Abstract

WITTING, BROOKE ELLEN. Evaluation of Floral Habitat as a Food Source for Natural Enemies of Insect Pests in North Carolina. (Under the direction of David B. Orr and H. Michael Linker).

A field study was conducted in 2004 and 2005 to observe flower-feeding of potential beneficial insectary plants by insects. Sixteen flower species were individually observed once weekly for two minutes beginning between 12 and 1 pm in 2004. Five species were observed twice weekly beginning at 9:30 am and 12 pm in 2005. Insects were identified to family level and analyzed by feeding guild. In both years, predators were observed feeding from fennel (*Foeniculum vulgare* P. Mill.) flowers in greater abundance than from any other flowers observed. Fennel also was fed upon most often by parasitoids in 2005. Pollinators were observed feeding most often from Indian blanket (*Gaillardia pulchella* Foug.) in 2004 and from black-eyed Susan (*Rudbeckia hirta* L.) and buckwheat (*Fagopyrum esculentum* Moench) in 2005. In both years, herbivorous crop pests, deleterious and non-crop parasitoids, and deleterious predators were not significantly affected by flower species.

A field study was conducted in August 2005 to determine the relative attractiveness of floral habitat to three families of microhymenopteran egg parasitoids: Mymaridae, Scelionidae, and Trichogrammatidae. Habitat plants were yarrow (*Achillea millefolium* L.), celosia (*Celosia cristata* L.), buckwheat, fennel, daisy (*Leucanthemum* x *superbum* (J. W. Ingram) Berg. ex Kent.), and black-eyed Susan. Non-flowering crabgrass (*Digitaria sp.* Haller) served as a control. Sticky traps were used to monitor microhymenoptera and were placed at three heights: flower height, 0.5 times flower height, and 1.5 times flower height. Flower heads were removed from half of each plot and traps were placed in the center of each subplot. Results from this experiment show that flower species and height affected all

three families of microhymenoptera but flower removal only affected scelionids. At flower height, scelionids were trapped in greater abundance in celosia plots at flower height in flowers-present versus flowers-removed treatments. Trichogrammatids were trapped in greatest abundance at 0.5 times flower height in un-mowed crabgrass plots and mymarids were most abundant at 0.5 times flower height in black–eyed Susan plots. Our results indicate that habitat plantings may attract microhymenoptera but that flowers themselves do not appear to be responsible for this attraction.

A combined laboratory and field study was conducted to determine the effect of different food sources on the longevity and fecundity of *Trichogramma exiguum* Pinto & Platner and the longevity of *Cotesia congregata* (Say). Newly eclosed (<12 h) female wasps were provisioned with one of two treatments: fennel or buckwheat flowers, or one of two controls: honey or water. Wasps were monitored daily until all had died. Fecundity of *T. exiguum* was monitored using *Ephestia kuehniella* Keller egg cards. Longevity was greatest in *T. exiguum* provisioned with honey and in *C. congregata* provisioned with buckwheat flowers. Buckwheat provisioned *T. exiguum* exhibited greater longevity than those provided fennel. Longevity of *C. congregata* provisioned with fennel and honey was approximately equal. Water provisioned *T. exiguum* and *C. congregata* exhibited the shortest longevity. Total fecundity was greatest in *T. exiguum* provisioned with honey or buckwheat. Average female to male ratio over the lifetime of each female was greatest in *T. exiguum* provisioned with water alone, likely because of sperm limitation in wasps exhibiting greater longevity. Total average number of female offspring produced was greatest in *T. exiguum* provided honey or buckwheat flowers although no difference in total female offspring were observed between adults provisioned with buckwheat or fennel flowers. Our results show that

provisioning *T. exiguum* with honey and buckwheat flowers caused greater longevity, total fecundity, and lifetime production of female offspring than water alone. Buckwheat flowers also lead to greater longevity in *C. congregata.*

EVALUATION OF FLORAL HABITAT AS A FOOD SOURCE FOR NATURAL ENEMIES OF INSECT PESTS IN NORTH CAROLINA

by

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DEDICATION

I am pleased to dedicate this thesis to my mom, Sylvia Lynn Witting, whose stubborn determination for her children's success has led me here. Thank you.

BIOGRAPHY

Brooke Ellen Witting was born in Norman, Oklahoma in 1978. She moved to Durham, North Carolina with her family in 1981. Brooke attended Warren Wilson College in Swannanoa, NC and received her B.A. in Environmental Studies with a minor in Biology in 2000 under Dr. Louise Weber. It was at WWC that she discovered her love for the life sciences and entomology. In 2004 she began work on a Master's of Science degree at North Carolina State University in the Department of Entomology under the direction of Drs. David Orr and H. Michael Linker.

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Finally, I would like to thank Dr. Louise Weber for igniting the spark that fueled my interest in entomology. I will never forget her first lecture as she described spending hours examining insect boxes and being astounded by the diverse and beautiful world of insects. It was at that moment that I knew I had found my calling.

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Observations of Insect Flower-Feeding

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Abstract

Habitat plantings may be used to increase diversity of natural enemies to enhance biological control of agricultural pests by providing nectar and pollen, an appropriate microclimate, or hosting alternative prey. This study was conducted in 2004 and 2005 to observe flower-feeding of potential beneficial insectary plants by insects. Sixteen flower species were individually observed once weekly for two minutes beginning between 12 and 1 pm in 2004. Five species were observed twice weekly beginning at 9:30 am and 12 pm in 2005. Insects were identified to Family level and analyzed by feeding guild. In both years more predators were observed feeding from fennel (*Foeniculum vulgare* P. Mill.) flowers than from any other flowers. Fennel also was fed upon most often by parasitoids in 2005. Pollinators were observed feeding most often from blanket flower (*Gaillardia pulchella* Foug.) in 2004 and from black-eyed Susan (*Rudbeckia hirta* L.) and buckwheat (*Fagopyrum esculentum* Moench) in 2005. In both years, herbivorous crop pests, deleterious and non-crop parasitoids, and deleterious predators were not significantly affected by flower species.

Introduction

Insects and flowering plants are believed to have relied on one another for the last 125 million years. Palynivory (feeding on pollen) is considered to be the evolutionary forerunner to pollination and was followed by nectarivory where plants ensured their reproduction by enticing pollinators with nectar rewards (Labandeira 1998). While less frequently noted, plants may attract insects using nectar for another reason. By luring predatory insects with sweet secretions, plants can potentially encourage predators to feed on herbivorous insects (Wäckers 2005).

Plants can assist natural enemies by providing appropriate microclimates, food resources, such as nectar and pollen, or by hosting alternative prey (Landis et al. 2000). While many natural enemies are carnivorous as larvae, the adults are often omnivorous or herbivorous and rely on plant foods to promote increased longevity and fecundity (Jervis and Kidd 1986; Cortesero et al. 2000; Wäckers 2005). The 'enemies hypothesis' (Root 1973) implies that natural enemies are more effective at reducing crop pest numbers in diverse rather than simple habitats. In modern cropping systems, plant diversity tends to be low, reducing plant resources such as sugar, which may impact beneficial insects. Habitat management is a type of conservation biological control that employs the use of plant resources to enhance the effectiveness of natural enemies and can be an important tool in suppressing agricultural pest insect populations by increasing diversification of plants in agricultural systems (reviewed by Coll 1998).

Plant-provided resources can increase effectiveness of natural enemies by generating greater longevity, fecundity, or host-searching ability. The effectiveness of insectary habitat has been shown in many cases (e.g. Irvin et al. 2000; Stephens et al. 1998). However, only

about half of the studies comparing diversified cropping systems to monocultures have yielded positive results in terms of reduced pest numbers (Heimpel et al. 2005). Failures in the field may be caused in part to plants' varied abilities to provide natural enemies with food resources due to plant physiology and morphology. Many factors influence the suitability of floral habitat as food sources to natural enemies including availability of flowers in time and space, floral architecture, floral odor, and nutritional composition of nectar and pollen (Wäckers 2005). For example, buckwheat (*Fagopyrum esculentum* Moench) flowers are readily fed upon by many natural enemies (English-Loeb 2003); however, nectar production ceases in the afternoon (Olson et al. 2005). Patt et al. (1997) found that floral architecture and odor played important roles in the foraging efficiency of two parasitoids (Hymenoptera: Eulophidae). Analysis of gut sugars of parasitic ichneumonoid and chalcidoid wasps showed significantly higher amounts of fructose, a sugar not naturally present in insect bodies, in wasps collected from flowering buckwheat borders than from soybean borders (Lee and Heimpel 2003). Wäckers (2004) screened eleven species of flowering plants for suitability as food sources to an ichneumonid and two braconid parasitoids. Only four plant species were found to be attractive, while three plant species were actually determined to be repulsive.

It is generally accepted that natural enemies forage effectively on non-specialized flowers such as composites and umbels which contain compact groups of small florets with accessible nectaries (Proctor et al. 1996). Field observations have shown that natural enemies exhibit preferential feeding behavior to various species of flowering plants. In a study conducted by Colley and Luna (2000), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* P. Mill.), and Korean mint (*Agastache rugosa* Fischer & C. A. Meyer)

elicited the greatest number of feeding visits from beneficial hoverflies visiting eleven flowering plant species. Carreck and Williams (1997) recorded insect visits to individual flowering species of two commercial flower mixes and found *Phacelia tanacetifolia* Benth to be most attractive to hoverflies and hymenoptera. However, the hymenoptera observed were predominately members of the family Apidae, not parasitoids. Lövei et al. (1993) observed feeding by hoverflies from several species of flowering plants and determined that coriander provided food resources to the greatest number of hoverflies of the plants observed. Al-Doghairi and Cranshaw (1999) observed insect visits to 150 plant species in 37 families and found that members of Asteraceae, the aster family; Apiaceae (formerly Umbelliferaceae), the carrot family; Brassicaceae, the mustard family; Lamiaceae, the mint family; Scrophulariaceae, the figwort family; and Crassulaceae, the stonecrop family received the most visits by natural enemies.

This study was designed to determine which flowers attracted the greatest numbers of parasitoids and predators of crop pests in North Carolina. The observational studies mentioned previously were conducted in Oregon, the United Kingdom, New Zealand, and Colorado respectively. To our knowledge, few if any observational studies of flower-feeding by natural enemies have been conducted in the southeastern United States. Forehand (2004) recorded abundance of natural enemies collected from flowering habitat in North Carolina using a vacuum sampler; however direct observations of flower-feeding by natural enemies were not made.

We were also interested in recording the numbers of herbivorous crop pests feeding from flowers. One risk associated with placing flowering plants near crop fields is that pest insects could potentially gain a fitness benefit from floral resources. Baggen and Gurr (1998)

found that while buckwheat and coriander flowers increased longevity of an encyrtid parasitoid, flowers also increased longevity and fecundity of the parasitoid's herbivorous host. Pest numbers and crop damage in the field were also amplified as proximity to flowering habitat increased.

Finally, it is important to quantify members of other feeding guilds in addition to beneficial parasitoids, predators, and crop pests. Many insects can be observed feeding from flowers that to farmers may appear to be beneficial. These insects include hymenoptera that are pollinators, predators of beneficial spiders (e.g. members of Pompilidae) and parasitoids of pollinators or natural enemies (e.g. members of Chrysididae). The previously mentioned study by Carreck and Williams is a good example of documentation of hymenopteran pollinators rather than predators and parasitoids being considered beneficial insects. Stephens et al. (1998) provide an important case where numbers of *Anacharis sp.* (Hymenoptera: Figitidae), a parasitoid of the beneficial brown lacewing, were increased in orchards sown with buckwheat than in herbicide treated control plots. We hope that this study can elucidate the preferences of different feeding guilds to floral habitat to provide growers with a preliminary recommendation of insectary habitat for natural enemies in North Carolina.

Materials and Methods

Research site. This study was conducted at the Center for Environmental Farming Systems near Goldsboro, N.C. on the Small Farm Unit. The Small Farm Unit is a highly diverse, organic farm approximately 6.07 ha in size. A wide variety of commodities are grown at the Small Farm Unit including vegetable, grain, flower, forage, and small fruit

crops. Some livestock production, including chickens, turkeys, and goats also occurs on the Small Farm Unit.

Experimental design. Observational data were collected from three flower strips in three distinct locations on the Small Farm Unit that were established the previous year. Flower strips were separated by an average distance of 48.2 m. For all studies, flower strips measuring approximately 56.4 x 2.7 m were divided into 6.1 x 2.7 m plots.

In 2004, each flower strip contained five plots, three of which were commercially available beneficial insectary plantings and two that contained pure stands of fennel and buckwheat (Table 1.1). Greenhouse grown plants were transplanted using a grid to achieve an ideal plant community according to seed companies' instructions in a complete block design with selective placement of plots (Forehand 2004).

In 2005, flower strips contained seven plots laid out using a complete block design with selective placement of plots. Fennel, daisy (*Leucanthemum* x *superbum* (J. W. Ingram) Berg. ex Kent.), yarrow (*Achillea millefolium* L.), and black-eyed Susan (*Rudbeckia hirta* L.) were planted because natural enemies were observed feeding from these plants most often during the 2004 study. Celosia (*Celosia cristata* L.) appeared to attract and feed a large number of natural enemies when observed anecdotally. Buckwheat was chosen because of its prevalence in scientific literature as being an insectary plant attractive to natural enemies (Colley and Luna 2000; Irvin et al. 2000; Stephens et al. 1998), although for the most part, results have been variable (Irvin et al. 1999; Berndt et al. 2002).

Plant management. In 2004 and 2005, plants were watered as needed and weeds were managed with hand-weeding inside plots and mechanical mowing around plots. In 2005, plots containing previously established fennel and yarrow were utilized because plant

densities were high enough that a pure stand had already been obtained. All other plants were either transplanted or directly seeded into plots. In 2005, flower strips each contained seven plots. Plots were planted with greenhouse-grown celosia, daisy, and black-eyed Susan transplants on 25 May, 2005. Fifty-four plants of each species were planted per plot in three rows with 30.5 cm between each plant and 46 cm between each row using hand trowels and bulb diggers. Buckwheat was directly seeded into plots at a rate of 56.04 kg/ha and raked in using a steel rake. Buckwheat seed was purchased from Jeffrey's Seed Co. (1608 US 117 South, Goldsboro, NC 27503). The remaining seeds were purchased from Germania (5978 N Northwest Hwy, PO Box 31787, Chicago, IL 60631-0787) (See Table 1.1 for cultivars).

Celosia, black-eyed Susan, and daisy transplants were grown in the Biological Control Greenhouse at North Carolina State University, Raleigh, NC. Plants were started in 96-cell round plug trays (3.8 by 3.9 cm, Hummert International, 4500 Earth City Expressway, Earth City, MO 63045) filled with moistened Metro-Mix 200 potting soil (Scotts-Sierra Horticulture Products Co., The Scotts Company, 1411 ScottsLawn Rd., Marysville, OH 43041) on 25 and 28 March, 2005. Four trays were planted per species with two seeds planted per cell thinned to one plant per cell. Plants were grown in a greenhouse with a heating set point of 21.1º C and a ventilation set point of 26.7º C. Plants were watered as need with a misting bed and/or hand watering. Trays were placed under high intensity metal halide lights with an 11 h photophase. The photophase was extended to 16 h on 22 April, 2005. When roots were established and the aboveground portion was of sufficient size, plants were transplanted to 473 ml plastic cups (Kmart Corporation, Troy, MI 48084) with a drainage hole drilled in the bottom using a 1.3 cm drill bit. Prior to transplanting, plots were tilled and all plot borders as well as celosia, black-eyed Susan, and daisy plots

were covered with woven black plastic ground cover (Wyatt-Quarles Seed Company, 730 Hwy 70 West, Garner, NC 27529) secured with landscape anchor pins (DuPont™ Garden Products™, Chestnut Run Plaza, Bldg. 728, PO Box 80728, Wilmington, DE, 19880-0728) to suppress weeds and preserve soil moisture.

Sampling. In both years, one observation of insect flower-feeding per plant species was made in each replicate on each sampling date. Observations of insect feeding were conducted on seven dates in 2004 (2 June, 9 June, 24 June, 8 July, 14 July, 22 July, and 4 August) and on thirteen dates in 2005 (21 June, 24 June, 28 June, 1 July, 5 July, 12 July, 15 July, 18 July, 2 August, 5 August, 9 August, 12 August, and 16 August). Observations in 2004 began between 12 and 1 pm. This time was chosen after performing a daylong observation of insect activity on 31 May, 2004 from dawn to dusk where we found the greatest amount of activity to occur midday. Observations were made at 9:30 am and 12:00 pm in 2005. The 9:30 observation was added due to low numbers of insects found feeding midday on buckwheat in 2004, presumably because peak nectar production in buckwheat occurs in the morning (Olson et al. 2005; Free 1993).

A single observer called out identified insects to a recorder who also kept time. This approach allowed the observer to watch flowers for the prescribed period without interruption. For a single observation, the observer constantly scanned an approximately 0.3 m^2 area of actively blooming flowers of a single plant species for two minutes. Insects observed directly feeding from flower heads were recorded to family level. Feeding was considered to be direct application of the insects' mouthparts to the area of the plant producing nectar and/or pollen or apparent application of the mouthparts to this region accompanied by movement of the head or body into the floral structures. Insects that moved

from flower to flower within the area of observation were counted once. Insects that left the area and returned were counted a second time, similar to methods described by Colley and Luna (2000).

All insects that were too small to be identified in the field were removed with an aspirator and transferred to a vial containing 50% ethanol and returned to the laboratory for identification. Preliminary identification of specimens from each insect family was performed by Dr. David Orr. Mr. David Stephan verified identification and specimens were placed in the NCSU museum as vouchers.

Data analysis. Insects observed feeding on flowers were grouped according to feeding guilds (Table 1.3). The number of insects observed feeding at each plant species were square root transformed then analyzed using general linear and mixed models for each feeding guild (PROC GLM, PROC MIXED, SAS Institute 2003). Plant species that flowered in only one replicate or received no feeding visits from members of a specific feeding guild were omitted prior to analyses to avoid skewing results. Dates of observations that fell within the same week in 2005 were combined prior to analyses to reduce imbalance in data due to differences in blooming period among plant species.

Results

In 2004, numbers of parasitoids, predators, pollinators, and non-crop herbivores observed were significantly affected by flower species (F = 6.60, df = 3, 5, P = 0.0344; F = 10.45, df = 9, 16, $P < 0.0001$; F = 12.43, df = 9, 16, $P < 0.0001$; F = 4.05, df = 9, 16, P = 0.0073) (Appendix 1.1). Herbivorous crop pests, deleterious and non-crop parasitoids, and deleterious predators were not significantly affected by flower species (F = 1.57, df = 9, 16, P = 0.2064; F = 0.12, df = 5, 9, P = 0.9849; F = 2.99, df = 5, 9, P = 0.0731; F = 2.56, df = 1,

2, $P = 0.2506$). In 2005, flower species significantly affected the numbers of parasitoids, non-crop parasitoids, predators and pollinators ($F = 41.79$, df = 2, 4, $P = 0.0021$; $F = 27.45$, df = 4, 8, $P < 0.0001$; $F = 9.08$, df = 4, 8, $P = 0.0045$) but not non-crop herbivores, herbivorous crop pests, deleterious parasitoids, or deleterious predators ($F = 1.23$, df = 3, 6, $P = 0.3773$; $F = 2.67$, df = 4, 8, $P = 0.1104$; $F = 2.86$, df = 3, 6, $P = 0.1267$; $F = 9.74$, df = 1, 2, $P = 0.0891$). Pollinators and deleterious parasitoids were affected by time of day observations were made ($F = 12.69$, df = 1, 10, $P = 0.0052$; $F = 9.86$, df = 1, 10, $P = 0.0105$) while parasitoids, non-crop parasitoids, predators, deleterious predators, non-crop herbivores and herbivorous crop pests were not ($F = 1.16$, df = 1, 6, $P = 0.3235$; $F = 3.19$, df = 1, 10, $P = 0.1042$; $F = 0.16$, df = 1, 10, $P = 0.6966$; $F = 0.70$, df = 1, 4, $P = 0.4487$; $F = 0.50$, df = 1, 8, $P = 0.4994$; $F = 0.95$, df = 1, 10, $P = 0.3528$). The interaction between time of day and flower species significantly affected pollinators, deleterious parasitoids, and predators ($F = 16.58$, df = 4, 10, $P = 0.0002$; $F = 7.07$, df = 4, 10, $P = 0.0057$; $F = 8.85$, df = 4, 10, $P = 0.0025$) but not parasitoids, non-crop parasitoids, deleterious predators, non-crop herbivores and herbivorous crop pests ($F = 1.00$, df = 2, 6, $P = 0.4207$; $F = 0.98$, df = 4, 10, $P = 0.4620$; $F = 0.18$, df = 1, 4, $P = 0.6907$; $F = 0.94$, df = 3, 8, $P = 0.4664$; $F = 1.10$, df = 4, 10, $P = 0.4062$).

In 2004, overall parasitoid feeding was low (Table 1.3). Parasitoids were only observed feeding from four flowers: celery (*Apium graveolens* L.), daisy, fennel, and yarrow. Of these flowers, significantly more parasitoids were found feeding from celery. The remaining flowers did not differ in the numbers of parasitoids observed feeding from them. However, because celery was observed on relatively few occasions, results are not highly conclusive due to lack of robustness in the data. In 2005, parasitoids were found feeding

from fennel in higher numbers than from any of the other plant species observed (Table 1.4). Approximately equal numbers of parasitoids fed from yarrow, celosia, buckwheat, and black-eyed Susan.

In 2004, predators were observed feeding in significantly higher numbers from fennel than the remainder of the flowers observed (Table 1.3). Predators fed from celery and yarrow at higher levels than from clover (*Trifolium repens* L.), blanket flower (*Gaillardia pulchella* Foug.), California poppy (*Eschscholzia californica* Cham.), and tickseed (*Coreopsis lanceolata* L.). In 2005, significantly more predators fed from fennel than the other flower species regardless of time of day (Table 1.4). Buckwheat was fed upon to a lesser degree than fennel, however significantly more predators were present on buckwheat at 9:30 than at 12:00.

Flowers in this study varied greatly in the numbers of pollinators that fed from them. In 2004, higher numbers of pollinators were found feeding from blanket flower, although numbers did not significantly differ from pollinators feeding from tickseed (Table 1.3). Numbers of pollinators feeding from tickseed, fennel, yarrow, daisy, black-eyed Susan, and California poppy were approximately equal while celery, clover, and buckwheat were fed upon least. In 2005, more pollinators were observed feeding from black-eyed Susan and buckwheat than all other plant species (Table 1.4). More pollinators were observed at both black-eyed Susan and buckwheat at 9:30 than at 12:00.

In 2004, non-crop herbivores fed most from celery flowers (Table 1.3). Yarrow was fed upon more frequently than California poppy but no significant difference was found among the remainder of the flower species. Non-crop herbivores were not significantly affected by flower species in 2005 (Table 1.4).

The effects of replication and date on numbers of insects feeding from flowers in 2004 were significant for parasitoids ($F = 3.98$, df = 2, 11, $P = 0.0383$; $F = 14.48$, df = 6, 7, $P < 0.0001$). Date also significantly affected non-crop herbivores ($F = 2.37$, df = 6, 13, $P = 0.0401$). However, this was probably due to unevenness in the data as parasitoids and non-crop herbivores were found feeding most often from celery, which was present in only two of the three replicates for two weeks. Replication did not effect deleterious or non-crop parasitoids, deleterious predators, predators, pollinators, non-crop herbivores, or herbivorous crop pests ($F = 0.54$, df = 2, 13, $P = 0.5883$; $F = 0.59$, df = 2, 13, $P = 0.5568$; $F = 3.33$, df = 2, 10, $P = 0.0779$; $F = 0.23$, df = 2, 17, $P = 0.7948$; $F = 2.91$, df = 2, 17, $P = 0.0619$; $F = 0.87$, df = 2, 17, $P = 0.4228$; $F = 0.50$, df = 2, 17, $P = 0.6100$). Date played a significant role in the number of pollinators and non-crop parasitoids found feeding from flowers ($F = 9.16$, df = 6, 13, $P < 0.0001$; $F = 3.13$, df = 6, 13, $P = 0.0139$) but not deleterious parasitoids, deleterious predators, predators, or herbivorous crop pests ($F = 1.06$, df = 6, 13, $P = 0.4063$; $F = 2.40$, df = 6, 13, $P = 0.1056$; $F = 1.86$, df = 6, 13, $P = 0.1029$; $F = 1.16$, df = 6, 13, $P = 0.3377$). Upon closer observation we noted that blanket flower and fennel harbored higher numbers of pollinators during the middle of our sampling dates while other flower species were fed upon by approximately equal numbers of pollinators throughout the study. Numbers of non-crop parasitoids observed feeding from flowers were low throughout the entire study. Because we were able to identify probable causes leading to a significant effect, data were averaged across both replication and date.

In 2005, no effect of replication was found for any of the feeding guilds. Week significantly affected the number of pollinators and non-crop parasitoids observed on flowers ($F = 34.21$, df = 5, 10, $P < 0.0001$; $F = 7.89$, df = 5, 10, $P = 0.0030$) but did not affect

numbers of non-crop herbivores, herbivorous crop pests, predators, deleterious predators, parasitoids, non-crop or deleterious parasitoids (F = 2.15, df = 4, 8, P = 0.1654; F = 0.50, df = 3, 6, P = 0.6977; F = 0.83, df = 5, 10, P = 0.7181; F = 2.68, df = 5, 10, P = 0.0865; F = 2.03, df = 5, 10, P = 0.1599; F = 1.31, df = 5, 10, P = 0.3356). The number of pollinators visiting flowers decreased steadily with the progression of weeks, likely because peak flowering occurred at the beginning of the study and flower-production declined as weeks passed. Non-crop parasitoids were observed feeding from flowers infrequently.

Discussion

In this study we were primarily interested in determining which flowering plants provided floral food resources to beneficial insects. We regarded only two feeding guilds, parasitoids and predators, as beneficial insects because of their ability to reduce numbers of agricultural pests. We were also interested in recording all other insects feeding from floral structures to determine whether or not crop pests fed from flowers and to separate insects which may appear to be beneficial to farmers because they belong to the Order Hymenoptera. The latter have species that may be deleterious because of their potential to reduce numbers of pollinators or spiders through predation or parasitization (e.g. Pompilidae and Chrysididae) (Triplehorn and Johnson 2005). We also recorded numbers of pollinators which are beneficial to the farm but play no role in crop pest management.

Results from this study show that insects belonging to different feeding guilds preferentially feed from different flower species. Although sampling was conducted in a similar manner from year to year planting design was considerably different and plant species observed differed making direct comparison of the two study years impossible. However, in both years, the same feeding guilds were affected by flower species with the

exception of non-crop herbivores and non-crop parasitoids. Numbers of deleterious parasitoids and predators, and herbivorous crop pests were not affected by flower species. Additionally, some overall trends in the frequency of feeding visits made by beneficial insects can be seen in both years. Fennel received the greatest number of feeding visits from predators both years and in 2005 fennel was frequented most often by beneficial parasitoids. In 2004, celery was visited most often by parasitoids. This study reinforces the observation that umbelliferous flowers which have easily accessible nectaries are often frequented by beneficial insects (Patt et al. 1997). Celery however only bloomed for three weeks in only two of the three replicates. Additionally, celery is a biennial and therefore would unlikely be a desirable beneficial insectary plant as growers would have to wait a full year for flowering to commence. Fennel bloomed continuously and aggressively throughout both years of the study.

In 2004, few insects were found feeding from buckwheat when all observations were conducted at noon. Buckwheat tends to wilt in hot weather and does not produce nectar in the afternoon (Lee and Heimpel 2003; Olson et al. 2005). By adding a morning observation we were able to see that buckwheat was attractive to pollinators and predators after finding the previous year that buckwheat attracted relatively low numbers of members of all feeding guilds.

We found no significant effect of flower species on numbers of herbivorous crop pests observed feeding from floral structures. Additionally, overall numbers of crop pests feeding from flowers were low for both years. This does not mean, however that crop pests did not feed from the flowers in this study. Time of day could have played an important role in our findings as many lepidopteran pests are active in the evening. For example, Forehand

(2004) observed crepuscular feeding habits of noctuid and sphingid moths and found that moths fed most heavily from celosia flowers.

This study does not allow us to provide a definitive recommendation for beneficial insect habitat to growers in North Carolina. In 2004, flowering was inconsistent across replications and dates causing many gaps in the data. Siberian wallflower (*Erysimum hieracifolium* L.) and dame's rocket (*Hesperis matronalis* L.) were eliminated from data analysis because they received so few feeding visits. This shows that these flowers are likely poor choices as habitat planting to attract natural enemies in North Carolina. Other plants, such as celery and cilantro (*Coriandrum sativum* L.) exhibited a short blooming period, making them unsuitable insectary habitat plants as well. As was previously mentioned, the biennial nature of celery is undesirable. In 2005 a similar problem was encountered with Shasta daisy plants when blooming failed to commence the same season daisies were planted. Fennel showed promising characteristics both phenologically and in its ability to attract beneficial insects. Fennel, however, can be invasive and is listed on the California Exotic Plant Pest List (1999). Fennel also causes contact and photodermatitis in humans and should be handled only when wearing gloves (Simon et al. 1984). Because of the lack of complete knowledge of biology and phenology of plants used in this study, future research that is more exhaustive than the present study is needed. We hope that the current findings can be a starting point for future observational studies of beneficial insect flower-feeding in North Carolina.

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Table 1.1 Plant species observed in each beneficial insect habitat flower strip. Goldsboro, NC

2004					
Common Name	**Scientific Name**	**Plant Family**	**Weeks in Bloom**	**Replicates in Bloom**	**Cultivar**
Alfalfa	*Medicago sativa* L.	Fabaceae	2	1	
Black-eyed Susan	*Rudbeckia hirta* L.	Asteraceae	7	2-3	
Blanket flower	*Gaillardia pulchella* Foug.	Asteraceae	7	2-3	
Blazing star	*Liatris spicata* (L.) Willd.	Asteraceae	3	1	
Buckwheat	*Fagopyrum esculentum* Moench	Polygonaceae	7	2-3	
California poppy	*Eschscholzia californica* Cham.	Papaveraceae	5	3	
Celery	*Apium graveolens* L.	Apiaceae	3	1-2	
Cilantro	*Coriandrum sativum* L.	Apiaceae	3	1	
Dame's rocket	*Hesperis matronalis* L.	Brassicaceae	4	2-3	
Fennel	*Foeniculum vulgare* P. Mill.	Apiaceae	7	3	'Smokey Bronze'
Purple prairie clover	*Dalea purpurea* Vent.	Fabaceae	2	1-2	
Red clover	*Trifolium repens* L.	Fabaceae	7	3	
Shasta daisy	*Leucanthemum* x *superbum* (J.W. Ingram) Berg. ex Kent.	Asteraceae	6	1-3	
Siberian wallflower	*Erysimum hieracifolium* L.	Brassicaceae	5	1-2	
Tickseed	*Coreopsis lanceolata* L.	Asteraceae	5	1-2	
Yarrow	*Achillea millefolium* L.	Asteraceae	7	3	
2005					
Black-eyed Susan	*Rudbeckia hirta* L.	Asteraceae	3	3	'Indian Summer'
Buckwheat	*Fagopyrum esculentum* Moench	Polygonaceae	3	3	
Celosia	*Celosia cristata* L.	Amaranthaceae	5	3	'Cramer's Crested Series Burgundy'
Fennel	*Foeniculum vulgare* P. Mill. x Rubrum	Apiaceae	7	3	'Smokey Bronze'
Shasta daisy	*Leucanthemum* x *superbum* (J.W. Ingram) Berg. ex Kent.	Asteraceae	0	0	'Alaska'
Yarrow	*Achillea millefolium* L.	Asteraceae	4	3	'Silver Queen'

Table 1.2 List of insect families by feeding guild

Feeding Guild	Families Observed
Herbivore – Crop Pest	Chrysomelidae, Coreidae, Curculionidae, Hesperiidae, Miridae, Papillionidae, Pentatomidae, Pieridae, Scarabaeidae
Herbivore – Non-Crop	Ctenuchidae, Geometridae, Mordellidae, Nymphalidae, Thyreocoridae
Parasitoid – Non-Crop	Scoliidae, Tephiidae
Parasitoid – Beneficial	Eulophidae, Figitidae, Tachinidae
Parasitoid – Deleterious	Chrysididae
Pollinator	Anthophoridae, Apidae, Halictidae, Megachilidae
Predator – Beneficial	Anthocoridae, Cantharidae, Coccinellidae, Chrysopidae, Lygaeidae, Sphecidae, Staphylinidae, Syrphidae, Vespidae
Predator – Deleterious	Pompilidae

Table 1.3 Mean ± SD number of insects per two minutes in each feeding guild observed feeding from flowers 2 June – 4 August. Goldsboro, N.C. 2004

Plant Species	Parasitoids	Non-Crop Parasitoids	Deleterious Parasitoids	Herbivores Non-Crop	Herbivores Crop Pests	Deleterious Predators	Predators	Pollinators
Black-eyed Susan	$0.0 \pm 0.0_{B}$	$0.6 \pm 1.1_{A}$	$0.2 \pm 0.6_{A}$	$0.2 \pm 0.4_{BC}$	$0.1 \pm 0.3_{A}$	$0.0 \pm 0.0_{A}$	$0.5 \pm 0.7_{BCD}$	$1.3 \pm 2.4_{CDE}$
Blanket flower	$0.0 \pm 0.0_{B}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{A}$	$0.2 \pm 0.7_{BC}$	$0.2 \pm 0.4_{A}$	$0.0 \pm 0.0_{A}$	$0.2 \pm 0.5_{CD}$	$6.1 \pm 4.6_{A}$
Buckwheat	$0.0 \pm 0.0_{B}$	$0.5 \pm 0.5_{A}$	$0.0 \pm 0.0_{A}$	$0.2 \pm 0.5_{BC}$	$0.4 \pm 1.0_{A}$	$0.1 \pm 0.2_{A}$	$0.5 \pm 1.0_{BC}$	$0.0 \pm 0.0_{F}$
California poppy	$0.0 \pm 0.0_{B}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{A}$	$0.1 \pm 0.3_{C}$	$0.2 \pm 0.4_{A}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{D}$	$0.8 \pm 0.8_{CDE}$
Celery	$2.4 \pm 3.6_{A}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{A}$	$12.2 \pm 18.6_{A}$	$0.5 \pm 0.6_{A}$	$0.0 \pm 0.0_{A}$	$1.2 \pm 1.3_{B}$	$0.4 \pm 0.9_{DEF}$
Clover	$0.0 \pm 0.0_{B}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{A}$	$1.0 \pm 3.15_{BC}$	$0.5 \pm 0.6_{A}$	$0.0 \pm 0.0_{A}$	$0.4 \pm 0.6_{CD}$	$0.3 \pm 0.2_{EF}$
Daisy	$0.5 \pm 1.4_{B}$	$0.8 \pm 1.2_{A}$	$0.2 \pm 0.6_{A}$	$0.5 \pm 0.7_{BC}$	$0.6 \pm 0.8_{A}$	$0.0 \pm 0.0_{A}$	$0.9 \pm 1.5_{B}$	$1.5 \pm 1.6_{CD}$
Fennel	$0.5 \pm 0.9_{B}$	$0.2 \pm 0.5_{A}$	$0.1 \pm 0.4_{A}$	$0.4 \pm 0.5_{BC}$	$0.4 \pm 0.8_{A}$	$0.6 \pm 1.2_{A}$	$3.2 \pm 2.6_{A}$	$2.8 \pm 3.4_{BC}$
Tickseed	$0.0 \pm 0.0_{B}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{A}$	$0.7 \pm 1.0_{BC}$	$0.3 \pm 0.5_{A}$	$0.0 \pm 0.0_{A}$	$0.0 \pm 0.0_{D}$	$3.1 \pm 2.4_{AB}$
Yarrow	$0.1 \pm 0.2_{B}$	$0.4 \pm 0.5_{A}$	$0.1 \pm 0.4_{A}$	$2.0 \pm 3.2_{B}$	$1.6 \pm 1.5_{A}$	$0.0 \pm 0.0_{A}$	$0.7 \pm 1.0_{BC}$	$1.8 \pm 1.97_{C}$

Means within the same column followed by the same letter are not significantly different. Means separated using LS means (SAS Institute 2003)

Table 1.4 Mean ± SD number of insects per two minutes in each feeding guild observed feeding from flowers[1] 21 June – 16 August. Goldsboro, N.C. 2005

Plant Species	Time of Day	Parasitoids	Non-Crop Parasitoids	Deleterious Parasitoids	Herbivores Non-Crop	Herbivores Crop Pests	Deleterious Predators	Predators	Pollinators
Fennel	9:30	$1.0 \pm 1.3_{A, A}$	$0.1 \pm 0.3_{AB, AB}$	$0.0 \pm 0.3_{A, A}$	$0.2 \pm 0.4_{A, A}$	$0.3 \pm 0.5_{A, A}$	$0.2 \pm 0.5_{A, A}$	$3.0 \pm 2.4_{AB, AB}$	$2.5 \pm 2.5_{B, BC}$
Buckwheat	9:30	$0.2 \pm 0.6_{B, A}$	$0.8 \pm 1.5_{A, A}$	$0.1 \pm 0.3_{A, A}$	$0.1 \pm 0.2_{A, A}$	$0.2 \pm 0.4_{A, A}$	$0.0 \pm 0.0_{A, A}$	$2.0 \pm 1.8_{BC, B}$	$5.1 \pm 3.2_{A}$
Yarrow	9:30	$0.0 \pm 0.0_{B, A}$	$0.0 \pm 0.0_{B, B}$	$0.0 \pm 0.1_{A, A}$	$0.1 \pm 0.3_{A, A}$	$0.3 \pm 0.4_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.2 \pm 0.4_{F, F}$	$1.1 \pm 1.4_{CD, CD}$
Celosia	9:30	$0.0 \pm 0.0_{B, A}$	$0.0 \pm 0.0_{B, B}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.2 \pm 0.3_{A, A}$	$0.0 \pm 0.0_{A, A}$	$1.5 \pm 0.8_{C, D}$	$0.9 \pm 1.0_{D, D}$
Black-eyed Susan	9:30	$0.0 \pm 0.0_{B, A}$	$0.0 \pm 0.1_{B, B}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.4 \pm 0.6_{F, F}$	$4.3 \pm 2.2_{A}$
Fennel	12:00	$0.3 \pm 0.4_{A, B}$	$0.7 \pm 0.9_{AB, AB}$	$0.0 \pm 0.0_{A, A}$	$0.2 \pm 0.5_{A, A}$	$0.1 \pm 0.3_{A, A}$	$0.4 \pm 0.5_{A, A}$	$3.8 \pm 2.0_{A, AB}$	$2.2 \pm 1.8_{BC, B}$
Buckwheat	12:00	$0.0 \pm 0.0_{A, B}$	$0.9 \pm 1.8_{A, A}$	$0.3 \pm 0.5_{A, A}$	$0.1 \pm 0.2_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.1_{A, A}$	$1.2 \pm 1.2_{E, D}$	$0.7 \pm 1.0_{D, D}$
Yarrow	12:00	$0.0 \pm 0.1_{A, B}$	$0.1 \pm 0.3_{B, B}$	$0.2 \pm 0.4_{A, A}$	$0.4 \pm 0.7_{A, A}$	$0.4 \pm 0.4_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.7 \pm 1.5_{EF, F}$	$2.6 \pm 2.8_{BC, B}$
Celosia	12:00	$0.0 \pm 0.0_{A, B}$	$0.1 \pm 0.3_{B, B}$	$0.0 \pm 0.0_{A, A}$	$0.1 \pm 0.2_{A, A}$	$0.2 \pm 0.3_{A, A}$	$0.0 \pm 0.0_{A, A}$	$2.0 \pm 1.7_{C, BC}$	$1.0 \pm 1.2_{CD, CD}$
Black-eyed Susan	12:00	$0.0 \pm 0.0_{A, B}$	$0.2 \pm 0.3_{B, B}$	$0.1 \pm 0.2_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.0 \pm 0.0_{A, A}$	$0.1 \pm 0.2_{F, F}$	$2.3 \pm 1.2_{B, B}$

Means within the same column followed by the same letter are not significantly different. Means separated using LS means (SAS Institute 2003)

Relative Attractiveness of Habitat Plantings to Microhymenoptera

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Abstract

Flowering habitat is used in cropping systems to provide a food source in the form of nectar or pollen to natural enemies of agricultural insect pests. This study was conducted in August 2005 to determine the relative attractiveness of floral habitat to three families of microhymenopteran egg parasitoids: Mymaridae, Scelionidae, and Trichogrammatidae. Habitat plants were yarrow (*Achillea millefolium* L.), celosia (*Celosia cristata* L.), buckwheat (*Fagopyrum esculentum* Moench), fennel (*Foeniculum vulgare* P. Mill.), daisy (*Leucanthemum* x *superbum* (J. W. Ingram) Berg. ex Kent.), and black-eyed Susan (*Rudbeckia hirta* L.). Non-flowering crabgrass (*Digitaria sp.* Haller) served as a control. Sticky traps were used to monitor microhymenoptera and were placed at three heights: flower height, 0.5 times flower height, and 1.5 times flower height. Flower heads were removed from half of each plot and traps were placed in the center of each subplot. Trapped microhymenoptera were counted with the expectation that greater numbers would be trapped in subplots with flowers intact at flower height if flowers were indeed attractive. Results from this experiment show that flower species and height affected all three families of microhymenoptera but flower removal only affected scelionids. At flower height, scelionids were trapped in greater abundance in celosia plots at flower height in flowers-present versus flowers-removed treatments. Trichogrammatids were trapped in greatest abundance at 0.5 times flower height in un-mowed crabgrass plots and mymarids were most abundant at 0.5 times flower height in black–eyed Susan plots. Our results indicate that habitat plantings may attract microhymenoptera but that flowers themselves do not appear to be responsible for this attraction.

Introduction

A wide variety of predators and parasitoids are relied upon for biological control of insect crop pests. Microhymenopteran parasitoids, in particular, can play a crucial role in reducing crop pest numbers. Egg parasitoids can be especially important since pests are killed before feeding-damage to crops can occur. Numerous studies have been conducted using direct observation to determine food preferences of predators and parasitoids (Jervis et al. 1993; Carreck and Williams 1997; Al-Doghairi and Cranshaw 1999; Colley and Luna 2000). However, microhymenopteran parasitoids are minute, making direct observation of feeding very difficult.

Parasitic microhymenoptera have short mouthparts. Because of this, floral architecture and nectar accessibility play an important role in determining the attractiveness and suitability of flowering habitat to microhymenoptera. Plants in the carrot family (Apiaceae) and the buckwheat family (Polygonaceae) have been determined to successfully provide resources to microhymenoptera and other short-tongued beneficial insects such as hoverflies, because of their small florets and exposed nectaries (Lövei et al. 1993; Proctor et al. 1996; Tooker and Hanks 2000). In a study examining floral architecture preferences of two microhymenopteran parasitoids in the family Eulophidae Patt et al. (1997) found parasitoids foraged more effectively on flowers with open, easily-accessible nectaries. Maingay et al. (1991) collected hundreds of individuals of numerous species of entomophagous and parasitic hymenoptera feeding from sweet fennel (*Foeniculum vulgare* P. Mill. var. *dulce* Battandier & Trabut) (Apiaceae). Stephens et al. (1998) found increased parasitism and higher numbers of a braconid parasitoid in orchard understories sown with buckwheat (*Fagopyrum esculentum* Moench) than in bare ground controls. In a greenhouse

experiment, English-Loeb et al. (2003) found higher egg parasitism by *Anagrus* parasitoids (Hymenoptera: Mymaridae) caged on buckwheat flowers with flowers present than those caged on buckwheat with inflorescences removed. Irvin et al. (2000) found parasitoids to be seven times more abundant in buckwheat plantings with flowers present than in those with flowers removed. No difference was found between buckwheat plants with flowers removed and an herbicide-treated control, indicating that floral resources rather than vegetative properties of buckwheat were responsible for attraction of parasitoids to the plants.

However, not all studies using flowering plants to enhance numbers of microhymenopteran parasitoids have proved successful. For example, Berndt et al. (2002) examined abundance of two leafroller parasitoids in buckwheat plantings compared to grass and clover controls. No difference in abundance of *Glyptapanteles demeter* (Wilkinson) (Hymenoptera: Braconidae) was found and only significantly higher numbers of male *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) parasitoids were trapped in buckwheat plantings. In a review by Heimpel and Jervis (2005) an increase of parasitism was observed in only seven out of twenty studies comparing floral habitat to controls. Additionally, only one out of the twenty studies showed a decline in pest numbers. This indicates that even if microhymenoptera are attracted to flowering habitat, a decrease in pest density is not guaranteed.

The present study was conducted to indirectly measure the relative attractiveness of different flowering plants to microhymenopteran egg parasitoids in the families Mymaridae, Scelionidae, and Trichogrammatidae in North Carolina. Plants for this study were chosen because of their floral morphologies or because of their prevalence in scientific literature.

Materials and Methods

Research site. Research was conducted on the Small Farm Unit at the Center for Environmental Farming Systems near Goldsboro, N.C. The Small Farm Unit is a highly diverse organic farm approximately 6.07 ha in size. A wide variety of commodities are grown at the Small Farm Unit including vegetable, flower, and small fruit crops. Some livestock production, including chickens, turkeys, and goats also occurs on the Small Farm Unit.

Experimental design. Measurements of the abundance of microhymenoptera in habitat plantings were collected from three replicates, each measuring 56.4 x 2.7 m, divided into seven 6.1 x 2.7 m plots. Replicates were separated from one another by an average distance of 48.2 m. Plots were laid out in the following order from the northeast to the southwest: celosia (*Celosia cristata* L.), fennel (*Foeniculum vulgare* P. Mill.), yarrow (*Achillea millefolium* L.), black-eyed Susan (*Rudbeckia hirta* L.), buckwheat (*Fagopyrum esculentum* Moench), and daisy (*Leucanthemum* x *superbum* (J. W. Ingram) Berg. ex Kent.). A plot at southwest end of each replicate dominated by naturally occurring crabgrass (*Digitaria sp.* Haller) served as a control.

Celosia, black-eyed Susan, and daisy plants were transplanted into plots in three rows with plants spaced 30.5 cm apart and 46 cm between each row using hand trowels and bulb diggers on 25 May, 2005. Buckwheat (Jeffrey's Seed Co., 1608 US 117 South, Goldsboro, NC 27503) was hand-seeded at a rate of 56.04 kg/ha. Fennel and yarrow plants were planted in 2003 as previously described (Chapter 1).

All flower heads were removed from half of each treatment plot using pruning shears and half of each control plot was mowed on 29 July, 2005. A coin toss was used to

determine from which side of the plot to remove plants. Flower removal prior to bud-break and mowing occurred for the remainder of the study.

Plant management. Celosia, black-eyed Susan, and daisy plants (See Table 1.1 for cultivars) were grown in the Biological Control greenhouse at North Carolina State University, Raleigh, NC. Heating and ventilation set points were 21.1º C and 26.7º C, respectively. Seeds (Germania, 5978 N Northwest Hwy, PO Box 31787, Chicago, IL 60631-0787) were planted in 96-cell round plug trays (3.8 x 3.9 cm, Hummert International, 4500 Earth City Expressway, Earth City, MO 63045) filled with moistened Metro-Mix 200 potting soil (Scotts-Sierra Horticulture Products Co., The Scotts Company, 1411 ScottsLawn Rd., Marysville, OH 43041) in late March of 2005. Plants were watered as needed with a misting bed and/or hand watering. Trays were placed under high intensity metal halide lights with an 11 h photophase. Photophase was extended to 16 h on 22 April, 2005. Plants were transplanted to 473 ml plastic cups (Kmart Corporation, Troy, MI 48084) with a drainage hole drilled in the bottom using a 1.3 cm drill bit when roots were established and aboveground portions were of sufficient size.

Prior to transplanting, plots were tilled and celosia, black-eyed Susan, and daisy plots as well as the borders surrounding all plots were covered with woven black plastic ground cover (Wyatt-Quarles Seed Company, 730 Hwy 70 West, Garner, NC 27529) secured with landscape anchor pins (DuPont™ Garden Products™, Chestnut Run Plaza, Bldg. 728, PO Box 80728, Wilmington, DE, 19880-0728) to suppress weeds and preserve soil moisture. Plants were planted through holes cut in the ground cover. Watering occurred as needed and weeds were managed with hand-pulling inside plots and mechanical mowing around plots.

Sampling. Microhymenoptera were monitored with traps made from 51 mm sections

of 19 mm diameter PVC pipe spray painted with yellow plastic enamel (The Valspar Corporation, Wheeling, IL 60090) and wrapped with tanglefoot-coated clear acrylic sheets (Great Lakes IPM, 10220 Church Rd. NE, Vestaburg, MI 48891-9746). In each subplot, traps were placed on a single stake at three heights: 0.5 the height of flowers, flower height, and 1.5 times flower height. Traps were secured to plastic stakes and were changed twice weekly from 9 August to 16 August, 2005.

Immediately following collection, traps were returned to the laboratory where tanglefoot-coated acrylic sheets were removed from PVC sections, sandwiched between two sheets of clear plastic wrap (Kmart Corporation, Troy, MI 48084), and placed in plastic freezer bags (1 qt., Hefty®, Pactiv Corp., 1900 W Field Ct., PO Box 5032, Lake Forest, IL 60045) for storage in a freezer at -20º C. Using a dissecting microscope (Leica, Wild MZ8, Leica Microsystems GmbH, Ernst-Leitz-Strasse 17-37, 35578 Wetzlar) the number of individuals in the families Mymaridae, Scelionidae, and Trichogrammatidae on each sheet was recorded.

Data analysis. Abundance data were square root transformed prior to analyses. Data were analyzed to determine the effects of flower species, flower removal, and trap height on abundance of microhymenoptera in habitat plantings using general linear models (PROC GLM) and least significant difference (LSD) tests of means (SAS, 2003). Type III Sums of Squares are presented in Appendix 2.1-2.2 and t-groupings from LSD tests are presented in Table 2.1.

Results

Flower species significantly affected abundance of mymarids and trichogrammatids but not scelionids (F = 11.81, df = 5, 10, P = 0.0006; F = 13.45, df = 5, 10, P = 0.0004; F =

1.83, df = 5, 10, $P = 0.1947$) (Appendix 2.1). Height ($F = 21.47$, df = 2, 44, $P < 0.0001$; $F = 25.51$, df = 2, 44, $P < 0.0001$; $F = 8.25$, df = 2, 44, $P = 0.0009$) and the interaction between flower species and height played a significant role in abundance of mymarids, scelionids, and trichogrammatids ($F = 7.24$, df = 10, 44, $P < 0.0001$; $F = 6.69$, df = 10, 44, $P < 0.0001$; $F = 4.17$, df = 10, 44, $P = 0.0004$). The interaction between flower species and flower removal significantly affected trichogrammatids ($F = 7.16$, df = 5, 12, $P = 0.0026$) but not mymarids or scelionids ($F = 0.56$, df = 5, 12, $P = 0.7280$; $F = 1.35$, df = 5, 12, $P = 0.3104$). Flower removal and the interaction between flower removal and height significantly affected abundance of scelionids ($F = 6.76$, df = 1, 12, $P = 0.0232$; $F = 6.20$, df = 2, 44, $P = 0.0042$). Flower removal and the interaction between flower removal and height did not significantly affect abundance of mymarids ($F = 1.62$, df = 1, 12, $P = 0.2266$; $F = 2.26$, df = 2, 44; $P = 0.1167$) or trichogrammatids ($F = 0.18$, df = 1, 12, $P = 0.6818$; $F = 0.41$, df = 2, 44, $P = 0.6672$). There was a significant three way interaction between flower species, flower removal, and height for scelionids and trichogrammatids ($F = 2.64$, df = 10, 44, $P = 0.0130$; $F = 2.28$, df = 10, 44, $P = 0.0298$), but not for mymarids ($F = 1.69$, df = 10, 44, $P = 0.1123$).

Among the different heights, a significant flower effect was found for mymarids, scelionids, and trichogrammatids at height 2 (flower height) ($F = 5.08$, df = 5, 10, $P = 0.0141$; $F = 4.70$, df = 5, 10, $P = 0.0182$; $F = 5.78$, df = 5, 10, $P = 0.0092$) and height 1 (0.5 times flower height) ($F = 12.55$, df = 5, 10, $P = 0.0005$; $F = 3.24$, df = 5,10, $P = 0.0536$; $F = 22.38$, df = 5, 10, $P < 0.0001$) (Appendix 2.1). At the height 3 (1.5 times flower height), there was a significant flower effect on abundance of trichogrammatids ($F = 5.58$, df = 5, 10, $P = 0.0103$) but not on abundance of mymarids ($F = 2.56$, df = 5, 10, $P = 0.0965$) or scelionids ($F = 1.04$, df = 5, 10, $P = 0.4479$).

Discussion

Abundance of microhymenoptera caught on sticky traps was used as an indirect indicator of relative attractiveness of each plant species to the three parasitoid families studied. The assumption was made that if flowers were attractive to microhymenoptera, a greater number would be caught at height 2 (the height of flower heads) in the subplots where flowers had not been removed. Crabgrass was chosen as the control for this study because it offered a vegetative habitat without flowers. It was assumed that if flowers were attractive, more microhymenoptera would be caught in plots containing flowering habitat than in non-flowering controls.

Each microhymenopteran family responded differently to the plants in this study (Table 2.1). Mymarids were found in greatest abundance at height 1 in black-eyed Susan plots. Scelionids were most abundant in celosia plots at height 2. The greatest number trichogrammatids were trapped in crabgrass control plots both at height 1 and height 3. None of the flowers determined to attract microhymenoptera belong to the families Apiaceae or Polygonaceae. These findings are significant because both fennel and buckwheat have been heralded as suitable beneficial insect habitat (Maingay et al. 1991; Stephens et al. 1998; Irvin. et al. 2000; English-Loeb et al. 2003). Similar to the present findings, past work on the Small Farm Unit found abundance and diversity of natural enemies sampled from various cut flower and herb species to be lowest in plots containing pure stands of fennel and highest in celosia (Forehand 2004).

Little evidence was found in this study that flower removal affected the number of wasps caught on traps. For the majority of the plant species tested, numbers of trapped microhymenoptera were the same in subplots where flowers were present compared to

subplots where flowers had been removed. Only scelionids were found in greater abundance at flower height in celosia plots where flowers remained intact (Table 2.1). This finding was similar to that of Rebek et al. (2005) who found that the removal of inflorescences from four species of flowering plants in an ornamental landscape had no effect on abundance of natural enemies collected on sticky cards. Both these studies contradict results of Irvin et al. (2000) who found greater abundance of the leafroller parasitoid *Dolichogenidea tasmanica* in buckwheat plantings with flowers present than in plantings where flowers had been removed indicating an attraction to floral structures.

Overall, the abundance of sampled microhymenoptera in this study was not different in flower plots compared to control (crabgrass) plots. Scelionids and mymarids were found in greater numbers in a few plots containing flowering plants than in the control plots. Of these plots, mymarids were solely found in higher numbers halfway below the flower of black-eyed Susan and scelionids in greater abundance in celosia plots at height 2 (Table 2.1). These findings suggest the flowers themselves were not attractive to mymarids. English-Loeb et al. (2003) found parasitism by mymarids to increase in the presence of buckwheat flowers. However, mymarids were caged on buckwheat putting them in close proximity to flowers. In the field, mymarids may not be able to locate flowers because of their reduced wings. Scelionids showed preferential attraction to celosia plantings at flower height indicating a possible attraction to floral structures. Overall, scelionids are larger in body size and have more well-developed wings than mymarids or trichogrammatids. This could allow scelionids to preferentially locate floral food resources due to greater flight ability. At height 2, trichogrammatids were most abundant in yarrow plots where flowers had been removed (Table 2.1). Trichogrammatids were most abundant at height 1 in un-mowed control plots

but were also highly abundant in mowed crabgrass control plots and buckwheat plots where flowers had been removed (Table 2.1). This shows that while trichogrammatids appeared to be attracted to some habitats, flowers were clearly not responsible for this attraction.

Future field studies could be conducted to investigate which vegetative qualities of plants, rather than flowers, determine relative attraction to microhymenoptera. If vegetative habitat is attractive to different microhymenoptera, it would be useful to determine which habitats are preferred. In the current study, mean numbers of trichogrammatids were significantly greater within the canopy (height 1) of un-mowed crabgrass plots than in the canopy of any other plant species studied (Table 2.1). Using paper models of plant foliage Lukianchuk and Smith (1997) determined that female *T. minutum* Riley had a greater foraging success on simple rather than complex surfaces. It may be that the vegetative qualities of grass in this study exhibited a less complex structure than the foliage of the flowering plants. Trichome-density on plant surfaces could have played a role in preference of some plants over others. Keller (1987) determined that walking speed of *T. exiguum* was influenced by leaf-trichome form and density, with less-densely pubescent leaves permitting the fastest walking speeds. Measures of trichome-density and type are generally used to evaluate host-finding ability of parasitoids but could be important if trichomes impede location of food sources. Quantification of foliar trichomes could also be valuable since trichomes can provide shelter to microhymenoptera (Cortesero et al. 2000). In the present study, mymarids were found in greatest abundance in black-eyed Susan plots at height 1 regardless of flower presence or absence. Black-eyed Susan and celosia in our plots were similar with regard to height, leaf size and shape, amount of foliage, and canopy closure. Black-eyed Susan foliage was densely covered with trichomes while celosia foliage was

glabrous. This observation further strengthens the argument that presence of foliar trichomes may be a factor in attraction of mymarids to black-eyed Susan.

In a study by Thorpe (1985) vegetation type (soybeans vs. weedy margins) was not determined to be an important factor in parasitism rates by *Trichogramma minutum* Riley or *T. pretiosum* Riley. However, height was determined to be important, with higher levels of egg parasitism by *T. minutum* at greater heights and higher parasitism by *T. pretiosum* at lower heights. Microhymenoptera in the current study were trapped at different heights relative to the height of flowers. Because different plant species bloomed at different heights, conclusions about flight-level preferences of different microhymenoptera could not be drawn. Future research could be conducted with traps placed at constant heights relative to ground-level in plots containing flowering and non-flowering plants. This would allow one to analyze flight behavior of different microhymenoptera in varied habitats relative to a constant height. In the present study, celosia and black-eyed Susan bloomed at approximately equal heights. Fennel flowers were well-above and yarrow flowers well below celosia and black-eyed Susan inflorescences. The effects of these height differences could have played a role in the results obtained in the present study if microhymenoptera were present in fennel plots at the approximate height of celosia flowers but traps were not placed there to monitor activity.

While this study did not directly quantify attraction of microhymenoptera to habitat, some insight to habitat preference was obtained using relative measures of abundance. Microhymenoptera were not found in greater abundance in plantings containing members of the families Apiaceae or Polygonaceae. Higher numbers of only one microhymenopteran family were found at flower height in only one plant species, celosia, a member of the

pigweed family, Amaranthaceae. Abundance of trapped microhymenoptera varied with plant species at different trap heights for each hymenopteran family. This indicates that while habitat appears to play an important role in abundance of microhymenoptera for the most part floral food resources do not appear to be the causative agent.

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Table 2.1 Mean ± SD number of parasitoids caught on yellow sticky traps placed at three different heights in plots with flowers present or mechanically removed from five plant species

Parasitoid Family	Plant Species	Trap Placed at 0.5 Plant Height		Trap Placed at Plant Height		Trap Placed at 1.5 Plant Height	
		Flowers	No Flowers	Flowers	No Flowers	Flowers	No Flowers
Mymaridae							
	Black-eyed Susan	$10.8 \pm 5.6_{A}$	$11.7 \pm 7.7_{A}$	$2.0 \pm 1.6_{A}$	$3.2 \pm 2.8_{A}$	$2.3 \pm 1.36_{A}$	$1.3 \pm 1.3_{A}$
	Buckwheat	$2.1 \pm 2.3_{B}$	$3.4 \pm 2.3_{B}$	$2.6 \pm 2.7_{A}$	$1.4 \pm 1.4_{BC}$	$1.0 \pm 0.87_{BC}$	$1.4 \pm 1.1_{A}$
	Celosia	$2.6 \pm 1.2_{B}$	$2.8 \pm 2.4_{B}$	$2.2 \pm 1.1_{A}$	$2.2 \pm 1.3_{AB}$	$0.8 \pm 0.83_{C}$	$1.3 \pm 1.4_{A}$
	Crabgrass (Control)*	$2.2 \pm 2.4_{B}$	$2.7 \pm 1.7_{B}$	$1.4 \pm 1.2_{A}$	$2.2 \pm 1.3_{AB}$	$1.8 \pm 1.39_{AB}$	$0.6 \pm 1.3_{A}$
	Fennel	$1.6 \pm 1.3_{B}$	$1.3 \pm 1.0_{B}$	$0.8 \pm 1.6_{A}$	$0.7 \pm 0.7_{C}$	$1.1 \pm 1.17_{BC}$	$1.3 \pm 1.4_{A}$
	Yarrow	$1.4 \pm 1.4_{B}$	$1.7 \pm 1.2_{B}$	$1.8 \pm 1.4_{A}$	$4.0 \pm 2.1_{A}$	$1.4 \pm 0.73_{ABC}$	$2.1 \pm 2.8_{A}$
Scelionidae							
	Black-eyed Susan	$5.1 \pm 3.2_{A}$	$2.6 \pm 2.6_{A}$	$16.8 \pm 7.5_{AB}$	$10.0 \pm 3.0_{AB}$	$4.0 \pm 2.9_{A}$	$3.1 \pm 1.5_{A}$
	Buckwheat	$3.9 \pm 2.1_{A}$	$4.3 \pm 3.5_{A}$	$5.0 \pm 4.2_{C}$	$2.3 \pm 2.4_{C}$	$2.3 \pm 1.5_{A}$	$2.7 \pm 1.3_{A}$
	Celosia	$6.1 \pm 6.1_{A}$	$2.2 \pm 2.2_{A}$	$26.3 \pm 21.6_{A}$	$6.1 \pm 4.4_{ABC}$	$2.3 \pm 2.6_{A}$	$3.1 \pm 2.5_{A}$
	Crabgrass (Control)*	$3.7 \pm 3.3_{A}$	$5.3 \pm 4.6_{A}$	$6.6 \pm 6.0_{BC}$	$2.9 \pm 2.7_{C}$	$3.2 \pm 3.3_{A}$	$3.6 \pm 3.2_{A}$
	Fennel	$9.0 \pm 3.7_{A}$	$5.3 \pm 4.2_{A}$	$4.2 \pm 3.9_{C}$	$4.4 \pm 3.1_{B}$	$2.9 \pm 2.2_{A}$	$4.3 \pm 1.9_{A}$
	Yarrow	$11.6 \pm 6.3_{A}$	$5.9 \pm 3.7_{A}$	$7.4 \pm 4.4_{BC}$	$11.2 \pm 6.0_{A}$	$4.3 \pm 2.7_{A}$	$4.2 \pm 2.7_{A}$
Trichogrammatidae							
	Black-eyed Susan	$5.6 \pm 2.6_{BC}$	$5.6 \pm 4.8_{AB}$	$4.2 \pm 2.2_{BC}$	$5.6 \pm 4.4_{B}$	$1.9 \pm 0.9_{B}$	$2.0 \pm 1.8_{C}$
	Buckwheat	$4.8 \pm 3.1_{BC}$	$14.9 \pm 10.4_{A}$	$9.7 \pm 4.9_{A}$	$6.1 \pm 2.5_{B}$	$3.3 \pm 1.3_{AB}$	$5.0 \pm 3.3_{B}$
	Celosia	$0.9 \pm 1.17_{C}$	$1.7 \pm 1.0_{B}$	$3.8 \pm 3.2_{BC}$	$4.9 \pm 3.2_{B}$	$2.6 \pm 2.1_{B}$	$3.3 \pm 1.4_{BC}$
	Crabgrass (Control)*	$32.4 \pm 19.1_{A}$	$4.9 \pm 18.9_{A}$	$9.3 \pm 6.6_{AB}$	$7.2 \pm 3.5_{A}$	$12.7 \pm 26.5_{A}$	$4.8 \pm 3.7_{B}$
	Fennel	$2.4 \pm 2.1_{C}$	$2.8 \pm 2.5_{B}$	$2.2 \pm 1.5_{C}$	$2.3 \pm 2.50_{C}$	$2.0 \pm 1.5_{B}$	$2.3 \pm 1.0_{BC}$
	Yarrow	$13.9 \pm 8.0_{B}$	$6.7 \pm 4.2_{AB}$	$5.1 \pm 3.7_{B}$	$13.67 \pm 6.7_{A}$	$4.4 \pm 2.5_{AB}$	$8.6 \pm 3.8_{A}$

Means within the same column followed by the same letter are not significantly different. Means separated using LS means (SAS Institute 2003)

* Crabgrass served as a non-flowering control. 'Flowers' in the column-heading represent un-mowed subplots and 'no flowers' represent mowed subplots.

Effects of Food Type on Longevity and Fecundity of

Trichogramma exiguum and Longevity of *Cotesia congregata*

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Abstract

Hymenopterous parasitoids useful to biological control of agricultural pests can gain fitness benefits when provided with food resources. This study was conducted to determine the effect of different food sources on the longevity and fecundity of *Trichogramma exiguum* Pinto & Platner and the longevity of *Cotesia congregata* (Say). Newly eclosed (<12 h) female wasps were provisioned with one of two treatments; fennel (*Foeniculum vulgare* P. Mill.) or buckwheat (*Fagopyrum esculentum* Moench) flowers or one of two controls; honey or water. Wasps were monitored daily until all had died. Fecundity of *T. exiguum* was monitored using *Ephestia kuehniella* Keller egg cards. Longevity was greatest in *T. exiguum* provisioned with honey and in *C. congregata* provisioned with buckwheat flowers. Buckwheat provisioned *T. exiguum* exhibited greater longevity than those provided fennel. Longevity of *C. congregata* provisioned with fennel and honey was approximately equal. Water provisioned *T. exiguum* and *C. congregata* exhibited the shortest longevity. Total fecundity was greatest in *T. exiguum* provisioned with honey or buckwheat. Average female to male ratio over the lifetime of each female was greatest in *T. exiguum* provisioned with water alone, likely because of sperm limitation in wasps exhibiting greater longevity. Total average number of female offspring produced was greatest in *T. exiguum* provided honey or buckwheat flowers although no difference in total female offspring were observed between adults provisioned with buckwheat or fennel flowers. Our results show that provisioning *T. exiguum* with honey and buckwheat flowers caused greater longevity, total fecundity, and lifetime production of female offspring than water alone. Buckwheat flowers also lead to greater longevity in *C. congregata.*

Introduction

The majority of adult hymenopterous parasitoids benefit from carbohydrate food resources. Both longevity and fecundity of parasitoids can increase in the presence of non-host food sources. Feeding is even obligatory in some parasitoids before egg maturation can occur (Jervis and Kidd 1986). Parasitoids can obtain carbohydrates in the field from homopteran honeydew, floral, and extrafloral nectar. These foods are often presented to parasitoids in the form of floral habitat as many flowers produce nectar and may host insects that exude honeydew.

Several laboratory studies have shown that adult hymenopterous parasitoids exhibit increased longevity when provisioned with a sugar source. In a 2001 study, Wäckers tested fourteen sugars to determine the effects on longevity of *Cotesia congregata* (Say). Longevity of wasps provisioned with the three most commonly occurring nectar-sugars sucrose, glucose, and fructose was determined to be 15 times greater than that of wasps provided water alone. In 1999, Baggen et al. determined that longevity of an encyrtid parasitoid was increased significantly when caged on flowers of dill (*Anethum graveolens* L.), buckwheat (*Fagopyrum esculentum* Moench), and faba bean (*Vicia faba* L.). In a previous study, Baggen and Gurr (1998) found a significant increase in longevity of the same parasitoid species caged on dill, borage (*Borago officinalis* L.), or coriander (*Coriandrum sativum* L.) and found higher rates of parasitism in potato crops located adjacent to these flowering plants than in crops 20 m from flowers. Irvin et al. (1999) found that mixed bouquets of buckwheat and coriander increased the longevity of the males but not females of a leafroller parasitoid, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae).

Both males and females had significantly higher longevity when provided honey-water or buckwheat flowers compared to those provided water alone.

A number of studies have shown that an increase in fecundity can occur when wasps are provided food (e.g. Ashley and Gonzalez 1974; Yu et al. 1984; Hagley and Barber 1992; Idris and Grafius 1995; Leatemia et al. 1995; Shearer and Atanassov 2004). While providing food resources to pro-ovigenic species may not lead to a direct increase in fecundity, increased longevity may indirectly lead to increased parasitism due to an extension of the amount of time available to encounter hosts (Thompson 1999). This has been illustrated in several studies examining the reproductive output of hymenopteran parasitoids during their first few days of life. Berndt and Wratten (2005) examined the effects of sweet alyssum (*Lobularia maritima* (L.) Desv.) flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. They found that longevity increased seven-fold when female parasitoids were provisioned with alyssum plants with flowers compared to those provided plants with flowers removed. They also found that lifetime fecundity increased significantly when flowers were present. However, daily fecundity in the first three days of life (the time period that females in controls survived) remained approximately equal. Results of a study conducted by Leatemia et al. (1995) found similar results when studying *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) provided different food sources. Lifetime fecundity was increased dramatically in honey-fed versus unfed females; however reproductive output was approximately equal during the first two days of a female's life in both treatments and controls.

In biological control programs, female parasitoids are much more valuable than males because only females are able to directly reduce pest numbers through parasitization. In

addition to examining fecundity of parasitoids provided different food sources, the studies by Berndt and Wratten (2005) and Leatemia et al. (1995) examined the male to female ratio of offspring produced. Berndt and Wratten found a strong bias towards male offspring when female parents were denied sweet alyssum flowers. Female parents provided sweet alyssum flowers produced an approximately equal number of males and females at the beginning of the study but produced more male offspring as they aged. The findings of this study contrast a previous study conducted by Berndt et al. (2002) where *D. tasmanica* reared from leafroller cocoons in close proximity to flowering buckwheat exhibited a higher female to male ratio than those reared from cocoons collected from control plots. Leatemia et al. found approximately equal male to female ratios in fed and unfed *T. minutum* females early in life. However, a strong male bias was observed in the lifetime sex ratio in offspring produced by parents who were provided a carbohydrate source than unfed and water-fed parents because of exclusive production of male offspring after the 6th day of oviposition. A shift towards male offspring later in life is not uncommon in parasitoids. Female parasitoids are limited in the amount of sperm available for fertilization and tend to use sperm shortly after mating (King 1987).

The objective of this study was to examine the effects of different carbohydrate food sources on the longevity, fecundity, and sex ratio of *Trichogramma exiguum* Pinto & Platner (Hymenoptera: Trichogrammatidae) and the longevity of *Cotesia congregata* (Say) (Hymenoptera: Braconidae). *Trichogramma exiguum* is an egg parasitoid of many agricultural insect pests, including tomato and tobacco hornworms, *Manduca sexta* L. and *M. quinquemaculata* (Haworth) (Suh et al. 2000). *Cotesia congregata* is a larval parasitoid of caterpillar pests in the family Sphingidae (Le et al. 2003). Results from this study could help

growers in North Carolina choose floral habitat that could benefit these parasitoids and subsequently reduce agricultural pest numbers by providing food resources.

Materials and Methods

Source of Seed. Buckwheat seed was purchased from Jeffrey's Seed Co. (1608 US 117 South, Goldsboro, NC 27503. Daisy (*Leucanthemum* x *superbum* (J. W. Ingram) Berg. ex Kent.) and fennel (*Foeniculum vulgare* P. Mill.) seed were purchased from Germania (5978 N Northwest Hwy, PO Box 31787, Chicago, IL 60631-0787).

Experimental design. Longevity and fecundity of *T. exiguum* were estimated in cages using a randomized complete block design, with position of cages on shelves in a rearing-room acting as blocks. The experiment was repeated forty-three times. Provision of fennel or buckwheat flowers were the two treatments, with provision of water or honey-water solution acting as controls.

Longevity of *C. congregata* was determined in cages at the North Carolina State University Horticultural Field Laboratory in Raleigh, NC. The experiment was repeated fifty-two times using a randomized complete block design with position in the field acting as blocks. The same treatments and controls as the *T. exiguum* study were used. A daisy treatment was eliminated from both *T. exiguum* and *C. congregata* studies because plants could not be forced to bloom in sufficient numbers their first year.

Plant Maintenance. Plants were grown in a greenhouse with a heating set point of 21.1º C and a ventilation set point of 26.7º C. Plants were watered as needed with a misting bed and/or hand watering. Trays were placed under high intensity metal halide lights with an 11 h photophase extended to 16 h on 22 April, 2005 to promote reproductive development.

Daisy and fennel plants were started in 96-cell round plug trays (3.8 by 3.9 cm, Hummert International, 4500 Earth City Expressway, Earth City, MO 63045) filled with moistened Metro-Mix 200 potting soil (Scotts-Sierra Horticulture Products Co., The Scotts Company, 1411 ScottsLawn Rd., Marysville, OH 43041) and approximately 2 g of Osmocote fertilizer (19-6-12) per cell (Scotts-Sierra Horticulture Products Co., The Scotts Company, 1411 ScottsLawn Rd., Marysville, OH 43041) on 4 February and 10 March, 2005. Daisy and fennel plants were transplanted to quart-sized terra cotta pots on 15 March, 2005 and later to 11.35 L plastic pots (Wyatt-Quarles Seed Company, 730 Hwy 70 West, Garner, NC 27529) on 26 May, 2005. Fennel was staked using bamboo and plastic-coated twist wire (Hillman, Cincinnati, OH, 45231) on 7 June, 2005 and was cut back to the second node on 20 June, 2005 to extend blooming period. Buckwheat was planted weekly beginning 18 April, 2005 into moistened Metro-Mix 200-filled 473 ml plastic cups (Kmart Corporation, Troy, MI 48084) with a drainage hole drilled in the bottom using a 1.3 cm drill bit. Seedlings were thinned to one plant per cup.

Half the fennel and daisy plants for *C. congregata* field trials were transferred from the greenhouse to the Horticultural Field Laboratory on 20 June, 2005. Fennel was held outdoors in a sheltered area for 24 h to harden off before being transplanted into beds. In an attempt to promote blooming, a quarter of the total daisy plants were held in a walk-in cooler at 4.4° C.

Beds were tilled prior to transplanting. Fennel and daisies were planted in two rows per bed with approximately 46 cm spacing between plants on 27 June, 2005. Buckwheat seed was broadcast weekly into 0.3 m^2 plots and incorporated using a steel rake. Plants were watered three times weekly via drip tape with supplemental water added as necessary.

Plant Pest Management. Plants in the greenhouse were monitored for signs of pest infestation. Daisy foliage was sprayed for aphids with 3% ai Sunspray® ultra-fine horticultural oil (Sure-Grow Research, 7265 Hwy 95, Centre, AL 35960) on 5, 9, and 31 May, 2005. Fennel foliage was sprayed on 31 May, 2005. Fennel and daisy plants were sprayed with Safer® insecticidal soap (69 N Locust St, Lititz, PA 17543) on 7, 16, 24, and 28 June, 2005. Daisies were sprayed with 15 ml/3.78 L 2.5% ai Permethrin (Spectracide® Bug Stop, Spectrum Group, Division of United Industries Corp., PO Box 142642, St. Loius, MO, 63114-0642) on 5, 11, and 29 July, 2005.

Source of insects. *Trichogramma exiguum* used in this study were reared from hornworm (*Manduca spp.*) eggs collected from tomato plants at the Small Farm Unit of the Center for Environmental Farming Systems, near Goldsboro, NC. Eggs were placed in plastic vials (12 by 75 mm, Fisher Scientific, Pittsburgh, PA) capped with cotton plugs then held in an environmental chamber (Percival Scientific Incorporated, 505 Research Dr., Perry, IA 50220) at 20° C, 80% RH, and a 14 h photophase until emergence. Newly eclosed (=12 h) individual *T. exiguum* females were transferred to plastic vials capped with a cotton plug. A single male from each replicate was collected so that species identity could be confirmed.

Cotesia congregata for this study were reared from field-collected tomato and tobacco hornworms (*Manduca quinquemaculata* (Haworth) and *M. sexta* (L.) respectively). Larvae were collected from the Small Farm Unit, tobacco fields (Union Church Rd (SR1805) and Grady-Frye Rd (SR 1809); NC 22 at Star Ridge Road (SR 1834)), and a tomato garden (1145 Union Church Road, Carthage, NC).

Hornworm larvae were placed individually in 240 ml plastic containers covered with cheesecloth secured with a rubber band and held at 20° C, 80% RH, and a 14 h

photophase. Newly eclosed *C. congregata* (= 12 h) were placed individually in plastic vials capped with cotton. Sex was determined using a binocular microscope (Leica Wild MZ8, Leica Microsystems GmbH, Ernst-Leitz-Strasse 17-37, 35578 Wetzlar). Sub-samples were collected so that species identification could be confirmed. Females were immediately transferred to the Horticultural Field Laboratory and placed individually into an experimental cage.

Experimental cages. Food sources were provided to individual female *T. exiguum* and *C. congregata* wasps within an enclosed experimental cage (Figure 3.1). Cages were made from 7.6 cm sections of 3 mm thick clear rigid acrylic tube (Plastics and Fiberglass Products Co., 1505 Capital Blvd., Raleigh, N.C. 27603). Tops and bottoms of cages were covered with synthetic mesh screen (35 mesh/cm) (JoAnn Stores, Inc., 5555 Darrow Rd., Hudson, OH, 44236) attached to tube rims with Super Glue (Henkel Consumer Adhesives, Inc, 32150 Just Imagine Dr., Avon, OH 44011). An approximately 1 cm diameter hole was cut in the screen top to allow daily replacement of *E. kuehniella* egg cards and at the opposite side of the screen bottom an approximately 2 cm diameter hole was cut to allow insertion of flowers. The edges of screen holes were reinforced using Super Glue. These holes were plugged with cheesecloth-wrapped cotton to prevent escape of wasps.

Longevity. All experiments using *T. exiguum* were carried out in a rearing room at 25° C, 75% RH, and a 16 h photophase. Potted plants were placed on shelves so that flowers were inserted between slots in the shelf above. Flower heads were positioned so that head touched the inside of the lower screen and side of cages. Flowers were replaced when approximately 50% of florets had senesced. Wasps were visually monitored when flowers were changed in order to avoid escape.

Cages holding *T. exiguum* were misted once daily using a spray bottle to provide wasps with free water by spraying perpendicularly across the top of the cage to avoid pooling. Cages selected to be water controls were provided no additional inputs in addition to the aforementioned misting and *E. kuehniella* eggs. Honey controls in *T. exiguum* trials were provided a single streak of pure honey (Best Yet, Fleming Companies, Inc., Oklahoma City, OK, 73126) approximately 50 mm long applied with an insect pin to the internal wall of the cage on the first day of each replication.

Cages holding *C. congregata* were placed in a cradle constructed from plastic-coated floral wire (1825-T Joyce Ave., Panacea Products Corp., Columbus, OH, 43219) secured to 1.2 m wooden tobacco stakes using 20.3 x 3.2 cm zinc-plated wood screws (Hillman, Cincinnati, OH, 45231) at the Horticultural Field Laboratory. A protective cover was constructed for each cage to prevent entry of rain and direct sunlight. The cover was a 26.7 cm plastic plate (Kmart Corporation, Troy, MI 48084) with a hole cut in it to allow insertion of the tobacco stake. Gray nylon tent fabric (JoAnn Stores, Inc., 5555 Darrow Rd., Hudson, OH, 44236) was secured to the edge of the plate using hot glue. Metal washers (31 mm diameter) were secured to the lower edge of the fabric using duct tape to prevent fabric from being raised by the wind. Tobacco stakes were driven into the ground using a mallet so that cages were placed at flower height and faced southwesterly.

One compound buckwheat or fennel flower was inserted into each treatment cage. Honey was swabbed onto the inside of honey-control cages using a cotton-tipped swab (Q-tip, Unilever, Trumbull, CT, 06611) in a band approximately 15 x 30 mm wide. All cages were misted twice daily at approximately 12 and 17 h using a spray bottle to provide wasps

with free water. Cages were monitored daily until all wasps had died. Longevity was recorded for each female.

Fecundity. Each female *T. exiguum* was provided one *Ephestia kuehniella* Keller egg card per day suspended from the top of the cage by a no.1 enamel insect pin (Morpho no.1, BioQuip, 2321 Gladwick St., Rancho Domingo, CA, 90220). Egg cards were fashioned from Avery® self-adhesive labels (Avery-Dennison Corp., Brea, CA) cut into sections approximately 5 x 10 mm. Labels were dipped into a vial containing 25 g UV-sterilized *E. kuehniella* eggs (Beneficial Insectary, 14751 Oak Run Rd., Oak Run, CA, 96069) in order to evenly coat the adhesive side of the label with eggs. Weekly shipments of *E. kuehniella* eggs were received and unused eggs were stored for one week in a sealed plastic container (1.4 L Serve-n-Save, Rubbermaid, 3320 W. Market St., Fairlawn, OH, 44333) suspended above a saturated salt-water solution (Top-Flo evaporated salt, Cargill, Inc., Minneapolis, MN, 55440) using shaped plastic-coated wire.

After exposure to *T. exiguum* in cages, cards were held individually in cotton-capped plastic vials at 20° C, 80% RH, and 14 h photophase. Cards were placed in a freezer at -20° C when all adults had emerged and died. The number of black eggs and adults (by sex) were counted in each vial to estimate daily fecundity. Total fecundity was estimated by summing daily fecundity over the life span of each female. Only those females that produced both female and male progeny (i.e. mated) were included in data analysis.

Data Analysis. Prior to analyses, *T. exiguum* longevity and total fecundity data were square root transformed and sex ratio data for fecundity were arcsine transformed. *Cotesia congregata* longevity data were log transformed prior to analysis. Data were analyzed to determine the effects of food source on the longevity and fecundity of *T. exiguum*

and longevity of *C. congregata.* Data were analyzed using least squares means and general linear models (PROC GLM, SAS Institute, 2003). Replicates where two or more treatments were lost (i.e. wasp escaped or fate was unknown) were not included in data analyses. Tables 3.1 and 3.2 show total n-values used in analyses for each treatment.

Results

***T. exiguum* longevity.** Food type was determined to be a significant factor in the longevity of *T. exiguum* ($F = 34.07$, df = 3, 100, $P < 0.0001$). All food sources provided to *T. exiguum* significantly differed from one another with respect to longevity (Table 3.1). *Trichogramma exiguum* provided honey had the greatest longevity while those provided only water had the lowest longevity. Buckwheat increased longevity 8.4-fold while fennel increased longevity 4.3-fold, when compared to females provided with water.

***C. congregata* longevity.** The food type provided to *C. congregata* females significantly affected longevity ($F = 47.58$, df = 3, 141, $P < 0.0001$). Longevity was on average 2.6 times greater in *C. congregata* provided buckwheat flowers than those given honey and 8.5 times greater than those given only water (Table 3.1). Longevity of *C. congregata* provided fennel and honey was not significantly different.

***T. exiguum* fecundity.** Food type provided to *T. exiguum* significantly affected total fecundity, total female offspring, and the ratio of female to male offspring produced ($F = 10.16$, df = 3, 54, $P < 0.0001$; $F = 5.98$, df = 3, 54, $P = 0.0013$; $F = 2.87$, df = 3, 36, $P = 0.0499$). Significantly more offspring were produced by females provided honey and buckwheat than those provisioned with fennel or water alone (Table 3.2). Buckwheat increased fecundity 6.3-fold while fecundity of *T. exiguum* provided fennel increased 2.5-fold. The mean percentage of female offspring produced per female was significantly higher

in *T. exiguum* provisioned solely with water than in *T. exiguum* provided honey or buckwheat. The mean percentage of females produced by *T. exiguum* provisioned with fennel did not significantly differ from any of the other treatments. The average total number of females was greatest for females provisioned with honey and buckwheat (Figures 3.4 and 3.2). Total average number of female offspring produced per adult was not significantly different between honey and buckwheat treatments or between buckwheat and fennel treatments. Fewer and approximately equal numbers of females were produced by parents provisioned with fennel or water alone (Figures 3.3 and 3.5).

Discussion

The assumption was made at the beginning of this study that wasps provisioned with water alone would display the shortest longevity while wasps provided honey would live the longest. While this was the case with *T. exiguum*, honey controls in *C. congregata* trials exhibited a much shorter longevity than expected. This was probably due to harsh environmental conditions in the field since the experiment was carried out in late summer when the weather was hot and dry. It appeared that honey crystallized on arena walls, likely rendering it unavailable as a food source to *C. congregata.*

C. congregata provided buckwheat flowers were able to survive under the previously described harsh conditions. This could be because in addition to supplying sugar, buckwheat nectar may also supply water to the wasps. It also may be that buckwheat provided *C. congregata* with a favorable microclimate. Baggen et al. (1999) conducted a similar study where *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae) parasitoids were caged on flowering plants with plants devoid of their flowers serving as a control. For future studies, we recommend using this approach in order to reduce differences in microclimates.

Buckwheat also caused increased longevity in *T. exiguum* compared to fennel and water. In this case, sugar or water supplied by the nectar was the likely cause of this increase as microclimate was less important since this study was carried out in the laboratory under ideal conditions. Although buckwheat was found to increase longevity of *T. exiguum* under laboratory conditions, this does not mean that *T. exiguum* preferentially feed from buckwheat in the field. Relative attraction of trichogrammatids and two additional microhymenopteran parasitoids was not found to be significantly higher in flowering buckwheat plots compared to plots where flowers had been removed or to non-flowering crabgrass controls (Chapter 2). Trichogrammatids may not have been attracted to buckwheat in the field because of competitive exclusion by other insects. If buckwheat nectar is depleted due to feeding by other insects, such as large hymenopteran pollinators and hoverflies (Diptera: Syrphidae) then this nectar may not be available to parasitoids even if they show improved longevity and fecundity when provisioned with buckwheat in the laboratory (Lee and Heimpel 2003).

We were interested in overall fecundity, the percentage of females produced, and the total number of females produced per *T. exiguum* female provided different food sources. While overall fecundity is important, the number of females produced is vital to biological control since only female offspring are able to reduce crop pest numbers by parasitizing of eggs. A 25-30% higher ratio of females to male offspring was observed in parents provided water than those provided buckwheat or honey (Table 3.2). This may seem counterintuitive; however females have a limited amount of sperm available for fertilization after mating. Therefore, more female offspring tend to be produced early in a female's life when sperm are most abundant (King 1987). Because *T. exiguum* provided water exhibited the shortest lifespan and lowest total fecundity, the overall percentage of female offspring produced was

high relative to total offspring produced. Our findings were similar to those of previous studies where total female to male sex ratio was higher in unfed treatments than in treatments where female parents were provided a sugar source (Leatemia et al. 1995; Berndt and Wratten 2005).

In summary, buckwheat flowers can increase longevity and fecundity of *T. exiguum* and longevity of *C. congregata.* These findings correspond with numerous other successful studies (e.g. Ashley and Gonzalez 1974; Yu et al. 1984; Hagley and Barber 1992; Idris and Grafius 1995; Leatemia et al. 1995; Gurr and Nicol 2000; Shearer and Atanassov 2004). While information on the effects of food resources on longevity and reproductive output of parasitoids are important to the study of biological control, additional studies on feeding behavior of parasitoids in the field is needed.

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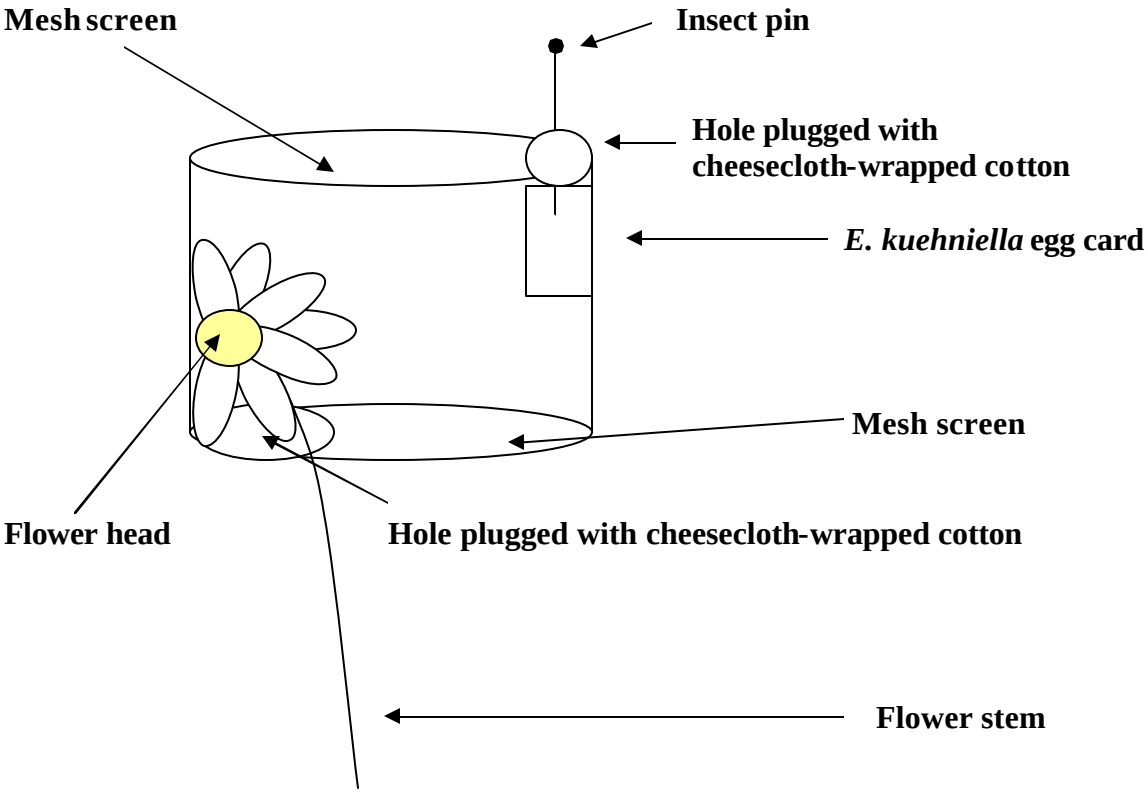

Figure 3.1 Diagram of experimental cage

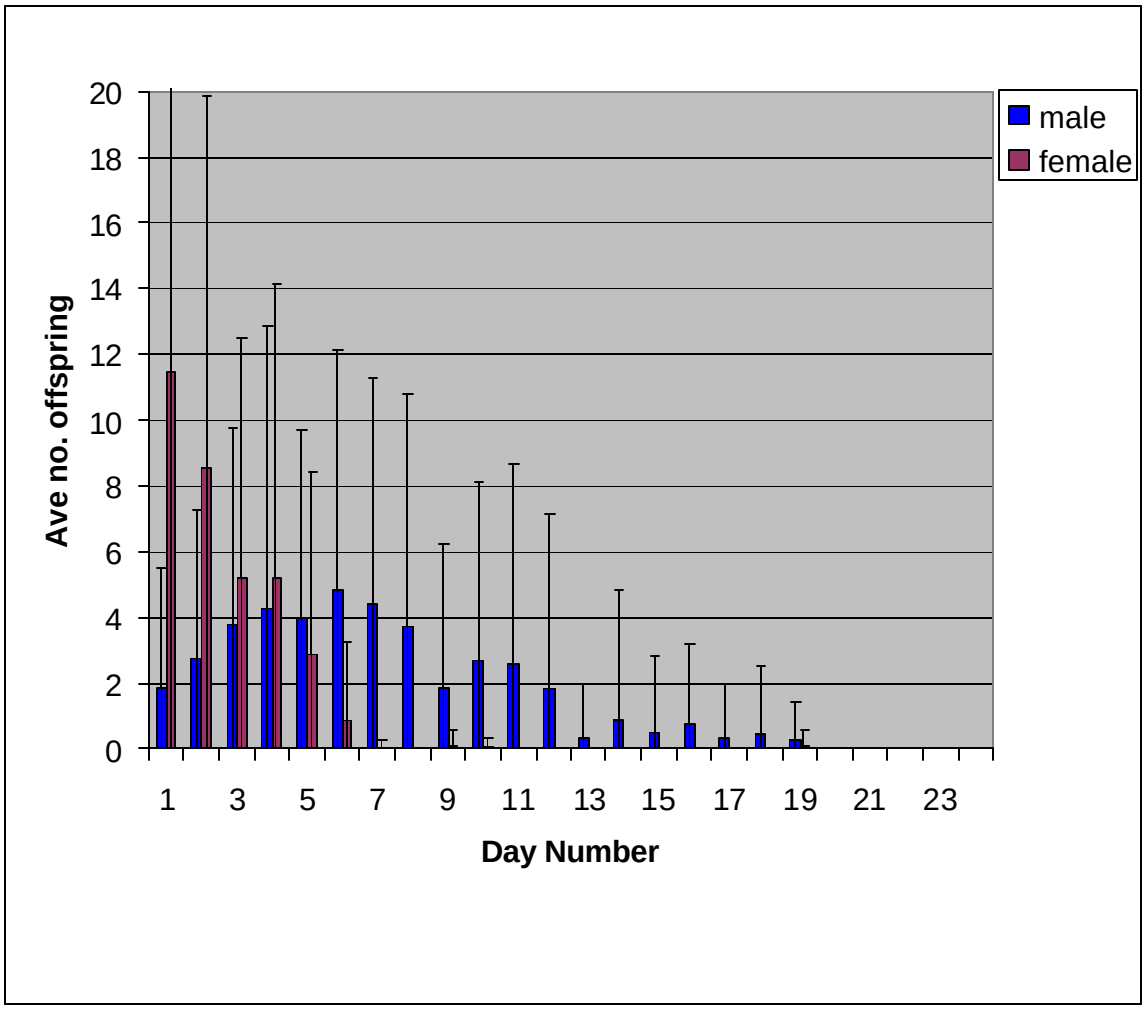

Figure 3.2 Mean ± SD daily number of offspring produced by *T. exiguum* provisioned with buckwheat flowers and water in a laboratory study

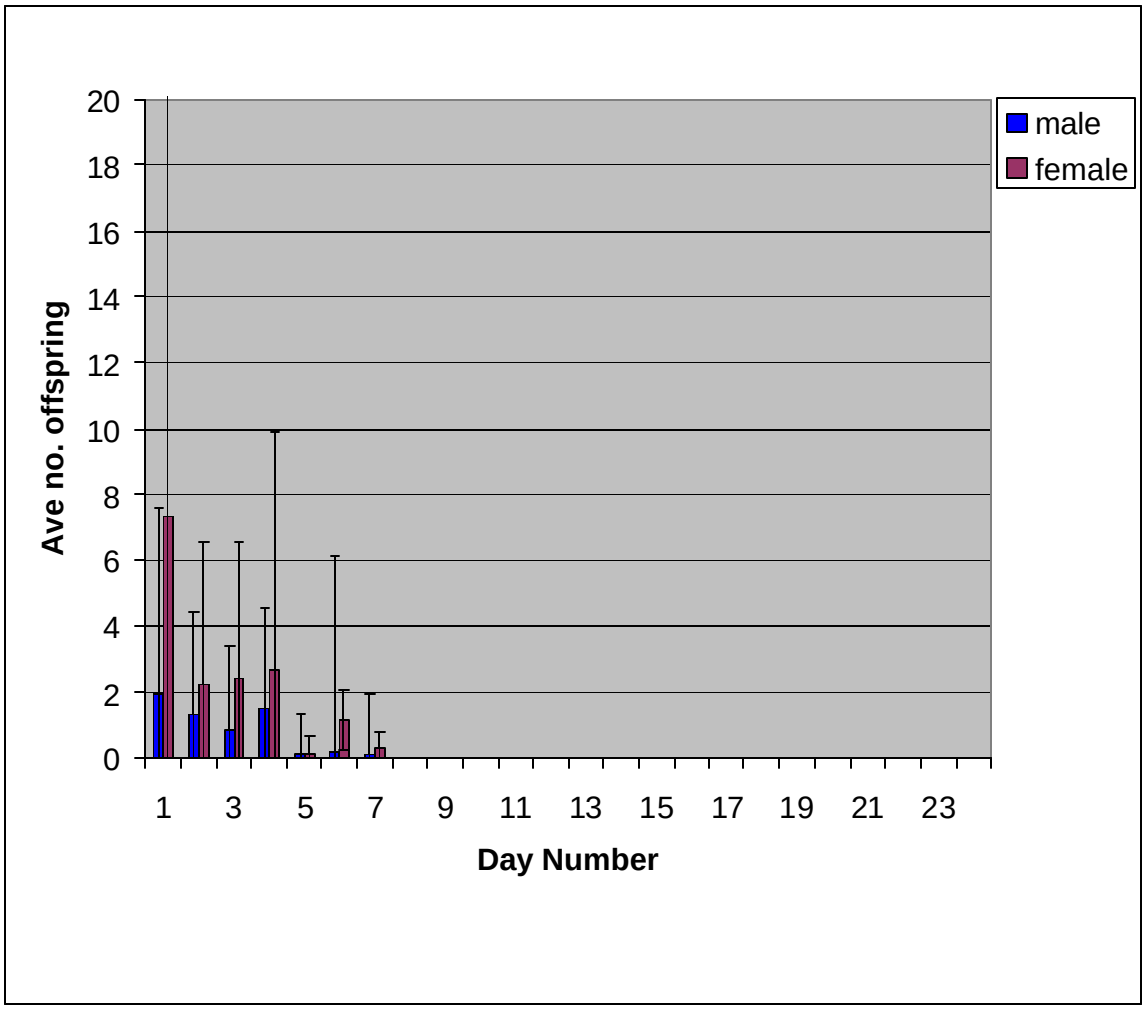

Figure 3.3 Mean ± SD daily number of offspring produced by *T. exiguum* provisioned with fennel flowers and water in a laboratory study

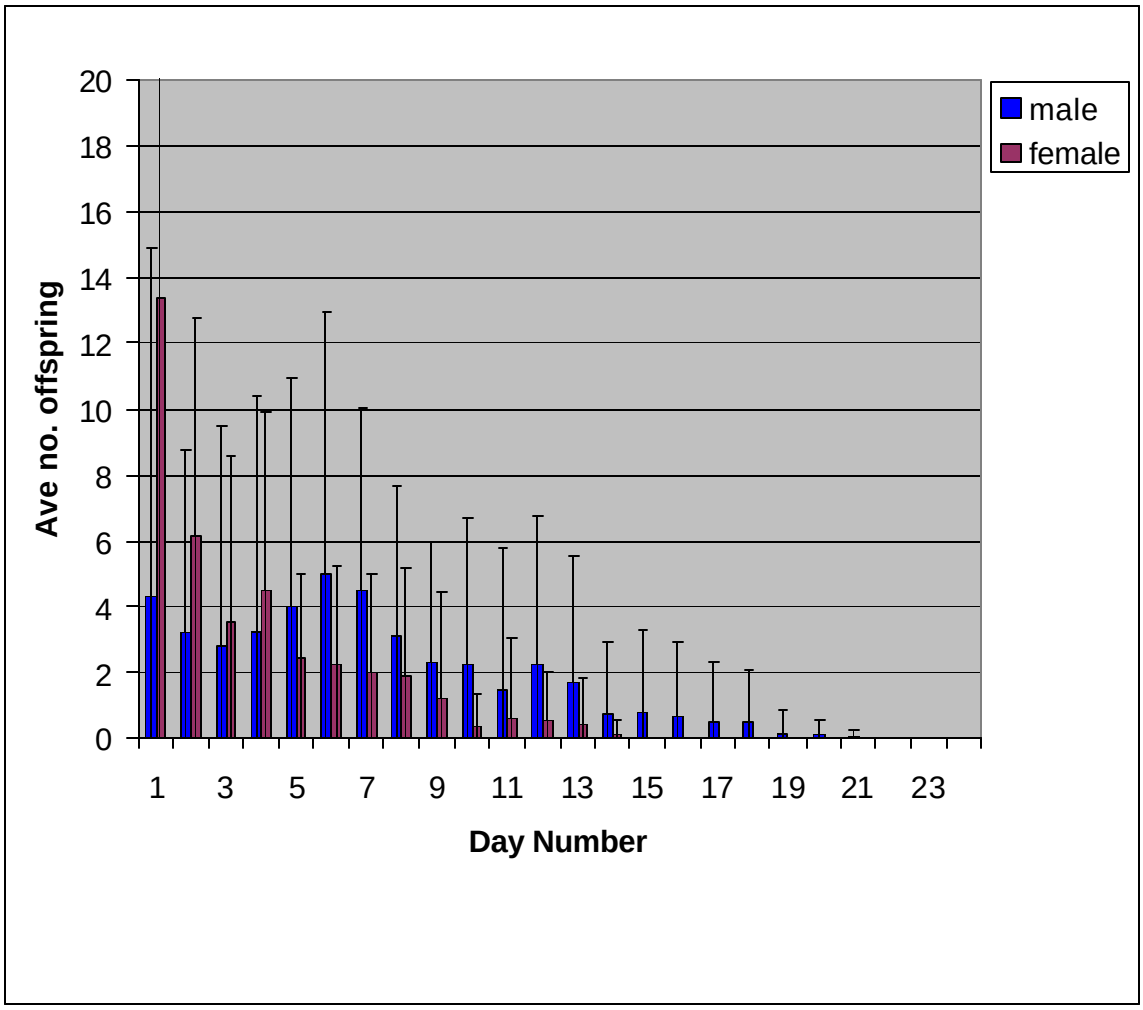

Figure 3.4 Mean ± SD daily number of offspring produced by *T. exiguum* provisioned with honey and water in a laboratory study

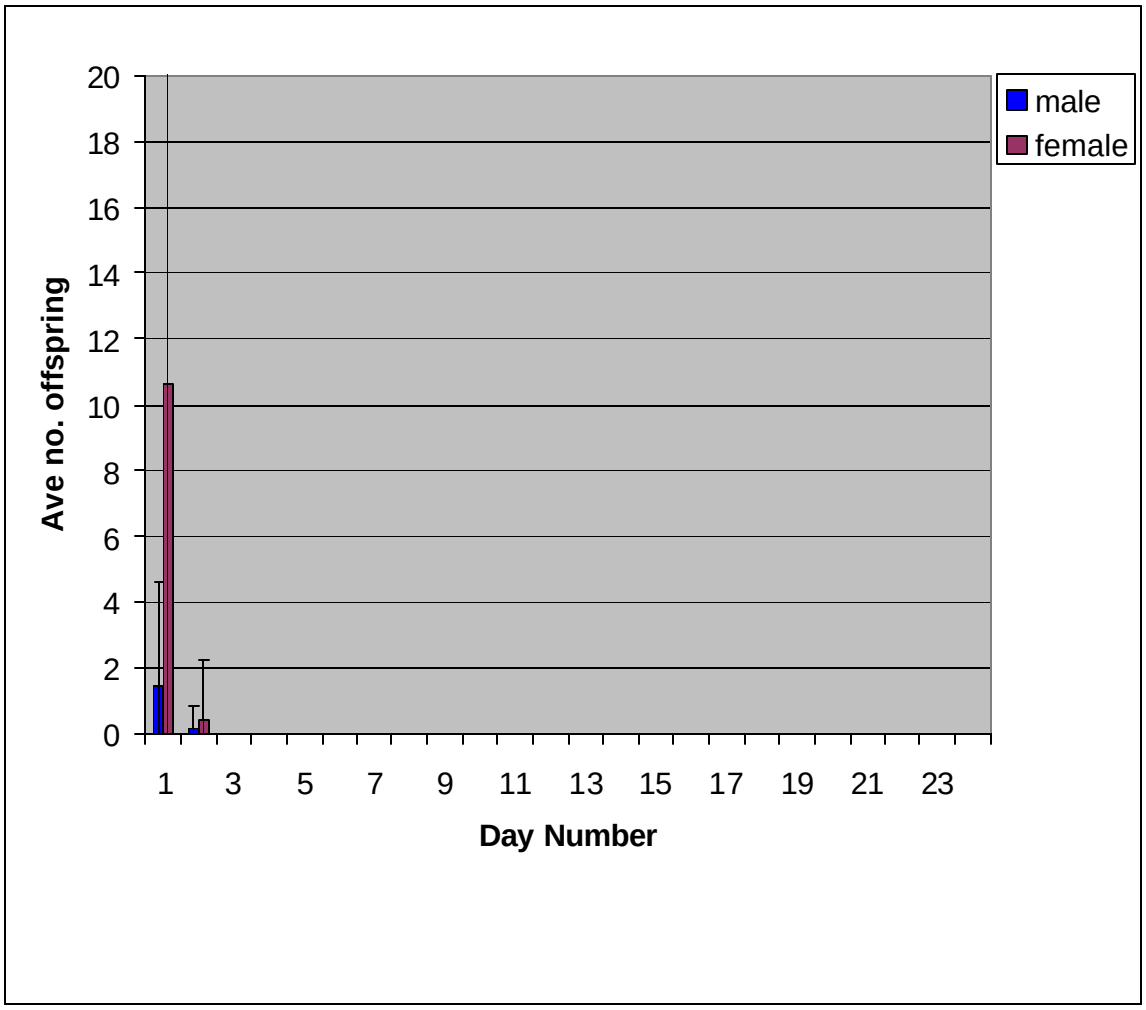

Figure 3.5 Mean ± SD daily number of offspring produced by *T. exiguum* provisioned with water in a laboratory study

Table 3.1 Mean ± SD longevity of *T. exiguum* and *C. congregata* provided different food sources

Parasitoid	Food Source	Mean ± SD	n
T. exiguum[*]	Buckwheat	$6.7 \pm 5.6_B$	23
	Fennel	$3.4 \pm 3.0_C$	23
	Honey	$10.1 \pm 6.2_A$	26
	Water	$0.8 \pm 0.6_D$	32
C. congregata[*]	Buckwheat	$5.1 \pm 3.3_A$	34
	Fennel	$1.7 \pm 1.4_B$	32
	Honey	$2.0 \pm 1.4_B$	39
	Water	$0.6 \pm 0.2_C$	40

Means within the same column followed by the same letter are not significantly different. Means separated using LS means (SAS Institute 2003)

[*] *T. exiguum* longevity determined under laboratory conditions, *C. congregata* longevity determined under field conditions

Table 3.2 Mean ± SD number of offspring, females, and percent females produced of *T. exiguum* provided different food sources

Food Source	Total number offspring	Total number females	% Female offspring	n
Buckwheat	$102.1 \pm 97.3_{A}$	$46.3 \pm 42.5_{AB}$	$54.5 \pm 31.0_{B}$	19
Fennel	$40.2 \pm 37.3_{B}$	$25.6 \pm 24.1_{BC}$	$76.3 \pm 17.6_{AB}$	19
Honey	$113.9 \pm 72.1_{A}$	$52.1 \pm 33.8_{A}$	$59.5 \pm 27.3_{B}$	20
Water	$16.1 \pm 25.8_{B}$	$13.9 \pm 22.5_{C}$	$88.3 \pm 7.5_{A}$	20

Means within the same column followed by the same letter are not significantly different. Means separated using LS means (SAS Institute 2003)

APPENDICES

APPENDIX - CHAPTER 1

Appendix 1.1 Type III Sums of Squares for flower species

Feeding Guild	Year	df	F value	Pr>F
Parasitoids	2004	3, 5	6.60	0.0344
Predators	2004	9, 16	10.45	<0.0001
Pollinators	2004	9, 16	12.43	<0.0001
Herbivore-crop pest	2004	9, 16	1.57	0.2064
Herbivore-non-crop	2004	9, 16	4.05	0.0073
Parasitoid-non-crop	2004	5, 9	2.99	0.0731
Deleterious Parasitoids	2004	5, 9	0.12	0.9849
Deleterious Predators	2004	1, 2	2.56	0.2056
Parasitoids	2005	2, 4	41.79	0.0021
Predators	2005	4, 8	27.45	0.0001
Pollinators	2005	4, 8	9.08	0.0045
Herbivore-crop pest	2005	4, 8	2.67	0.1104
Herbivore-non-crop	2005	3, 6	1.23	0.3773
Parasitoid-non-crop	2005	4, 8	5.65	0.0185
Deleterious Parasitoids	2005	3, 6	2.86	0.1267
Deleterious Predators	2005	1, 2	9.74	0.0891

Appendix 1.2 SAS (2003) input code for data analysis, 2004 observational data

```
data a; input DATE  TRT  $ REP BPAR HCPT HNCP NEUT PANC
PARA POLL PRBA PRED;
cards;
data b; set a;
sqPRED = sqrt(PRED);
sqPARA = sqrt(PARA);
sqHCPT = sqrt(HCPT);
sqHNCP = sqrt(HNCP);
sqBPAR = sqrt(BPAR);
sqPOLL = sqrt(POLL);
sqPANC = sqrt(PANC);
sqPRBA = sqrt(PRBA);
*** use if unbalanced ****;
proc glm data=b; class rep Trt Date; where trt ^ in
('DAP','ERH','ALF','LIS')
and date ^ in (1,6);
model  PARA  sqpara = rep |Trt date date*rep date*trt;
means  rep*trt rep*date trt date;

output out=p p= ppara psqpara r=rpara rsqpara;
run;

proc gplot; plot rpred*ppred=trt
rpara*ppara=trt rhcpt*phcpt =trt/vref=0;
run;

proc glm data=b; class rep Trt Date;
where trt ^ in ('DAP','ERH','ALF','LIS','CIL','HES');
model PRED sqpred HCPT sqhcpt PANC sqpanc POLL sqpoll HNCP
sqhncp
 = rep |Trt date date*rep date*trt;
test h=trt e=rep*trt;
means  rep*trt trt*date;
means trt /lsd e=rep*trt lines;

run;

proc gplot; plot rpred*ppred=trt
rsqpred*psqpred=trt rhcpt*phcpt rsqhcpt*psqhcpt=trt
rpoll*ppoll rsqpoll*psqpoll /vref=0;
run;

proc mixed data=b; class rep Trt Date; where trt ^ in
('DAP','ERH','ALF','LIS','CIL');
```

```
model sqpred  = Trt date date*trt;
random rep rep*trt rep*date;
lsmeans   trt*date;
lsmeans trt;
run;
*** sqPoll, does show interaction but use means over time
anyway? *********;
proc mixed data=b; class rep Trt Date;
where trt ^ in ('DAP','ERH','ALF','LIS','CIL','HES');
model sqpoll = Trt date date*trt;
random rep rep*trt rep*date;
lsmeans   trt*date;
lsmeans trt;
ods output lsmeans=lsm;
run;

proc gplot data=lsm; plot estimate*date=trt; run;

*** GLM for PANC, BPAR **************************;
proc glm data=b; class rep Trt Date;
where trt ^ in
('DAP','ERH','ALF','LIS','CIL','HES','CLO','CAP','TIC','GAI');
model  PANC sqpanc BPAR sqbpar
= rep |Trt date date*rep date*trt;
test h=trt e=rep*trt;
means  rep*trt rep*date trt*date;
means trt /lsd e=rep*trt lines;
run;

*** GLM for PRBA ************************;
proc glm data=b; class rep Trt Date;
where trt ^ in
('DAP','ERH','ALF','LIS','CIL','HES','CLO','CAP','TIC','GAI','
CEL', 'BES','YAR', 'DAI');
model   PRBA sqprba
= rep |Trt date date*rep date*trt;
test h=trt e=rep*trt;
means  rep*trt rep*date trt*date;
means trt /lsd e=rep*trt lines;
*;
run;

*** GLM for PARA ************************;
proc glm data=b; class rep Trt Date;
where trt ^ in
('DAP','ERH','ALF','LIS','CIL','HES','CLO','CAP','TIC','GAI','
BES','BWT');
```

```
model  Para sqpara
= rep |Trt date date*rep date*trt;
test h=trt e=rep*trt;
means  date rep*trt  trt*date;
means trt /lsd e=rep*trt lines;
run;

*** ANOVA by date ***********;
data c; set b; if trt  in ('DAP','ERH','ALF','LIS') then
delete;
proc sort data=c; by date;
proc glm data=c; by date;
class rep trt;
model PRED sqpred HCPT sqhcpt para sqpara PANC sqpanc BPAR
sqbar POLL sqpoll HCNP sqhcnp =rep trt;
means trt /lsd lines;
lsmeans trt /pdiff;
run;
```

Appendix 1.3 SAS (2003) input code for data analysis, 2005 observational data

```
data a; input TIME DATE TRT $ REP BPAR HCPT HNCP NEUT PANC
PARA POLL PRBA PRED;
cards;

data b; set a;
sqPRED = sqrt(PRED);
sqPARA = sqrt(PARA);
sqHCPT = sqrt(HCPT);
sqHNCP = sqrt(HNCP);
sqBPAR = sqrt(BPAR);
sqPOLL = sqrt(POLL);
sqPANC = sqrt(PANC);
sqprba = sqrt(prba);
if date in (1,2) then week=1;
if date in (3,4) then week=2;
if date in (5,6) then week=3;
if date in (7,8) then week=4;
if date in (9,10) then week=5;
if date in (11,12,13) then week=6;
*** use if unbalanced ****;
proc sort data=b; by rep trt week time;
proc means noprint data=b; by rep trt week time;
output out=m mean =  ;
var PRED sqpred HCPT sqhcpt PANC sqpanc POLL sqpoll
PARA  sqpara  HNCP sqhncp bpar sqbpar prba sqprba;
run;

data m; set m;
trt_time = trim(time)||"_"||trim(trt);
proc print data=m; run;

proc glm data=m; class rep Trt week time;
model  PRED sqpred HCPT sqhcpt PANC sqpanc POLL sqpoll
PARA  sqpara  HNCP sqhncp bpar sqbpar prba sqprba = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
lsmeans trt*time;
output out=p p= ppred psqpred phcpt psqhcpt ppanc psqpanc
ppoll psqpoll
```

```
ppara psqpara
r=rpred rsqpred rhcpt rsqhcpt rpanc rsqpanc rpoll rsqpoll
rpara rsqpara;
run;

proc gplot; plot rpred*ppred=trt RSQPRED*PSQPRED
rpara*ppara=trt rsqpara*psqpara rhcpt*phcpt =trt
rsqhcpt*psqhcpt /vref=0;
run;

title 'HCPT';
proc glm data=m; class rep Trt week time; where  week <5;
model   HCPT sqhcpt  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
lsmeans trt*time;
output out=p2 p=  phcpt psqhcpt
r= rhcpt rsqhcpt ;
run;

proc glm data=m; class rep Trt_time week ;where week <5;
model  HCPT sqhcpt   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;
proc gplot data=p2; plot rhcpt*phcpt =trt rsqhcpt*psqhcpt
/vref=0; run;

title 'PARA';
proc glm data=m; class rep Trt week time; where trt ^ in
('BES','CEL');
model  PARA  sqpara  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
```

```
means trt /lsd e=rep*trt lines;
run;

proc glm data=m; class rep Trt_time week ;where trt ^ in
('BES','CEL');
model  PARA sqpara   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

title 'PRBA';
proc glm data=m; class rep Trt week time; where trt ^ in
('BES','CEL','YAR');
model  PRBA  sqprba  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
means trt /lsd e=rep*trt lines;
run;

proc glm data=m; class rep Trt_time week ;where trt ^ in
('BES','CEL','YAR');
model  PRBA sqprba   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

title 'BPAR';
proc glm data=m; class rep Trt week time; where trt ^ in
('CEL');
model  BPAR  sqbpar  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
```

```
means  rep*trt trt*time trt week;
means trt /lsd e=rep*trt lines;
run;

proc glm data=m; class rep Trt_time week ;where trt ^ in
('CEL');
model  BPAR sqbpar   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

title 'PANC';
proc glm data=m; class rep Trt week time;
model  PANC sqpanc  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
means trt /lsd e=rep*trt lines;
run;

proc glm data=m; class rep Trt_time week ;
model  PANC sqpanc   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

title 'HNCP';
proc glm data=m; class rep Trt week time; where trt ^ in
('BES') and week ne 3;
model  HNCP  sqhncp  = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
```

```
means trt /lsd e=rep*trt lines;
run;

proc glm data=m; class rep Trt_time week ; where trt ^ in
('BES') and week ne 3;
model  HNCP sqhncp   = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

title 'PRED, POLL';
proc glm data=m; class rep Trt_time week ;
model  PRED sqpred  POLL sqpoll  = rep |Trt_time
week week*rep  trt_time*week ;
test h=trt_time e=trt_time*rep;
test h=week e=rep*week;
means   trt_time  week;
means trt_time /lsd e=trt_time*rep lines;
run;

proc glm data=m; class rep Trt week time;
model   PANC sqpanc
 HNCP sqhncp bpar sqbpar prba sqprba = rep |Trt
week week*rep week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=time time*trt e=time*rep*trt;
test h=week e=rep*week;
test h=week*trt e=rep*week*trt;
means  rep*trt trt*time trt week;
means trt /lsd e=rep*trt lines;
run;

title 'PRED, POLL';
proc glm data=m; class rep Trt time week ;
model  PRED sqpred  POLL sqpoll  = rep |Trt
week week*rep  week*trt rep*trt*week
time  time*trt time*rep*trt time*week time*week*TRT;
test h=trt e=trt*rep;
test h=week e=rep*week;
means   rep*trt trt*time  trt week;
means trt /lsd e=rep*trt lines;
run;
```

APPENDIX - CHAPTER 2

Appendix 2.1 Type III Sums of Squares

Flower species	**df**	**F value**	**Pr>F**
Mymarids	5, 10	11.81	0.0006
Scelionids	5, 10	1.83	0.1947
Trichogrammatids	5, 10	13.45	0.0004
Height	**df**	**F value**	**Pr>F**
Mymarids	2, 44	21.47	<0.0001
Scelionids	2, 44	25.51	<0.0001
Trichogrammatids	2, 44	8.25	0.0009
Flower removal	**df**	**F value**	**Pr>F**
Mymarids	1, 12	1.62	0.2266
Scelionids	1, 12	6.76	0.0232
Trichogrammatids	1, 12	0.18	0.6818
Flower species by Height	**df**	**F value**	**Pr>F**
Mymarids	10, 44	7.24	<0.0001
Scelionids	10, 44	6.69	<0.0001
Trichogrammatids	10, 44	4.17	0.0004
Removal by Height	**df**	**F value**	**Pr<F**
Mymarids	2, 44	2.26	0.1167
Scelionids	2, 44	6.20	0.0042
Trichogrammatids	2, 44	0.41	0.6672
Flower by Removal	**df**	**F value**	**Pr<F**
Mymarids	5, 12	0.56	0.7280
Scelionids	5, 12	1.35	0.3104
Trichogrammatids	5, 12	7.16	0.0026
Flower x Removal x Height	**df**	**F value**	**Pr<F**
Mymarids	10, 44	1.69	0.1123
Scelionids	10, 44	2.64	0.0130
Trichogrammatids	10, 44	2.28	0.0298

Appendix 2.2 Type III Sums of Squares for each plant species at each of three heights

Insect Family	Height*	df	F value	Pr>F
Mymarids	1	5, 10	12.55	0.0005
Scelionids	1	5, 10	3.24	0.0536
Trichogrammatids	1	5, 10	22.38	<0.0001
Mymarids	2	5, 10	5.08	0.0141
Scelionids	2	5, 10	4.70	0.0182
Trichogrammatids	2	5, 10	5.78	0.0092
Mymarids	3	5, 10	2.56	0.0965
Scelionids	3	5, 10	1.04	0.4479
Trichogrammatids	3	5, 10	5.58	0.0103

* Height 1 = 0.5 plant height; trap height 2 = flower height; trap height 3 = 1.5 plant height

Appendix 2.3 SAS (2003) input code for data analysis

```
data a; input
Date Flower $  Trt  Height Rep MYM SCE TRI;
cards;
proc print;

data b; set a;
sqMYM = sqrt(MYM);
sqSCE = sqrt(SCE);
sqTRI = sqrt(TRI);
*** proc Glm with All data ************;
proc glm data=b; class rep flower trt height date;
model MYM sqMYM SCE sqSCE TRI sqTRI = rep flower rep*flower
                trt trt*flower rep*trt(flower)
                    height|flower|trt height*rep
height*rep*trt*flower
                    date |height|flower|trt rep*date;
test h=flower e=rep*flower;
test h= trt trt*flower e= rep*trt(flower);
test h= height height*flower height*trt height*flower*trt
     e= height*rep*trt*flower;

means trt*flower trt*height;
lsmeans trt*flower*height /slice =flower*trt out=lsm;
output out= p p =  pMYM psqMYM pSCE psqSCE pTRI psqTRI
              r =  rMYM rsqMYM rSCE rsqSCE rTRI rsqTRI;

proc gplot data=p; plot  rMYM*pmym  rsqMYM*psqmym rSCE*psce
rsqSCE*psqsce
rTRI*ptri rsqTRI*psqtri / vref=0;
run;

proc sort data=lsm; by _name_;
proc gplot data=lsm; by _name_; where _name_ ^ in ('sqMYM',
'sqTRI','sqSCE');
plot lsmean*flower = trt ;
symbol1 c=blue v=circle;
symbol2 c=blue v=dot;
run;

proc gplot data=lsm; by _name_; where _name_ ^ in ('sqMYM',
'sqTRI','sqSCE');
plot lsmean*flower = height ;
symbol1 c=blue v=circle;
symbol2 c=blue v=dot;
```

```
symbol3 c=red v=square;
run;
*** proc mixed with All data ************;
proc mixed data=b; class rep flower trt height date;
model  sqMYM   =  flower |trt|height|date/ ddfm=satterth;

 random rep rep*flower rep*trt(flower) height*rep
height*rep*trt*flower rep*date;
lsmeans trt*flower /slice =flower;
lsmeans height*flower /slice =height;
run;
proc mixed data=b; class rep flower trt height date;
model  sqSCE   =  flower |trt|height|date/ ddfm=satterth;

 random rep rep*flower rep*trt(flower) height*rep
height*rep*trt*flower rep*date;
lsmeans trt*flower /slice =flower;
lsmeans height*flower /slice =height;
run;
proc mixed data=b; class rep flower trt height date;
model    sqTRI =  flower |trt|height|date/ ddfm=satterth;

 random rep rep*flower rep*trt(flower) height*rep*trt*flower ;
lsmeans trt*flower /slice =flower;
lsmeans height*flower /slice =height;
run;

proc sort data=b; by height;
*** ANOVA by Height to get LSD on flowers ********;
proc glm data=b; by height;
class rep flower trt  date;
model MYM sqMYM SCE sqSCE TRI sqTRI = rep flower rep*flower
                trt trt*flower rep*trt(flower)
                    date |flower|trt rep*date;
test h=flower e=rep*flower;
test h= trt trt*flower e= rep*trt(flower);
means flower /lsd e= rep*flower;
run;

*** Compare Trts at Height = 2;
proc mixed data=b; class rep flower trt date; by height; where
height = 2;
model  sqMYM   =  flower |trt|date/ ddfm=satterth;
 random rep rep*flower rep*trt(flower) height*rep
height*rep*trt*flower rep*date;
lsmeans trt*flower /slice =flower;
run;
```

```
proc mixed data=b; by height; where height = 2;
class rep flower trt height date;
model  sqSCE   =  flower |trt|date/ ddfm=satterth;

 random rep rep*flower rep*trt(flower)  height*rep*trt*flower
rep*date;
lsmeans trt*flower /slice =flower;
run;
proc mixed data=b; by height; where height = 2;
class rep flower trt height date;
model    sqTRI =  flower |trt|date/ ddfm=satterth;

 random rep rep*flower rep*trt(flower) height*rep*trt*flower ;
lsmeans trt*flower /slice =flower;
run;
proc print;

data b; set a;
sqMYM = sqrt(MYM);
sqSCE = sqrt(SCE);
sqTRI = sqrt(TRI);
run;
*** proc Glm with All data ************;


proc sort data=b; by height;
*** ANOVA by Height to get LSD on flowers ********;
proc glm data=b; by height;
class rep flower trt  date;
model MYM sqMYM SCE sqSCE TRI sqTRI = rep flower rep*flower
                trt trt*flower rep*trt(flower)
                    date |flower|trt rep*date;
test h=flower e=rep*flower;
test h= trt trt*flower e= rep*trt(flower);
means flower /lsd e= rep*flower;
run;
*** ANOVA by Height and trt to get LSD on flowers ********;
proc sort data=b; by height trt;
proc glm data=b; by height trt;
class rep flower trt  date;
model MYM sqMYM SCE sqSCE TRI sqTRI = rep flower rep*flower
                date |flower rep*date;
test h=flower e=rep*flower;
test h= date  e= rep*date;
means flower;
means flower /lsd e= rep*flower;
run;
```

APPENDIX - CHAPTER 3

Appendix 3.1 SAS (2003) input code for data analysis, *T. exiguum* and *C. congregata* longevity

```
data a; input FOOD REP LONG ;
cards;
data b; set a; if rep in (1,4,9,10,16,19,22,23) then delete;
loglong = log(long);
sqlong = sqrt(long);

*** use if unbalanced ****;
proc glm data=b; class rep food;
model long loglong sqlong = rep food;
means food rep;
lsmeans food/pdiff;
means food /lsd lines;
output out=p p=predlong ploglong psqlong r=rlong rloglong
rsqlong;
run;


proc gplot; plot rlong*predlong=food
rloglong*ploglong rsqlong*psqlong =food/vref=0;
run;


data a; input FOOD  REP  LONG ;
cards;
data b; set a;
loglong = log(long);
proc glm; class rep food;
model long loglong= rep food;
means food rep;
means food /lsd;
output out=p p=predlong ploglong r=rlong rloglong;run;

proc gplot; plot rlong*predlong=food
rloglong*ploglong=food/vref=0;
run;
*** use if unbalanced ****;
proc glm data=b; class rep food;
model long loglong= rep food;
lsmeans food rep;
lsmeans food/pdiff;
means food /lsd lines;
output out=p p=predlong ploglong r=rlong rloglong;
run;
```

Appendix 3.1 SAS (2003) input code for data analysis, *T. exiguum* fecundity

```
data a; input TRT $ REP DAYNO DAYFEC MALE FEMALE;
cards;
title 'Using all days ';
proc sort data=a; by trt rep;
proc means; by trt rep;
output out=m  sum= totfec totmale totfemale;
var dayfec male female;

data r; set m;
pctfemale= 100*totfemale/(totfemale+totmale);
longev = _freq_;
totsexed = totfemale+totmale;
apctfem = arsin(sqrt(pctfemale/100));
sqtotfec = sqrt(totfec);
logfec = log10(totfec+.5);
sqtotfem = sqrt(totfemale);
proc print; run;

proc glm; class trt;
model pctfemale apctfem totfec sqtotfec logfec totfemale
sqtotfem = trt;
means trt;
means trt/lsd lines;
output out=p p=pfem pafem pfec psqfec plfec ptfem psqtfem
r=rfem rafem rfec rsqfec rlfec
rtfem rsqtfem;
run;

proc gplot data=p; plot rfem*pfem rafem*pafem rfec*pfec
rsqfec*psqfec
rlfec*plfec rtfem*ptfem rsqtfem*psqtfem /vref=0;
run;

*** to compare with Anova ***;
proc genmod data=r; class trt;
model totfemale/totsexed = trt /d=bin type3 pscale;
contrast 'wat vs fen' trt 0 -1 0 1;
contrast 'wat vs hon' trt 0 0 1 -1;
contrast 'wat vs bwt' trt 1 0 0 -1;
contrast 'hon vs bwt' trt 1 0 -1 0;
contrast 'fen vs bwt' trt 1 -1 0 0;
run;
```

```
title 'Using first 2 days only';

proc sort data=a; by trt rep;
proc means; by trt rep; where dayno<3;
output out=m  sum= totfec totmale totfemale;
var dayfec male female;

data r2; set m;
pctfemale= 100*totfemale/(totfemale+totmale);
longev = _freq_;
totsexed = totfemale+totmale;
apctfem = arsin(sqrt(pctfemale/100));
sqtotfec = sqrt(totfec);
logfec = log10(totfec+.5);
sqtotfem = sqrt(totfemale);
proc print; run;

proc glm data=r2; class trt;
model pctfemale apctfem totfec sqtotfec logfec totfemale
sqtotfem = trt;
means trt;
means trt/lsd lines;
output out=p p=pfem pafem pfec psqfec plfec ptfem psqtfem
r=rfem rafem rfec rsqfec rlfec
rtfem rsqtfem;
run;
proc gplot data=p; plot rfem*pfem rafem*pafem rfec*pfec
rsqfec*psqfec
rlfec*plfec rtfem*ptfem rsqtfem*psqtfem /vref=0;
run;

*** to compare with Anova ***;
proc genmod data=r; class trt;
model totfemale/totsexed = trt /d=bin type3 pscale;
contrast 'wat vs fen' trt 0 -1 0 1;
contrast 'wat vs hon' trt 0 0 1 -1;
contrast 'wat vs bwt' trt 1 0 0 -1;
contrast 'hon vs bwt' trt 1 0 -1 0;
contrast 'fen vs bwt' trt 1 -1 0 0;
run;

title 'Using first day only';

proc sort data=a; by trt rep;
proc means; by trt rep; where dayno<2;
output out=m  sum= totfec totmale totfemale;
var dayfec male female;
```

```
data r2; set m;
pctfemale= 100*totfemale/(totfemale+totmale);
longev = _freq_;
totsexed = totfemale+totmale;
apctfem = arsin(sqrt(pctfemale/100));
sqtotfec = sqrt(totfec);
logfec = log10(totfec+.5);
sqtotfem = sqrt(totfemale);
proc print; run;

proc glm data=r2; class trt;
model pctfemale apctfem totfec sqtotfec logfec totfemale
sqtotfem = trt;
means trt;
means trt/lsd lines;
output out=p p=pfem pafem pfec psqfec plfec ptfem psqtfem
r=rfem rafem rfec rsqfec rlfec
rtfem rsqtfem;
run;
proc gplot data=p; plot rfem*pfem rafem*pafem rfec*pfec
rsqfec*psqfec
rlfec*plfec rtfem*ptfem rsqtfem*psqtfem /vref=0;
run;
```