OBSERVATIONS ON NECTAR AVAILABILITY AND BEE VISITATION AT PATCHES OF YELLOW STAR-THISTLE AND CHASTEBERRY ON THE NORTHEAST AEGEAN ISLAND OF LESVOS (GREECE)

Kuzeydoğu Ege Lesvos (Yunanistan) Adasında Güneş Çiçeği ve Hayıt Bitkisi Alanlarında Nektar Varlığı ve Arı Ziyaretleri Üzerine Gözlemler

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

We examined numbers of honey bees (*Apis*) versus non-*Apis* bees at patches of yellow star-thistle, *Centaurea solstitialis*, and chasteberry, *Vitex agnus-castus*, on the Northeast Aegean Island of Lesvos in Greece. The resulting visitation patterns are associated with average nectar volumes measured from florets of each plant species. Honey bees visit *V. agnus-castus* more frequently when bees are sampled from adjoining floral displays of both species. In addition, the average size of bee pollinators (as measured by head capsule width) collected during this time was always larger from *V. agnus-castus* relative to *C. solstitialis*, and significantly so in the majority of cases. These patterns contrast with findings from previous studies in the western USA (California) where yellow star-thistle is not a native plant species but honey bees commonly visit it. Indeed, in North America, the abundant honey derived from yellow star-thistle is often considered a desirable product for marketing by beekeepers.

Key words: *Centaurea solstitialis*, chasteberry, competition, invasive species, Lesvos, *Vitex agnus-castus*, yellow star-thistle.

INTRODUCTION

Invasive plant species influence the environments they invade in many ways, including through the introduction of new food resources such as nectar (Ghazoul, 2002). The highly invasive yellow star-thistle, *Centaurea solstitialis* L., for example, attracted honey bees in high numbers in an island ecosystem, Santa Cruz Island, California, in the western USA, resulting in greater seed production for this plant species than would be possible without them (Barthell et al., 2001). Honey bees on Santa Cruz Island show a much higher proportion of visits, relative to native bees, to *C. solstitialis* than to the native gumplant, *Grindelia camporum* E. Greene (Barthell et al., 2000). This species has also been recognized as a good honey plant to North American beekeepers (Pellet 1976), many of whom have taken advantage of its presence to sell its honey as a product at markets (Fig. 1).

Figure 1. Yellow star-thistle honey that is commonly found in the northwestern USA.

Observations of the bee fauna at yellow star-thistle in the western USA (Santa Cruz Island and elsewhere) describe high proportions of honey bees relative to native bees (Thorp et al. 1994 and 2000; Maddox et al. 1996, McIver et al. 2009). However, our initial observations on the island of Lesvos (Greece), where honey bees and yellow star-thistle are native, showed a very diverse native bee guild with honey bees being relatively uncommon visitors to *C. solstitialis* (Barthell et al. 2009). Populations of *C. solstitialis* also appear to be relatively less dense on Lesvos and adjoining areas in Turkey (Uygur et al. 2004), compared to rapid range expansion in the western USA (Pitcairn et al. 2006). In addition, the recognition of a yellow star-thistle honey is unknown in the native range of this plant species.

In 2009 (Barthell et al.), we began to study ways in which nectar may influence pollinator visitation patterns and how negative interactions (phytophagy, competition, etc.) might influence visitation and seed set in yellow star-thistle in its native range. Herein we provide preliminary observations of visitation patterns by bees to *C. solstitialis* and a natural competitor for pollinators, chasteberry, or *Vitex agnus-castus* L. The latter species is common in the region where we studied *C. solstitialis* on Lesvos, and early observations suggested it may be attractive to honey bees and other large-bodied bee species (Figs. 2a and b). We predicted that eusocial and other large-bodied bee species would prefer *V. agnus-castus* over *C. solstitialis* due to larger volumes of nectar produced by florets in the former species. Below, we provide results comparing *C. solstitialis* and *V. agnus-castus* for proportions of honey bees versus other bee species at flower patches, nectar levels (Nectar Flow and Standing Crop) as well as the average size of the bees visiting each flower species.

Figure 2. Honey bee (a) and carpenter bee (b) visiting *V. agnus-castus*.

STUDY LOCALE

Lesvos is located among the seven Northeast Aegean Islands and is about 70 km long by 40 km wide and approximately 1,630 square km in area. Between 1,500 and 1,600 plant taxa are thought to reside on the island, many of which were described originally by the ancient Greek botanist Theophrastus over 2,000 years ago (Theophrastus 1916; Bazos 2005, Petanidou and Lamborn, 2005). Indeed, the biological history of the island is rich, being the site where Aristotle did his influential marine biological studies along the shore of Kalloni Bay (Tipton, 2006). A previous study describes this locale and a corresponding island in the Pacific Ocean, Santa Cruz Island, USA, in additional detail (Barthell et al., 2009). Our study occurred in the vicinity of the Ancient City of Pyrra on the eastern banks of Kalloni Bay.

MATERIALS AND METHODS

Nectar

From 18:00 to 20:00 on 12 June, 2008, two inflorcences on each of at least 18 plants of both *C. solstitialis* and *V. agnus-castus* (selected along a belt transect) were covered with small mesh bagging material to prevent visitation by flying insects or other potential pollinators. The following morning, just prior to 07:00, the mesh material was permanently removed from one inflorescence per plant, providing a measure of Standing Crop. The other inflorescence was left covered between readings, making it a measure of Nectar Flow. A fresh floret from each inflorescence of each plant and of each species was then checked every two hours: 07:00, 09:00, 11:00, 13:00, 15:00, 17:00, and 19:00. The volumes from these readings were added cumulatively to give a total volume for each inflorescence of each treatment on every plant.

Bee Collections

In an effort to assess the types and average size of flower visitors, bees were collected during 15-minute periods at adjoining (< 50 m) patches of *C. solstitialis* and *V. agnus-castus*. To do so, each collector was equipped with a standard insect aerial net and asked to obtain specimens (regardless of duplicates) *ad libitum*. Once the first collection set was finished, the two collectors immediately exchanged patches (flower species) and began again for 15 minutes. This exchange was meant to assure that no collector bias was entering into the sampling process. A total of four such exchanges (30 minutes in duration, each) were conducted at three-hour intervals, beginning at 09:00, 12:00, 15:00 and 18:00.

All bees collected during the aforementioned time periods were immediately placed in cyanide vials and pinned the same afternoon to retain the integrity of the specimens for identification and measurement under a dissection scope. Bee size was estimated using a scope equipped with an ocular micrometer. The widest transverse diameter of the head capsule was measured with the ocular micrometer to the nearest 0.1 mm as an estimate of body size (e.g., Barthell et al. 2006).

Analyses

Differences in nectar volumes per plant species were assessed with a one-tailed t-test. Proportions of honey bees relative to other types of bees visiting at the respective plant species were analyzed for differences with a Chi Square test. Differences in bee sizes (head capsule widths) were tested with a one-tailed t-test. Where the assumption of homogeneity of variances was not met between the aforementioned groups, a pooled variance was used in t-tests.

RESULTS

Nectar Flow and Standing Crop

Average Nectar Flow (NF) volume per floret of *C. solstitialis* was 0.092 (± 1 SE = ± 0.012) µl versus 0.176 (± 0.040) µl for *V. agnus-castus*; this difference is significant according to a one-tailed t-test ($t = 1.94$, $df = 18.7$, $P = 0.033$). For Standing Crop (SC) values, the average volume per floret for *C. solstitialis* was 0.026 (± 0.007) versus 0.092 (± 0.034) for *V. agnus-castus*; this difference is also significant according to a one-tailed t-test ($t = 1.79$, $df = 18.7$, $P = 0.045$). The average NF was therefore 3.54 times higher than SC for *C. solstitialis* while *V. agnus-castus* NF was 1.91 times greater than its SC. NF for *V. agnus-castus* was nearly twice (1.91-fold) that of *C. solstitialis* while for SC this value was 3.54 times greater for *V. agnus-castus* than *C. solstitialis*.

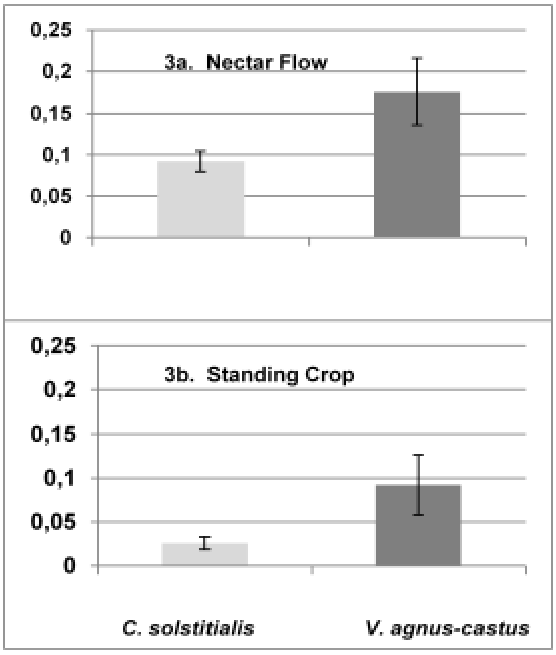

Figure 3. Average (mean ± 1 SE) nectar flow (a) and standing crop (b) volumes (in µl) for *C. solstitialis* (light shading) and *V. agnus-castus* (dark shading).

Visitation Patterns

During two hours (total duration) of collection over the entire day of 13 June, and among 80 total specimens collected, no honey bees were detected at *C. solstitialis* plants (Table 1). However, a total of 55 honey bees were collected at nearby *V. agnus–castus* plants, in addition to 78 other bees. Within the large-bodied genera of *Bombus* and *Xylocopa*, we collected only one specimen, *Xylocopa iris* (Christ), foraging at *C. solstitialis*. Honey bees and non-*Apis* bees did not forage in the same proportions on the two plant species ($\chi^2 = 29.51$, df = 1, P < 0.001). Honey bees preferred *V. agnus-castus* over *C. solstitialis* plants (goodness of fit to 1:1 ratio; $\chi^2 = 34.00$, df = 1, P < 0.001). As a multi-species group, non-*Apis* bees displayed no preference for either plant (goodness of fit to 1:1 ratio; $\chi^2 = 0.03$, df = 1, P = 0.874).

Table 1. Numbers of Non-*Apis* and *Apis* (honey) bees collected from flowers of *C. solstitialis* and *V. agnus-castus* with mean (±1 SE) head capsule widths in mm; t-test results concern body sizes and are presented for each 15-min collection interval. (An asterisk indicates a significant difference.)

Time	*C. solstitialis* Non-*Apis* (*Apis*) (Mean ± SE mm)	*V. agnus-castus* Non-*Apis* (*Apis*) (Mean ± SE mm)	t, df, P
09:00-09:15	14 (0) (2.96 ± 0.79)	8 (5) (4.01 ± 1.42)	2.00, 10.70, = 0.038*
09:15-09:30	9 (0) (2.59 ± 0.86)	9 (6) (4.02 ± 1.34)	5.45, 11.20, < 0.001*
12:00-12:15	10 (0) (3.00 ± 1.52)	7 (4) (4.03 ± 1.52)	4.33, 15.00, < 0.001*
12:15-12:30	6 (0) (3.32 ± 1.35)	10 (2) (3.88 ± 1.17)	0.86, 8.70, = 0.206
15:00-15:15	11 (0) (3.12 ± 0.94)	13 (6) (4.02 ± 1.11)	2.66, 19.30, = 0.008*
15:15-15:30	13 (0) (3.05 ± 0.84)	10 (2) (4.28 ± 1.35)	1.75, 10.00, = 0.056
18:00-18:15	11 (0) (3.06 ± 0.92)	10 (4) (4.22 ± 1.33)	3.65, 15.30, = 0.001*
18:15-18:30	6 (0) (2.67 ± 1.09)	11 (6) (4.06 ± 1.22)	8.45, 13.60, < 0.001*
TOTAL	**80 (0) (-)**	**78 (55) (-)**	**N.A.**

Bee Size

In each collection period, regardless of collector, the average size of bees collected at *V. agnus-castus* was always larger than those collected during the same time at *C. solstitialis*. The average head capsule width of bees in each group collected from *C. solstitialis* never exceeded 4.0 mm, whereas all but one of the eight groups collected from *V. agnus-castus* did exceed 4.0 mm. According to a one-tailed t-test, the difference in size of bees collected from the two plant species was statistically significant in six of the eight 15-minute collection periods (Table 1). An increased representation of large-bodied bees in the Apidae (subfamilies Apinae and Xylocopinae) was observed in collections from *V. agnus-castus* (as noted above for the genera *Apis*, *Bombus* and *Xylocopa*)

DISCUSSION

Regardless of the time of day, honey bees were never collected at *C. solstitialis* during our 15-minute sampling periods (totaling two hours per person or four person hours). However, adjacent flowering plants of *V. agnus-castus* yielded nearly as many honey bees as other bee species combined. When large-bodied species in the genera *Bombus* and *Xylocopa* are considered, the pollinator guilds appear very distinct. Indeed, as in other systems, the distinction between large- and small-bodied bee pollination systems may have good descriptive if not biological value (e.g., Frankie et al. 1983); the biological basis for this difference in size may simply be in foraging energetics of the respective species as mediated through some form of exploitative competition (Schaffer et al. 1979).

Unlike the North American ecosystems where the areas and densities of *C. solstitialis* patches are impressive (Pitcairn et al. 2006), native populations are often less so (Uyger et al. 2004). It stands to reason that non-native populations of self-incompatible species such as *C. solstitialis* will require even greater visitation rates to establish viable and expanding populations (Sun and Ritland 1997, Gerlach and Rice 2003). However, there is also emerging evidence that some newly invasive plant species, including *C. solstitialis*, show higher levels of self-compatibility in the process of becoming invasive (Pyšek et al. 2011, Petanidou et al. In Press), in keeping with Baker's hypothesis (Baker 1965); additional evidence from Santa Cruz Island (USA) concerning self-compatibility in the invasive and sympatric congener *Centaurea melitensis* L. is also consistent with this hypothesis (Barthell et al. 2005).

The difference in foraging by honey bees at flowers between its native and non-native ranges may explain the relative lack of familiarity of yellow star-thistle honey in Turkey and Greece relative to the western USA where it is so appreciated for its flavor (Pellet 1976). In addition, the ecologically newfound association between honey bees and *C. solstitialis*, may explain this plant's success in invading the western USA as an otherwise largely self-incompatible plant species, while also providing a novel source of nectar for commercial honey sales. Ironically, it is also increasingly clear that natural competitors such as *V. agnus-castus* also dampen visitation by honey bees to this species in its native range; future studies will explore this phenomenon more closely with consideration for its impact on local beekeeping practices vis-á-vis honey production.

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GENİŞLETİLMİŞ ÖZET

Giriş: İstilacı bitki türleri istila ettikleri çevrelere nektar gibi yeni besin kaynakları getirerek büyük etkiler yapmaktadır. Batı Amerika'da Kaliforniya'daki Santa Cruz adasında örneğin güneş çiçeği *Centaurea solstitialis* ada ekosisteminde balarılarını daha yüksek sayıda cezbetmekte ve kendisine çekmektedir, sonucunda ise bu türün fazla sayıda tohum üretmesine neden olmaktadır. Bu çalışmada benzer durum iki farklı türde güneş çiçeği ve hayıt bitkisinin ürettiği nektar miktarı ile tozlaştırıcıları kendilerine farklı sayılarda çekip çekmediği konusu araştırılmıştır.

Materyal ve Metod: Çalışma Yunanistan'ın Kuzeydoğu ege adası olan Lesvos'da gerçekleştirilmiştir. Çalışma da adada bulunan güneş çiçeği *Centaurea solstitialis*, ve hayıt bitkisi *Vitex agnus-castus* ve bu alanlardaki *Apis* cinsi balarıları ve *Apis* cinsi olmayan balarıları kullanılmıştır. Çalışma 3 aşamada gerçekleştirilmiştir. İlk aşamada nektar miktarı belirlenmiştir. Miktar belirlenirken her iki türden 18 adet bitki seçilmiş ve bunlardan da 2'şer çiçekten birisi tozlayıcıların ziyaretini engellemek için bir örtü ile kapatılmıştır. Bu çiçeklerden ise saat 07:00 ile 19:00 arası her iki saatte bir üretilen nektar ölçülmüştür. İkinci aşamada ise 2 farklı örnekleyici 3 saatlik aralıklarla 09:00 ile 18:00 arasında farklı alanları değişerek böcek toplama kepçesi ile bitkileri ziyarete gelen arıları örneklemişlerdir. Üçüncü aşamada ise elde edilen veriler uygun istatistiksel

(t-testi, Ki-kare testi) analizlere tabi tutulmuşlardır. Sonuç olarak elde edilen ziyaret örüntüsü her bir bitki çiçeklerinden ölçülen ortalama nektar miktarı ile karşılaştırılmıştır.

Sonuç ve Tartışma: İlk aşama sonucuna göre elde edilen nektar miktarı her iki farklı ölçüm içinde hayıt (*V. agnus-castus*) bitkisinde çok daha yüksek olduğu kaydedilmiştir. Ziyaret örgüsü tespitinde ise balarılarının iki bitkiyi istatistiksel olarak anlamlı bir şekilde ziyaret ettiği bulunmuştur. Her iki türün bulunduğu komşu alanlardan yapılan örneklemelerde *Apis* cinsi balarıların *V. agnus-castus*'u daha fazla ziyaret ettikleri belirlenmiştir. Ek olarak, bu zaman içerisinde örneklenen ortalama tozlaştırıcı arı büyüklüğü (kafa kapsülü genişliği ile ölçülen) *V. castus-agnus*'dan toplananların *C. solstitialis*'a göre daima büyük olduğu ve birçok durumda anlamlı bir şekilde benzer sonuçlar bulunmuştur. Bu bulunan örüntüler Amerika'nın batısında (Kaliforniya) güneş çiçeğinin doğal bir bitki olmadığı alanlarda yapılan önceki çalışmalar ile zıtlık oluşturmaktadır. Bu alanlardaki güneş çiçeğinden elde edilen bal ekosistem üzerine yaptığı etki yanında ayrıca arıcılık marketine de etki yapmaktadır.

Anahtar Kelimeler: *Centaurea solstitialis*, hayıt, rekabet, istilacı tür, Lesvos, *Vitex agnus-castus*, güneş çiçeği.