

NECTAR PLANT PREFERENCES OF *HEMARIS* (SPHINGIDAE: LEPIDOPTERA) ON CO-OCCURRING NATIVE *CIRSIUM* AND NON-NATIVE *CENTAUREA* (ASTERACEAE) INFLORESCENCES

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Abstract—Preferences for certain nectar plants by moth pollinators have not been extensively studied, particularly seasonal switches in nectar diet. The genus *Hemaris*, found throughout North America and parts of other continents, is a useful new system to address questions of flower-insect interactions as the moths are relatively common and day-flying. In this study we address the following questions with a group of three *Hemaris* species. (1) Does *Hemaris* display a preference for foraging on the native swamp thistle, *Cirsium discolor*, over the non-native invasive knapweeds, *Centaurea* spp.? (2) What factors might be driving *Hemaris*' preferences? The bloom periods of *C. discolor* and *Centaurea* overlap in the northeastern United States, with *Centaurea* blooming from approximately June through September and *C. discolor* blooming for approximately the first three weeks of August. *Hemaris* typically ecloses in the first week of July and fed on *Centaurea* until *C. discolor* bloomed. We tracked and recorded *Hemaris* visits to both plant species. During the co-blooming period of *C. discolor* and *Centaurea*, *Hemaris* visits significantly more *C. discolor* inflorescences, demonstrating a possible preference for the native species. *Hemaris* reverts to nectaring at the non-native *Centaurea* after native *C. discolor*'s bloom period ends. The nectar of *C. discolor* has a significantly higher sugar concentration than does the nectar of *Centaurea*; this difference may account for part of the moths' preference for *C. discolor*.

Keywords: *Centaurea*; *Cirsium discolor*; *Hemaris*; nectar plant preferences; non-native species

INTRODUCTION

Preferences for certain nectar plants by pollinators has been examined in bees (Wykes 1952; Roubik & Buchmann 1984; Alm et al. 1990; Leong & Thorp 1999; Schemske & Bradshaw 1999), butterflies (Wiklund et al. 1979; Alm et al. 1990; Grundel et al. 2000), birds (Bolten & Feinsinger 1978; Roberts, 1996; Schondube & Del Rio 2003) and, more rarely, in moths (Riffell et al. 2008). The relative abundances of floral resources can change throughout a pollinator's life, necessitating seasonal switches in nectar diets and many types of pollinators have displayed these nectar diet shifts (Feinsinger & Swarm 1982).

Food preference may also change based on a pollinator's capacity to learn and seasonal resource availability. Ensuing shifts in diet are commonly described for a variety of organisms, though rarely in moths (e.g. Tinbergen 1960; Persson & Hansson, 1999; Sydeman et al. 2001). An emerging issue in ecology is the role of non-native plant species in the diets of native pollinators. Interactions between pollinators and non-native plants vary greatly among invaded systems (Parker & Haubensack 2002; Mitchell et al. 2009). In many cases non-natives have been shown to directly

compete with natives and draw pollinators away from native plants thereby reducing native seed set (Mosquin 1971; Brown & Mitchell 2001; Brown et al. 2002; Bell et al. 2005; Kandori et al. 2009) while in other cases, the presence of non-natives has no effect on or may even facilitate native pollination (Lavery & Hiemstra 1998; Moragues & Traveset 2005; Bartomeus et al. 2008).

This study focuses on the nectar feeding behaviour of *Hemaris*, a genus of diurnal moths (Lepidoptera) of the family Sphingidae, at native and non-native Asteraceae species. Although they are relatively common organisms, little is known of the behaviour or function of *Hemaris* as a flower visitor. As caterpillars, the niches of *Hemaris* species overlap. *Hemaris thysbe* and *H. diffinis* feed on woody vines such as *Vitis* spp., *Lonicera sempervirens*, shrubs such as *Symphoricarpos* spp. and forbs such as *Apocynum cannabinum*. *Hemaris gracilis* larval host plants include shrubs of the heath family (Ericaceae) such as *Kalmia* spp. and *Vaccinium vacillans* (Butterflies and Moths of North America 2013; Tuttle 2007). These ericaceous hosts are not found in abundance at our site, so the lack of larval hosts may explain the dearth of *H. gracilis* individuals encountered. Adult nectar resources overlap broadly. Adults have been observed nectaring from a wide variety of native and horticultural flowers including *Cirsium discolor*, *Monarda* spp., *Petunia x hybrida*, *Kalmia latifolia*, and *Asclepias syriaca* (Tartaglia 2013).

Received 29 October 2013, accepted 5 May 2014

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In this study, we address the following questions: (1) Does *Hemaris* display a preference for foraging on the native swamp thistle, *Cirsium discolor*, over the non-native invasive knapweeds, *Centaurea* spp.? (2) What factors might be driving *Hemaris*' preferences? We expected that our findings would improve our understanding of community pollination dynamics with this little-studied group of pollinators.

MATERIALS AND METHODS

Study system

Hutcheson Memorial Forest (HMF), in Franklin, Somerset County, New Jersey, is a habitat consisting of several early successional meadows interspersed between forest patches of various age and species composition. HMF consists of 226 ha of meadows and upland temperate forest surrounded by various landscape matrix types including urban and suburban areas, as well as being connected to a corridor network of open space throughout central NJ. We made our observations in one of HMF's early-successional meadows. This meadow is permanently maintained in an early successional sere and is surrounded by stands of *Juniperus virginiana*.

We made observations from 16-July until 28-August, 2012. Throughout the study period, the most abundantly blooming plants were *Centaurea* spp., knapweeds native to Eurasia. *Centaurea* was first reported in North America in the early 1890's (Morisawa 1999). There are two species of non-native *Centaurea* at HMF, *C. stoebe* spp. *micranthos* (Gugler) Hayek and *C. nigrescens* Willd (ITIS 2014). As the two species are nearly indistinguishable, we did not differentiate between them in this study. *Cirsium discolor*, a thistle native to the northeast blooms abundantly for a short period of time during the summer, so the bloom periods of these plants overlap for only approximately the first three weeks of August. Thistle grows to an average height of 1.8 m at HMF while knapweed is shorter (average height 1.2 m). Both plants belong to the Asteraceae and have similar floral forms consisting of purple/lavender inflorescences of densely packed discoid florets. However, knapweed also possesses ray florets, which are lacking in thistle. We had established previously that *Hemaris* is attracted to purple flowers over other colours (Tartaglia 2013).

Data Collection

We netted *Hemaris* of all three species on two 20 m unidirectional transects, one running through a patch of *Cirsium discolor* plants and one running through a patch of *Centaurea* plants. Upon capture, each moth was manually dusted with fluorescent dye powder (Shannon Luminous Materials, Inc., Santa Ana, California via BioQuip, Rancho Dominguez, California) to track which flowers they landed on (Kearns & Inouye 1993). Care was taken to apply dye to both dorsal and ventral surfaces of the moths and to remove any powder that might obstruct their vision or antennae. Moths were released immediately after dye application. *Hemaris thysbe* individuals were dusted with orange dye powder, *H. diffinis* individuals were dusted with blue dye powder and *H. gracilis* individuals were dusted with yellow dye powder. At dusk, we returned to the site with blacklight

flashlights to track moth landings and recorded the number and species of inflorescences in the transects with orange, yellow or blue dye on them. We also examined other floral resources present in the field to determine if *Hemaris* utilises species besides *C. discolor* and *Centaurea* in this study system. Since the dye faded overnight to the degree that it was nearly indistinguishable the following day (particularly if rainfall occurred), we were able to make a distinction newly-visited inflorescences (i.e. visited during the course of that day) as discernibly brighter than previously visited inflorescences (i.e. dye was very faint or had disappeared totally).

We also counted thistle and knapweed inflorescences in our transects each day. Data for inflorescence number and *Hemaris* visitation was pooled for the two transects. We used t-tests to assess whether there were significant differences in *Hemaris* visitation to thistle and knapweed inflorescences.

To determine if sugar concentrations in nectar might be driving any observed preferences for thistle or knapweed, we sampled 20 disc florets from 10 inflorescences each for thistle and knapweed plants growing adjacent to our transect. We extracted nectar by separating individual disc florets, removing the bottom of the tube and squeezing nectar onto a Reichert I0431 hand refractometer surface. Nectar extracted from individual florets from separate inflorescences was pooled. We sampled nectar once at mid-day (between 1200 and 1300h) and once in afternoon (between 1500 and 1600h). We used t-tests to assess whether there were significant differences in mean nectar concentrations of thistle and knapweed.

RESULTS

In the northeastern United States, the bloom periods of knapweed and thistles coincide for only a few weeks (Fig. 1). Knapweed blooms for nearly the entire summer (from June through September) and has a much longer bloom period than *Cirsium discolor*, which blooms for approximately the first three weeks of August. Knapweed is also more abundant than thistle. During the overlapping bloom period, there were on average 45.3 thistle inflorescences (115 at peak) and 157 on average (175 at peak) knapweed inflorescences present in our transects. Prior to thistle blooming at the study site, *Hemaris* ($N = 50$) was netted from knapweed and fluorescent dye tracking revealed that they nectared exclusively at knapweed during this time. When thistle bloom began, *Hemaris* ($N = 64$) was netted from both thistle and knapweed and fluorescent dye tracking revealed that they nectared at both thistle and knapweed. However, the moths visited significantly more thistle inflorescences ($t = 2.82$, $df = 24$, $P = 0.007$) during overlapping bloom times, despite the difference in abundance between the two plant species at the site. During the co-blooming period, *Hemaris* made 351 total visits to *Cirsium* inflorescences vs. 78 visits to *Centaurea* inflorescences (Fig. 1). When thistles ceased blooming, fluorescent dye tracking revealed that *Hemaris* ($N = 11$) reverted to nectaring at knapweed inflorescences (58 visits).

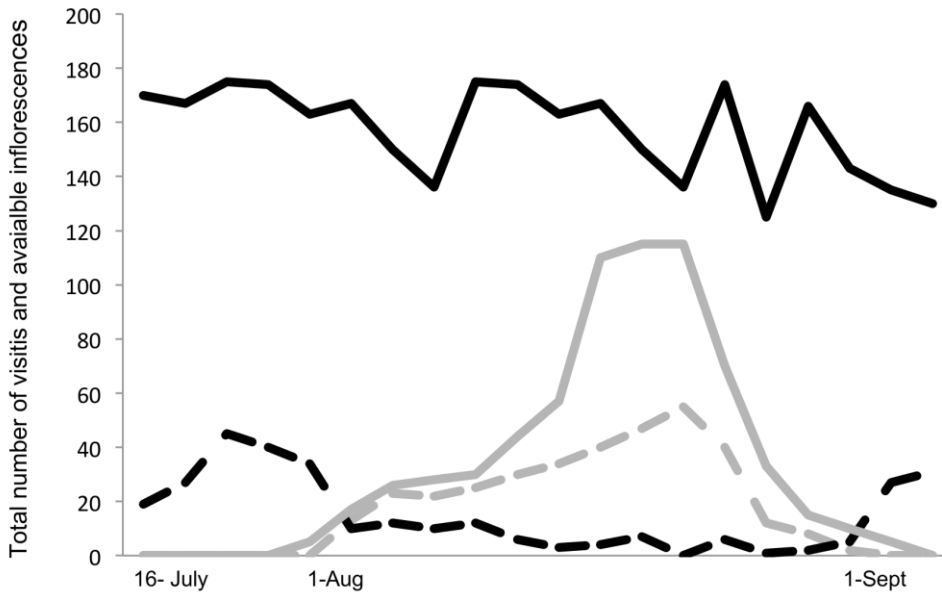


FIGURE 1: Total visits of *Hemaris* to thistle and knapweed inflorescences vs available thistle and knapweed inflorescences in two 20m transects over the course of the study. Solid black line represents available knapweed inflorescences; solid gray line represents available thistle inflorescences; dotted black line represents visits to knapweed; dashed gray line represents visits to thistle.

Three *Hemaris* species are present at HMF. Of the 114 individuals dusted with fluorescent powder in this study, 65 were *H. diffinis*, 43 were *H. thysbe*, and 6 were *H. gracilis*. Among *Hemaris* species there were no significant differences ($F_{2,34} = 0.395$, $P = 0.68$) in visitation to thistle and knapweed. In a previous study specifically examining *Hemaris* foraging behaviours (Tartaglia 2013), we also found no differences between the three *Hemaris* species, so we have treated them at the genus level for our study. Other floral resources (*Asclepias syriaca*, *Convolvulus* spp., *Erigeron philadelphicus*, *Leucanthemum vulgare*, *Penstemon hirsutus*, *Solidago* spp.) existed in the field but were ignored by the moths.

Refractometry to determine sugar concentration differences between the two preferred nectar resources revealed that thistle (56.2% sugar) has significantly ($t = 7.22$, $df = 38$, $P < 0.01$) more sugar-rich nectar than knapweed (47.1% sugar).

DISCUSSION

Our results add to the growing body of literature regarding the role of non-native plant species in plant-pollinator relationships. The diurnal *Hemaris* genus is an active and selective visitor to floral communities. It seems that the non-native *Centaurea* serves as a “place-holder” food resource for *Hemaris* until the preferred nectar source, *C. discolor*, blooms, creating an apparent sequential mutualism between the moths, the non-native *Centaurea*, and the native *C. discolor*. *Centaurea* may help sustain the *Hemaris* population, allowing sufficient numbers of the moths to survive until their preferred food source becomes available. Our data set did not address the parallel question of whether *C. discolor* benefits as well. Waser & Real (1979) were among the first to consider in detail the concept that sequentially flowering species may sustain pollinator populations throughout seasons, ensuring reproductive success for both plants and pollinators and our data lends

support to this concept as *Hemaris* heavily utilises *Centaurea* until *C. discolor* becomes available.

No specific research has been done addressing colour preference in *Hemaris*, but it has been established that naive *Macroglossum stellatarum* is able to learn and retain visual colour cues (Kelber 1996). Comparisons between diurnal and nocturnal Sphingidae have revealed that diurnal hawkmoths do rely more heavily on visual cues (colour and light intensity) to detect resources, while nocturnal species relied on odor cues (Balkenius et al., 2006) so colour may indeed be a critical factor in these moths’ selection preferences.

Though the reason why *Hemaris* seems to prefer *C. discolor* may be due to the higher sugar concentration in the native thistle, other factors influence the differential attraction. Hawkmoths rely on multiple sensory inputs to detect nectar resources (Raguso 2004; Balkenius et al. 2006), and Sphingidae have keen olfactory capacities in addition to their visual capacities. In fact, resource switching in Sphingidae has been documented by Riffell et al. (2008) when *Manduca sexta* switched from flowers of *Agave* flowers to those of *Datura* in their study system. Though *Datura* flowers have less nectar than *Agave*, the moths utilised them due to an innate preference for *Datura* (Riffell et al. 2008). Although the plants seem odorless to humans, *Hemaris* may be able to detect different more- or less-attractive volatiles from the two plants. Additionally, *C. discolor* does not possess ray florets, so differences in ray floret reflectance, inflorescence shape or inflorescence size may drive differential visitation to the flowers. Diurnal Sphingidae have been shown to rely heavily on visual cues when foraging (Kelber 1996; Balkenius et al. 2006) and Dafni et al. concluded that a wide variety of floral spatial factors contribute to insects’ foraging behaviours (1997).

ACKNOWLEDGEMENTS

The authors thank the Hutcheson Memorial Forest Center for funding. C. Holzappel, R.A. Raguso and L. Struwe provided

valuable feedback on this manuscript. S. Wehman and E. Golan assisted with fieldwork.

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