are more attractive host plants to M. ochroloma should be tested.

While beetle populations varied in space and time, *Microtheca ochroloma* could be collected in this particular field year-round. It seems likely that volunteer plants (or those which are no longer being harvested but haven't been removed from the field) could be providing a suitable habitat for a small number of beetles within this agricultural field. Removing old host plants as soon as possible may delay the re-introduction of YMLB into new plantings.

I am continuing to examine the dormancy patterns of the yellowmargined leaf beetle as well as chemically mediated host finding mechanisms. I hope that these studies will yield more biological and ecological information that can further inform the design of intercropping techniques.

I presented the results of this research at the Florida Academy of Sciences meeting on March 18, 2005. Since the inception of this project, I have spoken with many farmers about their experiences with this insect and provided as much information as possible to them about the yellowmargined leaf beetle. I have recently been contacted by a community college student whose term project involves *M. ochroloma* and I hope to have a productive collaboration with him.

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Block of oak leaf lettuce intercropped with mizuna

Interior plot A, with severe damage from yellowmargined leaf beetle, *M. ochroloma*.

Adult yellowmargined leaf beetles colonizing a volunteer arugula plant.

Adult yellowmargined leaf beetle

Early instar yellowmargined leaf beetle

Cluster of yellowmargined leaf beetle eggs

Yellowmargined leaf beetle larvae feeding on mizuna