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REPRODUCTIVE BIOLOGY OF *CHEIROSTEMON PLATANOIDES* (STERCULIACEAE): I. FACTORS INFLUENCING NECTAR PROPERTIES

Ron Scogin

Introduction

Cheirostemon is a monotypic genus consisting of the single species *Cheirostemon platanoides* Humbolt & Bonpland (= *Chiranthodendron pentadactylon* Larreat.) which is native to montane cloud forests of southern Mexico and Guatemala. *Cheirostemon platanoides* is regarded as a "classical" bat-visited plant (Vogel 1958), although Toledo (1975) has reported frequent visitation by perching birds as well. Floral pigments and nectar constituents of this taxon were reported by Scogin (1980). The present work was undertaken in an effort to better understand the mechanisms regulating nectar constituents.

Materials and Methods

Experiments were performed on and materials collected from two mature trees of *Cheirostemon platanoides* cultivated on the campus of California Polytechnic University at Pomona, CA. Nectar constituents were determined as described by Scogin (1980). Nectar buffering capacity was determined by titration of nectar with standardized solutions of HCl and NaOH while hydrogen ion concentration changes were monitored on a laboratory pH meter.

Two drops of invertase inhibitor solution or pH altering solution were introduced into each sepal cup in experimental treatments of flowers. Inhibitor concentrations (as cation normalities) were Ag (as AgNO_3), 2×10^{-3} N; Cu (as CuSO_4), 6×10^{-3} N; Pb (as PbNO_3), 6×10^{-3} N, and Zn (as ZnSO_4), 7×10^{-3} N (Myrback and Willstaedt 1955).

For temperature-dependence studies flowers were removed from the plants and placed in refrigerators or ovens of suitable controlled temperatures in the laboratory.

Results and Discussion

It has been postulated that nectar sugar parameters (sugar concentration, sugar composition, and caloric content per flower) have adaptively changed among flowering plants to best suit the energetic requirements and taste preferences of particular pollinator classes (Heinrich and Raven 1972; Baker

1978). Little information is available, however, regarding the environmental factors or bioregulatory mechanisms which control these nectar sugar parameters. In the present study the effects of three experimental variables (temperature, nectar pH, and presence of enzyme inhibitors) upon three nectar properties (sugar concentration, nectar volume, and nectar sugar composition) were examined.

Sugar concentration.—For a given nectar volume, the nectar sugar concentration depends upon the rate of sugar flux through the nectary surface. This sugar secretion is generally accepted to be an active, energy-requiring process and nectar sugar concentration has been found to be sensitive to numerous biological and environmental factors. Among taxa these factors include pollinator class (Baker 1978) and the type and extent of nectary vascularization (Agthe 1951). Within a taxon sugar concentration may depend upon such biological factors as the height of the flower above ground level (Baker 1978) and simple intraspecific variation (Brink 1982) or upon such environmental factors as intrafloral relative humidity (Corbet et al. 1979) and ambient humidity (Plowright 1981).

In the present work nectar sugar concentration was decreased by all experimental treatments. Since sugar secretion by nectaries is an active biological process, it is reasonable to expect that heavy-metal ions with known inhibitory effects on biologically active proteins might also inhibit the process of active sugar secretion. This was observed in all cases, but most notably with the introduction of Ag and Pb ions (see Table 1).

The secretion of nectar sugar has recently been proposed (Luttge 1977) to be energetically driven by chemiosmotic coupling to a proton pump, rather than by sugar dephosphorylation. If this were the case, one would predict that drastic changes in extracellular pH would disrupt the proton pump and inhibit sugar transport (i.e., reduce nectar sugar concentration). This effect was observed at both strongly acid and alkaline pH's and thus supports a mechanism of sugar secretion energized by a proton pump.

Nectar volume.—The daily volume of nectar production (i.e., rate of solute passage), in conjunction with the rate of sugar secretion, defines the physicochemical properties of nectar such as viscosity, energy content per unit volume of nectar, and energy content per flower, all of which vary from taxon to taxon, are more or less taxon specific, and are postulated to represent adaptation to a pollinator class (Baker 1978). The volume of nectar per flower can vary over four orders of magnitude among taxa and is presumably under genetically based physiological control. Apart from the regulatory physiology of the particular taxon, nectar volume is influenced by such environmental factors as ambient humidity (Shuel 1956), temperature (Fahn 1949), and mineral nutritional status of growth substrate (Shuel 1967).

Table 1. Experimental modification of nectar properties of *Cheirostemon platanoides*.

Treatment	Sugar concentration (%)	Nectar volume (ml)	Energy per flower (cal)	Nectar pH (final)	Nectar sugar composition
None	10.2	3.9	1545	7.8	hexose dominated, some sucrose
pH modification					
0.5 N NaOH	7.5	1.4	398	>10	unchanged
0.01 N NaOH	7.5	4.0	1170	8.7	unchanged
0.5 N HCl	9.8	0.2	76	1.0	unchanged
0.01 N HCl	7.8	2.4	724	7.0	unchanged
Buffered at 4	9.1	5.8	2047	7.9	unchanged
Buffered at 10	7.6	2.1	622	7.9	unchanged
Invertase inhibitors					
Cu	9.4	1.6	587	7.0	unchanged
Zn	9.6	2.6	966	6.9	unchanged
Ag	7.4	3.6	1039	7.7	unchanged
Pb	8.0	1.6	499	7.7	unchanged
Temperature (C)					
5	4.1	0.8	128	n.d.	n.d.
20	3.8	1.0	148	n.d.	n.d.
30	5.4	0.4	84	n.d.	n.d.
45	4.4	0.1	17	n.d.	n.d.

n.d.: not determined.

Daily volume of nectar production in *Cheirostemon* is consistent with previous reports in that it is dependent upon stage of floral maturity and temperature (Table 1). Nectar volumes were reduced in all flowers which were removed from the plant and transferred into the laboratory, but nectar volumes were reduced most at both lowered and elevated temperatures (relative to the 20° control at room temperature). Optimum nectar production temperatures have been previously reported to lie within the range of 12°–25° (Fahn 1979).

Nectar volume was decreased in varying amounts by invertase inhibitors. The reason for this is not immediately apparent unless the cationic enzyme inhibitors also interfere with osmotic regulation of water transport from the nectary surface. Extreme extracellular pH values also reduce nectar volume, probably by disruption of the same water-releasing physiological processes. Dilute acids and bases (0.01 N) have a less-marked effect on nectar volume. In fact, their presence appears to be overcome by the buffering capacity of the nectar. Nectar production was enhanced by buffering at pH 4. This suggests that the optimum extracellular pH for the water-releasing

processes from nectaries may be slightly acidic, although the buffering capacity of the added solution was exceeded by that of the natural nectar to yield a final pH of 7.9.

Nectar sugar composition.—Nectar sugars consist almost universally of glucose, fructose, and sucrose. The varying relative amounts of these constituents define nectar sugar composition, which appears to be under genetic control and of adaptive significance (Baker 1978). There is little explicit discussion in the literature regarding the physiological-biochemical mechanism for the regulation of nectar sugar composition, although data relevant to this problem exist. Two possible models exist for the regulation of nectar sugar composition 1) a secretory/reabsorption specificity model and 2) an invertase activity regulation model.

In the secretion/reabsorption specificity model each nectar sugar component is proposed to have an independent secretory and/or reabsorption apparatus with its own independent regulatory mechanism. These three sugar-regulating systems (one each for glucose, fructose, and sucrose) might exist in the nectary cell plasmalemma or in intracellular membrane systems engaged in "packaging" prenectar.

In the invertase activity model all nectar sugars originate in a prenectar secretion as sucrose and the final composition is determined by the degree of activity of the hydrolytic enzyme, invertase. The localization and site of activity of invertase could be in the nectar itself, in the nectary cell plasmalemma or cell wall, in intracellular membrane systems within the nectary cells, or in the nectary cell cytoplasm.

Various arguments can be marshalled for and against the suggested models. In support of the specific secretion/reabsorption model, it is well established that nectar secretion is an energy-requiring, active process which is inhibited by decouplers of cellular respiration (Ziegler 1956). The occurrence of high concentrations of phosphatase activity (and therefore presumed energy utilization) in the plasmalemma of nectary cells (Figier 1968) is circumstantial evidence in support of active sugar secretion at the plasmalemma. These observations are a necessary, but not sufficient, proof of active specific secretion at the cell surface.

The reabsorption of compounds (other than sugars) concomitant with secretion has been demonstrated (Ziegler and Lüttge 1959; Shuel 1961; Lüttge 1962), but has not specifically been demonstrated for sugars in nectar.

A formal argument against the secretion/reabsorption model is its complexity. It would require three independent, but coordinated, regulatory systems, one for each sugar constituent. This is not evidence against this model, but the model does not satisfy a "simplest case" prerequisite for a mechanism.

The specific secretion model has also been invoked to explain differences among plant taxa in nectar amino acid content (Lüttge and Schnepf 1976).

The frequent observation that the molar concentration ratio of glucose to fructose in nectar is near unity (Furgala et al. 1956; Maurizio 1959; Lüttge 1961; Van Handel et al. 1972) indirectly supports the invertase activity model. Precisely that result would be predicted if nectar sugars were derived directly from sucrose hydrolysis. Notable exceptions to the unity ratio do occur (e.g., *Robinia pseudoacacia* L. in Maurizio 1959; Baker and Baker 1982) and these deviations may serve to flag taxa in which a specific secretion/reabsorption process is occurring.

An additional, although not compelling, argument in favor of the invertase activity model is its simplicity and ease of possible control. Evolutionary modification of nectar composition could be accomplished by regulating the activity of a single enzyme which is sensitive to such universal enzymatic activity controls as enzyme concentration, temperature, enzyme configuration, and pH.

If invertase activity were involved in nectar sugar regulation, four sites of activity are possible: 1) in the nectar itself, 2) in the secretory cell plasmalemma or cell wall, 3) in an intracellular membrane system (probably endoplasmic reticulum (ER)) of the nectary cells, and 4) in the cytoplasm of nectary cells.

Sugar-modifying enzymatic activity has been reported in nectar. Zimmermann (1954) and Frey-Wyssling et al. (1954) reported transglucosidase and transfructosidase activity in nectar. However, Van Handel et al. (1972) were unable to detect invertase activity in nectar samples.

The present work tests the second possible location for invertase activity, viz., in the plasmalemma or cell wall of the nectary secretory cells.

Postulate: The invertase activity responsible for determination of nectar sugar composition is localized in the plasmalemma or cell wall of the exterior cells of the nectary and these sites are in chemical contact with the nectar solution.

Test: To test this postulate, known invertase inhibitors and pH-modifying chemicals were placed in the evacuated sepal cups and the composition of subsequently produced nectar was compared with untreated samples for any change in the sugar composition.

The above postulate seemed possible since 75–100% of total cellular invertase activity has been shown to reside in the cell wall structures (Straus, 1962).

As shown in Table 1, neither invertase inhibitors nor pH modifiers qualitatively affected *Cheirostemon* nectar sugar composition. Angiosperm and fungal invertases have pH optima near 5.0 and activity drops to less than 20% of maximum at pH values less than 3 or greater than 7 (Myrback and Willstaedt 1955; Pressey 1966). Yet at experimentally modified nectar pH values as extreme as 1 and 10, *Cheirostemon* nectar composition was not detectably altered. Similarly, no change in nectar sugar composition could be detected in the presence of heavy metal ion inhibitors at concentrations

sufficient to reduce invertase activity to 20% of its maximum (Myrback and Willstaedt 1955). The possibility of slight quantitative changes in nectar sugar composition by experimental treatments cannot be discounted using the present techniques, but substantial increases in nectar sucrose content due to invertase inhibition did not occur.

The natural pH of *Cheirostemon* nectar is slightly alkaline at 7.8, so that the nectar itself would have an inhibitory effect on invertase if this activity site proposal were correct. Similarly, it might be expected that if nectar pH were crucial in maintaining a selectively important nectar composition, then the nectar would be strongly buffered at that pH to prevent enzyme activity variations due to pH value fluctuations. The buffering capacity of *Cheirostemon* nectar was determined to be 0.016 milliequivalents per ml. This is lower by an order of magnitude than most physiological buffer systems (Williams and Wilson 1975), which suggests that nectar pH is not biologically significant in this system.

The remaining, and currently most likely, model for nectar sugar composition regulation is that of intracellularly localized invertase. The results of DeFekete et al. (1967) are consistent with that model. These workers demonstrated the presence of invertase (and other sugar metabolism enzymes) in fractionated nectary tissues of *Deutzia scabra* Thunb. and *Convolvulus sepium* L. Invertase activity was partitioned in *Deutzia* as follows: 10.3% in cell wall, nucleus, and chloroplast; 2.2% in mitochondria; 0.2% in microsomal membranes (including ER); and 87.3% as soluble enzyme. In *Convolvulus* 21.2% of activity was in the cell wall, nucleus, and chloroplast; 63.6% in the mitochondrial-microsomal fraction; and 14.3% as soluble enzyme. The discrepancy between distributions of activity between the two taxa was not discussed by the authors. The results with *Convolvulus* are consistent with ultrastructural studies which reveal tritiated sucrose (presumed in prenectar) accumulating in ER cisternae of nectar-secreting hairs of *Lonicera japonica* Thunb. (Fahn and Rachmilevitz 1975). Invertase present in the microsomal membrane fraction (ER) could act upon this prenectar to give it its final sugar composition prior to secretion by a granulocrine (reverse pinocytotic) mechanism.

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