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Graphical abstract

Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): Effects on longevity and body nutrients

Timothy D. Nafziger Jr., Henry Y. Fadamiro *

Research highlights

► We examine the effects of the nectar of three flowering plant species, sweet alyssum, buckwheat, and licorice mint, on the lifespan and body nutrient levels of the caterpillar parasitoid wasp, *Microplitis croceipes*. ► Buckwheat increases the lifespan of female and male wasps by at least two-fold relative to those provided water only. ► Buckwheat also increases the amount of body sugars and glycogen in the wasp. ► Females live longer than males on all diet treatments. ► Sweet alyssum and licorice mint are not good nectar sources for the wasp.
Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): Effects on longevity and body nutrients

Timothy D. Nafziger Jr., Henry Y. Fadamiro *

Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

In support of an ongoing study to evaluate potential farmscaping plants for utilization in organic vegetable production systems, we examined the effects of the nectar of three flowering plant species, sweet alyssum (*Lobularia maritima*), buckwheat (*Fagopyrum sagittatum*), and licorice mint (*Agastache foeniculum*), on the lifespan and body nutrient levels of the wasp, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a key parasitoid of some caterpillar pests of vegetable crops in the USA. The greatest longevity (~16 days) was recorded for honey-fed wasps (positive control). Buckwheat significantly increased the lifespan of female and male wasps by at least two-fold relative to wasps provided water only (longevity ~2–4 days). Licorice mint significantly increased female longevity and numerically increased male longevity. Sweet alyssum slightly increased longevity of both sexes but this was not significantly different from the water only control. Females had a significantly longer longevity than males on all the diet treatments. The greatest carbohydrate nutrient levels (sugar content and glycogen) were recorded in honey-fed wasps followed by wasps fed buckwheat, whereas very little nutrients were detected in wasps provided sweet alyssum, licorice mint or water only. However, female wasps were observed to attempt to feed on all three flowering plant species. Thus, the low nutrient levels detected in wasps provided sweet alyssum or licorice mint may be because the nectars were not accessible or were of poor quality. Further studies will evaluate the effects of the promising farmscaping plants on the beneficial and pest insect communities in the field.

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1. Introduction

Farmscaping is an ecological approach to pest management that usually involves planting hedgerows, insectary, or flowering plants to attract and support populations of beneficial organisms (Dufour, 2000). Farmscaping plants are planted to attract and provide resources to beneficial insects that may not otherwise be available in monoculture crop field. These resources provided to natural enemies can include shelter, reproductive habitat, and alternative or supplemental food sources (Dufour, 2000; Landis et al., 2000; Lee et al., 2006).

The concept of farmscaping is partly based on the knowledge that natural enemies require supplemental food sources to achieve maximum fitness. For example, adult parasitoid wasps of many species are known to require sugar meals for maximum longevity (Heimpel et al., 1997; Olson et al., 2000; Fadamiro and Heimpel, 2001; Wäckers, 2001; Berndt et al., 2002; Hogervorst et al., 2007; Evans et al., 2010). Increased longevity may enhance reproduction by allowing parasitoids more time for host location and attack (Heimpel and Jervis, 2005; Vollhardt et al., 2010). The positive effect of sugar feeding on longevity and/or fecundity has been demonstrated for many parasitoid species in the laboratory (Heimpel et al., 1997; Olson et al., 2000; Heimpel, 2001; Lee et al., 2004; Fadamiro and Chen, 2005; Fadamiro et al., 2005). Additional studies have demonstrated the ability of parasitoids to feed on nectar sources (Lee et al., 2004; Johananowicz and Mitchell, 2000; Fadamiro and Chen, 2005; Evans et al., 2010). Since nectar sugars consist primarily of sucrose, and its two monosaccharide components, glucose, and fructose (Wäckers, 2001), nectar should be a good food source for parasitoids. Studies have shown that the ability of parasitoids to utilize different nectar sources is dependent on several factors including flower attractiveness, floral morphology, nectar accessibility, and parasitoid mouthpart morphology (Jervis, 1998; Patt et al., 1997; Wäckers, 2004). Therefore, the ability of key species of parasitoids to utilize various nectar sources cannot be assumed and must first be evaluated prior to the recommendation of farmscaping plant species in any particular agroecosystem.

The goal of this study was to evaluate the suitability of some potential farmscaping plants as floral nectar sources for *Microplitis croceipes* (Hymenoptera: Braconidae): Effects on longevity and body nutrients. Biological Control (2010), doi:10.1016/j.biocontrol.2010.11.005
crocippe (Cresson) (Hymenoptera: Braconidae), an important specialist parasitoid of Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) caterpillars and related species in the southern USA (Lewis et al., 1991). Helicoverpa zea, also referred to as tomato fruitworm or cotton bollworm, is a major pest of tomatoes in Alabama and other parts of the U.S. (Flanders and Smith, 2008). Adult M. cucerpes are usually provided with artificial sugar solutions during rearing but little is known about its ability to utilize floral nectar as food in the field. The farmscaping plants tested in this study were sweet alyssum (Lobularia maritima), buckwheat (Fagopyrum sagitatum), and licorice mint (Agastache foeniculum). Buckwheat and sweet alyssum were selected based on previous studies which showed their potential as effective farmscaping plants (Stevens et al., 1999; Johanowicz and Mitchell, 2000; Berndt et al., 2002; Fadamiro and Chen, 2005; Lee and Heimpel, 2008). Licorice mint was selected based on its availability and abundant flowers. We recently undertook a preliminary field study which tested the potential of these plants as farmscaping plants in central Alabama (Nafziger and Fadamiro, in press). The results showed relatively higher numbers of parasitoids on some of the farmscaping plants than neighboring plants, suggesting that they are attractive to parasitoids. However, it was not clear if the parasitoids could successfully obtain nectar from these plants or if the plants could enhance parasitoid lifespan. Specifically, we tested (1) the effects of feeding on these flowering plants on the longevity of M. cucerpes females and males, and (2) if M. cucerpes females have the ability to obtain nutrients from the nectar of the farmscaping plants.

2. Materials and methods

2.1. Farmscaping plants

Buckwheat, sweet alyssum, and licorice mint were tested for their suitability as nectar sources for M. cucerpes. The plants were sown weekly at the Auburn University Plant Science greenhouse on earthworm castings in 4-inch diameter plastic pots at 26 ± 2 °C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Newly emerged adult parasitoids were collected daily, sexed and assigned to the different treatments.

2.2. Parasitoids

The parent culture of M. cucerpes was provided by the USDA-ARS, Insect Biology and Population Management Research Laboratory (Tifton, Georgia, USA; contact: Dr. Dawn Olson) and reared on caterpillars of Heliothis virescens Fab. (Lepidoptera: Noctuidae), as described by Lewis and Burton (1970). Eggs purchased from Benzory (Tifton, Georgia, USA; contact: Dr. Dawn Olson) and reared on earthworm castings in 4-inch diameter plastic pots at 26 ± 2 °C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Newly emerged adult parasitoids were collected daily, sexed and assigned to the different treatments.

2.3. Longevity

The survivorship of adult female and male M. cucerpes was compared when provided the following five treatments as food: (1) sweet alyssum plant, (2) buckwheat plant, (3) licorice mint plant, (4) honey, and (5) water only. In this and the sugar feeding experiments, honey was used as a positive control (e.g., Johanowicz and Mitchell, 2000), whereas water was the negative control. Newly emerged parasitoids were placed in groups of 6 individuals (3 per sex) in a 30 × 30 × 30 cm screen cage (Bug-Dorm-1, Megaview Science Education Services Co., Ltd., Taichung, Taiwan) and provided with one of the treatments. All treatments were provided with water. Sweet alyssum was presented as whole small potted plants (2 per cage) while buckwheat and licorice mint were presented as cut flowers with the stems placed in water and replaced every three days. Using freshly cut flowers has been shown to have little differences on the longevity of parasitoid wasps compared to intact plants (Wade and Wratten, 2007). The cages were checked daily for parasitoid survival and dead wasps were removed. The experiment was replicated five times for a total of 15 individuals per sex per treatment. Survivorship data for each sex was analyzed separately by using analysis of variance (ANOVA) followed by the Tukey-Kramer honestly significant difference (HSD) test to determine significant treatments effects (P < 0.05, JMP Version 7.01, SAS Institute, 2007). Significant differences between the sexes were determined for each diet treatment using the Student’s t-test (P < 0.05, JMP Version 7.01, SAS Institute, 2007).

2.4. Body nutrient assays

M. cucerpes females were put in a screen cage (same type used for the longevity tests) and provided with sweet alyssum, buckwheat, licorice mint, honey, or water only, as described for the longevity experiment. All treatments were provided with water. The wasps were allowed to feed for three days after which they were removed, frozen at −20 °C, and bioassayed to analyze the gut contents. The amount of sugar content, glycogen, and lipids in the individual wasps was estimated using previously described biochemical tests and procedures (van Handel, 1965; Olson et al., 2000; Fadamiro and Heimpel, 2001; Fadamiro and Chen, 2005; Fadamiro et al., 2005). Ten replicates were tested per treatment. Briefly, an individual wasp was crushed in a 1.5 mL microcentrifuge tube containing 50 mL of 2% sodium sulfate solution and placed on ice. The dissolved nutrients were then extracted with 450 mL of chloroform: methanol (1:2), after which the tube was vortexed. The tube was then centrifuged and 200 mL of the resulting supernatant was transferred to a glass tube for the sugar assays. Another 200 mL was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90 °C until approximately 50 mL of solution was left in the sugar tube and all solution was evaporated from the lipid and glycogen tubes.

2.4.1. Fructose

To estimate the amount of fructose, 950 mL anthrone reagent was added to the sugar tube, mixed thoroughly and left to react at room temperature for 1.5 h (cold anthrone reading). After the reaction time elapsed, the solution was poured into a 1.5 mL methacrylate cuvette and the optical density (absorbance) measured at 625 nm using a spectrophotometer. To convert absorbance readings to absolute fructose amounts (mg), standard curves were generated by determining the cold anthrone absorbance (at 625 nm) of different amounts (1–50 mg) of pure fructose (Fisher, Fairlawn, New Jersey). A linear regression was the best fit and generated the linear equation: \[ \text{Fructose (mg)} = \frac{72.917 \times \text{absorbance}}{1.506} \]. The sugar content in each wasp was estimated by multiplying the fructose amount by 2.5, since only 200 mL of the original 500 mL was used for the fructose (cold anthrone) assay, (Fadamiro and Heimpel, 2001).

2.4.2. Glycogen

One mL of anthrone reagent was added to the microcentrifuge tube containing the precipitate. After centrifugation, the tube was heated at 90 °C for 10 min and then cooled on ice and the absorbance read at 625 nm. (Glycogen standard calibration) curves were
generated by determining the absorbance of oyster glycogen (ICN Biomedicals, Aurora, Ohio) at a range of 1–50 μg (three replicates per dose). A linear regression was the best fit and generated the linear equation: (Glycogen (μg) = 78.332 × absorbance – 2.149). The equation was used to convert absorbance readings to absolute glycogen amount (μg). The amount of glycogen estimated above was considered to be representative of the whole wasp because all glycogen in the sample is presumed to precipitate to the bottom of the tube.

2.4.3. Lipids

The amount of lipids in each wasp was determined by adding 40 mL of sulfuric acid to the tube containing the lipid precipitate. The tube was then heated at 90°C for 2 min, cooled on ice, and 960 mL of a vanillin phosphoric acid reagent was added. The solution in the tube was left to react at room temperature for 30 min, mixed, and the absorbance read at 525 nm. To convert absorbance values to absolute lipid amounts (μg), lipid standard curves were generated by determining the absorbance of pure vegetable oil at a range of 1–50 μg (three replicates per dose). A linear regression was the best fit and generated the linear equation: (Lipids (μg) = 81.010625 × Lipid (absorbance) + 1.6917706). This equation was used to convert absorbance readings to absolute lipid amount. To estimate the total amount of lipids present in each wasp, the lipid amount was multiplied by 2.5 because 200 mL of the original 500 mL was used for the assay (Fadamiro et al., 2005).

3. Results

3.1. Longevity

Diet had a significant effect on the longevity of M. croceipes females (F = 23.088, df = 4, P < 0.0001) and males (F = 35.043, df = 4, P < 0.0001) (Fig. 1). Both sexes were observed to attempt to feed on all three flowering plants tested in this study. Female wasps that fed on honey lived significantly longer than those that fed on any other treatment. Females that fed on buckwheat and licorice mint lived significantly longer than wasps that fed on sweet alyssum or water only. Female wasps that fed on sweet alyssum lived longer than wasps that fed on water only but the difference was not statistically significant. In general, the greatest survivorship was recorded on honey followed by buckwheat and licorice mint.

Comparing the sexes, female wasps lived significantly longer than males in all five treatments: honey (F = 4.642, df = 1, P = 0.0403), licorice mint (F = 10.993, df = 1, P = 0.0028), buckwheat (F = 7.031, df = 1, P = 0.0130), sweet alyssum (F = 4.258, df = 1, P = 0.0500), and water only (F = 8.802, df = 1, P = 0.0062) (Fig. 1).

3.2. Body nutrient assays

The gut sugar and glycogen data were log transformed because they were not normally distributed.

3.2.1. Sugar content

Significant differences were found in the sugar content of female wasps provided the different diet treatments (F = 6.63, df = 4, P = 0.003) (Fig. 2A). Wasps fed on honey had significantly higher sugar content levels than wasps fed on licorice mint, sweet alyssum or water only. Wasps that fed on buckwheat had at least three times as much sugar as wasps provided licorice mint, sweet alyssum or water only, but these differences were not statistically significant (Fig. 2A).

3.2.2. Glycogen

Significant differences were recorded in the amounts of glycogen detected in wasps in the different diet treatments (F = 8.021,
Higher glycerol levels than those that fed licorice mint, sweet alyssum, or water only (Fig. 2B).

3.2.3. Lipids

Significant differences were also recorded in the amounts of lipids detected in wasps in the different diet treatments (F = 5.165, df = 4, P = 0.002) (Fig. 2C). Wasps fed on honey had significantly higher lipid levels than wasps on the remaining treatments (Fig. 2C).

4. Discussion

4.1. Sources of Nutrients

Adult M. croceipes were shown in this study as capable of feeding on some of the farmscaping plants with increased longevity and slightly higher body nutrient levels. In the longevity experiment, both sexes of M. croceipes were observed to forage on the farmscaping plants but only buckwheat and licorice mint significantly enhanced their longevity. Females had a significantly longer lifespan than males on all the diet treatments. This was especially evident in the buckwheat and licorice mint treatments. Similar results showing greater longevity for females than conspecific males have been reported for some other parasitoid species (Olson et al., 2000; Fadamiro and Heimpel, 2001). Higher female longevity may simply be because females have more need for nutrients than males in order to provide energy for host location and oviposition.

The results of the body nutrient assays showed significantly higher carbohydrate nutrient levels in female wasps provided with honey but none of the flowering plants caused a significant increase in body nutrient levels, compared to the water only control. However, wasps provided with buckwheat had numerically much higher carbohydrate nutrient levels than wasps provided water only or the other farmscaping treatments. These results together with the significant increase in longevity of buckwheat-fed wasps confirm the ability of M. croceipes to utilize buckwheat nectar. Other authors have also reported on the ability of several parasitoid species to utilize buckwheat (Stephens et al., 1998; Nicholls et al., 2000; Lee et al., 2004; Fadamiro and Chen, 2005).

Sucrose and its monomer components, glucose and fructose, have been shown to have the greatest effect on the lifespan of parasitoids (Wächters, 2001; Chen and Fadamiro, 2006; Luo et al., 2010). Nectar sugar contains primarily these same components (Wächters, 2001), which may explain the increased longevity recorded with buckwheat nectar in this study. In contrast, very low amounts of sugar and glycogen were detected in wasps provided licorice mint, despite our results which showed increased longevity with this flowering plant. The reasons for these somewhat inconsistent results are not clear, but it is plausible that the wasps were able to obtain from licorice mint just enough nutrients to enhance their longevity but not enough to remain in their gut or for storage as glycogen. Alternatively, it may be that the wasps were able to obtain from licorice mint some non-sugar nutrients, such as pollen or other resources, which may enhance their lifespan. Pollen has been reported to be beneficial to some parasitoid species (Jervis et al., 1996). Future studies are necessary to determine the basis for the increased longevity obtained with licorice mint. In general, the results of the longevity and body nutrient tests confirmed the inability of the wasps to utilize sweet alyssum nectar.

Our results on the differential utilization of the three farmscaping plant species by M. croceipes may be explained by several factors, in particular differences in floral morphology and nectar accessibility. Both factors may affect foraging behavior of parasitoids and their ability to obtain nectar nutrients from flowering plants (Patt et al., 1997). The positive results obtained with buckwheat are not surprising because of its known relatively accessible nectar (Stephens et al., 1998; Lee et al., 2004; Fadamiro and Chen, 2005). Our observations showed that M. croceipes adults foraged on sweet alyssum, but apparently were unable to utilize the nectar. This may indicate that sweet alyssum florets are morphologically incompatible with the mouthparts of M. croceipes, as has been suggested for other insect and plant interactions (Patt et al., 1997; Jervis, 1998; Fadamiro and Chen, 2005). The nectar could also be inaccessible due to high viscosity as has been reported for certain other parasitoids (Winkler et al., 2009). However, it is more likely that sweet alyssum nectar is of inferior quality to M. croceipes. Future studies on the nectar composition of the different farmscaping plants would be helpful in determining if the negative results recorded with sweet alyssum in this study are related to inferior nectar quality.

Feeding on buckwheat caused a modest increase in body carbohydrate nutrient levels but did not result in increased lipid levels, suggesting that adult M. croceipes are incapable of converting dietary sucrose to lipids, as has also been reported for several other parasitoid species (Olson et al., 2000; Fadamiro and Heimpel, 2001; Giron and Casas, 2003; Lee et al., 2004; Fadamiro et al. 2005). However, wasps that fed on honey had significantly higher lipid levels. To test whether this was due to contamination, we carried out a basic test for lipids with the honey and found that the honey used in the tests contained some lipids as a component (Nafziger and Fadamiro, 2006). This indicates that the increased lipid level obtained for honey-fed wasps was not an indication of the ability of M. croceipes to convert sugars to lipids. In general, the enhancement of longevity of M. croceipes by some of the diet treatments may suggest a synovigenic (i.e. emergence with some immature eggs) life history strategy for this species, which is true for most parasitoid species (Ellers and Jervis, 2004). Synovigenic species usually need a sugar resource to fully develop eggs (Bezemer et al., 2005), while provovigenic species are typically short-lived and have little need for sugar feeding in the field (Jervis et al., 2001).

In conclusion, buckwheat and licorice mint were identified in this study as beneficial to M. croceipes, and may also be suitable to some other parasitoids of tomato pests. Buckwheat is readily established in the field, requires little maintenance, and flowers for three to four weeks. Licorice mint is relatively more difficult to establish in the field but flowers for nearly two months as long as it is maintained. Sweet alyssum does not grow well in the field and requires extensive maintenance due to its susceptibility to be overgrown with weeds. Furthermore, sweet alyssum provided little benefit to M. croceipes in either nutritional value or longevity, indicating that it is not a good farmscaping plant for enhancing parasitoid fitness. Ongoing field studies will further evaluate the suitability of these flowering plants as farmscaping plants.

5. Conclusion

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