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Characteristics of Nectar Production and Standing Crop in *Campsis radicans* (Bignoniaceae).

Andrea Alexis Edge
East Tennessee State University

Characteristics of Nectar Production and Standing Crop in *Campsis radicans* (Bignoniaceae)

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Andrea A. Edge

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Darrell Moore, Chair

Thomas C. Jones

Michael S. Zavada

Keywords: pollination syndromes, *Campsis radicans*, nectar production

ABSTRACT

Characteristics of Nectar Production and Standing Crop in *Campsis radicans* (Bignoniaceae)

by

Andrea A. Edge

We examined several aspects of nectar production in *Campsis radicans* to better understand how standing crop is affected and how production affects pollinator visitation. In all experiments, nectar and concentration of flowers were measured and total sugar was calculated. Flowers do not produce additional nectar unless nectar is removed, and it is not resorbed. Volume of standing crop and total sugar fluctuates throughout the day, whereas concentration remains constant. Age and time of day significantly affect regeneration of nectar and sugar. The number of removals did not significantly affect the amount of nectar or sugar regenerated; however, concentration declined significantly after the initial removal. We have established several factors affecting nectar production, although clearly there are other aspects influencing the production of nectar in *C. radicans*. Focus should be placed on determining the physiological aspects of secretion as well as studying the role that environmental factors have on physiological aspects.

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CHAPTER 1

INTRODUCTION

Angiosperms have developed specific floral traits used to attract a variety of animals as potential pollinators. Some of these floral systems are general and attract many different visitors that are effective pollinators, whereas some plants have coevolved with specific visitors which are now the most (and possibly the only effective) pollinator to the plant. Depending on the combination of traits (corolla color, scent, nectar/pollen rewards, stigma/anther position, etc.) some animals are more likely than others to visit and successfully pollinate certain flower species. For example, many flowers that display red, tubular flowers with large amounts of dilute nectar and little to no scent are typically pollinated by birds. According to this ornithophilous pollination syndrome, birds are attracted to the red corollas and use the large amounts of dilute nectar as a food source. Flowers that are bee-pollinated typically exhibit the melittophilous syndrome in which corollas are marked with bright yellow or blue nectar guides to help visitors find their ways to the rewards (Faegri and van der Pijl, 1979).

While pollination syndromes are helpful in generally classifying groups of pollinators, it has been shown that many plant species are visited by an array of animal visitors (McCall and Primack, 1992; Herrera, 1996; Hingston and McQuillan, 2000). Herrera (1996) cautions that plant-pollinator interactions are too complex to place into general pollination syndromes. For example, while the classical bee syndrome is evident in Fabaceae species in Tazmania, other flower species exhibiting zygomorphic yellow flowers with nectar guides are ignored by bees and are visited by flies, especially in higher elevations. While this study finds pollination

syndromes to be poor predictors of animal visitors, Wilson et al. (2004) finds that flower color was a good variable in predicting hummingbird vs. bee visited flower species.

Campsis radicans exhibits the apparent ornithophilous pollination syndrome displaying long, orange-reddish flowers. Bertin (1982*a*) described the floral visitors to this woody-climbing vine, among these being ruby-throated hummingbirds *(Archilochus colubris)*, honey bees *(Apis mellifera)*, bumble bees (*Bombus* spp.), and sweat bees (Halictidae). Although there are several visitors to this plant, it is thought that hummingbirds are the primary pollinators because they are capable of carrying 10 times as many pollen grains per visit as honeybees or bumblebees (Bertin, 1982*a*). Despite this much greater potential for pollen deposition, honey bees, bumble bees, and sweat bees have a much higher visitation rate than that of the hummingbirds. Other studies show flowers that exhibit the ornithophilous pollination syndrome may benefit from these high rates of visitation by invertebrates. Cumulative visits by bees may deposit as much, if not more, pollen than one or two bird visits, especially if those birds are scarcer in some areas relative to others (Kraemer and Schmitt, 1997; Wooller and Wooller, 2002; Roberston et al., 2005). Mistletoes (*Peraxilla* spp.) in New Zealand display the ornithophilous syndrome but a native short-tounged bee (*Hylaeus agilis*) is capable of supplementing, or even partially replacing birds as pollinators (Robertson et al., 2005). Similarly, Kraemer and Schmitt (1997) did not observe birds visiting *Echium wildpretii,* a flower that exhibited the ornithophilous syndrome: they found that honey bees and a short-tounged solitary bee, *Anthophora alluaudi*, were the most common visitors with the latter being the most effective pollinator. However, a later study discovered that birds visited *E. wildpretii* early in the season when bee visits were low, but this visitation pattern reversed significantly by the end of the season (Valido et al., 2002). Conversely, studies also show that

flowers exhibiting the bee-pollination syndrome can also be visited and pollinated by birds (Stone et al., 2003; Raju and Rao, 2006).

Environmental variables such as community structure, elevation, weather, season, and time of day can affect visitation activity to flowers better than flower morphology (McCall and Primack, 1992; Primack and Inouye, 1993; Hingston and McQuillan, 2000). It may be unlikely in some areas that have lower densities of hummingbirds than others that *C. radicans* can rely on one hummingbird visit alone because it requires high amounts of pollen (upwards of 4,000 grains, although 400 can be sufficient) from outcrossed donors or mixed pollinations with outcrossed donors and self pollen in order to successfully set fruit (Bertin and Sullivan, 1988; Bertin et al., 1989). While evidence suggests that honey bees have replaced hummingbirds as the main pollinator in cultivated *C. radicans* populations in Poland, fruit set was very low (Kolodziejska-Degorska and Zych, 2006) and similar to the fruit sets in the study done by Bertin (1982*a*). However, these numbers are much different from an earlier study done by Elias and Gelband (1975) in which they cited an average of 2.6 fruits per inflorescence. If the flower receives insufficient pollinations, fruit abortion will be induced (Bertin, 1982*a*; Bertin, 1982*b*; Bertin, 1985; Bertin and Sullivan, 1988; Bertin, et al., 1989; Bertin, 1990*a*; Bertin, 1990*b*).

In order to gain a better understanding of the pollination biology of *C. radicans* and what the most effective pollinators are, a comprehensive knowledge of the visitation patterns and behaviors of these potential pollinators is needed, as well as understanding the patterns of production and availability of food sources (i.e. nectar and pollen). A fundamental understanding of nectar production patterns and how environmental and physiological processes affect these patterns will provide valuable insights into the interactions between plant and pollinator. This manuscript focuses primarily on the availability and production of nectar as a

food source and investigates several environmental and physiological factors that influence nectar production.

Nectar production can be influenced by a variety of factors such as locale (Ono *et al.*, 2008), flower age (Torres and Galetto, 1998; Valtuena et al., 2007), time of day (Macukanovic-Jocic et al., 2004; Valtuena et al., 2007) visitation frequency (Torres and Galetto, 1998; Valtuena et al., 2007; Kaesar et al., 2008), flower size (Longo and Fischer, 2006), plant population density (Klinkhamer, 2004), floral display (Biernaskie and Cartar, 2004), sexual dimorphism (Torres and Galetto, 1998; de Castro and de Oliveira, 2001; Liu et al., 2002; Carlson, 2007), CO_2 levels (Lake and Hughes, 1999), and many others (see references in Zimmerman, 1988; Rathke, 1992). A potential pollinator's assessment of nectar quality at any point in time occurs upon encountering the standing crop. The distribution of standing crop within a population is a function of the variability of nectar production within the population and the rate of visitation by foragers (Zimmerman, 1988). Nectar sources that are more variable are more likely to result in potential pollinators visiting inflorescences significantly less than constant nectar resources. This may help reduce potential inbreeding caused by visitors trap-lining from flower to flower on a single plant with many large flowers (Biernaskie et al., 2002). Such variability would benefit *C. radicans* because it cannot rely on self-pollen alone (Bertin, 1982*a*; Bertin, 1982*b*; Bertin, 1985; Bertin and Sullivan, 1988; Bertin et al., 1989; Bertin, 1990*a*; Bertin, 1990*b*).

Flowers of *C. radicans* have been described as being primarily adapted for hummingbird pollination (Bertin, 1982). However, if flowers are visited primarily by hummingbirds, we would expect that there would be large quantities of dilute nectar with a concentration around 20-25 %, or even lower, as this is typical nectar concentration for "bird-pollinated" flowers (Pyke, 1981; Nicolson, 2002; McDade and Weeks, 2004; Mendonca and Anjos, 2006).

However, despite claiming bird-pollination of *C. radicans*, Bertin (1982) found concentrations of about 30 % in standing crop and also observed a very large number of bumblebees, honeybees, and halictids as visitors and showed that bumblebees and honeybees could adequately pollinated *C. radicans* flowers. If *C. radicans* is primarily pollinated by bees, then we would expect that volume be relatively low and nectar concentration would be much higher so that the nectar would be more characteristic of "bee-pollinated" flowers. On the other hand, it would not be irrational to consider that *C. radicans* would adapt to more than one pollinator. Many studies show that plants are pollinated by visitors other than what their syndromes suggest (McCall and Primack, 1992; Waser et al., 1996; Herrera, 1996; Kraemer and Schmitt, 1997; Hingston and McQuillan, 2000; Wooller and Wooller, 2002; Roberston et al., 2005; Wilson et al., 2007), so it is reasonable to consider that a plant existing in a habitat with many potential pollinators would adapt to have more than one pollinator. If this hypothesis is true, we would expect that nectar concentration would be attractive to both hummingbird and bee visitation, by being on the higher end of "bird-pollinated" flowers and the low end of "bee-pollinated" flowers, and flowers would contain an intermediate volume of nectar. Nectar production has rarely been comprehensively studied in any one species (Zimmerman, 1988; Rathcke, 1992; Torres and Galetto, 1998). Bertin (1982*a*) characterized standing crop in *C. radicans* for flowers open to pollination of known and unknown age that were open to pollination, as well as nectar regeneration in repeatedly sampled flowers that were not open to pollination. Because nectar production consequently affects pollinator behavior (Cartar, 2004; Leiss et al., 2009) and pollination rate (Silva and Dean, 2000), a more detailed understanding of the nectar secretion patterns in these flowers is needed.

Our study also aims to quantify several factors of nectar production. The first is to determine when nectar and sugar production begins and how much is present at various stages of

development and during anthesis. Elias and Gelband (1975) noted that *C. radicans* begins production at some point before anthesis, but it was not noted when. One possibility is that *C. radicans* produces enough nectar before anthesis and produces no additional nectar as long as no visitors remove the nectar. However, it is also possible that additional nectar is produced throughout anthesis regardless of whether a visitor has removed any nectar. At the end of anthesis, the flower can either resorb the remaining nectar in order to conserve and reallocate resources (Nepi and Stpiczyńska, 2008*a*, and references therein), or the nectar is simply wasted (Búrquez and Corbet, 1991). Another aspect of nectar production is the effect of multiple removals. It may be expected that the more times nectar is removed from a flower, the more nectar the flower will produce in response (Galetto et al., 1994; Castellanos et al., 2002) in order to continue attracting pollinators.

An additional aspect that we want to consider is the rate of nectar and sugar regeneration in continually sampled flowers. If flowers are being visited the most in the morning, nectar regeneration may be highest at that time in order to keep up with the constant removal of nectar. If nectar visitation is highest in the late afternoon, then we might expect that regeneration would be highest late in the afternoon. Alternatively, nectar regeneration may be highest at the hours when pollinators are not as active in order for the plant to regenerate nectar for the next round of heavy pollinator activity. Because flowers are open continuously for 2-3 d, it is also a possibility that there are nocturnal visitors to *C. radicans*. Bertin (1982*a*) did not investigate this in depth, only noting some, but no extensive, nocturnal sphingid visitation. If these flowers are nocturnally pollinated, then regeneration may be high at night, which *Bertin* (1982*a*) did not find. Bat-pollination is not likely because flowers that have adapted to this pollinator are typically dull in color and produce a fruity or musty smell (Baker, 1961). Moth pollination is

more likely as moths visit narrow corolla tubes that are usually, but not exclusively, white in color. They are also nocturnal, but some species are diurnal or crepuscular (Baker, 1961).

Previous work by Bertin (1982*a*) determined that nectar production had a higher rate in the late morning hours and became low in the late evening/early morning hours. However, flowers of the same age were used, conflating the results, making it impossible to determine whether the affect was due to the time of day, age of the flower, or some interaction between the two. Our study uses several groups of flowers in order to determine how both age and time of day affect regeneration. Many studies show that both factors can affect rate of regeneration (Torres and Galetto, 1998; Macukanovic-Jocic et al., 2004; Valtuena et al., 2007), so it is likely that one or both play some part in regeneration. Plants may have adapted to regenerate more nectar at specific times of day in response to pollinator activity, either to produce more nectar at times when pollinator activity is high or produce more nectar to replenish flowers for the next round of pollination. If there are few to no nocturnal pollinators, *C. radicans* may be taking the opportunity during the night hours to replenish its nectar for the next day. We also expect that flowers will produce less nectar as they age because flowers may reallocate resources for other functions such as seed development.

Although standing crop has already being characterized for *C. radicans*, it is important to determine this characteristic in our study, as Bertin (1982*a*) used populations in Illinois and Missouri and only looked at standing crop of flowers of unknown age once in one population. Variation with respect to time of day, population structure, and visitation frequency is certainly expected. It is, therefore, important to sample standing crop at different sites at different times throughout the flowering season. Assuming that volume fluctuates with degree of visitation, standing crop will be inversely proportional to the amount of visitation throughout the day. If

visitation rate is highest early in the day, then standing crop should be the lowest at that time; however, if visitation is highest late in the day then standing crop should be lowest in the evening.

This study aimed to investigate 1) nectar availability in closed and open flowers and determine if there is a diel rhythm based on time of day or age of flower, 2) if time of day or age of flower influences nectar regeneration, 3) how cumulative nectar and total sugar production are affected by multiple removals, and 4) the volume, concentration, and total sugar of the standing crop at various time of day.

CHAPTER 2

ORIGINAL ARTICLE

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Characteristics of nectar production and standing crop in *Campsis radicans* (Bignoniaceae)

Andrea A. Edge, Byron N. Van Nest, Michael Feathers, and Darrell Moore*

East Tennessee State University, Department of Biological Sciences, Box 70703, Johnson City, TN 37614, USA

Nectar production in *Campsis radicans*

*Corresponding author: moored@mail.etsu.edu

ABSTRACT

- *Background and Aims* Understanding the factors that affect standing crop is important, as this is how any potential pollinator assesses a prospective food source. We examined several aspects of nectar production in *Campsis radicans* to better understand how standing crop is affected and how production affects potential pollinator visitation.

- *Methods* In all experiments nectar and concentration were measured and total sugar was calculated. Flowers were sampled only once to determine the general nectar secretion patterns of buds and bagged open flowers at various times and ages as well as standing crop of flowers of unknown ages open to pollination throughout the day. Repeatedly sampled, bagged flowers were used to determine the effects that age and time of day had on nectar regeneration as well as the effects that number of removals had on cumulative nectar and sugar regeneration.

- *Key Results* Flowers do not produce additional nectar unless removed and nectar is not resorbed. Standing crop data showed that volume fluctuates throughout the day and has a highly significant correlation with sugar, whereas concentration remains constant regardless of volume (about 30 %), which is higher than most hummingbird pollinated flowers. Age and time of day show a significant interaction affecting regeneration of nectar and sugar. The number of removals did not significantly affect the amount of nectar or sugar regenerated; however, concentration declined significantly after the initial removal.

- *Conclusions* We have established several factors that affect nectar production, although it is clear that there are other aspects influencing the production of nectar in *C. radicans*. Although concentration declines significantly after the first experimental removal to 16 % - 24 %, the concentration of standing crop is consistently 30 %. Homeostatic mechanisms

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likely play a large role in nectar production; therefore, it is important that more focus be placed on determining the physiological aspects of nectar secretion, as well as comprehensively studying the role that environmental factors have on the physiological aspects.

Keywords: Nectar production, standing crop, nectar, nectar regeneration, *Campsis radicans*, Bignoniaceae

INTRODUCTION

Campsis radicans is a member of the very large, predominantly neotropical family, Bignoniaceae. It produces wind-dispersed seeds characteristic of its subfamily, Tecomeae. *Campsis radicans* also shares a disjunct distribution with its only sister species, *Campsis grandiflora*, in eastern Asia. Its historical habitat is likely a dry forest, which is suitable for winged seed dispersal (Gentry, 1983), although it also occurs widely in disturbed areas such as edges of woods and fences and along roadsides and streams (Wen and Jansen, 1995).

Members of the Bignoniaceae family that are endemic to neotropical areas employ a wide variety of pollination strategies. In any given area it is possible for about 20 different Bignoniaceae species to live within the same habitat due to a large number of pollinators and wide diversity of morphological characteristics (Gentry, 1979). Each of these species within the community exploits different types of pollinators such as birds, bats, hummingbirds, bees, and hawkmoths, thus deterring hybridization between closely related individuals. Many of the sympatric Bignoniaceae are pollinated by the same guild of intermediate to large solitary bees. To further separate the pollination niches, about half of the 20 species within the area have different peak flowering times within the flowering season (Gentry, 1980). Pollinators rely heavily on these plants as a food source, as Bignoniaceae makes up a large portion of some forest areas (Gentry, 1980; Gentry, 1990).

While specialization can be more beneficial in cases of high potential pollinator diversity, generalization may be more beneficial in areas with lower pollinator abundance. Bertin (1982*a*) describes *C. radicans* as being hummingbird pollinated, although in this study it was clear that there was a low abundance of hummingbirds, but a large number of bee visitors capable of pollination (Bertin, 1982*a*). While *C. radicans* demonstrates characteristics of the "bird-

pollinated" syndrome (e.g. large, reddish-orange corollas and copious amounts of nectar), it also

shows at least one morphological characteristic of the "bee-pollinated" syndrome (e.g. the

presumptive bright yellow nectar guides on the inside of the corolla). *Campsis* may have

adapted to the low abundance of hummingbirds in open and disturbed areas by allowing other

effective pollination by more abundant pollinators such as bees. It would not be unreasonable to

consider that a plant would adapt to exploiting two or more types of pollinators even though

some predominant morphological features imply that it is pollinated by only one visitor (Waser

et al., 1996; Kraemer and Schmitt, 1997; Wooller and Wooller, 2002; Robertson *et al.*, 2005).

Perhaps the most important characteristics involved in attracting potential pollinators are those

concerned with nectar—its concentration, volume, and rate of production and how these factors

vary with time of day and age of flower.

Because nectar is an important reward that many animals rely on as a food source,

potential pollinators place selective pressures on plants to produce nectar at optimal times, rates,

volumes, and concentrations. In turn, plants must secrete the appropriate quantity and quality of

nectar in order to attract the most effective pollinators to facilitate pollination (Zimmerman,

1988). These intricate relationships have caused many plants and animals to evolve

complementary morphological traits. In addition to many documented cases of these

evolutionary relationships (Macior, 1971; Kiester *et al.*, 1984; Zimmerman, 1988), some specific

examples include fig wasps and their host species (Marussich and Machado, 2007) as well as the

Madagascar star orchid and its pollinator, the hawkmoth, *Panogena lingens*, (Sphingidae)

(Nilsson *et al.*, 1985).

A potential pollinator's assessment of nectar quality at any point in time occurs upon

encountering the standing crop. The distribution of standing crop within a population is a

function of the variability of nectar production within the population and the rate of visitation by foragers (Zimmerman, 1988). A fundamental understanding of nectar production patterns and how environmental and physiological processes affect these patterns will provide valuable insights into the interactions between plant and pollinator.

Nectar production can be influenced by a variety of factors such as locale (Ono *et al*., 2008), flower age (Torres and Galetto, 1998; Valtuena *et al*., 2007), time of day (Macukanovic-Jocic *et al*., 2004; Valtuena *et al*., 2007), visitation frequency (Torres and Galetto, 1998; Valtuena *et al*., 2007; Kaesar *et al*., 2008), flower size (Longo and Fischer 2006), plant population density (Klinkhamer, 2004), floral display (Biernaskie and Cartar, 2004), sexual dimorphism (Torres and Galetto, 1998; de Castro and de Oliveira, 2001; Liu *et al*., 2002; Carlson, 2007), CO_2 levels (Lake and Hughes, 1999), and many others (see references in Zimmerman, 1988; Rathke, 1992).

Flowers of *C. radicans* have been described as being primarily adapted for hummingbird pollination (Bertin, 1982). However, if flowers are visited primarily by hummingbirds, we would expect that there would be large quantities of dilute nectar with a concentration around 20-25 %, or even lower, as this is typical nectar concentration for "bird-pollinated" flowers (Pyke, 1981; Nicolson, 2002; McDade and Weeks, 2004; Mendonca and Anjos, 2006). However, despite claiming bird-pollination of *C. radicans*, Bertin (1982) found concentrations of about 30 % in standing crop and also observed a very large number of bumblebees, honeybees, and halictids as visitors and showed that bumblebees and honeybees could adequately pollinated *C. radicans* flowers. If *C. radicans* is primarily pollinated by bees, then we would expect that volume be relatively low and nectar concentration would be much higher so that the nectar would be more characteristic of "bee-pollinated" flowers. On the other hand, it would not be

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flowers that were not open to pollination. Because nectar production consequently affects

pollinator behavior (Cartar, 2004; Leiss *et al*., 2009) and pollination rate (Silva and Dean, 2000),

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throughout anthesis, regardless of whether a visitor has removed any nectar. At the end of

anthesis, the flower can either resorb the remaining nectar in order to conserve and reallocate

resources (Nepi and Stpiczyńska, 2008*a*, and references therein), or the nectar is simply wasted (Búrquez and Corbet, 1991). Another aspect of nectar production is the effect of multiple removals. It may be expected that the more times nectar is removed from a flower, the more nectar the flower will produce in response (Galetto *et al*., 1994; Castellanos *et al*., 2002) in order to continue attracting pollinators.

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study uses several groups of flowers in order to determine how both age and time of day affect regeneration. Many studies show that both factors can affect rate of regeneration (Torres and Galetto, 1998; Macukanovic-Jocic *et al*., 2004; Valtuena *et al*., 2007), so it is likely that one or both play some part in regeneration. Plants may have adapted to regenerate more nectar at specific times of day in response to pollinator activity, either to produce more nectar at times when pollinator activity is high or produce more nectar to replenish flowers for the next round of pollination. If there are few to no nocturnal pollinators, *C. radicans* may be taking the opportunity during the night hours to replenish its nectar for the next day. We also expect that flowers will produce less nectar as they age because flowers may reallocate resources for other functions such as seed development.

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The goals of this study were to establish a more detailed understanding of nectar production in *C. radicans* by 1) determining basic nectar secretion patterns in closed flowers and unvisited open flowers, 2) examining the rate of regeneration with respect to time of day and

flower age, 3) determining the cumulative amount of nectar and sugar regenerated after various

numbers of removals, and 4) assess standing crop at different sites and at different times of the

blooming season and how it may be affected by environmental factors.

MATERIALS AND METHODS

Study Site and Species

This study was conducted during the months of June, July, and August of 2007, 2008, and 2009

at two different locations in Johnson City, Tennessee. Site 1, used during all 3 years, was the

longtime abandoned Marine Corps Armory, now an overgrown meadow with clusters of large

trees. Black locusts (*Robinia pseudoacacia*), blackberry (*Rubus* spp.), goldenrod (*Solidago* spp.),

Virgin's Bower (*Clematis virginiana*), and honeysuckle (*Lonicera* spp.) were common. *Campsis*

radicans grew primarily along a fence line bordering this site but also grew throughout the

blackberries and honeysuckle and within trees. Site 2 (approx. 4 km from Site 1), used during

June and July of 2009, was an overgrown, urban, roadside embankment in Johnson City, TN.

Campsis radicans also grew along the fenceline here; honeysuckle and bush clover (*Lespedeza*

cuneata) were abundant at this site.

    Campsis radicans is a woody climbing vine that relies on outcrossing as well as

vegetative propagation (Bertin, 1982). Although it is not self-compatible, it is capable of setting

fruit with self pollen if donor pollen is also present (i.e. cryptic self-incompatibility) (Bertin and

Sullivan, 1988; Bertin *et al*., 1989). It grows in disturbed areas and flowers between May and

late September, with the highest flowering period spanning the end of June and the beginning of

July. Flowers typically are open only for 2-3 days. The flowers are protandrous with anthers

that are dehiscent at the time of anthesis and stigmas that become receptive about 10 hours after

anthesis and continue to be receptive for about 55 h (Bertin, 1982). At least 450 pollen grains are needed to set fruit although more than 4,000 will not increase seed set (Bertin, 1990). Flowers are considered to be bird-pollinated (Bertin, 1982).

Nectar secretion patterns in closed and open flowers

Using capillary micropipette tubes (10 µL and 20 µL), nectar was collected and measured from 15-20 closed buds of various lengths up to 60 mm (flowers open at 58 ± 5.41 mm SD; Edge *et al.*, unpublished data) at four different times of day (0300 h, 0900 h, 1500 h, and 2100 h). Bud lengths were measured from the tip of the longest sepal to tip of the corolla. Nectar concentration was measured using a pocket refractometer (Bellingham and Stanley, Turnbridge Wells, UK). Total sugar per sample (mg) was also calculated (Kearns and Inouye, 1993). Flowers were sampled only once. This was repeated four times on 27 July 2007, 29 June 2008, 17 July 2008, and 23 June 2009 at Site 1 and once on 13 June 2009 at Site 2. Flowers were sampled randomly with a number generator. General Linear Modeling (GLM) was used to analyze the effects of time of day, age of flower, and day of collection on volume, concentration, and total sugar. Corrections for heterogeneity were made by taking the square root of the data sets for volume and total sugar and the cube root of the data set for concentration. Comparison of means was performed using Scheffe's *post-hoc* test.

Flowers being sampled for nectar secretion patterns in open flowers were bagged prior to anthesis with a fine mesh bridal veil to prohibit any visitors from collecting nectar. Bagged flowers were inspected at 0700 h, 1500 h, and 2300 h to determine the approximate time of anthesis. Flowers were considered open once any petals began to unfurl: a variety of insects were capable of entering the corolla at this point. A 24-h period was allowed to pass before any

nectar sampling began. Flowers were sampled randomly and chosen only from the group of flowers found to be open during the inspection survey.

Volume, concentration, and total sugar were measured in a similar fashion to the closed flower study, and flowers were sampled at 0315 h, 0915 h, 1515 h, and 2115 h. Each flower was sampled once. Flower age was estimated from the difference between the approximate time of anthesis (0700 h, 1500 h, or 2300 h) and the sampling. We determined a categorical age of each flower (i.e. 0-8 h, 8-16 h, etc.) because exact age could not be known. Sampling was conducted three times in 2008 (18, 23, and 27 July) at Site 1 and once on 11 June 2009 at Site 2. GLM was used to analyze the effects of time of day, age of flower, and day of collection on volume, concentration, and total sugar. Heterogeneity was corrected by taking the square root of the data set for volume and total sugar and the square of the data set for concentration.

Effects of nectar removal

Three different treatments (Control, 1X and 2X) were used to assess the effect of nectar removal on the secretion patterns of individual flowers. Flowers that appeared close to anthesis were bagged with fine bridal mesh on the evening (approx. 1700 h) prior to the day of the experiment. On the following morning, at approximately 0830 h, bagged flowers were inspected and all opened flowers were discarded: because they had opened sometime overnight, a precise age could not be determined. Bagged flowers were inspected again at 0930 h and 1030 h. Any flowers that had opened since the initial inspection were used for the study. 1100 h was designated the start time ("time 0") and all flowers of this same age class were known to be of similar age (within 2.5 hours of one another).

The sampling regime for each treatment was similar to the methods of Galetto *et al*. (1994). Flowers in the 1X treatment were sampled 10 h after "time 0" and again at 34 h after "time 0". Flowers in the 2X treatment were sampled 2, 10, and 34 h after "time 0." The control group was sampled only once at 34 h after "time 0." Volume, concentration, and total sugar were measured as in the open and closed flower studies. Cumulative volume and sugar were calculated for each flower for each of the three treatments. One-way ANOVA was used to determine if treatment affected cumulative sugar and volume, and ANOVA for repeated measures was used to assess the effects of nectar removals on volume, concentration, and total sugar. Pair-wise comparisons of means were performed using Scheffe's *post-hoc* test. This experiment was performed three times at Site 1 on 21 June, 2 July, and 10 July, 2009.

Nectar regeneration as a function of age or time of day

Unopened buds approaching anthesis were bagged the evening prior to the first day of sampling. On day one of sampling, 30 min prior to each sampling time (0230 h, 0830 h, 1430 h, and 1830 h), bagged flowers were inspected for newly opened flowers (identified from unfurled petals). Of those newly opened flowers, 10-15 were chosen randomly at each of the four sample times. Flowers were sampled in the same order every 6 hours following their initial sample. Newly opened flowers were selected only on day 1, except for flowers in the 0300 h category, which also had to be obtained on day 2, due to a low rate of anthesis in the early morning hours. This sampling regime allowed us to examine flowers of different, known ages at the same times of day in order to determine the relative influences of time of day and age of flower on nectar regeneration. Nectar volume, concentration, and total sugar were measured as before. This study was performed only once on 14 June 2009 at Site 1. The data were analyzed using two-

way ANOVA for repeated measures. Pair-wise comparisons of the treatment means were performed using Scheffe's *post-hoc* test.

Standing crop

Twelve to 15 open flowers of unknown age were sampled every 4 hours for a 24 h period starting at 0200 h. Each flower was sampled once. Volume, concentration, and total sugar were measured as described above. Standing crop was measured twice at Site 1 (25 June and 7 July 2009) and three times at Site 1 (19 June, 30 June, and 14 July 2009). Linear regression was used to determine any correlations between dependent variables and one-way ANOVA was used to determine any differences in concentration, total sugar, or volume with respect to time of day.

RESULTS

Nectar secretion in closed flowers and open flowers

The two studies from the same year at Site 1 on 28 June and 18 July 2008 were analyzed together to report here. The data from 2008 showed that time of day was not a significant factor affecting volume ($P=0.823$, $F_{3,118} = 0.303$) (Fig. 1A), concentration ($P = 0.340$, $F_{3,50} = 1.156$) (Fig. 1B), or total sugar ($P = 0.658$, $F_{3,118} = 35.246$) (Fig. 1C). However, age (the length of the bud) exhibited a significant effect on volume ($P < 0.0001$, $F_{5,118} = 26.438$) (Fig. 2A), concentration of unopened flowers ($P = 0.03$, $F_{2,50} = 3.873$) (Fig. 2b), and total sugar ($P < 0.0001$, $F_{5,118} = 35.246$) (Fig. 2C). Because of the small amount of nectar in the smaller buds (0-10 mm, 0.47 ± 0.47 µL s.e.; 10-20 mm, 0.07 ± 0.07 µL s.e.; 20-30 mm, 0.43 ± 0.24 µL s.e.), it was difficult to obtain a large enough sample for the refractometer to read the concentration.

Lengths < 30 mm were excluded from the analysis because there were not enough buds in these categories with an observable amount of nectar to obtain a concentration.

Scheffe's *post-hoc* test showed that with respect to volume, ages 40-50 mm (23.82 ± 3.37 µL s.e.) and 50-60 mm (59.87 ± 7.95 µL s.e.) were significantly different from all other age categories and from each ($P < 0.05$). With respect to total sugar, flowers 40-50 mm (7.16 ± 1.03 mg s.e.) and 50-60 mm (21.01 ± 3.42 mg s.e.) were significantly different from all other age categories ($P < 0.05$), except each other ($P > 0.05$). With respect to concentration, ages 40-50 mm (26.95 ± 0.93 % w/w s.e.) and 50-60 mm (30.39 ± 1.01 % w/w s.e.) were significantly different from 30-40 mm (8.45 ± 3.64 % w/w s.e.). Day of collection was a significant factor only with respect to concentration ($P = 0.015$), and there was a significant interaction between time, age, and day of collection with respect to volume ($P = 0.048$).

TABLE. 1 Significance values for closed and open flower studies (2007, 2009).

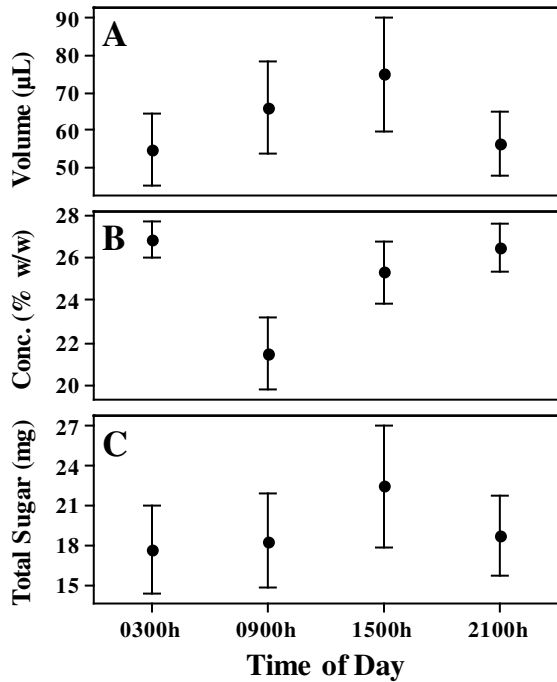|	Volume (µL)	Concentration (% w/w)	Sugar (mg)
CLOSED			
Site 1, 2007			
Time	N/S	< 0.0001	0.035
Age	< 0.0001	< 0.0001	< 0.0001
Time*Age	N/S	N/S	0.023
Site 1, 2009			
Time	N/S	< 0.0001	0.59
Age	< 0.0001	N/S	< 0.0001
Time*Age	N/S	N/S	N/S
Site 2, 2009			
Time	N/S	< 0.0001	N/S
Age	< 0.0001	0.005	< 0.0001
Time*Age	N/S	N/S	N/S
OPEN			
Site 2, 2009			
Time	N/S	N/S	N/S
Age	0.012	N/S	N/S
Time*Age	N/S	N/S	N/S

FIG. 1 Affect of time of day on nectar secretion in open and closed flowers (2008). Differences in volume (A), concentration (B), and total sugar (C) of open and closed flowers from five replicates in 2008 with respect to time of day. There are no significant differences in volume and total sugar with respect to time of day when closed and open flowers are analyzed together ($P > 0.05$); although, there is a significant difference in concentration ($P = 0.016$). However, when open and closed flowers are analyzed separately, there are no significant differences in volume, concentration, or total sugar with respect to time of day ($P > 0.05$). Error bars are one s.e. from the mean.

Open flowers were sampled three times at Site 1 during 2008. Time of day had no effect on volume (115.5 ± 9.36 µL s.e., $P = 0.294$, $F_{3,113} = 1.262$) (Fig. 1A), concentration (26.74 ± 0.54 % w/w s.e., $P = 0.432$, $F_{3,109} = 0.928$) (Fig. 1B), or total sugar (35.43 ± 2.97 mg s.e., $P = 0.467$, $F_{3,114} = 0.858$) (Fig. 1C). Age also had no effect on volume ($P = 0.727$, $F_{5,113} = 0.565$) (Fig. 2A), concentration ($P = 0.902$, $F_{5,109} = 0.315$) (Fig. 2B), or total sugar ($P = 0.692$, $F_{5,114} = 0.692$) (Fig. 2C). There was, however, a slightly significant interaction between time and age with respect to total sugar ($P = 0.043$, $F_{13,114} = 1.919$).
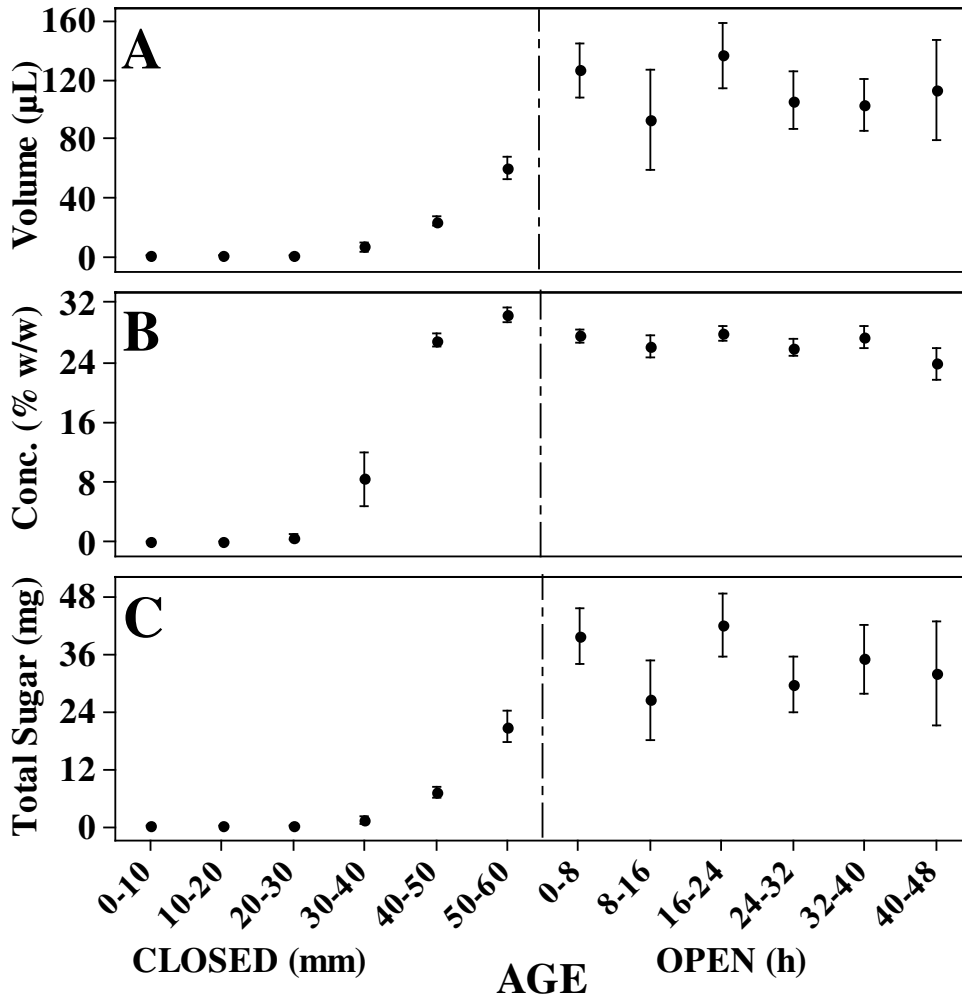
FIG. 2 Affect of age on nectar secretion in open and closed flowers (2008). Differences in volume (A), concentration (B), and total sugar (C) of open and closed flower from two closed and three open flower replicates in 2008 with respect to age of flower. Nectar and sugar do not begin to be produced until closed flowers reach 30-40 mm in length. Nectar and sugar are no longer produced in significant quantities once flowers open. Nectar reaches a concentration of 25 %- 30 % when the buds are 40-50 mm in length and concentration does not significantly change throughout anthesis of unsampled flowers. Error bars are one s.e. from the mean.

Effects of nectar removal

The number of removals did not significantly affect the amount of nectar regeneration

with respect to treatment for any of the three replicates (235.83 ± 22.74 µL s.e., $P = 0.62$, $F_{2,35} =$

0.484; 216.18 ± 11.12 µL s.e., $P = 0.782$, $F_{2,35} = 0.247$; 207.67 ± 21.16 µL s.e., $P = 0.822$, $F_{2,28} =$

0.198, for all three replicates) (Fig. 3A), and variation in volume within each treatment was quite

high. Variation in sugar between the three treatments was lower than it was for volume;

however, differences between treatments were also not significant (71.91 ± 7.57 mg s.e., $P =$

0.391, $F_{2,35} = 0.97$; 75.12 ± 3.97 mg s.e., $P = 0.162$, $F_{2,35} = 1.922$; 68.04 ± 6.47 mg s.e., $P = 0.213$,

$F_{2,28} = 1.643$, for all three replicates) (Fig. 3B).
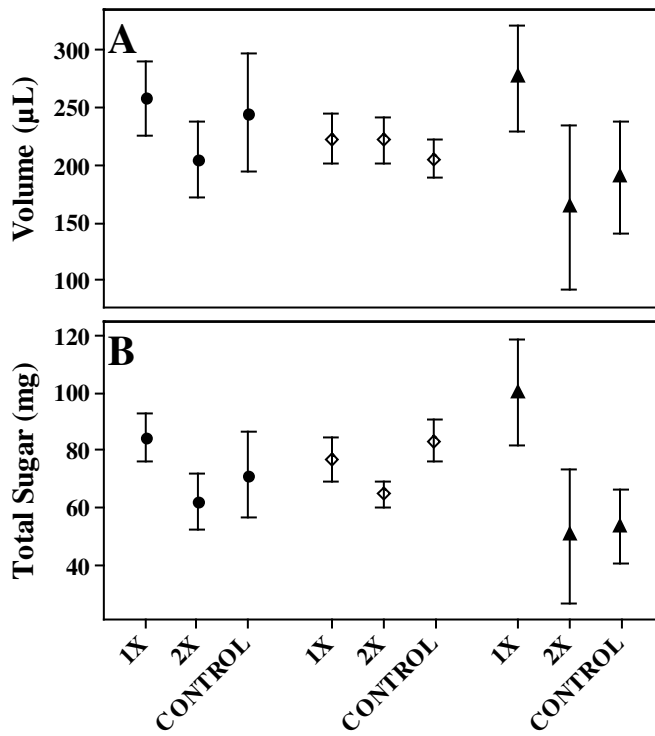
FIG. 3 Affect of repeated samplings on cumulative volume and total sugar. Mean cumulative volume (A) and mean cumulative total sugar (B) ± one s.e. for flowers sampled once, twice and never. There were no significant differences in volume or total sugar with respect to the number of times flowers were sampled. Solid circles represent the Replicate 1, open diamonds represent Replicate 2, and solid triangles represent Replicate 3.

The general trend for all of these replicates was for concentration to start out high at the

initial removal, and then decline with subsequent removals (Fig. 4B). For replicates 1 and 2,

concentration was between 30% and 35% during the first sampling regardless of the treatment

type. Scheffe's *post*-hoc tests showed the values for the initial samples of each treatment were not significantly different from each other ($P > 0.05$). Concentrations of subsequent samples were lower than the first removals (16 % - 24 %). In replicate 1, the concentrations of the second and third removals from treatments 2X and 1X were not significantly different from each other ($P > 0.05$); however, in replicate 2, concentration of only the second removals from the 2X and 1X treatments were significantly different from each other ($P < 0.05$). Treatment 2X showed no significant differences between removal 2 and removal 3 ($P > 0.05$). Replicate 3 showed the same general trend of replicate 1 and replicate 2, in that concentration started out highest for all of the initial removals. Concentration declined significantly with each removal during the 2X treatment ($P < 0.05$); although, concentration did not decline significantly at the second removal for the 1X treatment, and the concentration of sample 2 from treatment 2X was not significantly different from the concentration of the control ($P > 0.05$).
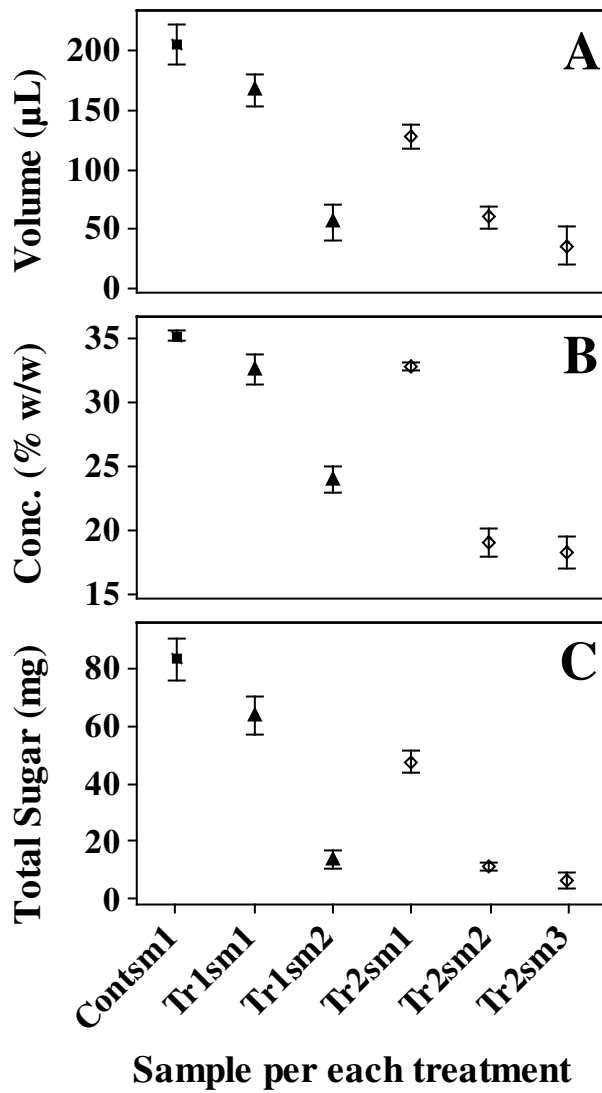
FIG. 4 Affect of repeated samplings on nectar and sugar regeneration. Mean volume (A), concentration (B), and total sugar (C) ± one s.e. produced for each treatment within Replicate 2 of the effects of nectar removal study. Volume, concentration, and total sugar regeneration decline significantly between the first and second removals regardless of treatment type. Solid squares represent the Control, solid triangles represent the 1X treatment, and open diamonds represent the 2X treatment.

Like concentration, volume also was higher at the initial removals (146-245 µL) (Fig.

4A). There were no significant differences among the initial volumes among the different

treatments in either replicate 1 or replicate 3. However, the initial removal between treatment

2X was significantly lower from the initial removal between the control in replicate 2 ($P =$

0.0172). All three replicates showed the same following trends (Fig. 4A). In treatment 1X, there was a significant decline in volume of nectar produced between the first removal to the second removal ($P < 0.05$); however, in treatment 2X the amount of nectar produced did not decline significantly between the first removal and the second removal and the second and third removals ($P > 0.05$). There was, however, a significant difference between volume of nectar produced between the first and third removals ($P < 0.05$).

Total sugar was highest at each initial removal (47-83 mg), and there were no significant differences in initial total sugar among the different treatments for any of the three replicates ($P > 0.05$) (Fig. 4C). In treatment 1X there was a significant decline in total sugar produced between the first and second removals for all replicates ($P < 0.05$). In treatment 2X the total amount of sugar produced was significantly different between the first and second removal ($P < 0.05$), but there were no significant differences between the second and the third removals for any replicates ($P > 0.05$). There were no significant differences between the second and third removals with respect to total sugar produced ($P > 0.05$).

Nectar regeneration as a function of age or time of day

Both age and time of day had significant effects on the regeneration of nectar (volume, $P < 0.0001$, $F_{7,273} = 13.18$; $P < 0.0001$, $F_{3,273} = 21.14$; concentration, $P < 0.0001$, $F_{7,264} = 81.32$; $P < 0.0001$, $F_{3,264} = 21.21$; total sugar, $P < 0.0001$, $F_{2,273} = 95.38$; $P < 0.0001$, $F_{3,273} = 12.49$, respectively) (Figs. 5-6). There was also a significant interaction between age and time of day on volume, concentration, and sugar ($P = 0.0019$, $F_{2,273} = 2.25$; $P < 0.0001$, $F_{2,264} = 5.4$; $P < 0.0001$, $F_{21, 273} = 4.17$, respectively). Concentration, volume, and sugar declined with age as well as time of day; however, regardless of age, concentration, volume, and total sugar showed some

similarities at certain times of day. It is interesting to note that regardless of the age, flowers within the same time category had very similar volumes and total sugar amounts. This is most noticeable at the first 1500 h sample. The other time samples have many similar clusterings; however, there are many time groups that are similar to other time groups. Despite these similarities, the adjusted p-values from the one-way ANOVA for repeated measures showed that only newly opened flowers (0-2 h) had significantly different volumes, concentrations, and total sugar from other age categories. No other age categories were significantly different from any other age categories except those that were newly opened regardless of the time that they opened. These results suggest that while there is a general decline in regeneration of volume, concentration, and total sugar, it is significantly higher at the initial sampling.
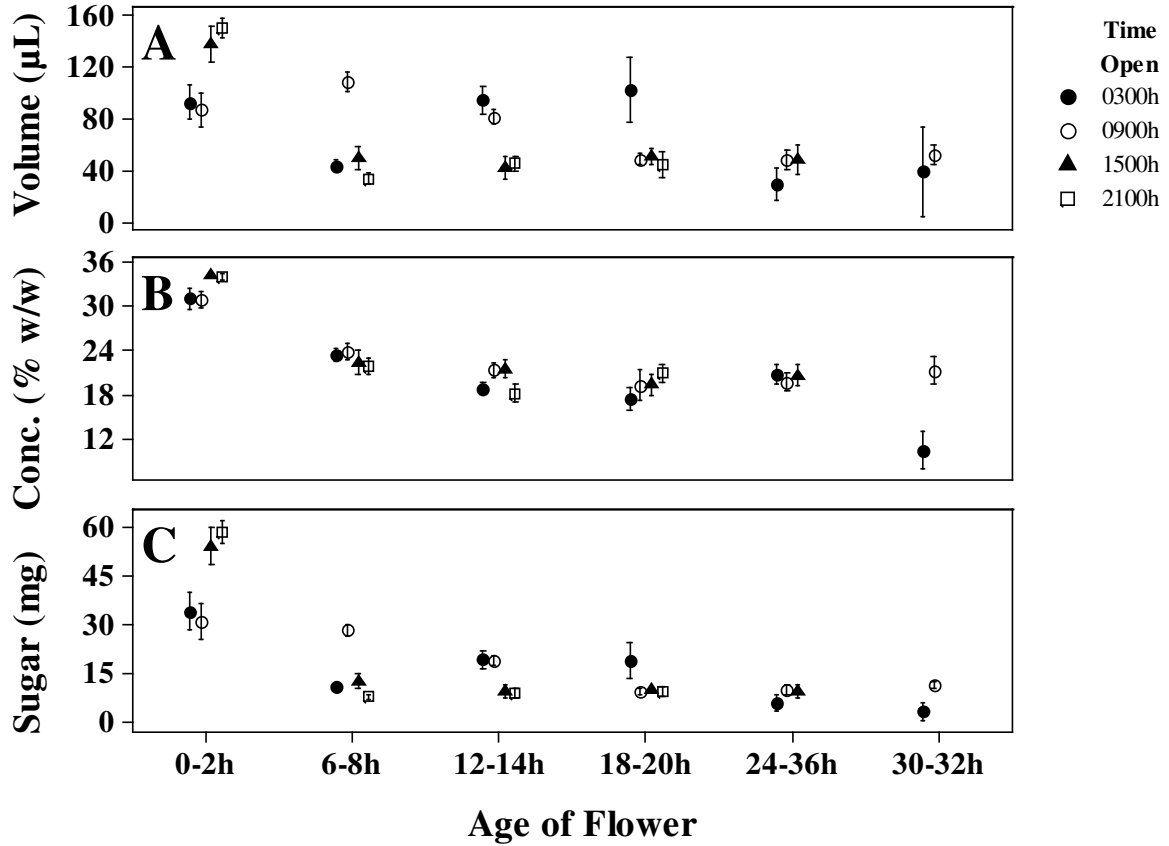
FIG. 5 Affect of age on nectar and sugar regeneration. Mean volume (A), concentration (B), and total sugar (C) ± one s.e. of groups of flowers that opened at different times to test for differences in nectar regeneration with respect to age of flower. Volume, concentration, and sugar declined significantly from the first sample regardless of the time that the flowers opened.
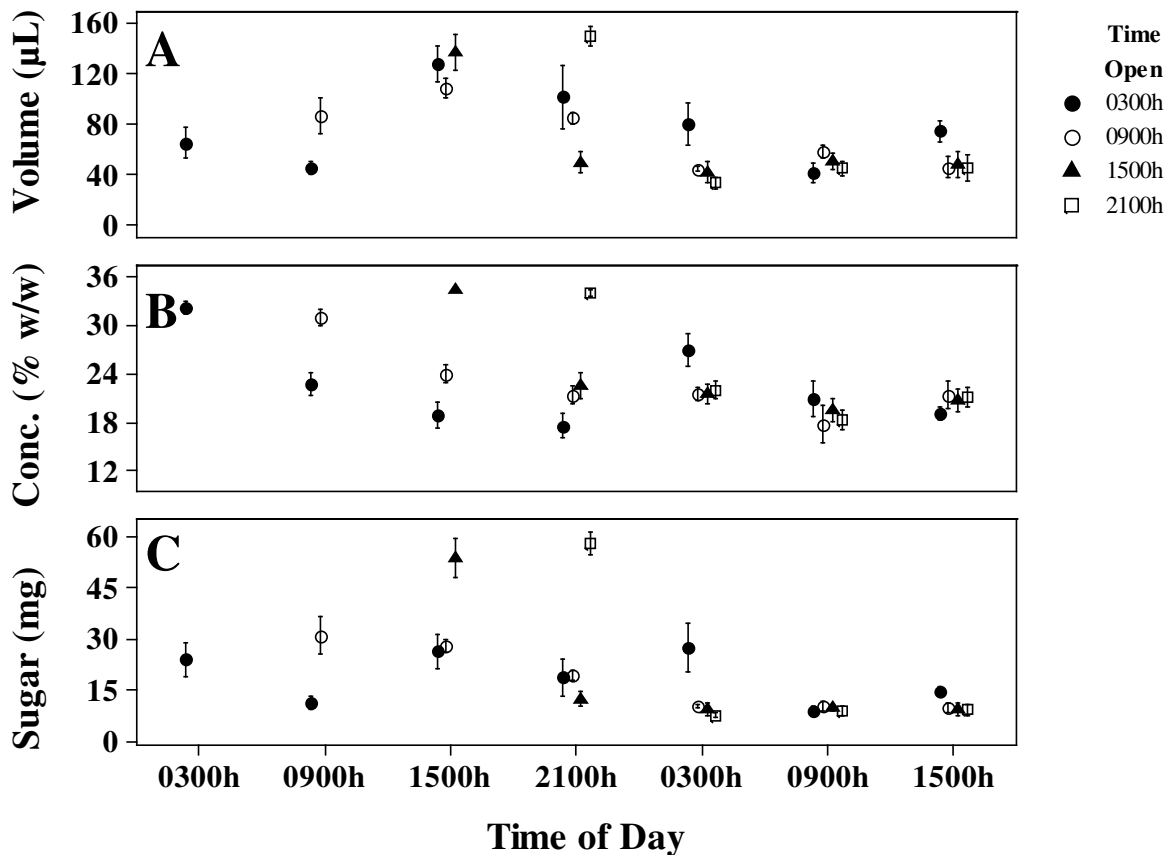
FIG. 6 Affect of time of day on nectar and sugar regeneration. Mean volume (A), concentration (B), and total sugar (C) ± one s.e. of groups of flowers that opened at different times to test for differences in nectar regeneration with respect to time of day. Volume, concentration, and total sugar tend to show more differences with respect to time of day than that of age of flower, although one-way ANOVA for repeated measures suggests that both age and time of day play a significant role in regeneration of nectar and total sugar.

Standing crop

All four replicates from 25 June, 30 June, 7 July, and 14 July 2009 showed a highly

significant correlation between volume and total sugar ($P < 0.0001$ for all, $r^2 = 0.992$, 0.945,

0.969, 0.987, respectively) (Fig. 7A). Conversely, volume and concentration showed no

significant correlation, (25 June, 30.48 ± 0.42 % w/w s.e., $P = 0.242$, $r^2 = 0.07$; 30 June, 31.25 ±

0.68 % w/w s.e., $P = 0.374$, $r^2 = 0.0$; 7 July, 29.45 ± 0.52 % w/w s.e., $P = 0.54$, $r^2 = 0.0$; 14 July,

31.26 ± 0.53 % w/w s.e., $P = 0.203$, $r^2 = 0.01$) (Fig. 7B). While there was a significant

correlation between volume and sugar for the 19 June 2009 experiment conducted at the Site 1

($P < 0.0001$, $r^2 = 0.74$), the correlation was not as strong as it was in the other four replicates.

Also, unlike the other four replicates, volume and concentration did show a significant

relationship, although the correlation was not as strong ($P = 0.001$, $r^2 = 0.11$). This is likely

because this experiment displayed a large variation in copious amounts of nectar (109.22 ± 9.21

µL s.e.), which may have been due to rain earlier in the day. Despite this large variation in

nectar, the average concentration of the standing crop on 19 June remained about 30%, which

was similar to that of the other replicates (30.09 ± 0.62 % w/w s.e.).
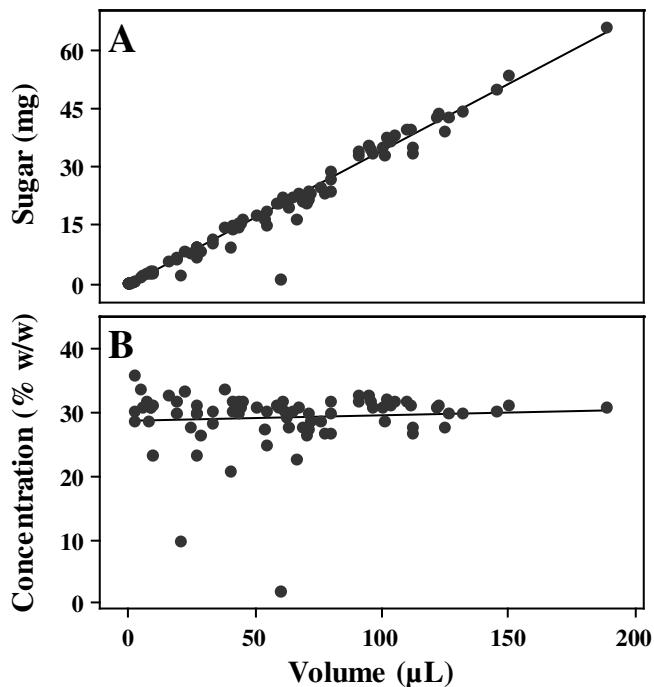

FIG. 7 Relationship of concentration and sugar with volume of standing crop. Regression of total sugar vs. volume (A) and concentration vs. volume (B) in standing crop on 7 July 2009. Sugar is highly correlated with volume ($P < 0.0001$); however, concentration remains constant throughout the day at about 30 % regardless of volume.

There were temporal variations throughout the day with respect to the volume of standing crop, except for the replicate on 19 June in which time had no effect on volume ($P = 0.478$, $F_{5,89} = 0.911$). The general trend was higher volumes of nectar in the early morning, followed by a reduction in volume throughout the day, with nectar reaching its lowest point at 1800 h, and then a subsequent rebound later in the evening. This trend is evident in the replicates on 7 July at Site 2 ($P < 0.0001$, $F_{5,89} = 7.886$) (Fig. 8A), 14 July at Site 1 ($P < 0.0001$, $F_{5,89} = 9.279$), and to a lesser extent on 25 June 2009 at Site 2 ($P = 0.019$, $F_{5,71} = 2.941$). The replicate on 30 June at Site 1 shows a significant difference in volume with respect to time (39.02 ± 3.27 µL s.e., $P < 0.0001$, $F_{5,88} = 5.261$); however, there is only one significantly higher peak at 0600 h (70.99 ± 9.88 µL s.e., $P < 0.05$). Because total sugar is significantly correlated with volume, the temporal patterns of total sugar for each replicate were parallel to those of volume (Fig. 8B-8C). There were no significant differences with respect to time of day in total sugar on the 19 June experiment (35.14 ± 2.18 mg s.e., $P = 0.493$, $F_{5,89} = 0.887$). In all other replicates time did significantly affect amount of total sugar in a similar fashion as volume ($P < 0.05$, in all cases).
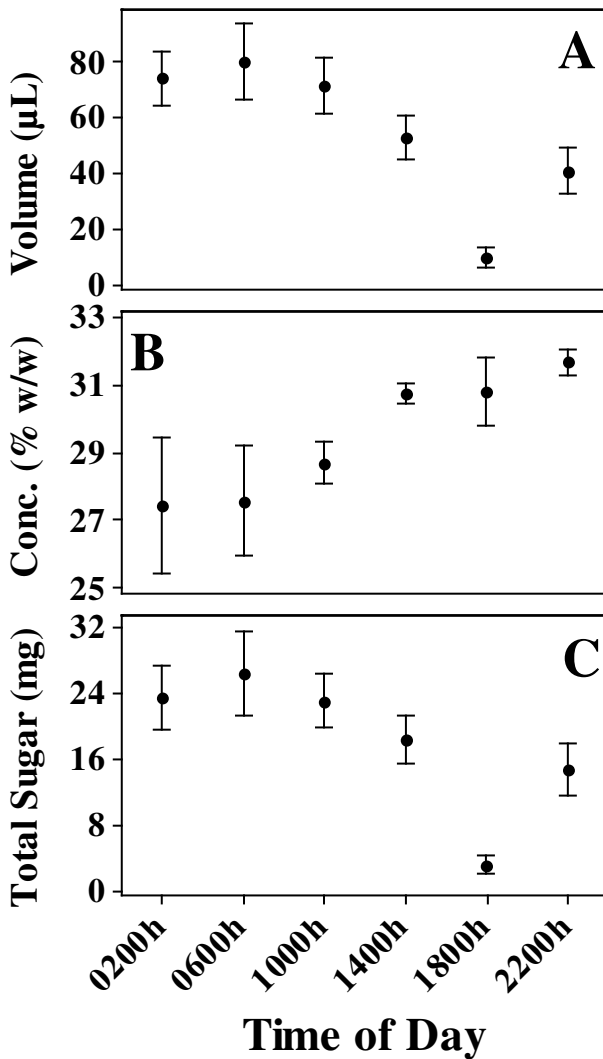
FIG. 8 Mean volume, concentration, and sugar of standing crop at various times of day. Mean volume (A), concentration (B), and total sugar (C) of standing crop on 7 July 2009. All values are ± one s.e. from the mean. Volume and sugar drop significantly at 1800 h, whereas concentration is significantly higher in the early evening hours.

Concentration did not differ significantly with time of day on 19 June at Site 1, 30 June at Site 1, or 7 July at Site 2 ($P = 0.408$, $F_{5,89} = 1.026$; $P = 0.916$, $F_{5,78} = 0.293$; $P = 0.051$, $F_{5,79} = 2.327$, respectively). Concentrations were higher at only one time of day on 25 June at Site 2 (1800 h, 32.8 ± 1.14 % w/w s.e. and 2200 h, 27.89 ± 1.23 % w/w s.e., $P = 0.05$, $F_{5,60} = 2.388$),

and 14 July at Site 2 (1400 h, 34.81 ± 1.04 % w/w s.e. and 0200 h, 28.89 ± 1.82 % s.e., $P = 0.037$,

$F_{5,65} = 2.557$).

DISCUSSION

Our study has contributed a significant extension of knowledge of nectar production for *C.*

radicans in addition to those of Bertin (1982). Our closed and open flower studies have

demonstrated that nectar and sugar began to be produced in significant quantities when the buds

attained a size of 40-50mm or 50-60mm in anticipation of anthesis (Fig. 2). Once open, there

were no significant changes in volume throughout the life of the flower, suggesting that in the

absence of pollinators flowers only produce a limited volume upon opening and will not produce

additional amounts of nectar unless removed. This is similar to another Bignoniaceae member,

Spathodea campanulata (Rangaiah *et al*., 2004). This may be a way for the plant to conserve

costly nectar resources, as producing excess nectar has been known to lower seed set in various

plants (Pyke, 1991; Ordano and Ornelas, 2005; Ornelas and Lara, 2009); however, Ordano and

Ornelas (2005) found that this is not always the case. In order to further avoid losing excess

energy, some plants are capable of nectar resorption (Nepi and Stpiczyńska, 2008*a*, and

references therein), although *C. radicans* does not show evidence of this, as volume and

concentration do not significantly change in older unsampled flowers. This also indicates that

additional sugar is not produced. This absence of change in volume and concentration at various

ages is contrary to what occurs in another Bignoniaceae member, *Pyrostegia venusta*

(Bignoniaceae), in which both concentration and nectar production increases as flowers age

(Galetto *et al*., 1994).

The number of nectar removals did not significantly affect the cumulative amount of nectar or total sugar that was produced (Fig. 3A and Fig. 3B). It was evident that nectar and sugar were regenerated after full removals; however, it was usually a significantly less amount than was originally produced and the concentration was significantly lower. *Pyrostegia venusta* exhibits a similar pattern in that total sugar did not change with respect to the number of removals and concentration declined after each removal. However, *P. venusta* produced significantly more nectar the more they were sampled (Galetto *et al*., 1994). It is interesting to note that we did not see a similar pattern in *C. radicans* for volume, even though it was clear that *C. radicans* flowers did regenerate nectar and total sugar after an initial sampling (Fig. 4). The significant addition of nectar may not be apparent because flowers regenerate only a small amount of nectar after the initial sampling, and many flowers within the 2X treatment did not produce any nectar by the last removal at 34 h. The absence of a significant additional secretion of nectar may be confounded by the high variation in nectar between individual flowers, as plants can have a substantial within-plant and between-plant variation in order to potentially increase outcrossing (Ott et al., 1985; Boose, 1997, but see Feinsinger, 1978).

Some protandrous plants have higher nectar production during the male-phase of flowering (Torres and Galetto, 1998; de Castro and de Oliveira, 2001; Liu *et al*., 2002; Carlson, 2007), although it is not likely that the high volume and concentration of nectar at the beginning of anthesis is due to this. Groups 2X were sampled 2, 10, and 34 h after anthesis and showed significant differences between 2 and 10 h, the time at which flowers were still in the male-phase (Bertin, 1982). Even if flowers went to the female-phase at the 10-h removal, a decline isn't likely due to the sexual-phase because we see the same type of decline in nectar and concentration after 2-h removals in this experiment and 6 h after the initial removals in the ATM

experiment, when the flowers in both experiments are still within the male-phase. Rarely the 2X treatments showed a significant difference between the 10-h and 34-h removals. There is an overall similar pattern of decline in concentration and volume, regardless of treatment, which suggests that the decline is due to the effect of sampling rather than sexual-phase. The ATM experiment also supports this conclusion, as it shows a similar pattern of significant decline in concentration after the initial removal followed by a general decline every 6 hours of sampling from the beginning of anthesis (Figs. 5 and 6).

At both sites, volume of standing crop exhibited similar temporal trends throughout the day. Volume was highest in the early morning hours, declined throughout the day, and then rebounded in the late evening hours (Fig. 8A). Total sugar was highly correlated with volume, thereby following similar temporal patterns (Fig. 7A). This is particularly interesting because the trends that we observed at our sites are very different from those observed by Bertin (1982), who found volume to be lowest in the late evening and early morning hours. The differences seen between the two studies may be due to the effects that time of day has on nectar production or the intensity of animal visitation. Our results suggest the decline in volume late in the afternoon and rebound in the late evening/early morning is due to possible repeated animal visitation throughout the day, although these results will need to be correlated to animal visitation rates (Edge *et al*., in prep.). It could be that nectar production is highest in the evening in order to prepare for the following day of visitation (Zimmerman and Pyke, 1986), although the results from the ATM study indicate that nectar production is highest between 0900 h and 2100 h (Fig. 6); therefore, it is likely that nectar accumulates during the evening hours in the absence of visitation (Bertin, 1982; Edge *et al*., unpublished data). Concentration remained relatively

constant at about 30 % regardless of time of day (Fig. 8B). Our data are consistent with the concentrations that Bertin (1982) recorded.

Our data illustrating the standing crop for volume and concentration throughout the day suggest that bee-pollination is a possibility, as standing crop concentration is relatively high. However, it does not rule out hummingbird pollination, as hummingbirds will still visit flowers of higher concentrations (Pyke, 1981; Nicolson, 2002; McDade and Weeks, 2004; Mendonca and Anjos, 2006). It is possible that *C. radicans* may have adapted to two primary pollinators, hummingbirds and bumble bees, which may explain why *C. radicans* does so well in open and disturbed areas where hummingbirds are not as abundant as bees. ATM experiments show that age of flower and time of day are both significant factors in controlling nectar and sugar production. In this study nectar and sugar was produced in higher quantities between 0900 h and 2100 h regardless of age, suggesting that nectar production is highest between these times in order to compensate for an increased amount of visitation by diurnal visitors.

ENR experiments show that concentration was high at initial nectar removals (30-35 %) and declined significantly with subsequent removals (16-24 %) (Fig. 4B). This pattern is also evident in the ATM study (Fig. 5B). It seems that because concentration declined significantly after a single nectar removal, it is expected standing crop continually open to pollination would also have a significantly lower concentration than 30 %. However, this is not the case, and it is likely that there are other physiological factors that are controlling sugar production in order to maintain concentration at a consistent level. It is also suggested that some type of homeostatic mechanism is operating that maintains volume production and nectar concentration in open flowers (Galetto *et al.*, 1994; Castellanos *et al*., 2002; Nepi and Stpiczynska, 2008*b*), but the physiological mechanisms controlling these aspects of nectar production are poorly understood.

Another possibility is that nectar evaporation could cause the concentration to increase in flowers exposed to direct sunlight (Rangaiah *et al*., 2004), although this may not be likely because *C. radicans* corollas are long, thereby creating a microclimate that prevents extensive evaporation (Plowright, 1987; Nicolson, 2002). If evaporation truly was a factor, we would expect a high level of variation in concentration due to different positions of flowers within the plant. Another thing to consider is that in our experiments nectar was completely drained. It is not likely that one visitor will drain an entire flower at each visit. It may be that sugar and nectar are regenerated at a constant rate in order to keep the concentration consistent. It may also be that no nectar is regenerated until the flower is completely drained. This latter possibility is unlikely, however, because nectar concentration decreases significantly below 30 % after one complete removal. It would be beneficial to know how nectar is produced after only a partial nectar removal, which would mimic a scenario more similar to a foraging event. This is difficult, however, because one would not know the amount of nectar or sugar present initially. In some large flowers such as *C. radicans* it may be possible to remove the nectar, measure the volume and concentration, and then replace a known portion of the nectar into the nectary. One would then be able to measure the amount of nectar regenerated after that time. Interpretation may be difficult, however, because it is not known how short period nectar removals of time would affect the rate of nectar secretion in *C. radicans*.

We have described a fundamental nectar secretion pattern in unsampled closed and open flowers, as well as determined how age and time of day affects nectar production and how production is affected by varying numbers of removals. While these aspects have expanded what is known about the nectar production of *C. radicans*, there are many other factors that must be investigated in order to gain a complete understanding of its nectar production.

Environmental variables are relatively easy to test, and many have been studied in other plants. However, physiological variables are not well understood, and testing them is much more difficult. It is likely that there is a 'sugar sensing' homeostatic mechanism that maintains the concentration of standing crop (Galetto *et al.*, 1994; Castellanos *et al*., 2002; Nepi and Stpiczynska, 2008*b*). Evidence shows that there are *in vivo* responses to different amounts of sugars in plant leaves of *Arabidopsis* as demonstrated by the up-regulation and down-regulation of specific genes (Gonzali *et al*., 2006), so it is plausible to consider that there are the same types of in *vivo* responses in nectaries. Clearly more research needs to be conducted to determine how these mechanisms work, as well as how environmental conditions affect them. A more complete understanding of nectar production in one species may provide insights on understanding the basic mechanisms in other flowering plants.

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VITA

ANDREA A. EDGE

EDUCATION**:**   M.Sc. (Biology) May 2010, East Tennessee State
                                    University, USA. Thesis: Pollination biology of
                                    Campsis radicans (Bignoniaceae), May 2010
                                  B.Sc. (Biodiversity and Conservation Biology) May 2006,
                                    Cedar Crest College, USA. Thesis: Variation in
                                    flower visitation among three species of bees in a
                                    Pennsylvania meadow, May 2006

EMPLOYMENT HISTORY**:**   Mississippi State University, Research Experience
                                    for Undergraduates, Research Intern, 2005
                                  Elmwood Park Zoo, Animal Care Intern, 2004

HONORS AND AWARDS: 2007-2009, Graduate Assistantship, East Tennessee State
                                    University
                                  2009, Research Assistantship, East Tennessee State
                                    University
                                  2005, Nomination by faculty in the Department of
                                    Biological Sciences at Cedar Crest College for
                                    Morris K. Udall Scholarship

PUBLICATIONS: Edge AA, Van Nest BN, Feathers M, Moore D. *In
                                    prep*. Characteristics of nectar production in
                                    Campsis radicans (Bignoniaceae).
                                  Edge AA, Van Nest BN, Feathers M, Moore D. *In
                                    prep*. Truly bird pollinated? Evidence for bumble
                                    bee pollination in *Campsis radicans* (Bignoniaceae).
                                  Moore D, Boyd S, Van Nest BN, Edge AA, Johnson JN,
                                    Naeger NL. *In prep*. Investigating nectar rhythms
                                    in squash (*Cucurbita pepo*): Effects on honey bee
                                    (*Apis mellifera*) foraging behavior.
                                  Jones TC, Akoury TS, Hauser CK, Neblett MF, Linville BJ,
                                    Edge AA, Weber NO. *In prep.* Octopamine and
                                    serotonin have opposite effects on anti-predator
                                    behaviour in the orb-weaving spider, *Larinioides
                                    cornutus*.

SUBMITTED ABSTRACTS: Pollination biology of *Campsis radicans* (Bignoniaceae).
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                                    Snowbird, Utah, 2009

Pollination biology of *Campsis radicans* (Bignoniaceae): Is it really bird pollinated? Annual Meeting for the Appalachian Student Research Forum, Johnson City, Tennessee, 2009

Investigating nectar rhythms in squash (*Cucurbita pepo*): Effects on honey bee (*Apis mellifera*) foraging behavior. Annual Meeting for the Entomological Society of America, Reno, Nevada, 2008

Variation in flower visitation among three species of bees in a Pennsylvania meadow. Annual Meeting for the Pennsylvania Academy of Science, Hershey, Pennsylvania, 2006

Variation in flower visitation among three species of bees in a Pennsylvania meadow. Lehigh Valley Ecology and Evolution Symposium, Lehigh, Pennsylvania, 2006

TEACHING EXPERIENCE: Biology III for majors labs- Spring and Fall 2008, Spring and Fall 2009
Biology I for majors labs- Fall 2007

ORGANIZATIONS: Botanical Society of America
Sigma Xi
Entomological Society of America
American Association for the Advancement of Science