JARQ 55 (4), 323-331 (2021) https://www.jircas.go.jp

Diurnal Dynamics of Nectar Secretion in *Cucurbita maxima* Duch: Implications for Effective Pollen Transfer by Japanese Wild Pollinators

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Abstract

To determine whether visits by wild pollinators correspond well with the physiological patterns of animal-pollinated crops, we investigated the diurnal dynamics of nectar secretion in the male and female flowers of cultivated kabocha squash *Cucurbita maxima* Duch, and its effect on the temporal visitation pattern by bumblebees, their Japanese wild pollinators, relative to visits by introduced honeybees *Apis mellifera* L. in Hokkaido, Japan. The amount of nectar secreted and the frequency of pollinator visits were monitored from floral opening at dawn until wilting in the early afternoon. Flowers of both sexes secreted nectar in a unimodal diurnal pattern, with secretion peaking during the middle stage of anthesis. The total production of nectar in female flowers was twice that in male flowers. Although the availability of pollinators varied between days, the frequency of visits by bumblebees corresponded well with the dynamics of nectar secretion by the flowers. The diurnal dynamics of nectar secretion and the large availability of nectar reward in female flowers would increase the probability of transferring viable pollen to female flowers and contributing to fruit production of this crop. Bumblebee visits were negatively correlated with honeybee visits. Although this negative correlation could be explained by the complementary effects of pollinators or competitive exclusion, the effects of introducing honeybees on wild pollinators cannot be easily evaluated and thus should be carefully examined by considering the local environmental context in future studies.

Discipline: Crop Science
Additional key words: *Apis mellifera*, bumblebees, entomophilous crops, male and female flowers, pollination services

Introduction

The fruits of *Cucurbita* spp. that include squash, pumpkin, and gourd are major agricultural products around the world. Originating in Meso- or South America (Sanjur et al. 2002), *Cucurbita* fruits are now produced in 119 countries and regions in every continent except Antarctica (FAO 2018). Because *Cucurbita* plants have monoecious flowers that require the transfer of pollen from male to female flowers for fertilization, pollination services provided by insects are crucial for fruit and seed production. Generalist wild pollinators such as the *Bombus*, *Eucera,* and *Apis* species contribute to *Cucurbita* production worldwide, especially in regions where *Cucurbita* specialist bees *Peponapis* and *Xenoglossa* are not distributed (Hurd et al. 1971, Knapp & Osborne 2019). However, since the decline of wild pollinators and their pollination services has become a worldwide concern, the production of crops that require animal pollinators may be at risk (IPBES 2016).

The availability of wild pollinators is often affected by the landscape context, such as the availability of nesting sites or floral resources and agricultural practices (Artz et al. 2011, Pfister et al. 2018). Even within the

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Received 25 September 2020; accepted 14 December 2020.

species *Cucurbita maxima*, the effective pollinator species differ (bees or beetles) depending on the environment (Ashworth & Galetto 2001). Honeybees (*Apis mellifera* L.), which are commonly introduced to compensate for insufficient pollination by wild insects, can modify plant–pollinator relationships in agricultural landscapes, and thus could adversely affect crop visits by wild pollinators (Magrach et al. 2017). However, such competitive interactions can also vary, depending on the local environmental context (Mallinger et al. 2017). Thus, site-specific information about pollinators is necessary for the sustainable use of wild pollinators for crop production.

Although the availability of wild pollinators may depend on the local environment, the offering of floral nectar is an important strategy for plants to control pollinators (Nepi et al. 2018, Stout & Goulson 2002). As pollinators rely on floral nectar for energy, they often respond to such nectar characteristics as volume, sugar concentration and composition, and temporal or spatial (including among-flower) variability (Biernaskie et al. 2002, Nunes et al. 2018, Rathcke 2012). Temporal and among-flower variation in nectar secretion would be quite important for *Cucurbita* spp., which have short periods of anthesis from dawn until early afternoon. Given the short duration of receptivity by the pistils and ovaries, and declining pollen viability during anthesis (Nepi 1993), an early transfer of pollen to female flowers is required for the setting of fruits. The accumulation patterns, compositions, and amounts of nectar sugars have been relatively well documented for *Cucurbita pepo*, *C. maxima,* and *C. moschata* (Canto-Aguilar & Parra-Tabla 2000, Dmitruk 2008, Nepi et al. 2011). In these species, female flowers offered larger amounts of sugar reward than male flowers by secreting larger volumes and/or higher concentrations of sucrose solution, and nectar is accumulated until around six hours of anthesis in both floral sexes (Ashworth & Galetto 2002, Nepi et al. 2001). However, two important factors affecting pollinator visits—the among-flower variability in available nectar and the temporal supplementation pattern of nectar after consumption by pollinators—have not been well documented (Biernaskie et al. 2002, but see Nepi et al. 2011).

We thus first aimed at describing the diurnal dynamics of nectar secretion and its variation among flowers in *Cucurbita maxima* Duch—the most commonly produced squash in Japan. Next, we investigated the responses of bumblebees (major wild pollinators) to the dynamics of nectar secretion relative to introduced *A. mellifera* in Hokkaido, Japan. As the Hokkaido region is the largest area of *Cucurbita* crop production in Japan, accounting for half of the country's total production (Japan Ministry of Agriculture, Forestry and Fisheries 2019), the responses of pollinators might be reflected in better management practices to maximize production.

Materials and methods

1. Study system

The field study was conducted in an open field (55 m × 260 m) of kabocha squash (*Cucurbita maxima* 'Kento') located in eastern Hokkaido, Japan (42°53′N, 143°08′E), on the windless fine days of August 6 and 11, 2009. The area was dominated by agricultural fields rotationally producing mainly corn, soybeans, potatoes, wheat, and beets. Small forest fragments of broad-leaved trees, small lots of conifer plantations, and farmers' houses were scattered within the landscape. The mean temperature between 4:00 and 13:00 was 22.5°C (16.2°C-29.9°C) on August 6, and 24.6°C (20.4°C-31.9°C) on August 11. The sun rose at around 4:30.

Cucurbita maxima is a self-compatible, climbing annual crop with short-lived, sexually dimorphic flowers (Paris 2016). The yellow, trumpet-shaped corolla has five lobes and is approximately 10 cm in diameter in both sexes. In addition to the presence or absence of an ovary under the corolla, male and female flowers differ in the nectary morphology and spatial arrangement and abundance within the plants. Whereas the nectaries of female flowers are open to pollinators at the base of the styles, those of male flowers are hidden under the united filaments, providing access to pollinators only through slits between the filaments, similar to those of *C. pepo* (Nepi et al. 2011). Male flowers are aggregated at the basal position of a lianoid plant with long peduncles, and female flowers are positioned close to the tip of the vine with short peduncles (about 2 cm). Generally, male flowers outnumber female flowers except for the first few days of flowering. Flowering in both males and females began synchronously at 4:00 and was completed by dawn at 4:30. Most flowers gradually began to wither at around 10:30 to noon and were closed by 13:30 to 14:00.

2. Measurement of nectar secretion

One day before the field investigation, we bagged female and male flower buds that were expected to bloom the next day with white organdy cloth to prevent insect visits. In total, 14 pairs of female and male buds were selected from 14 randomly selected plants. On the day of the investigation, nectar was extracted from each flower at one-hour intervals by Microcaps® (Drummond Scientific Company, Pennsylvania, USA) after removing the bag. Nectar was carefully extracted from the nectaries using 1-, 5-, 10-, and 100-μL capillary tubes depending on the remaining volume of nectar, and the flowers were bagged again until the next measurement. Nectar extraction was conducted from the opening (4:30) to the wilting (13:30) of flowers. The length of nectar in the capillary was measured by calipers and converted to volume in microliters. Given the destruction of floral structure during repeated bagging treatments or the limited time available for the hourly measurements, one male flower was sampled up to the first five hours, and two female and two male flowers were only sampled at the first hour (Table S1).

3. Observation of pollinator visits

We set two 7 m × 14 m plots separated by at least 14 m on both observation days. In order to include the flowers of both sexes, we located the two plots randomly within the field. We recorded the numbers of visits by bumblebees and honeybees within both plots during 10- to 19-minute observation trials from 4:25 to 13:41 on August 6, and from 4:46 to 14:25 on August 11. The total duration of the observations was 402 minutes (28 trials) on August 6, and 285 minutes (19 trials) on August 11.

4. Analysis

Sexual differences in the total production of nectar during anthesis were examined by a paired-sample *t*-test following the Browne–Forsythe test for homogeneity of variance. Sexual differences in the variation of hourly nectar secretion were also examined by the Browne–Forsythe test with Bonferroni corrections. To examine the diurnal pattern of nectar secretion and its difference between male and female flowers, we constructed a generalized linear mixed model (GLMM) with a gamma error distribution and log-link, for the response variable of hourly nectar secretion measured in volume, and conducted model selection based on Akaike's information criterion (AIC). The full model contained flower sex, time (decimal hour in 24-hour clock), squared time, and interactions between flower sex and time effects as fixed effects, and flower identity as a random effect. We selected a model with the lowest AIC as the best model. In case the difference between the AIC value of the second or subsequent models and the best model (δAIC) was within two, the simplest model was selected.

To examine the effects of the diurnal pattern of nectar secretion and the abundance of introduced *A. mellifera* on the visits of bumblebees to each flower, we constructed a GLMM assuming Poisson error distribution and conducted model selection based on AIC as described above. Flower sex, nectar volume (μL), frequency of visits by *A. mellifera*, and date were included as fixed effects, along with the offset terms duration of observations for each trial and available flower numbers in each plot, and a random effect of time. For this analysis, the numbers of bumblebee visits to male and female flowers in each observation trial were linked to the average volume of hourly nectar secretion of flowers of the corresponding date and sex. As visits by *Bombus diversus teresatus* Smith and invasive *B. terrestris* L. were quite rare relative to the dominant *B. hypocrita sapporoensis* Cockerell (Table 1), we pooled all visits by bumblebees for the analysis. The frequency of visits by *A. mellifera* was calculated as the number of visits to a single flower during a unit of time. All statistical analyses were conducted by R version 3.6.2 (R Core Team 2019).

Results

1. Flower and nectar production of *C. maxima*

The number of flowers increased between the first (August 6) and second (August 11) dates. Male flowers outnumbered female flowers on both days, with a female/male ratio of 14/151 on August 6, and 21/420 on August 11 (Table 1).

Female flowers produced approximately twice as much nectar (356.0 ± 61.0 μL, $n = 12$) as male flowers (179.5 ± 62.9 μL, $n = 11$) during anthesis ($t = 7.60$, $P < 0.01$) (Table S1). No sexual difference was detected for the variation in total production ($P = 0.79$). The mean hourly secretion of nectar showed unimodal temporal patterns in both male and female flowers. There was a significant positive effect of time, which represented an increasing trend with time in the early hours, and a significant negative effect of squared time, which represented a decreasing trend in the late hours (Table 2, Fig. 1). In female flowers, nectar secretion increased from flower opening at 4:30 (2.6 ± 5.7 μL (mean ± SD), $n = 14$), reached a maximum at 6:30 (85.7 ± 31.9 μL, $n = 12$), and then gradually decreased until wilting at 13:30 (10.7 ± 17.2 μL, $n = 12$). In male flowers, nectar secretion increased from flower opening at 4:30 (0.6 ± 1.1 μL, $n = 13$), reached a maximum at 8:30 (30.3 ± 23.8 μL, $n = 12$), and decreased until wilting at 13:30 (4.7 ± 3.6 μL, $n = 11$). Although the peak period of hourly secretion in female flowers was two hours earlier than that in male flowers, no differences in the pattern of hourly secretion were detected (and no interactions between flower sex and time effects were selected in the best model) (Table 2). The expected hour of maximum hourly secretion was at the middle stage of anthesis, around 9:00 in both sexes (Fig. 1). Variations in the hourly secretion of nectar did not differ between female and male flowers in any time period (Table S2).

Table 1. Numbers of visits by four bee species and numbers of flowers of *Cucurbita maxima* Duch in the 7 m × 14 m plots

Date	Plot	Female					Male					Observation duration (min)
		Bh	Bd	Bt	Am	Flower	Bh	Bd	Bt	Am	Flower	
2009/8/6	A	69	0	0	0	7	184	0	1	0	68	190
	B	83	0	1	0	7	177	3	4	1	83	212
2009/8/11	C	4	0	4	2	11	53	0	4	29	217	150
	D	3	0	0	0	10	52	0	0	11	203	135
Total		159	0	5	2	35	466	3	9	41	571	687

Bh: *Bombus hypocrita sapporoensis* Cockerell
Bd: *B. diversus tersatus* Smith
Bt: *B. terrestris* L.
Am: *Apis mellifera* L.

Table 2. Results of generalized linear mixed model (GLMM) and model selection for hourly secretion of nectar

		Intercept	Flower sex (male)	Time	Time^2	Time: Flower sex (male)	Time^2: Flower sex (male)	AIC	δAIC
Best model	Estimates	−6.33	−0.75	2.33	−0.13	-	-	1,638.9	0.0
	P-value	< 0.01	< 0.01	< 0.01	< 0.01	-	-		
2nd model	Estimates	−5.99	−1.42	2.29	−0.13	0.08	-	1,639.9	1.0
	P-value	< 0.01	0.04	< 0.01	< 0.01	0.31	-		
3rd model	Estimates	−6.16	−1.07	2.33	−0.13	-	0.00	1,640.0	1.1
	P-value	< 0.01	< 0.01	< 0.01	< 0.01	-	0.35		

Models with δAIC (Akaike's information criterion) < 2 are shown.

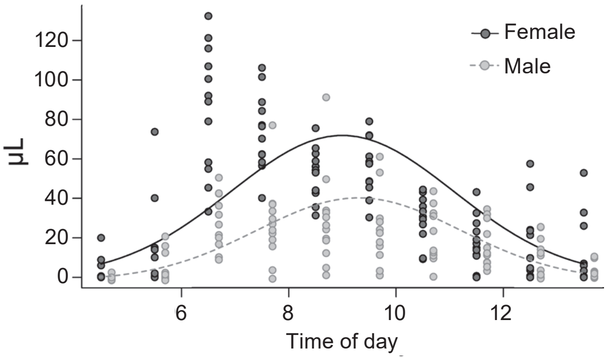

Fig. 1. Diurnal dynamics of hourly nectar secretion by male and female flowers of *Cucurbita maxima* Duch

Circles represent observed values, and lines indicate predicted values by the best fit model (Table 2). Dark gray represents females, and right gray represents males.

2. Visits by bumblebees

We recorded 685 visits by bees during the 687 minutes of observation, of which 625 were by *B. hypocrita sapporoensis*, 3 by *B. diversus tersatus*, 14 by invasive *B. terrestris*, and 43 by domesticated *A. mellifera* (Table 1). There were 166 visits to 35 female flowers and 519 visits to 517 male flowers. On a single flower basis, female flowers received 5.6 times more visits than did male flowers on average. The frequency of visits to female flowers generally increased in the first few hours after anthesis, and then subsequently decreased. The frequency of visits to male flowers was relatively stable and remained a low level even during the hours when female flowers received large numbers of visits (Fig. 2). Although movements of the bees between female and male flowers were not recorded, we occasionally observed such movements. Bumblebees did not actively forage for pollen on squash flowers, and often groomed their bodies, removing cucurbit pollen grains from body surfaces (Nakamura, unpublished data).

Although the frequency of visits by bumblebees differed between both observation dates, the bees positively responded to the amount of hourly nectar secretion (i.e., available nectar) (Table 3). Bumblebees preferred female flowers to male flowers, even after the effects of nectar availability were considered (significant effect of flower sex) (Table 3, Fig. 3). There was a negative correlation between the frequency of visits by bumblebees and honeybees.

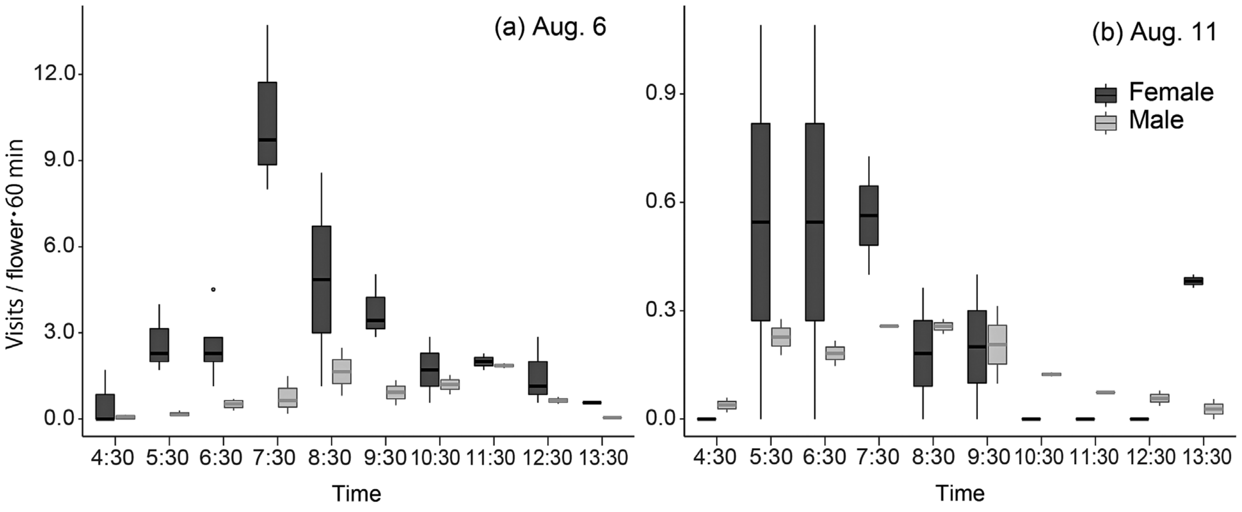

Fig. 2. Temporal visitation frequency of bumblebees to male and female flowers of *Cucurbita maxima* Duch

The bottom and top ends of the boxes indicate the first and the third quartiles, the segments inside the boxes indicate medians, the whiskers above and below the boxes indicate the minima and maxima within the range of 1.5 times of interquartile range from the upper or the lower quartiles, and the closed circles represent outliers.

Table 3. Results of generalized linear mixed model (GLMM) and model selection for visits by bumblebees

		Intercept (Female, Aug.6)	Nectar	Flower sex (Male)	Honeybee density	Date (Aug.11)	AIC	δAIC
Best model	Estimates	−6.38	0.03	−0.48	−201.50	−2.03	663.2	0.0
	P-value	< 0.01	< 0.01	< 0.01	0.03	< 0.01		

A model with δAIC (Akaike's information criterion) < 2 is shown.

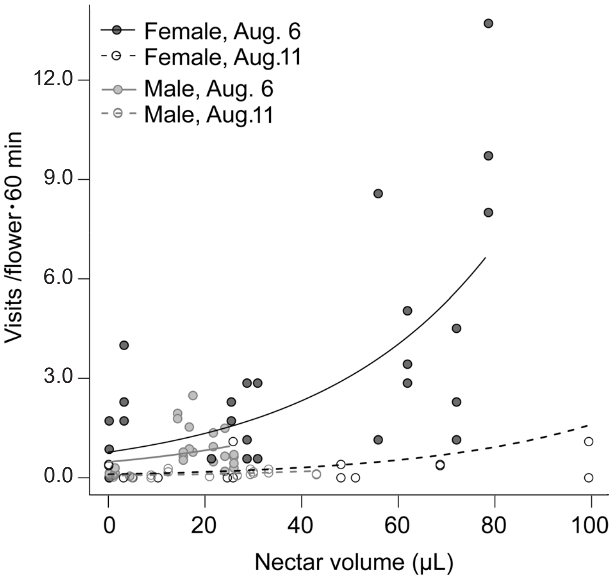

Fig. 3. Effects of nectar secretion by male and female flowers of *Cucurbita maxima* Duch on the visitation frequency of bumblebees to the flowers

Discussion

Hourly secretion of nectar increased in the first few hours after opening of the corolla, with expected peak periods of mean nectar secretion in the middle stage of anthesis in both male and female flowers. Although the pistils of *C. maxima* continue to be receptive to pollen even after wilting in the afternoon on the day of anthesis, the viability of pollen rapidly decreases during anthesis and falls to the minimum level for fertilization six hours after opening of the corolla (Hayase 1960). As the activity of bumblebees corresponded with the availability of nectar, the increasing trend in the hourly secretion of nectar until around five hours after the beginning of anthesis (9:00) might increase the chance of being pollinated by viable pollen.

Female flowers of *C. maxima* offered twice as much total nectar as that of male flowers. Although we did not measure sugar concentrations, Ashworth & Galetto (2002) reported equal sugar concentrations in male and female flowers of wild *C. maxima* ssp., except for the first hour after anthesis. An abundant production of nectar sugar in female flowers was also reported for *Cucurbita* spp. and other plant species (Dmitruk 2008, Pacini & Nepi 2007). Owing at least partially to their larger production of nectar, female flowers received about 5.6 times more visits than male flowers. As Ashworth & Galetto (2002) suggested, higher nectar production in female flowers of wild squash might be a reward-based strategy of the plant. Large amounts of nectar rewards might make female flowers more attractive than male flowers, even in this cultivated species.

Bumblebees preferred to visit female flowers, even when accounting for the effect of nectar availability. As we detected no significant sexual differences in the among-flower variation in the amount of nectar secretion in any time period, variation in nectar availability would not have affected the choice of male or female flowers by pollinators (Biernaskie et al. 2002). Differences between male and female flowers in the contents of metabolites and proteins in nectar (Chatt et al. 2018), accessibility of the nectaries (i.e., female flowers having nectaries open to pollinators, male flowers having hidden ones under united filaments; Nepi et al. 2011), and the presence or absence of pollen might have resulted in different preferences of the bumblebees. Because bumblebees rarely forage for cucurbit pollen (Brochu et al. 2020, Nakamura unpublished data), they may prefer female flowers that do not have pollen.

Despite the high attractiveness of female flowers, the total number of visits to male flowers was three times greater than that to female flowers, due to the highly male-biased floral production. As the number of male flowers was 16 times greater than the number of female flowers, and given our observation of pollinators moving between both female and male flowers, visitors to female flowers would have previously visited male flowers and carried pollen grains on their body surface. The male-biased flower ratio in *C. maxima,* together with the high attractiveness of female flowers owing partially to their large nectar presentation, might contribute to the pollination success of the species.

There was a negative correlation between the frequency of visits by bumblebees and honeybees. As there have been some reports of complementary effects on crop production by honeybees and other pollinator species due to their different foraging characteristics or responses to environmental factors, species-specific responses to local conditions, such as weather or co-flowering floral resources, might have resulted in this negative correlation (Brittain et al. 2013, Miñarro & García 2018). Conversely, other reports have suggested a competitive exclusion of wild pollinators by honeybees (Lindstrom et al. 2016, Nielsen et al. 2017, Wojcik et al. 2018). Because the species composition and interactions of wild pollinators vary spatially and temporally, depending on the local context (Peralta et al. 2020, Wignall et al. 2020), the effects of introducing honeybees should be carefully examined in more detail.

Acknowledgements

We are grateful to Mr. and Mrs. Hirose, who kindly allowed us to use their squash field. We also wish to thank Sa-aya Tsukuba and Megumi Yoshimoto for their support in the field survey. This work was supported by Japan's Ministry of Agriculture, Forestry and Fisheries through the research project, "Monitoring and enhancement of pollinators for crop production."

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Table S1. Dynamics of nectar secretion in female and male flowers of *Cucurbita maxima* Duch 'Kento'

		Time	4:30	5:30	6:30	7:30	8:30	9:30	10:30	11:30	12:30	13:30	Total
Female	6-Aug-09	F_01	0.00	0.56	45.36	84.28	62.60	71.96	43.16	14.92	45.62	7.00	375.45
		F_02	0.00	0.48	33.27	101.50	62.62	71.68	35.89	11.43	57.49	26.02	400.39
		F_03	0.00	1.87	58.24	70.24	56.09	61.20	30.59	32.55	24.05	52.85	387.68
		F_04	0.02	1.90	78.99	62.36	35.53	38.95	22.00	23.19	21.38	6.02	290.33
		F_05	0.01	0.04	115.97	77.09	75.53	78.97	44.41	27.41	0.03	32.77	452.25
		F_06	0.59	14.68	100.50	76.47	42.72	48.66	9.33	43.14	23.63	3.32	363.02
		F_07*[1]	0.60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11-Aug-09	F_08	0.19	15.00	92.01	88.77	65.42	58.53	39.32	17.09	3.65	0.00	379.97
		F_09	0.12	10.09	121.28	106.15	54.25	57.49	33.38	10.92	0.68	0.00	394.35
		F_10	0.00	1.99	54.88	56.49	53.00	48.62	29.38	13.83	4.70	0.47	263.37
		F_11	0.19	73.68	89.01	58.28	44.05	48.35	26.77	19.08	9.78	0.00	369.18
		F_12	6.03	40.11	132.40	62.18	58.82	45.45	9.23	0.74	0.00	0.00	354.94
		F_13*[1]	20.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		F_14	8.88	14.02	106.91	40.14	31.32	30.31	9.63	0.00	0.00	0.00	241.20
	Mean		2.62	14.53	85.73	73.66	53.50	55.01	27.76	17.86	15.92	10.70	356.01
	SD		5.68	21.85	31.92	19.35	12.97	14.40	12.80	12.44	19.24	17.23	61.02
	CV		2.17	1.50	0.37	0.26	0.24	0.26	0.46	0.70	1.21	1.61	0.17
	Maximum		20.00	73.68	132.40	106.15	75.53	78.97	44.41	43.14	57.49	52.85	452.25
	Minimum		0.00	0.04	33.27	40.14	31.32	30.31	9.23	0.00	0.00	0.00	241.20
Male	6-Aug-09	M_01	0.00	0.57	11.57	17.20	21.15	12.86	13.08	36.06	26.89	3.69	143.06
		M_02	0.21	1.88	11.73	5.16	25.78	2.50	1.83	4.74	1.60	4.48	59.92
		M_03*[2]	0.14	0.90	22.94	28.57	13.78	NA	NA	NA	NA	NA	NA
		M_04	0.02	1.32	21.62	31.12	6.36	25.73	11.12	9.12	0.93	1.24	108.60
		M_05	0.30	0.03	43.93	38.84	35.37	4.80	45.17	12.79	21.03	1.40	203.65
		M_06	0.16	3.59	44.11	23.82	2.60	62.67	12.56	8.94	27.09	11.30	196.85
		M_07*[1]	0.68	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11-Aug-09	M_08	0.34	17.41	33.22	0.80	92.80	31.41	34.03	6.88	12.59	12.05	241.52
		M_09	0.03	13.92	10.43	78.62	51.23	54.62	32.57	16.45	15.71	3.98	277.56
		M_10	0.01	3.85	28.24	38.52	27.91	29.63	25.00	13.75	13.89	4.22	185.02
		M_11	0.29	13.96	52.02	35.05	34.31	17.35	39.02	33.51	5.05	3.92	234.48
		M_12	4.00	22.21	37.80	20.56	31.96	24.01	14.19	23.47	2.98	1.96	183.14
		M_13*[1]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		M_14	1.48	2.93	18.34	25.41	20.18	19.39	15.12	31.52	2.84	3.46	140.68
	Mean		0.59	6.88	28.00	28.64	30.29	25.91	22.15	17.93	11.87	4.70	179.50
	SD		1.10	7.74	14.19	19.78	23.77	18.72	13.76	11.35	9.96	3.63	62.91
	CV		1.86	1.13	0.51	0.69	0.78	0.72	0.62	0.63	0.84	0.77	0.35
	Maximum		4.00	22.21	52.02	78.62	92.80	62.67	45.17	36.06	27.09	12.05	277.56
	Minimum		0.00	0.03	10.43	0.80	2.60	2.50	1.83	4.74	0.93	1.24	59.92

*[1] Limited due to the time constraints

*[2] Limited due to the destruction of flower during the bagging treatment

Bagged flowers were repeatedly sampled hourly from 04:30 to 13:30. Values represent the volume of nectar in microliters.

Table S2. Results of the Browne–Forsythe test to examine the equality of variances in the hourly secretion of nectar between male and female flowers

		Df	*F*-value	*P*
4:30AM	Between sexes	1	1.71	0.20
	Within sexes	25		
5:30AM	Between sexes	1	1.82	0.19
	Within sexes	22		
6:30AM	Between sexes	1	6.21	0.02
	Within sexes	22		
7:30AM	Between sexes	1	0.18	0.67
	Within sexes	22		
8:30AM	Between sexes	1	0.87	0.36
	Within sexes	22		
9:30AM	Between sexes	1	0.16	0.69
	Within sexes	21		
10:30AM	Between sexes	1	0.09	0.76
	Within sexes	21		
11:30AM	Between sexes	1	0.01	0.94
	Within sexes	21		
12:30AM	Between sexes	1	1.65	0.21
	Within sexes	21		
13:30AM	Between sexes	1	2.91	0.10
	Within sexes	21		

After Bonferroni correction, significant sexual differences in the variances were not detected in any time periods.