

Effect of nectar reabsorption on plant nectar investment in *Cerasus cerasoides*

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Flowering progress, floral morphology and nectar characteristics of *Cerasus cerasoides* were investigated from November 2011 to January 2012 in Kunming, Yunnan, China. We found that nectar removal had a positive effect on nectar production. The phenomenon of nectar reabsorption in *C. cerasoides* occurred at the end of the floral lifespan, on the tenth day after anthesis when allowing for nectar accumulation. A flower reabsorbs nectar when it has not been foraged, with the reabsorbed sugar content accounting for about 34% of the maximum sugar content. Therefore, nectar reabsorption can only reclaim a part of the energy allocated for nectar production.

Keywords: *Cerasus cerasoides*, floral morphology, flowering progress; nectar reabsorption.

PLANT visual and olfactory signals can convey certain information regarding nutritional rewards in fruits and flowers to visitors¹. Therefore, pollinators are attracted to flowers by floral traits, including morphology and rewards². Although the inherent complexity of relationships between plants and animals has attracted attention, their evolution and ecological significance is far from clear and deserves exploration³. Floral nectar is the main reward offered by plants to pollinators. The production, composition and presentation of nectar determine potential pollinators and are therefore important for plant reproduction⁴.

Nevertheless, nectar secretion may occur concomitantly with reabsorption and the latter process sometimes continues after secretion has stopped. The dynamic modulation of these two processes may be in response to ecological and physiological constraints, maintaining a relatively constant nectar concentration to ensure pollinator visits^{5–8}. Nectar reabsorption is a widespread but

poorly understood phenomenon. When a flower senesces, reabsorption of nectar constituents occurs, as was recently demonstrated in several species by various direct and indirect methodologies⁹. Reabsorbed nectar can be transported to the vegetative part of the plant⁸ or used by developing fruits or seeds. From the perspective of resource recovery, nectar reabsorption represents an important energy-saving strategy, recycling at least some materials invested in nectar production and thus may favour reduction of plant reproductive costs. This strategy may minimize the energy investment of a plant in nectar, but the extent of nectar that is reabsorbed remains unknown.

The sugar content of nectar is usually of primary interest, because energy is the currency usually considered by botanists, examining the costs and benefits of allocation of resources to pollinator attraction. So, from the ecological and evolutionary perspective, it is critically important to explore the dynamics and quantity of nectar secretion⁷.

Cerasus cerasoides (wild Himalayan cherry) is among the few alternative nectar plants in late winter. It bears thousands of flowers during peak flowering and each flower produces large amounts of nectar and pollen, which attract potential pollinators. Floral nectar can be produced in large quantities in *C. cerasoides*, so that it often spills over and falls-off flowers (pers. obs.). This phenomenon suggests that some flowers of *C. cerasoides* might have never been visited and floral nectar may not have collected and removed from the flower by pollinators, as pollinator abundance is lower in winter. Since nectar reabsorption is generally recognized as a strategy to recover resources, we wanted to investigate whether or not this phenomenon occurs in *C. cerasoides* and, if so, to what extent.

In the present study we investigate (1) the floral features and stages of floral development, and (2) the temporal pattern of nectar secretion and reabsorption in *C. cerasoides*. We discuss the extent of nectar reabsorption and its effects on plant nectar investment in *C. cerasoides*.

The genus *Cerasus* (Rosaceae) includes 40–50 species found in China¹⁰. *C. cerasoides* is the only winter-flowering species in this genus, with a centre of distribution in Yunnan, China¹¹. *C. cerasoides* has a close phylogenetic relationship with two species, *Cerasus avium* (L.) Moench (sweet cherry) and *Cerasus vulgaris* Mill. (sour cherry), both originating in Europe and West Asia¹². *C. cerasoides* is a perennial woody plant, and is commonly cultivated in some cities in southern China as an ornamental plant¹³ because of its attractive flowers and winter flowering. This study was carried out at the campus of Yunnan Agriculture University, Kunming, China.

The study took place from November 2011 to January 2012. In order to assess the flowering period for a given population of this species, we selected five trees of the same age and vigour and marked four branches pointing

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in different directions on these plants. We determined dynamic changes of flowering by observing and recording the quantity of buds, flowers and faded flowers. Simultaneously, we tagged 20 buds (flowering soon) per tree for measuring the floral lifespan and observing the development process of individual flowers. The floral lifespan was determined from 10 flowers per tree by following their development until they wilted. Floral lifespan is defined as the period from bud opening until corolla wilting. Changes in floral traits associated with floral development stages were observed from the other 10 tagged flowers per tree.

To determine flower morphology, 50 flowers from five plants (10 per tree) were randomly selected during the peak flowering of *C. cerasoides*. On all experimental plants, we counted the number of petals and stamens and used digital calipers to measure six other floral traits on fully open flowers: the diameter of the outer part of the corolla, length and width of petal, length of ovary, length of style and length of pedicel. To examine the pollen/ovule (P/O) ratio, 50 buds from five plants (10 per tree) were randomly chosen; these were near anthesis, i.e. the pollen was mature, but the anther had not dehisced. Pollen quantity was estimated using Anderson and Symon's¹⁴ modification of Lloyd's¹⁵ technique. With the aid of a dissecting microscope, all ovules were counted.

To determine the pattern of nectar secretion and variation in concentration and volume with flower age, we randomly tagged 12 bagged sets of 10 buds each (with the same age, flowering soon), using tulle bags of 0.5 mm diameter mesh to exclude visitors. After flower opening, we probed the nectar once for each set and one set per day, allowing the nectar to accumulate for different days for each set until the measurements. The measurements were performed at 13:00 h each day, covering the entire floral lifespan. Upon sampling, we immediately measured the volume (in microlitre) using graduated micropipettes, and sugar concentration in the extracted nectar (mg sucrose equivalent/mg solution) with a pocket refractometer (make: Bellingham and Stanley, UK; model: Brix Refractometer, 0–80%). We then calculated the total sugar by multiplying concentration by nectar volume by the density of the corresponding sucrose solution. The nectar secretion rate (NSR) per hour was calculated by dividing the amount of sugar (mg) produced by 24 h, i.e. the number of hours between the measurements. Nectar reabsorption rate (NRR) per hour was calculated by dividing the amount of sugar reabsorbed by 24 h, i.e. the number of hours between the measurements with which nectar reabsorption had been confirmed to occur.

To assess total nectar production without nectar reabsorption due to repeated removal each day, we randomly bagged 30 buds (same age, mature stage) in 0.5 mm diameter mesh tulle bags, on the same trees used for estimating nectar secretion. After opening, nectar was removed and measured from the same flower repeatedly,

at 13:00 h each day, throughout the floral lifespan. Each sample was extracted with a new capillary tube. Additionally, special care is critically important to avoid piercing the floral tissue and clogging the lumen when probing for nectar. We also determined the volume, concentration, total sugar content and NSR as described above.

Tests were performed using methods described by Sokal and Rohlf¹⁶. All distributions were tested for randomness of nominal data (runs test), homogeneity of variances, and departures from normality. The means of data for nectar volume, concentration and sugar content were compared with either two-sample *t*-test or one-way analysis of variance (ANOVA) with Duncan multiple comparisons test. Homogeneity of variances was tested with *F*-test or Bartlett's test. If the variances differed significantly, the Welch test was applied. The normality of the data series was checked using Kolmogorov–Smirnov test. If the normality assumption was violated, either Mann–Whitney test or Kruskal–Wallis test with Dunn's multiple comparisons post test was applied. Finally, the remaining sugar content per flower after reabsorption was compared using a *t*-test with the total sugar production per flower without reabsorption due to repeated removal each day. For the statistical evaluation of the results, the software SAS 9.0 was used.

C. cerasoides blooms once a year from late November to mid-January and the flowering period usually lasts about 50 days for a given population, but a single flower is only open for 8–12 days. Moreover, flowers emerge before leaves in this species.

The axes of inflorescences are typically umbellate with 2–5 flowers, and each flower is actinomorphic (Figure 1). They open diurnally, have a pleasant fragrance and are highly attractive to bees. Table 1 lists the floral traits.

We partitioned floral ontogeny into five stages, as illustrated in Figure 2: (i) prior to opening, bud tightly closed, with deep red calyx that has five free, purplish-red lobes at the top, and a carmine corolla (Figure 2*a*); (ii) corolla newly unfolding its petals, with pistil or some anthers partly released just visible; stigma yellowish-green in colour, no nectar (Figure 2*b*); (iii) flower similar to the previous stage, but petals spread to about 50% maximum corolla diameter, anthers mostly dehisced and still bright yellow, nectar just visible (Figure 2*c*); (iv) fully open flower, with petals entirely spread, corolla turned pink; some anthers vary greatly in colour and fade to yellowish-brown; rich nectar (Figure 2*d*); (v) the majority of anthers dried or fallen, leaving just the filaments and stigma present brown in colour, little nectar (Figure 2*e*).

Figure 3 is a plot of nectar secretion through the floral lifespan. Nectar production begins 24 h after flower opening. If the nectar is allowed to accumulate, the volume increases slowly from the second to the third day after anthesis, after which the volume increases rapidly

Table 1. Floral traits in *Cerasus cerasoides*

Organ	Component	Component size (mm)*	Colour	Other traits
Pedicel	–	Pedicel length: 19.52 ± 0.54	Purplish-red	–
Calyx	Five lobes	–	Purplish-red	Basal part fused to form a calyx tube
Corolla	Five petals	Corolla diameter: 25.4 ± 0.39 Petal length: 12.56 ± 0.18 Petal width: 9.46 ± 0.16	Pink	Obovate petal
Androecium	29–41 stamens	–	Yellow anthers	$30,500 \pm 45$ pollen grains per flower
Gynoecium	One ovary	Ovary length: 3.14 ± 0.12	Green	Superior ovary with 2 ± 1 greenish ovules
	One or rarely two styles	Style length: 15.26 ± 0.19	Yellowish-green	Three discoid stigmas per glabrous style

*Means (\pm standard error), $n = 50$; these values are the sizes of each component on fully open flowers.



Figure 1. Inflorescences of *Cerasus cerasoides* and honey bees foraging for nectar.

until it reaches a maximum volume of $45.3 \mu\text{l}$ at around the eighth day after anthesis (Figure 3 c). Subsequently, the volume decreases rapidly. During anthesis, the amount of sugar increases slowly until flowers are 5 days old (NSR = 0.01 mg/h), after which the sugar content increases rapidly (NSR = 0.17 mg/h) until it reaches maximum production (13.4 mg) on the ninth day after anthesis (Figure 3 e). Conversely, nectar sugar concentration almost always increases from when secretion starts (6.6%) until the flowers are about 9 days old (29.5%). Subsequently, there is a significant decrease in nectar concentration on the tenth day after anthesis (24.0%). After this, the concentration continues to increase (Figure 3 a). At the end of the floral lifespan, there is an active nectar re-absorption period just after the peak of sugar production (from the ninth to the tenth day after anthesis; Figure 3 e) with a NRR = 0.19 mg/h .

Regarding flowers from which nectar is extracted repeatedly during anthesis, the mean values of nectar concentration remain relatively constant (6.4% – 7.5% ; Figure 3 b). However, volume and sugar quantity increase progressively and then reduce (Figure 3 d and 3 f). The recurrent removal of nectar makes the nectar variables

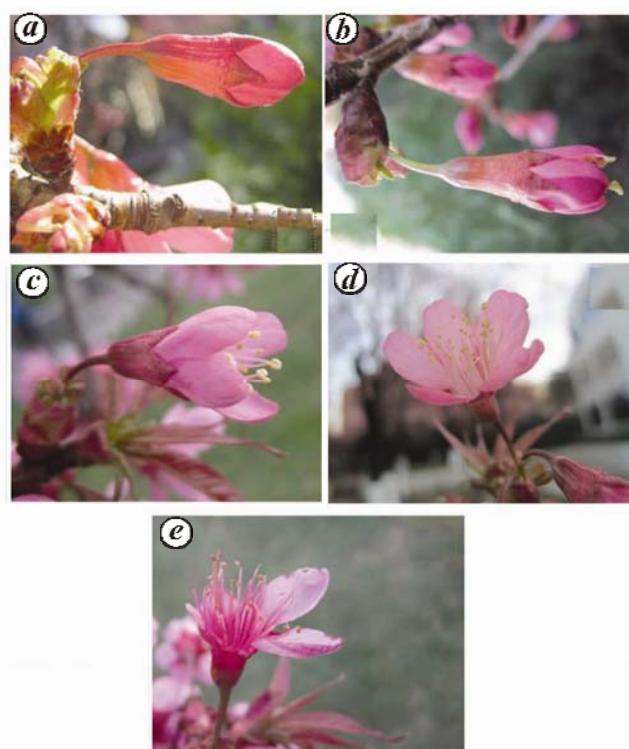


Figure 2. Flower stages of *C. cerasoides* as described in text. *a*, Prior to opening, bud tightly closed. *b*, Corolla slightly unfolded, pistil or some anthers partly released can be seen. *c*, Corolla diameter more or less 50% of maximum, anthers mostly dehiscent. *d*, Fully open flower with all anthers dehiscent and partly turned yellowish-brown. *e*, Withered flower with most anthers dried or even fallen.

(volume, concentration and sugar content) vary to a lesser extent.

Many plants can use nectar as a taste signal to encourage pollinators visiting flowers and this is also true for *C. cerasoides*. Flowers of *C. cerasoides* attract a diversity of generalist insects, including bees. According to Frisch¹⁷, sugar concentration in the nectar has to be at least 10% to be attractive to bees. Although nectar concentration in *C. cerasoides* is lower than 10% during the first few days, after the fifth day of flowering, it is higher than 10% and meets the demands from bees for sugar concentration (Figure 3 a). Both nectar production and concentration

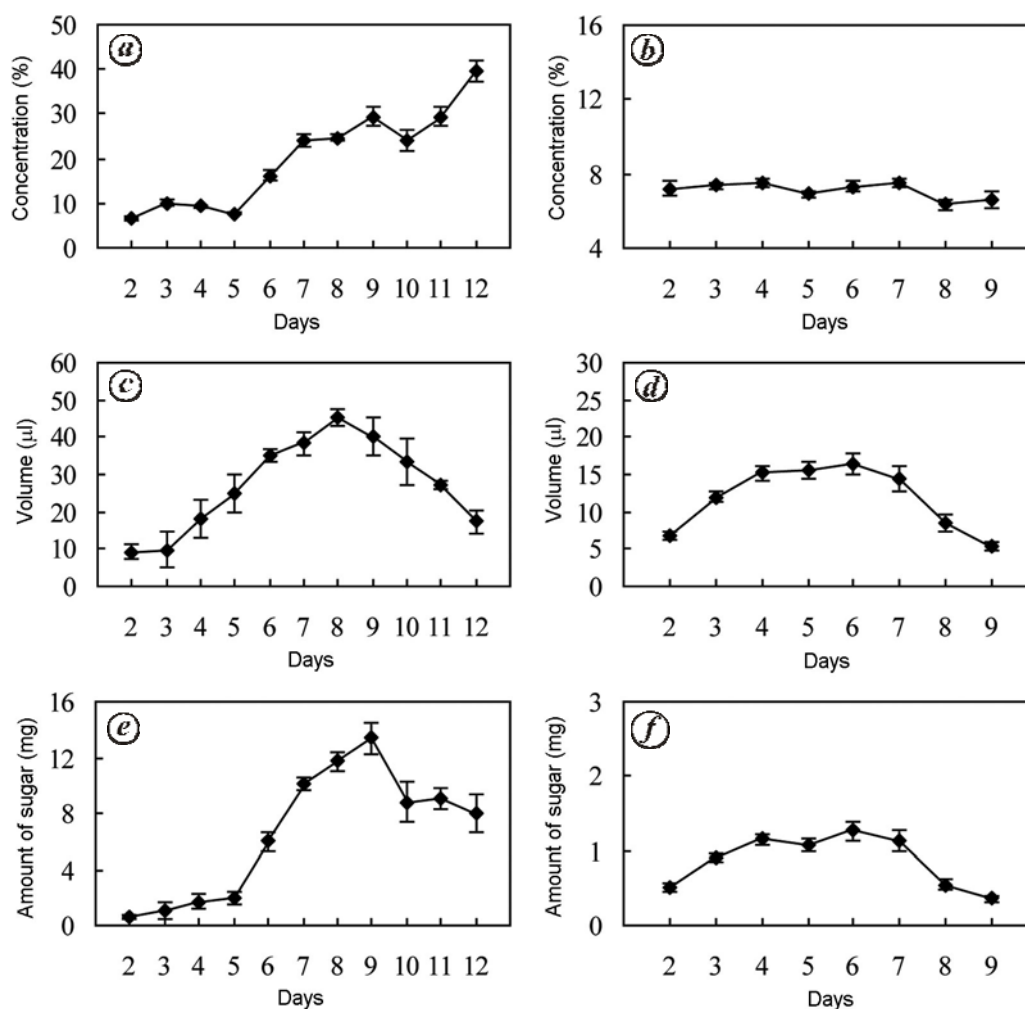


Figure 3. Nectar concentration (*a, b*), volume (*c, d*), and sugar content (*e, f*) during anthesis in *C. cerasoides* (*a, c, e*: Flowers for each set (total 12 sets) sampled only once during anthesis, allowing nectar to accumulate for different days until measurement. *b, d, f*: Each flower repeatedly sampled once per day during anthesis.) Lifespan of flowers sampled only once is 12 days and that of flowers repeatedly sampled is 9 days. No nectar is secreted on the first day of flower opening.

vary significantly if there is no artificial removal or foraging from pollinators. Because populations of pollinators are lower in winter than in the other seasons, some flowers of *C. cerasoides* may accumulate nectar for several days until they are foraged by pollinators. Therefore, flowers of *C. cerasoides* are attractive to many guilds of insects, and even birds with small body mass (Y. P. Ma, pers. obs.).

The decrease in nectar at the end of the floral lifespan in *C. cerasoides* can be attributed to nectar reabsorption. From the ninth to the tenth day after anthesis, the nectar sugar content decreases significantly. In the meantime, both nectar volume and concentration also decrease (Figure 3 *a, c* and *e*). We suggest that the decrease in nectar concentration and sugar is due to reabsorption of sugar from the nectar and that the decrease in nectar volume results mainly from reabsorption and partly from evaporation of water in the nectar. After the tenth day of

flowering, the nectar sugar content remains essentially constant, suggesting that both nectar secretion and reabsorption have ceased, while nectar volume decreases and nectar sugar concentration increases gradually due to evaporation until the flower withers (Figure 3 *a, c* and *e*). Cruden *et al.*¹⁸ showed that reabsorption occurs when maximum nectar production is reached and pollinators are inactive. For *C. cerasoides*, after the peak in sugar production, a flower reabsorbs nectar when it has not been foraged. The amount of sugar reabsorbed accounts for about 34% of the maximum amount of sugar (Figure 3 *e*). Therefore, nectar reabsorption can only reclaim a part of the energy allocated for nectar production. This strategy of resource recovery has been recently demonstrated or hypothesized in other plant species^{8,19,20}. Although there is relatively good evidence to show that nectar reabsorption occurs, the progress is far from clear and needs to be further investigated²¹⁻²⁴.

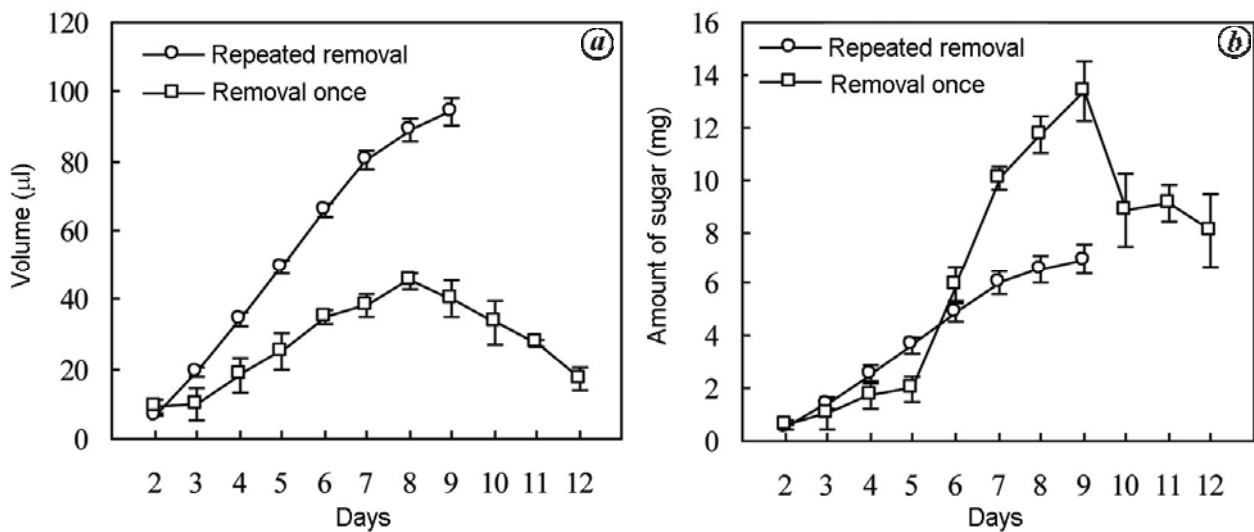


Figure 4. Comparison of (a) cumulative nectar volume (V_i) and (b) cumulative sugar content (S_i) in flowers repeatedly sampled (once per day during anthesis) with those in flowers sampled only once during anthesis (allowing nectar to accumulate for different days until measurement) in *C. cerasoides*. Blank square indicates removal once while blank circle indicates repeated removal. The cumulative nectar volume (V_i) is calculated using the following equation: $V_i = \sum_{j=2}^i (v_2 + v_3 + \dots + v_j)$ where v_i is the nectar volume of flowers repeatedly sampled on the i th day of flower opening. V_i is the cumulative nectar volume from the second day to the i th day of flower opening. The cumulative sugar content (S_i) is calculated using the following equation: $S_i = \sum_{j=2}^i (s_2 + s_3 + \dots + s_j)$, where S_i is the sugar content of flowers repeatedly sampled on the i th day of flower opening, S_i is the cumulative sugar content from the second day to the i th day of flower opening.

Removal of nectar affects its secretion in flowers of *C. cerasoides*. Although total nectar volume (94.2 μl) secreted by the repeatedly sampled flowers throughout their lifespan is significantly larger than the maximum nectar volume (45.3 μl) from the flowers allowed to accumulate nectar, the corresponding total sugar mass (6.9 mg) extracted from the flowers probed repeatedly is less than the maximum sugar mass (13.4 mg) from those accumulating nectar (Figure 4a and b). Relatively stable low sugar concentration in the fresh nectar from flowers subjected to recurrent probing throughout their lifespan may account for this (Figure 3b). Conclusively, repeated removal at 24 h intervals changes daily progression of floral nectar production or patterns of nectar presentation (nectar volume, concentration and sugar mass; Figure 3b, d and f). Meanwhile, removal of nectar affects its reabsorption in flowers of *C. cerasoides*. For the flowers repeatedly sampled, no reabsorption occurs throughout their lifespan. A possible reason is that nectar reabsorption is suppressed by removing available nectar from contact with the nectary soon after secretion. In fact, the daily progression of floral nectar production may differ between flowers due to the effects of uneven nectar removal by pollinators during anthesis. The amount of nectar removed will affect the occurrence and extent of reabsorption.

Nectar production entails a cost to the plant and nectar removal naturally occurs in all animal-pollinated species. It may therefore be important to know the effects of the removal of nectar on total nectar secretion in order to

accurately interpret the reproductive ecology of a plant species. So, if insects do not consume nectar, it may be actively reabsorbed by the nectary after anthesis. The quantity of nectar sugar in the flowers fluctuates through time as nectar is supplied by secretion or depleted by foraging animals or by reabsorption. It is interesting to note that in our study, the total amount of sugar for repeatedly sampled flowers is not significantly different from the amount of sugar remaining in the nectar in the flowers allowing it to accumulate, after they reabsorb nectar (Figure 4b). In other words, the amount of sugar a flower produces if it is never visited by pollinators would be equal to the total amount of sugar a flower produces throughout its lifespan if it is frequently visited by pollinators. This means that the minimum investment of a flower in *C. cerasoides* for attractive pollinators may be stable whether reabsorption occurs or not, even if perhaps as a consequence of intrinsic regulation or response.

Removal of nectar throughout the floral lifespan increases the total volume of nectar produced by each flower, but reduces the sugar concentration. Thus, nectar removal does not impose a high cost to the plants in nature. While there is a separate body of literature concerning nectar secretion, the mechanism underlying intrinsic regulation by the plant is generally regarded as 'marginal' in most studies and very few of them consider its ecological context⁹. Experimental approaches are necessary to further understand the role of constant amount of sugar produced by *C. cerasoides* flowers in the

interactions between successful reproduction of plants and pollinators.

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