

**Analysis of *Macrocentrus ancyliivorus* as a Natural Enemy of the Sunflower Moth,
*Homoeosomae electellum***

David Briggs

Abstract The sunflower moth, *Homoeosomae electellum*, is a particularly problematic sunflower pest in the Western and South Western areas of the United States. Natural enemies of *H. electellum* have been partially excluded by the significant morphological differences between wild and agricultural sunflowers, leaving some specialist parasitoids, like *Dolichogenidea homoeosomae*, only effective at parasitizing sunflower moth in the wild setting. This project looks at the foraging behavior of a generalist parasitoid, *Macrocentrus ancyliivorus*, on sunflower moth-infested sunflowers, to see if other possibilities for the use of natural enemies for sunflower pest management exist. Experimental trials showed that *M. ancyliivorus* does not have a significant behavioral response to sunflower type (wild versus agricultural), or a significant behavioral response as a result of experience. These results were unexpected based on the findings of similar studies. *M. ancyliivorus* did, however, respond to sunflower damage as a result of herbivore activity. More studies concerned with the responses of generalist and specialist parasitoid to sunflowers might elucidate the causes of differences between the behaviors of different parasitoids in the same setting.

Introduction

The sunflower, *Helianthus annuus* L., is a widely cultivated crop that is receiving increased attention as a source of oils. In the majority of the United States, however, chemical pesticides must be used to protect sunflower crops against the sunflower moth, *Homoeosoma electellum* Hulst (Schneider 1997). Because the sunflower moth larva attacks pollen, anthers, and ovaries throughout its development and because the damage increases the plant's susceptibility to fungal disease, sunflower moths can directly impact the value of a harvest and may cause the failure of a growing season (Schneider 1997). In some locations, larval densities have been recorded at levels close to 200 times greater in agricultural settings than in near-by fields of native sunflowers (Chen & Welter, 2002).

The prevalent use of agrochemicals for pest control has concerned environmental and public health scientists for decades (Hond et al. 2003; Mackauer et al. 1990). Despite greater recognition of the environmental and public health externalities posed by agrochemicals, it is often deemed a necessary evil due to the difficulty of developing alternate pest management strategies (Hond et al. 2003; Mackauer et al. 1990).

In some cases, communities of parasitoids have been used as part of a pest management program that avoids using these dangerous chemicals (Hond et al. 2003; Mackauer et al. 1990). Because sunflowers are native to the United States, they have well-established parasitoid communities in wild ecosystems (Schneider 1997). This presents the possibility for effective natural pest control within agricultural settings. Unfortunately, the specialist parasitoid of the sunflower moth, *Dolichogenidea homoeosomae* Muesebeck, has been shown to have limited success on agricultural flowers (Chen & Welter 2003).

In laboratory-based parasitism experiments, Chen and Welter found that *D. homoeosomae* was unable to parasitize effectively on agricultural flowers due to architectural interference (2003). Agricultural sunflowers can have a surface area close to 100 times greater than wild sunflowers and the depth of their seed set is substantially greater.

Although Chen and Welter show that the agricultural sunflower is a more challenging setting for a specialist, Charlet (1999) suggests that generalist parasitoids may be more effective in challenging phenotypic settings. Charlet notes that generalists with longer ovipositors may have equal or higher parasitism levels on cultivated sunflowers versus wild sunflowers.

Macrocentrus ancyliivorus Rowher is a generalist parasitoid, native to North America, and a documented parasitoid of sunflower moth in both wild and agricultural sunflower systems (Ahlstrom 2005; Charlet 1999; Teetes and Randolph, 1969). This combination of attributes suggests that *M. ancyliivorus* could be the basis for a pest management strategy that reduces the need for pesticides and avoids the introduction of non-native species. Despite this set of encouraging credentials, there are no studies examining the foraging behaviors of *M. ancyliivorus* in sunflowers.

Jones (1996) examined another generalist species within the *Macrocentrus* genus and found encouraging behavioral responses. Females responded positively to plant damage caused by European Corn Borer larvae in a variety of host plant species. Additionally, the parasitoids demonstrated the ability to learn through acquiring a positive association with host plants after exposure to damaged specimens of the plant. These findings suggest that *M. ancyliivorus* may also respond positively to plant damage and demonstrate a learned response. This study examines the behavioral response of naïve and experienced parasitoids on damaged and undamaged, wild and agricultural sunflowers.

Methods

To determine whether *M. ancyliivorus* responds behaviorally to flower type, plant damage, or experience, this experiment followed procedures similar to those used by Chen and Welter (2003): laboratory-based observational experiments. Experimental replicates provided information about the conditions of the flowers and parasitoid as independent variables and, as the dependent variable, data about how long the parasitoid spent on the flowers. We hypothesized that the parasitoid would spend greater amounts of time on agricultural sunflowers versus wild flowers, sunflower that were damaged versus undamaged, and experienced parasitoid would spend more time than naïve parasitoids.

Specimens of *M. ancyliivorus* were delivered as pupae from a colony maintained at the Kearney Agricultural Center in Kearney, CA. The pupae were kept in a sleeve-cage and, as they emerge, adults were collected into plastic vials streaked with honey. The sleeve cages were kept in a temperature and humidity controlled room with a 16:8 day-night ratio. The date of emergence was recorded as well as whether or not there were male parasitoids present when a

female emerged. This data allowed us to look for possible effects resulting from parasitoid age and whether it mated.

Sunflower moth larvae came from a laboratory colony maintained in Berkeley, CA. The colony was reared on an agar-based diet-solution containing all necessary dietary needs (Wilson, 1990). The colony was kept in a temperature and humidity controlled room that had a 16:8 day-night ratio. To maintain genetic diversity, the colony received inputs of wild adult specimens collected in both wild and agricultural sites in the Central Valley of California. Other efforts, such as using large numbers of adult moths when setting up mating chambers, also helped maintain genetic diversity in the colony.

Agricultural flowers were grown in greenhouses from the seeds of California oilseed sunflower populations. Oilseed flowers were a more logical choice for trials because they are the primary varieties used in agriculture in the United States (Charlet 1999). Wild flowers were also grown in the greenhouse, from seeds of self-seeding sunflower populations in the Central Valley of California. All plants grew in potting soil. Only healthy, blooming flowers were used for trials. All agricultural flowers used in experiments had a diameter larger than one inch and all wild flowers less than one inch.

Comparison of wild and agricultural flowers All flowers were infested with sunflower moth larvae that had developed on artificial diet for a period of 2-3 days. The amount of time sunflower moths fed on the flower ranged between 2-8 days. The number of larvae placed on agricultural flowers ranged between 10 and 20, in proportion to the size of the flower. Wild flowers received 4-8 larvae, also in proportion to the size of the flower.

Experimental replicates were run in chambers constructed with Plexiglas walls on the front and back and cloth walls on the sides. The cages were approximately 60x60x30 cm, providing ample space for a large sunflower head and a portion of its stem. Once a sunflower was placed in the Plexiglas chamber, a parasitoid was introduced. Parasitoid introduction consisted of removing the cotton from the end of the plastic vial and resting the vial's opening directly on the flower. Once the parasitoid crawled onto the flower, a stopwatch was used to record the amount of time it spent on the flower. The stopwatch was paused if the parasitoid crawled off the flower's head. If the parasitoid returned to the flower head in a short period of time (less than two minutes) timing continued. If the parasitoid left the flower's head and did not return, timing

ended. This form of timing provided a proxy for the amount of time that a parasitoid could potentially be finding and parasitizing hosts.

In total, forty trials were conducted, eighteen on agricultural flowers and twenty-two on wild flowers. The time spent by parasitoids was recorded in seconds and was \log_{10} transformed to fit conditions of normality. Individual parasitoids performed multiple trials separated by at least one hour of rest in their honey-streaked vial, and each trial was considered independently. Data comparing flower type was analyzed using a Student's t-test for unequal sample sizes.

Comparison of naïve and experienced parasitoids As mentioned above, individual parasitoids performed multiple replicates. The number of trials performed by each parasitoid was recorded to gauge the effect of experience. The learning response, like the flower type response, was examined only on infested flowers. Flower type was not kept constant for individual parasitoids, thus most parasitoids were randomly exposed to both flower types throughout their lifespan. Because order of exposure was random, a comparison that pooled the data across flower type could be done if there was no statistically observable difference of behavior caused by flower type. Since we used the trials from the flower type experiments to look at the effects of experience, there were also forty trials, thirteen for naïve parasitoids and twenty-seven for experienced. The data was analyzed using a Student's t-test for unequal sample sizes.

Comparison of infested and clean flowers The parasitoid's response to plant damage was examined by comparing time spent on infested and clean (or uninfested) flowers. The experimental procedures were the same as above, except that the clean flowers did not have sunflower moth larvae placed on them. Twenty-two trials were run in total, eleven infested flowers and eleven clean. Eight flowers in each group (infested and clean) were agricultural type, and 3 flowers in each group were wild type. The emphasis on agricultural flower in this set of experiments provides greater information about the behavior of the parasitoid in simulated agricultural conditions. The distributions of the two groups were not similar in this experiment, so a Welch's t-test was used to analyze the data.

Comparison of parasitism rate After every trial, the flower was dissected for sunflower moth larvae. Each larva was placed in the diet described above and reared until either a moth or parasitoid emerged, or death occurred. This data was the basis for rate of parasitism. Rate of parasitism was calculated by the equation $P/(P+A)$, where P is the number of adult parasitoids and A is the number of adult moths.

Results

Comparison of wild and agricultural flowers The time spent by parasitoids did not differ between wild and agricultural flower type.

Comparison of naïve and experienced parasitoids The time spent by naïve parasitoids did not differ significantly from that of experienced parasitoids.

Comparison of infested and clean flowers The time spent by *M. ancylivorus* on infested sunflowers ($471 \pm 2s$) (mean \pm s.e.) was significantly higher (p-value = 0.05) than the time spent on clean flowers ($129 \pm 2s$). Within this set of experiments, there also appeared to be a response to flower type. The time spent on agricultural flowers ($400 \pm 1s$) was significantly higher (p-value = 0.02) than the time spent on wild flowers ($68 \pm 2s$). To separate these two significant findings, we used a univariate fit model. This procedure simultaneously regressed the dependent variable with respect to both independent variables, and it looked at covariance in the independent variables. The results of this model are shown in Table 1.

Table 1: Effect test results of univariate fit model of the effect of plant damage and flower type on time spent by parasitoid.

	Sum of Squares	F-Ratio	Prob > F
Plant damage	1.75	6.37	.02
Flower type	2.60	9.46	.01

Comparison of parasitism rate Larvae were found in their second, third, and fourth instars when extracted from the sunflower. After being reared to adulthood, there were no instances of adult parasitoids emerging from the sunflower moth larvae. Larvae either emerged as adult moths or perished before pupation.

Discussion

Generalist parasitoids are expected to efficiently cope with large differences in plant structure and host location, such as those presented by the difference between wild and agricultural sunflowers. In this way, a generalist parasitoid might be expected to be a better biological control agent in agricultural sunflower production than a sunflower specialist parasitoid (Charlet 1999). We found that *M. ancylivorus* does not respond to the differences in sunflower plant structure.

Although the comparison of infested and clean flowers generated a significant response to flower type, the small number of replicates across flower type makes it unreasonable to favor this finding over the highly replicated one.

In addition to not responding to variations in flower type, *M. ancyliivorus* females were continually observed probing areas of the flower, such as the petals, that did not have any hosts or host cues. These observations indicate that factors outside of sunflower type may better explain generalist parasitoid behavior. Because generalists hit a wide range of host and host plants, selection pressure would likely promote behaviors that work in many settings rather than specific behaviors for specific plant types. Specificity would require a coevolved relationship with a plant type and host species.

In other studies, *Macrocentrus* species demonstrated a learning response (Jones 1996). My results indicate that *M. ancyliivorus* does not change its behavior after an initial exposure to a damaged sunflower. This may be another demonstration of the lack of specificity in generalist parasitoid behaviors. Instead of learning the features of a specific plant type and adjusting its behaviors to fit, the parasitoid might already have a full complement of adult behaviors before exposure to any plant. When the parasitoid is exposed to its first plant, it would behave the same as for all future exposures.

M. ancyliivorus had a significant behavioral response to plant damage. The response to plant infestation indicates that there may be a behavioral trigger associated with host cues. This accords with the idea that generalists respond only to the presence of host species, not plant type. Despite the response to herbivore presence, the actual behaviors that followed from their response remained ineffective. This also may be tied to a general set of parasitoid behaviors associated with the presence of hosts.

It was surprising to find that the parasitoids were unable to parasitize any larvae. Based on parasitism levels from field collections of sunflower moth, we know that *M. ancyliivorus* naturally parasitizes sunflower moth (Teetes and Randolph 1969; Beregovoy 1985). Although the laboratory setting used for these experiments was not a perfect replication of field settings, it seems reasonable to assume that the conditions of the experiment could have produced natural behavior. Chen and Welter (2003) found that *Dolichogenidea homoeosomae*, a specialist parasitoid of the sunflower moth, successfully parasitized under very similar conditions. It is

possible that the laboratory conditions affected the generalist parasitoid's ability to parasitize much more than the specialist used by Chen and Welter.

The results of this study present a number of unexpected and interesting observations. The findings even seem to conflict directly with some theoretical expectations for generalist parasitoids. More work with generalist and specialist parasitoids in this setting could shine light onto the subtleties, like behavioral efficiency and triggering, which currently remain hidden.

References

- Andow, D. A. and D. R. Prokrym, 1990. Plant structural complexity and host-finding by a parasitoid. *Oecologia* 82: 162-165.
- Beregovoy, V. H. 1985. Parasitism of the Sunflower Moth, *Homoeosoma electellum* (Hulst) (Lepidoptera: Pyralidae) in the Central United States. *Journal of the Kansas Entomological Society* 58: 732-736.
- Charlet, L. D., 1999. Biological control of sunflower pests: searching for new parasitoids in native *Helianthus*—challenges, constraints, and potential. In: Charlet, L. D., Brewer, G. J., *Biological Control of native or indigenous insect pests: Challenges, constraints, and potential.* Thomas Say Publications in Entomology. Lanham, MD, pages: 91-112.
- Chen, Y. H. and S. C. Welter. 2003. Confused by domestication: incongruent behavioral responses of the sunflower moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae) and its parasitoid, *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae), towards wild and domesticated sunflowers. *Biological Control* 28: 180-190.
- Chen, Y. H. and S. C. Welter. 2005. Crop domestication disrupts a native tritrophic interaction associated with the sunflower, *Helianthus annuus* (Asterales: Asteraceae). *Ecological Entomology* 30: 673-683.
- Finney, G. L., Flanders S. E. and H. S. Smith, 1947. Mass culture of *Macrocentrus ancylicivorus* and its host, the potato tuber moth. *Hilgardia* 17: 437-483.
- Jones, R. L. 1996. Semiochemicals in host and mate finding behavior of *Macrocentrus Grandii* Goidanich (Hymenoptera: Braconidae). *Florida Entomologist* 79: 104-108.
- Teetes, G. L. and N. M. Randolph, 1969. Seasonal abundance and parasitism of the Sunflower Moth, *Homoeosoma electellum*, in Texas. *Annals of the Entomological Society of America* 62: 1461-1465.
- Wilson, R. L. 1990. Rearing the sunflower moth (Lepidoptera: Pyralidae) for use in field evaluation of sunflower germplasm. *Journal of the Kansas Entomological Society* 63: 208-210.