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PATTERNS OF NECTAR PRODUCTION IN *ASCLEPIAS CURASSAVICA* (APOCYNACEAE)

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Abstract—Milkweeds are important nectar resources for insects in the New World. In addition, nectar is the germination medium for milkweed pollen. This study is the first controlled, greenhouse examination of patterns of nectar production in a milkweed species. We measured nectar volume, concentration, and mg of sugar in the pantropical, weedy milkweed *Asclepias curassavica*. Our results show that *A. curassavica* secretes nectar primarily during daylight hours and it continues at a constant daily rate for four to five days. Freshly secreted nectar is lower in sugar concentration than older nectar. This provides an opportunity for milkweed pollen to germinate throughout the day, but pollen germination could be inhibited at times when the sugar concentration increases. Nectar production in *A. curassavica* is adapted to attract diurnal insect pollinators over several days and to allow pollen germination to occur quickly. Significant differences in nectar production exist among plants and inflorescences within plants. Nectar production increases in flowers when nectar is extracted using paper wicks that simulate removal by insects in nature. Removal-enhanced nectar production in milkweeds may allow plants to adjust resources to inflorescences receiving insect visitation. Significant inter-plant differences in nectar production and the unique milkweed flower provides a model system for examining the role of pollinator-mediated selection on nectar traits.

Keywords: Asclepias; nectar production; neotropics; milkweed; pollination

INTRODUCTION

Variation in floral morphology, flower color, anthesis, and nectar production can be assembled into suites of traits recognized as pollination syndromes (Faegri & van der Pijl 1979). Selection for floral traits occurs through the interaction with pollinator functional groups whose unique behavioral and morphological attributes affect the pollination efficiency of the flowers (Fenster et al. 2004). Floral nectar is frequently the reward to pollinators and may be matched to the extraction mechanics and energetics of the pollinator. For example, butterfly flowers form a broad landing platform of brightly colored flowers that diurnally produce large volumes of dilute nectar that can be extracted using the tubular lepidopteran proboscis by active suction (Kim et al. 2011). Recent evidence also suggests that plants may respond to pollinator activity by increasing nectar production following insect visitation (Luo et al. 2014)

Patterns of nectar production influence several reproductive outcomes for the plant. Nectar production is often greatest in larger flowers, or flowers with unique attributes that pollinators learn to associate with greater nectar rewards (Cresswell & Galen, 1991; Fenster et al. 2006). Likewise, the variance in nectar production among flowers and inflorescences may be associated with sexual stage of the flower (Devlin et al. 1987), movement of pollinators (Zhao et al. 2016), pollination success (Pleasants & Chaplin 1983; Mitchell 1993), and levels of geitonomy/xenogamy (Hodges 1995; Biernaskie et al. 2002; Misaki et al., 2018). Nectar production appears to have a significant genetic component in some species (Campbell 1996; Boose 1997; Klinkhamer et al. 1999), although the experimental conditions rarely control for genotype x environmental interactions (Mitchell 2004). Thus, identifying patterns of nectar production in controlled conditions is essential for understanding how plant genotypes may interact with pollinators in natural populations.

Milkweeds (*Asclepias,* Apocynaceae) have long been recognized as important nectar sources to a large assemblage of native insects (Robertson 1929; Betz et al. 1994), beneficial insects for agriculture (Tillman & Carpenter 2014; James et al. 2016), and urban honeybees (MacIvor et al. 2017) in North America. Even though there are more than 140 milkweed species in the Americas, patterns of nectar production have been examined in only five species. Milkweed nectar appears to be sucrose rich (Southwick et al. 1981) with few other constituents. Milkweed cardenolides have been found in the nectar of species with high foliar and floral cardenolide content (Manson et al. 2012). Milkweeds species differ in their time of nectar secretion and nectar volume in field settings (see Pleasants and Chaplin 1983; Wilson et al. 1979; Wyatt & Shannon 1986; Wyatt et al. 1992). However, differences in nectar collection protocols, pollinator exclusion bag materials, and field settings have made it difficult to determine whether differences in nectar production exist between inflorescences on the plant and among plants. In addition, it is unknown if single sampling or repeat sampling of nectar from flowers affects the total nectar production in milkweeds. As demonstrated in other flower plants, nectar removal may enhance the rate of nectar secretion, thus flowers that are repeatedly sampled may secrete more total nectar than

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flowers that are sampled only once. (Castellanos et al. 2002; Ornelas et al. 2007; Luo et al. 2014).

In this study, we use *Asclepias curassavica* L. to examine patterns of nectar production in a common greenhouse environment. Specifically, we ask the following five questions. (1). Do individual plants differ in nectar production? (2) Do flowers on different umbels of the same plant have similar or different nectar production? (3) Do flowers secrete nectar over the duration of their 5-6 day (d) life span? (4) Do plants secrete nectar during the day or at night? (5) Does nectar removal affect the rate of nectar secretion?

MATERIALS AND METHODS

Study system

Asclepias curassavica is an annual milkweed native to the neotropics. It has been introduced elsewhere in the tropics where it occupies weedy sites such as moist ditches. The species is popular among monarch butterfly enthusiasts as it is easy to grow and readily available through many seed companies. *Asclepias curassavica* is self-compatible (Wyatt & Broyles 1997), although it maintains large colorful flowers, produces abundant nectar, and relies on insects for all pollinations. Principal pollinators have been identified as butterflies in its native range (Wyatt 1980; Bierzychudek 1981; Fuhro et al. 2010), but hymenoptera will visit and pollinate the flowers elsewhere in its naturalized range (Ward et al. 2012; Ward & Johnson 2013). *Asclepias curassavica* flowers and produces seeds year-round throughout the tropics (Kellett & Shefferson, 2018).

Milkweeds represent a monophyletic group with well-defined, unique flower morphology. The unique morphology has been summarized elsewhere (Wyatt & Broyles 1994) but the pertinent details include the following. Milkweed flowers have a showy corona of five hoods and horns modified from anther tissue. This corona encircles a gynostegium of five stigmatic chambers and styles that are partitioned between two ovaries. Pollen is packaged into sacs (i.e., pollinia) with an outer coat and a pore to funnel germinating pollen tubes towards the stigmatic chambers. Two pollinia from adjacent anthers are connected to a grooved corpusculum through thin bands of tissue known as translator arms. Therefore, the pollen dispersal unit is the pollinarium, which is composed of two pollinia connected by a common corpusculum. Nectar is produced from epithelial tissue lining the five stigmatic chambers. Nectar flows from the stigmatic chambers into the cuculli and hoods of the milkweed flower. Galil and Zeroni (1965) showed that nectar flow exist among hoods and stigmatic chambers is continuous by way of hidden passages.

Nectar has two primary roles in milkweed flowers. First, it is the primary reward for both diurnal and nocturnal insect visitors. According to data for *A. syriaca* (Southwick et al. 1981), the carbohydrate composition is nearly 100% sucrose. Second, nectar is the primary germination medium for milkweed pollen. After pollinia are deposited in stigmatic chambers, they are bathed in nectar, and pollen germination occurs 4-12 hours after pollinia insertion. Pollen germination is optimal in 5-30% sucrose solutions for *A. syriaca* (Kevan et al. 1989) and around 30% for *A. exaltata* (Shannon & Wyatt 1986). Preliminary trials on *A. curassavica* yield comparable results with the optimal range for pollen germination between 15 and 30% (unpub. obs.).

Growth conditions

Plants of *A. curassavica* were grown from seeds obtained from a variety of flower seed companies and from plants maintained from Anurag Agrawal at Cornell University. *Asclepias curassavica* seed germinates in 7-10 days, and these plants may produce flowers in six to ten weeks. Seeds were germinated in November 2017 at the SUNY Cortland greenhouses where the plants were maintained under artificial and natural light with a 14 hour light/10 hour dark photoperiod through May 2018. Plants were grown in a coconut coir soil mix (PRO-MIX HPCC) and watered twice daily. Plants were fertilized weekly with Peters 20-20-20 at the rate of 1 teaspoon per gallon of water.

Nectar extraction

We experimented with different nectar extraction techniques prior to beginning experiments. We used 10 µL calibrated micropipettes (Drummond Scientific, Broomall, PA, USA). The glass tips readily fit into the flower hoods and nectar readily ascended the pipettes by capillary movement. Two observations suggested that micropipette extraction was not optimal. First, we observed that hoods were sometimes damaged during the first day of nectar extraction. Damaged hoods were visibly withered the next day and without nectar. Second, additional nectar remained visible in the hoods after nectar extraction by pipette. This was true of nearly every flower sampled.

We conducted an experiment on flowers from three plants (two umbels per plant, seven flowers per umbel) to determine how much nectar remained in flowers after micropipette extraction. This experiment involved extracting as much available nectar via micropipette as possible, followed by removing the flower and inserting it upside down into a 0.5 mL microcentrifuge tube. Flowers were spun in a mini-microcentrifuge for 1-2 seconds. Flowers were removed from the tube and the expelled nectar drawn into the 10 µL micropipette for measurement. This allowed us to compare the volume and concentration of nectar extracted first using the micropipette followed by the centrifugation method. We adopted centrifugation as the method to extract all nectar because the volume remaining in the flower was as great as the volume initially extracted using the micropipettes (Fig. 1).

We measured the filled length of the 10 µL calibrated micropipette, then estimated nectar volume as a ratio of measured nectar column relative to the calibration mark on the micropipette. Nectar concentrations were determined using 0-50% and 45-85% hand-held refractometers (Bellingham & Stanley, Tunbridge Wells, U.K.). These refractometers measure nectar concentration as g per 100 g of nectar solution (C). The density (D) of sugar at each concentration was calculated using $D = 0.0000178\ C^2 + 0.00379201\ C + 0.9988603$ (Prŷs-Jones & Corbet 1991). Nectar sugar content per sample was then determined from the equations: Sugar Content (mg) = DVC/100 (Comba et al. 1999), where V is the volume of the sample in µL.

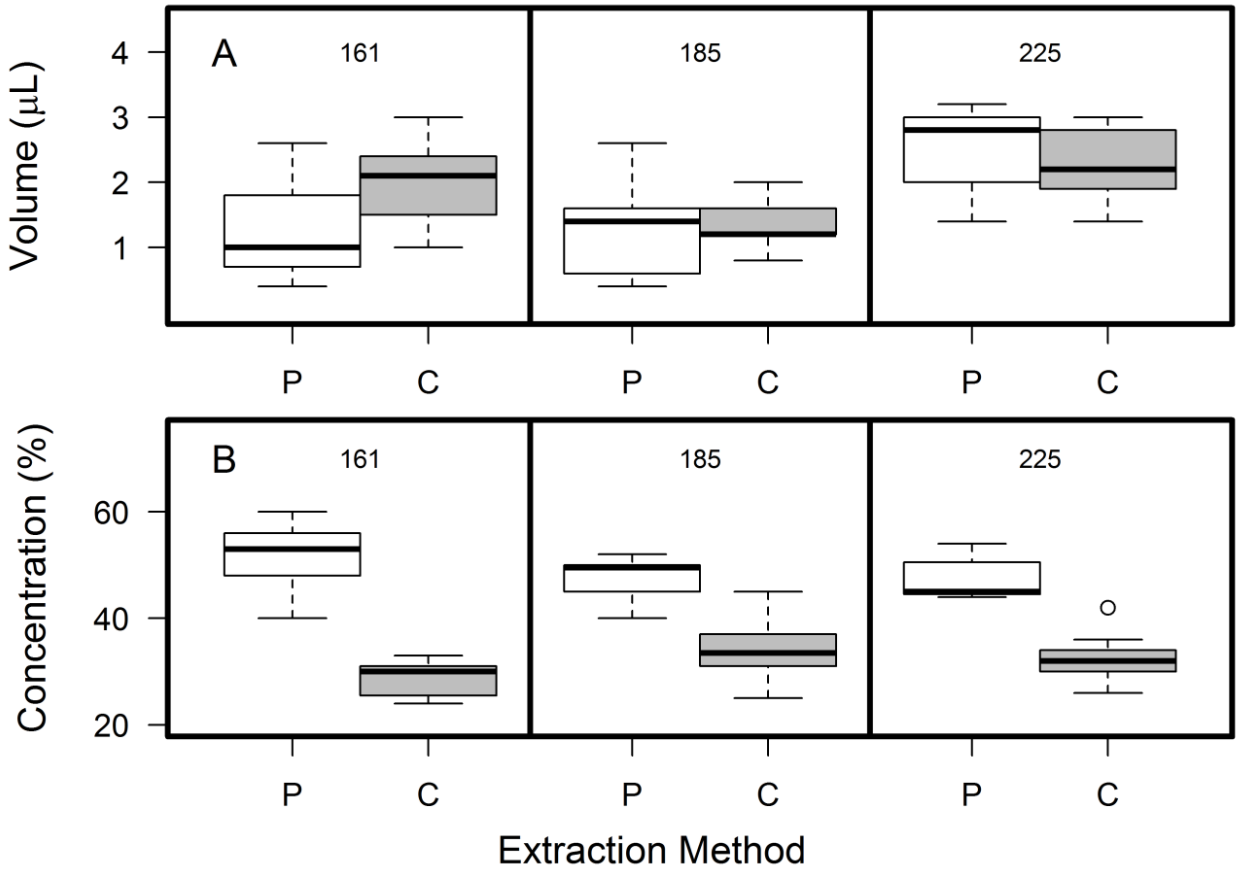

FIGURE 1. Median, quartiles, and outliers (open circles) for (A) nectar volume and (B) concentration for three plants (161, 185, and 225) sampled by10 µL micropipette (white bars, P) followed by centrifugation (gray bars, C).

Flower age and plant differences in nectar production

Twenty milkweed plants were used to examine nectar production over the life span of flowers, nectar production of flowers on different umbels, and nectar production differences among plants. Umbels were marked with a small, colored pipe cleaner 1-2 days before flowers began opening. As flowers opened, small black dots were placed on a single petal of each open flower using a black permanent marker with a felt tip. The number of dots on a petal identified the date the flower opened. Thus, we could determine the age of the flower when nectar was measured. We were able to measure the standing crop of nectar in flowers that were between 1 and 5 days old on most umbels. Flowers on two umbels per plant were measured one week apart during the experiment.

On nectar measurement days, all flowers from an umbel were placed individually upside down in pre-labelled 0.5 ml microfuge tubes. Tubes were placed into a mini-micro centrifuge and spun for one-two seconds. Flowers were removed from the tubes and nectar was collected into 10 µL calibrated micropipettes. Nectar volume, concentration, and sugar amount were determined as described above. We collected nectar data from 6-13 flowers per umbel for totals of 334 flowers on 40 umbels.

Time of nectar production

In early May 2018, we measured diel patterns of nectar production were determined on twelve plants grown from seed planted in January 2018. We wanted to measure new secretion from flowers that had been drained, so prior to starting the experiment, we removed nectar as completely as possible from flowers using 1mm X 10 mm filter paper wicks. Forceps were used to carefully insert the wicks into the base of each hood on the experimental umbels. Wicks were often replaced multiple times to remove all nectar. Care was used to not damage the flowers during this procedure. For a small subset of flowers, we extracted nectar first by filter paper wicks, then the remaining nectar was extracting using the one-two second centrifugation technique as described above. Using this method, we estimated that more than 90% of the standing nectar crop was removed using paper wicks prior to beginning the experiment.

Flowers were prepared by wicking nectar removal at 0500 h or 1700 h at the start of the experiment. Two rounds of the experiment were conducted. In the first round, two simultaneously open umbels were used per plant. The umbels were haphazardly assigned to the diurnal or nocturnal treatments. For the diurnal treatment, flowers from six plants were prepared at 0500 h to determine diurnal nectar production. Nectar was carefully removed using filter paper

wicks following the procedure described above. Nectar was allowed to accumulate in the flowers until 1800 h when the flowers were removed. Nectar was collected and measured using the mini-centrifuge method described above. For the nocturnal treatment, the second umbel on each plant was prepared at 1700 h by wicking nectar from the flowers. Nectar was collected the following morning at 0600 h to determine nighttime nectar production. In round two, the order of flower preparation and nectar collection was reversed so that night time nectar determination preceded daytime nectar determination. The mean diurnal and nocturnal nectar production rates (μL/h) were determined by dividing the flower nectar totals by the number of hours (i.e. 12 h). The average twenty-four hour production rate was determined by multiplying the hourly rate by twenty-four.

Effects of nectar removal on nectar production

Two 2-day-old flowers on 59 umbels were used to examine how nectar removal affected nectar production. Umbels were marked with colored pipe cleaners and the pattern of flower opening was observed and flowers marked as described above. For one of the flowers on each umbel, nectar was removed as completely as possible using filter paper wicks between 600 and 700 h. Nectar was then removed from both flowers that evening between 1800 and 1900 h. Our working hypothesis is that if nectar production rate remains constant, then the sampled flower should have approximately ½ the nectar as the second 2-d-old flower. Because mg of sugar production is the product of nectar volume and concentration, we chose mg of sugar as the estimate of nectar production.

Analysis

Analysis of variance (linear model, R Statistical Program 2018) was used to test for differences in nectar traits using umbel and plants as fixed effects. Similarly, analysis of variance was used to examine differences in nectar volume and concentration for nectar extraction methods and for differences in diurnal and nocturnal nectar production. In addition, R-studio was used to create boxplots showing the medians, quartiles and outliers in all experiments.

RESULTS

Nectar sampled by 10 μL micropipettes underestimates the true extractable standing crop by nearly 50% (Fig. 1, Tab. 1A). In all three plants where nectar was removed first by micropipette followed by centrifugation of flowers, the volume of nectar measured was different within plants, and a concentration gradient was revealed. The nectar sampled first by micropipette was more concentrated (40-60%) than the nectar remaining and extracted using centrifugation (15-45%). Nectar retrieved by micropipette was stored high in the nectar hoods and easily retrieved, but a thin film remained on hoods and subtle amounts of nectar could be seen at the base of the hood and in stigmatic chambers.

Differences in nectar production (μL/d), nectar concentration, and sugar content exist among plants of *A. curassavica* (Tab. 1B). Average nectar production across all plants was 1.08 ± 0.51 μL/flower/d, but ranged from a low of 0.65 ± 0.58 μL/d (plant 56) to 2.26 ± 0.51 μL/d (plant 133). In general, nectar production differences between umbels of the same plant were not significant (Tab. 1B and 2). However, six plants did exhibit significant differences among umbels, and the second umbel produced a greater volume of nectar in the second, later flowering, umbel. Sucrose production (mg/d) yielded similar production patterns to nectar volume, with five plants exhibiting among-umbel differences and four of the plants producing more sucrose per flower on the second umbel (Tab. 2, right column).

TABLE 1. (A) Analysis of variance for the effects of micropipetting versus centrifuging nectar volume, concentration, and sugar amount for *Asclepias curassavica*.

		Volume			Concentration			Sugar Content		
Model	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Plant	1	5.08	10.80	0.002	0.49	0.02	0.89	1.46	8.05	0.007
Method	2	2.27	4.83	0.013	1713.0	62.25	<0.001	0.80	4.42	0.019
Plant × Method	1	0.06	0.12	0.73	1.76	0.07	0.78	0.01	0.02	0.89
Error	37	0.47			28.21			0.18		
R^2		0.36			0.58			0.31		

TABLE 1. (B) Analysis of variance for the effects of plant identification and umbel on average daily nectar volume concentration and sugar amount for *Asclepias curassavica*.

		Volume			Concentration			Sugar Content		
Model	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Plant	19	2.39	19.39	< 0.001	128.33	4.54	< 0.001	1.14	19.96	< 0.001
Umbel	1	0.88	7.10	0.008	68.04	2.41	0.12	0.59	10.33	0.001
Plant × Umbel	19	0.224	1.81	0.021	4.91	4.91	< 0.001	0.15	2.56	< 0.001
Error	294	0.35			5.31			0.24		
R^2		0.58			0.38			0.60		

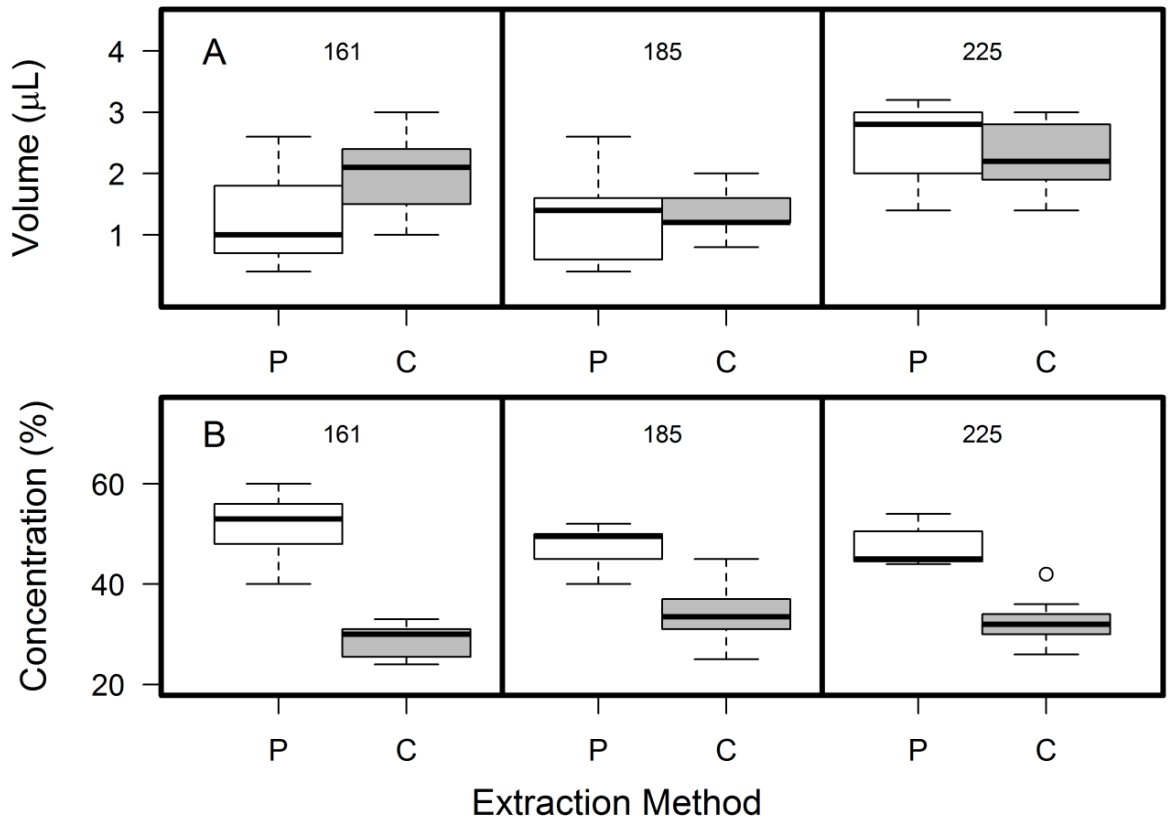

FIGURE 1. Median, quartiles, and outliers (open circles) for (A) nectar volume and (B) concentration for three plants (161, 185, and 225) sampled by10 µL micropipette (white bars, P) followed by centrifugation (gray bars, C).

Differences in nectar concentration were evident at the plant level, but not at the umbel level. Average nectar concentration across all plants was 46.9 ± 6.4 %, but ranged from a low of 42.7 ± 6.1% (plant 301) to a high of 54.1 ± 4.2% (plant 133). It is noteworthy that the plant with the greatest nectar concentration also had the greatest nectar volume. Three plants (133, 183, and 213) exhibited significant differences in nectar concentration but no clear pattern emerges on which umbel produced the higher or lower concentration.

For most plants, nectar production (µL/d) appeared constant across the four to five days of sampling (Fig. 2). Increases in nectar volume are incremental as flowers secrete roughly equivalent volumes of nectar on a daily basis. The majority of the flowers produced less than 6 µL over a 4-5 d period. In contrast, plant 133 produced so much nectar that it had overflown the hoods by days 3 and 4. Nectar was collected in all flowers sampled, and there were relatively few high/low volume outliers.

Nectar production is largely a diurnal process. Average nectar production during daylight was 0.31 ± µL/h (43.5 ± 7.2%) compared with nocturnal production of 0.08 ± µL/h (27.1 ± 5.0%). Mean hourly production rates ranged from 0.12-0.36 µL/h, which translates to a twenty-four hour production rate or 2.4-7.2 µL /d. In terms of sugar content secreted by flowers, nectar produced during the day contained more sugar than nectar produced at night (Fig. 3). On average, flowers secreted 0.17 mg/h during daylight hours but only 0.026 mg/h overnight. In one plant, the diurnal secretion of sugar was 13X greater than overnight secretion. Over the course of a 24 hour day, the average *A. curassavica* flower produces 4.7 µL with 3.08 mg of sugar.

Nectar removal by wicking appears to enhance the flow rate in milkweed flowers. Nectar replacement rate was equal to or greater than the rate of control flowers for 56 of the 59 umbels (Fig. 4). Nearly half (27 of 59) of the flowers had a replacement rate that was 2-4 times greater than the rate in control flowers.

DISCUSSION

Age, timing, and quantity of nectar production

Our results suggest that *A. curassavica* nectar production exhibits considerable variation among plants. Our use of same-age *A. curassavica* plants under common environmental conditions is most likely to demonstrate whether plant-to-plant differences in nectar production exist. Microclimate differences might exist in the SUNY Cortland greenhouse, especially when fans on heaters and the cooling system operate, that might contribute to faster evaporation on some plants. However, the nectar measurements occurred at a time of year when greenhouse temperature regulation occurred by opening/closing of roof vents rather than using cooling fans and heaters. Furthermore, it was often the case that a high nectar producing plant was next to plants with much lower nectar production. Interplant differences were most pronounced in nectar production rates (0.68-2.27 µL/d) and sugar concentration (43-53%). This level of variation is likely

TABLE 2. Nectar volume per d, concentration, and sugar content produced per d in flowers of twenty plants of *Asclepias curassavica*. Umbel pairs in bold are significantly different. *< 0.05, ** < 0.01, *** < 0.001.

Plant	Umbel	Flower Number	Volume (μL/d)	Concentration (%)	Sugar Content (mg/d)
9	1	9	0.81 ± 0.14	45.4 ± 2.7	0.45 ± 0.10
	2	8	0.94 ± 0.15	47.5 ± 3.1	0.54 ± 0.07
19	1	8	1.28 ± 0.64	41.5 ± 8.3	0.66 ± 0.45
	2	6	1.31 ± 0.49	49.0 ± 3.0	0.78 ± 0.30
24	1	6	0.65 ± 0.28	45.5 ± 5.1	0.37 ± 0.18
	2	7	0.72 ± 0.04	49.0 ± 6.7	0.44 ± 0.17
43	1	13	0.80 ± 0.03	47.4 ± 5.8	0.46 ± 0.11
	2	6	0.76 ± 0.02	42.8 ± 3.2	0.40 ± 0.16
56	1	6	**0.82 ± 0.03****	47.0 ± 5.6	**0.51 ± 0.22***
	2	9	**0.53 ± 0.11****	48.6 ± 4.3	**0.31 ± 0.06***
85	1	9	0.98 ± 0.58	46.0 ± 5.3	0.58 ± 0.42
	2	9	0.94 ± 0.44	46.2 ± 7.0	0.54 ± 0.28
109	1	9	1.42 ± 0.32	44.9 ± 4.7	0.77 ± 0.22
	2	8	1.44 ± 0.57	49.2 ± 6.2	0.91 ± 0.43
114	1	9	0.70 ± 0.39	42.0 ± 7.4	0.37 ± 0.23
	2	8	0.84 ± 0.22	45.8 ± 6.8	0.48 ± 0.19
127	1	9	0.87 ± 0.41	45.8 ± 4.0	0.49 ± 0.27
	2	7	1.04 ± 0.39	42.0 ± 2.9	0.53 ± 0.23
133	1	10	**2.05 ± 0.37***	**51.5 ± 3.7****	**1.32 ± 0.32****
	2	9	**2.49 ± 0.57***	**57.1 ± 2.7****	**1.81 ± 0.45****
143	1	9	0.82 ± 0.15	48.7 ± 6.0	0.50 ± 0.14
	2	8	0.96 ± 0.35	44.0 ± 3.6	0.50 ± 0.14
161	1	10	1.09 ± 0.32	45.8 ± 4.2	0.60 ± 0.21
	2	8	0.91 ± 0.36	46.6 ± 3.8	0.52 ± 0.23
180	1	8	1.17 ± 0.39	51.6 ± 5.3	0.75 ± 0.25
	2	5	1.17 ± 0.18	52.6 ± 1.7	0.76 ± 0.09
183	1	7	0.77 ± 0.13	**55.6 ± 4.2****	0.54 ± 0.14
	2	10	0.85 ± 0.25	**48.0 ± 6.6****	0.51 ± 0.20
200	1	10	0.99 ± 0.21	51.7 ± 4.3	0.63 ± 0.14
	2	7	0.71 ± 0.40	40.4 ± 3.8	0.33 ± 0.13
213	1	10	1.32 ± 0.33	**40.0 ± 11.5****	0.61 ± 0.23
	2	10	1.32 ± 0.56	**54.1 ± 2.8****	0.90 ± 0.38
225	1	9	**0.86 ± 0.18***	47.7 ± 3.2	0.51 ± 0.13
	2	8	**1.12 ± 0.31***	45.6 ± 3.0	0.62 ± 0.15
300	1	8	**0.71 ± 0.18*****	46.4 ± 6.1	**0.40 ± 0.11****
	2	6	**1.35 ± 0.31*****	45.6 ± 7.8	**0.74 ± 0.22****
301	1	8	**1.17 ± 0.33***	39.4 ± 5.9	**0.56 ± 0.21***
	2	10	**1.64 ± 0.49***	45.4 ± 6.2	**0.90 ± 0.34***
302	1	9	**1.14 ± 0.13***	45.9 ± 2.7	**0.63 ± 0.09***
	2	9	**1.40 ± 0.27**	47.1 ± 3.8	**0.81 ± 0.19**

to affect reproductive success and fitness in natural populations of *A. curassavica*. Nectar production has been shown to influence reproductive success by increasing pollinaria removal in *A. exaltata* (Wyatt & Shannon 1986) and *A. quadrifolia* (Pleasants & Chaplin 1983). High nectar production has been shown to negatively affect reproductive success by increasing self-pollination and reducing resources for seed and fruit production in some plants. For example,

increased nectar volumes on plants of *Mirabilis multiflora* increased flower visitation by hawkmoths, but it also increased self-pollination and resulted in decreased seed set (Hodges 1995). For the weedy, self-compatible *A.curassavica,* the importance of nectar production reproductive success may depend on the context of interspecific competition for pollinators in new habitats and increasing outcrossing in established populations.

For *A. curassavica,* nectar secretion occurs almost exclusively during daylight hours, and production continues into the fifth day of a flower life span. Although we did not investigate nectar reabsorption, we suspect that unused nectar is rapidly reabsorbed on the sixth and seventh day of the flower life span (pers. obs.). In contrast, other field experiments have reported that nectar secretion is overnight for *A. exaltata* (Wyatt & Shannon, 1986; Wyatt et al. 1992), *A. syriaca* (Willson & Bertin 1979; Southwick 1983; Wyatt et al. 1992), and *A. verticillata* (Willson et al. 1979).

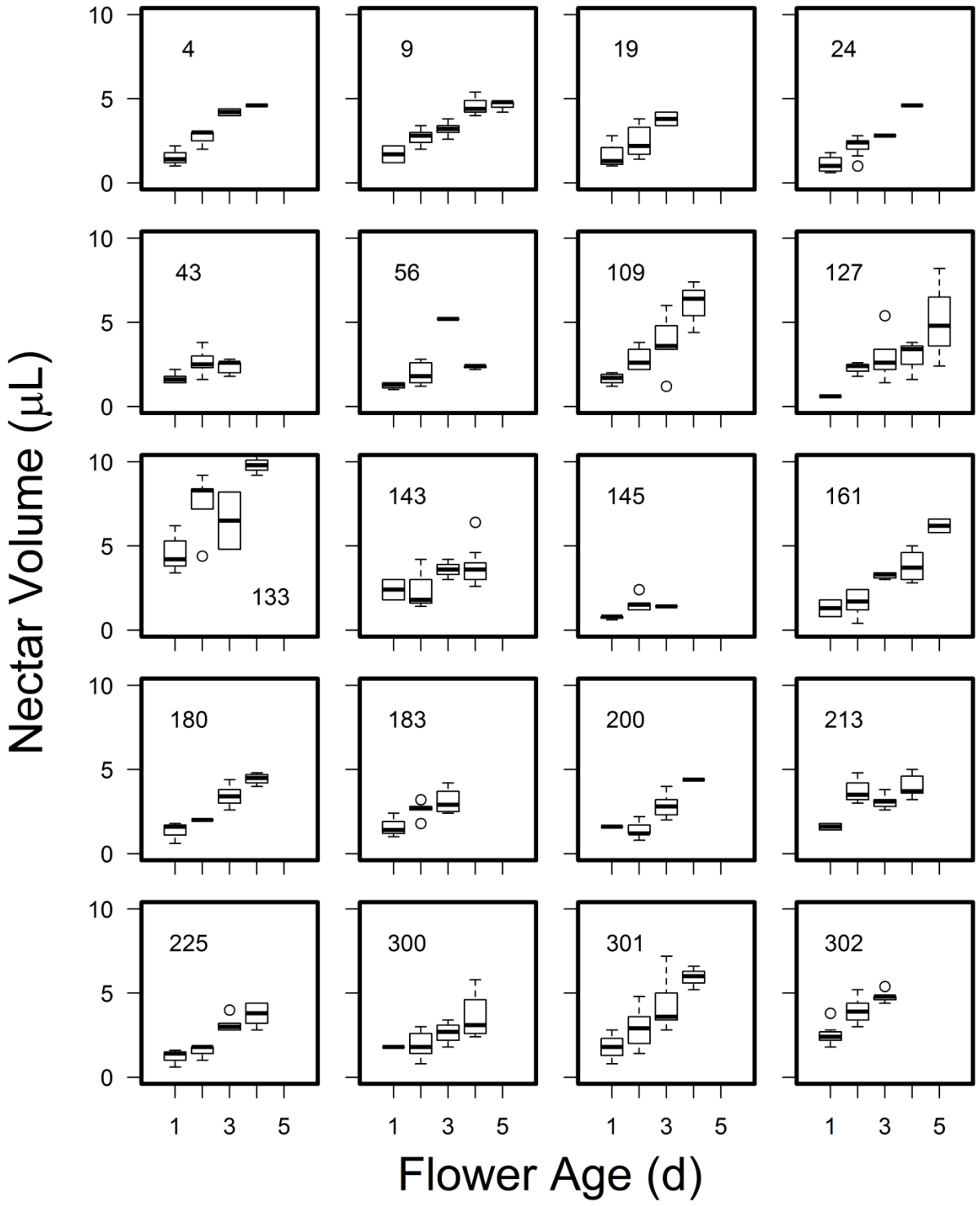

FIGURE 2. Median, quartiles, and outliers (open circles) for nectar volume by flower age for twenty plants of *Asclepias curassavica.*

Asclepias syriaca appears to reach peak production 2-3 d after anthesis, followed by diminished production and cessation by day five (Southwick & Southwick 1983). *Asclepias quadrifolia* (Pleasants & Chaplin, 1983) is known to secrete nectar during daylight, similar to *A. curassavica*. Although these patterns may be due to a biological clock as demonstrated in the sister taxon *Hoya* (Matile 2006), it is difficult to determine whether the timing is adaptive, as these milkweeds have generalist pollination systems that involve multiple pollinator functional groups.

Milkweeds produce abundant quantities of nectar relative to other species. *Asclepias curassavica* produced an average of 3.0 mg/d of sugar per flower in the greenhouse. In contrast, field grown flowers of *A. syriaca* produced between 0.9 and 1.9 mg/d (Southwick 1984). Although it would appear that nectar production is greater in *A. curassavica* than in *A. syriaca*, inflorescences of *A. curassavica* typically have 9-13 flowers, whereas umbels of *A. syriaca* often have more than a hundred flowers. Southwick (1984) determined that 4-37% of the daily assimilated photosynthate was allocated to nectar in flowers of *A. syriaca*. In contrast, *Pontederia cordata* allocates approximately 3% of its energy budget to nectar on a daily basis (Harder & Barrett 1992). Harder and Barrett (1992) show that the nectar production per flower of *A. syriaca* is nearly 15 times greater than nectar production in twenty-six other bee-pollinated flowers. Nectar production in greenhouse grown *A. curassavica* is greater than sixty other that have been included in wildflower seed mixes used in urban meadows of United Kingdom (Hicks et al. 2016).

Sugar concentration and its importance in milkweed nectar

The easily obtained nectar in the flower hoods has a higher sugar concentration than the nectar held deep within the cuculli (sensu Galil and Zeroni 1965) and stigmatic chambers. Water evaporation from nectar high in the hoods likely contributes to this gradient by forming a high-concentration nectar cap that insulates more recently produced nectar from evaporation. This observation is consistent with nectar production in tubular flowers (Fenster et al. 2004; Willmer 2011) or those of orchids with nectar spurs (Martins & Johnson 2007), where nectar is sheltered from environmental conditions that would evaporate water. Field studies on nectar production using mesh-material insect exclusion bags frequently measure concentrations above 40% for *A. curassavica* (Percival 1974; Ward & Johnson 2013), *A. exaltata* (Wyatt & Shannon 1986; Wyatt et al. 1992), and *A. syriaca* (Southwick & Southwick 1983). These high concentrations of field nectar are likely due to evaporation, and it is possible that a concentration gradient exists within these flowers as well.

To our knowledge, the Apocynaceae (*Asclepias* included) are the only flowering plants where floral nectar is the germination medium for pollen. Secretory tissues are found in the stigmatic chambers of *Asclepias* (Galil & Zeroni 1965), and pollen germination experiments confirm that freshly produced nectar approaches the optimal sugar concentration (5-30%) for pollen germination (Shannon & Wyatt 1986;

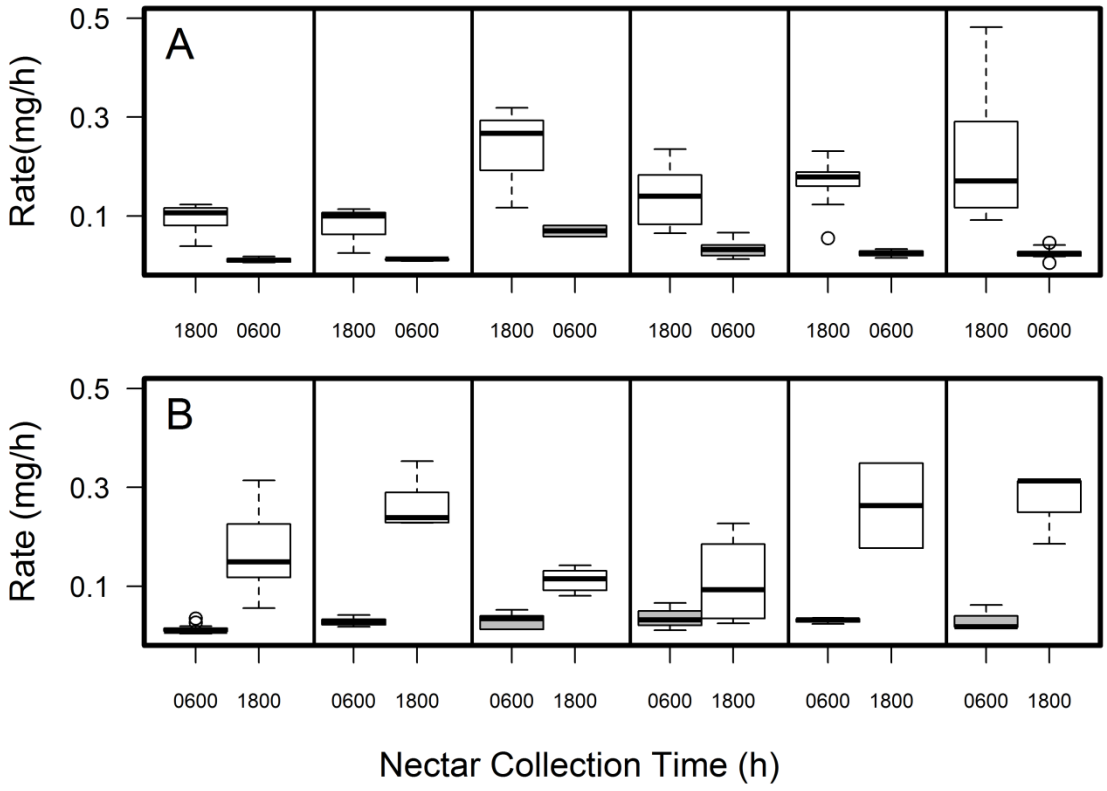

FIGURE 3. Median, quartiles, and outliers (open circles) for nectar sugar content collected at 0600 and 1800 h. (A) Nectar produced during daylight hours (white bars, 1800 h) measured prior to nectar produced during nighttime (gray bars, 0600 h) for six plants of *Asclepias curassavica*. (B) Nectar produced during nighttime hours measured prior to nectar produced during daytime for six additional plants

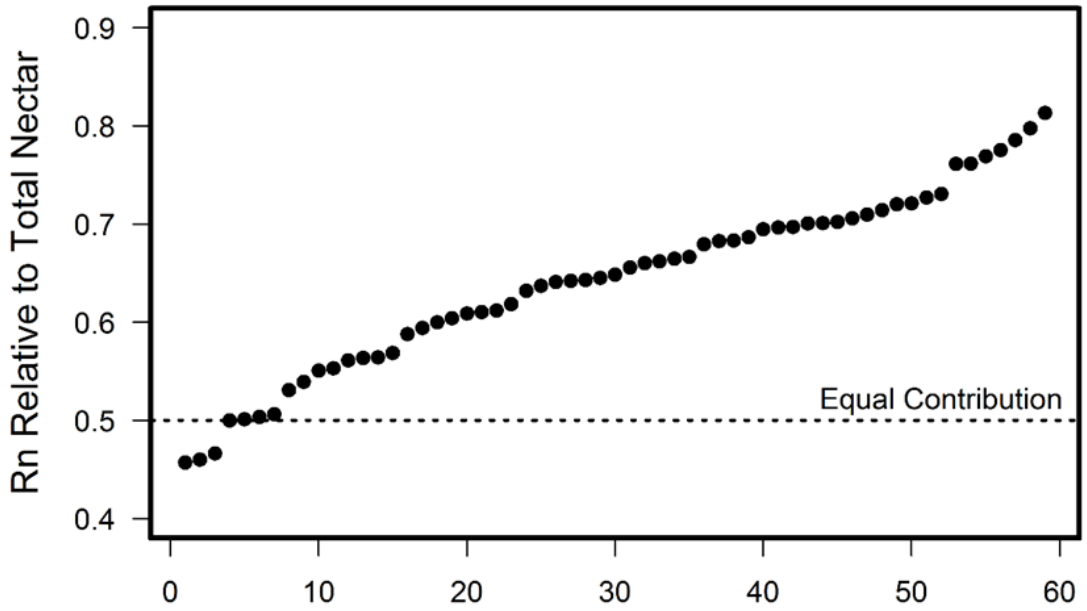

FIGURE 4. Replacement nectar (Rn) relative to total nectar production, Rn/(Rn+Cn), for flowers from 59 umbels. Sugar content (mg) was used for both replacement nectar and control nectar.

Wyatt & Shannon 1986; Kevan et al. 1989). In contrast, when anthers come into contact with nectar of *Paypayrola* and *Amphirrox* (Violaceae), pollen germination rates drop to 0% even though mean sugar concentration was only 24.2% (Braun et al. 2012). Kevan et al. (1989) suggested that concentrated nectar would inhibit pollen germination on hot days until freshly secreted nectar at night lowered the concentration and allow pollen to germinate. Our results show that an optimal nectar concentration for pollen germination is found throughout the day and night. Even when apparent nectar concentration is elevated above 50% during the day, nectar held deep in the flowers of *A. curassavica* has an optimal concentration for pollen germination.

Nectar and potential effects on pollinators

Several published reports identify lepidopterans as the principal pollinators of *A. curassavica* within its natural range of the neotropics (Wyatt 1980; Bierzychudek 1981; Fuhro et al. 2010). Lepidopterans use active suction through their proboscis to collect nectar at flowers. Using fluid dynamic models, Kim et al. (2011) predicted the optimal concentration for lepidopterans to be between 35 and 40%. Their findings are similar to those of Daniel Kingsolver, and Meyerhöfer (1989) where an optimal concentration of 31-39% was found for *Pieris* butterflies when fluid dynamics and muscular contractions cibarium were considered. Hymenopterans, on the other hand, extract nectar using viscous dipping of the proboscis and have an optimal concentration nearing 60%. Because *A. curassavica* secretes nectar throughout the day, a lower concentration of nectar suitable for lepidopterans is likely available as long as flowers are frequently visited and nectar is removed continuously. We suspect that similar concentration gradients may exist for other milkweed flowers and that the high concentration found in other species is an artifact of diurnal evaporation and prevention of nectar removal by using bagged flowers.

In addition to matching the nectar requirements for the lepidopterans, other floral traits suggest butterflies are the intended pollinators. The flowers have bright contrasting yellow-orange-red colors that are innately attractive to many tropical butterflies (Barp 2011; de Oliviera et al. 2015; Ramos et al. 2017). The flowers are odorless, but produce abundant nectar during the daytime. Flowers are clustered in umbels that are presented upward to form a flat landing platform. Although floral traits suggest butterfly pollination, we propose caution in adopting butterflies as the primary pollinators of *A. curassavica.* As described in Fenster et al. (2004), the most common floral visitor may not be the best pollinator if its efficiency at transporting pollen is lower than that of less common pollinators. We have observed that large butterflies with long legs rarely contact the corpuscular of small milkweed flowers. Nectar probing mistakes result in proboscis transport of milkweed pollinia by medium to large butterflies in a relatively haphazard manner. In *A. tuberosa*, another milkweed with yellow-orange flowers (commonly called butterfly weed), butterflies had moderately high levels of milkweed pollinaria removal, but much lower transport and insertion efficiency than hymenoptera (Fishbein & Venable 1996).

Removal-enhanced nectar production

Nectar removal appears to stimulate a high rate of replenishment in *A. curassavica.* The replacement rate far exceeds the baseline production of control flowers. Nearly half of the flowers in the nectar removal experiments responded by increasing the nectar flow rate 2-4X. Southwick (1983) reported that *A. syriaca* flowers sampled multiple times produced more than twice as much nectar in a 24 h period as those sampled only once. These data suggest that milkweeds can respond to increased pollinator activity by increasing the flow of nectar to flowers. Removal-enhanced nectar replenishment has been reported for nine of eleven

montane wildflowers in Colorado (Luo et al. 2014) that were both bee and hummingbird pollinated species. Removal-enhanced nectar replenishment in flowers of *Moussonia deppeana* increased total nectar production 2-4 times (Ornelas et al. 2007). The energetic trade-off of nectar replenishment and reproductive success is rarely investigated, but for *Penstemon roseus* increased nectar production resulted in lower seed mass (Ornelas & Lara 2009). Future studies on milkweed nectar production should consider that baseline nectar production likely underestimates actual nectar production in the field when insects are allowed to visit and remove nectar throughout the day.

Conclusions

Asclepias curassavica presents many opportunities for exploring the genetic basis and heritability of nectar production. Plants are reasonably easy to pollinate and seed germination is high. In addition, *A. curassavica* is one of the few milkweeds that can be propagated by vegetative means for investigating the environmental x genotype effects on nectar production. As a colonizing, weedy, self-compatible species, *A. curassavica* will permit experimental investigation on the role of nectar production and reproductive success, population establishment, and outcrossing. Our study shows that *A. curassavica* exhibits interplant variation for nectar production in a common garden experiment and that nectar has potentially important effects on pollen germination, reproductive success, and the energy budget through removal-enhanced nectar production.

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