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SELECTION FOR HIGH NECTAR PRODUCTION IN THREE SPECIES OF
LOTUS AND A STUDY OF THE EFFECTS OF HONEYBEE
VISITATION ON THREE CARBOHYDRATE PRODUCTION
PRODUCTION TYPES OF L. CORNICULATUS

by

Nancy J. Campbell

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ABSTRACT

SELECTION FOR HIGH NECTAR PRODUCTION IN THREE SPECIES OF
LOTUS AND A STUDY OF THE EFFECTS OF HONEYBEE VISITATION ON
THREE CARBOHYDRATE PRODUCTION TYPES OF L. CORNICULATUS

By

Nancy J. Campbell

A diverse population of Lotus were screened for nectar production. Receptacle diameter, height and volume were evaluated as potential indicants of carbohydrate production in 3 species. Receptacle volume was the best or equivalent to the best indicator of nectar carbohydrates for both L. tenuis and L. caucasicus. Receptacle height was the best indicator for L. corniculatus. A 14-fold range in nectar carbohydrates was observed. Further attempts at increasing carbohydrate production were made through breeding. Selected plants grouped into 3 carbohydrate production types, were found to be phenotypically stable for carbohydrate production at 2 locations. The effects of pollination at different floral ages on nectar production were examined for high, moderate and low nectar producing plants. Despite bee visitations, net nectar carbohydrates levels increased from days 1 to 3 for all carbohydrate production types before experiencing a decline on day 4.

Dedication

To my children

Jacob and Michelle

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GENERAL THESIS INTRODUCTION

Birdsfoot trefoil, (Lotus corniculatus) a perennial forage legume, is recognized both for its nutritional value to livestock (Hughes 1965) and its potential for production of surplus honey (Pellet 1976). In New York, birdsfoot trefoil ranks with alfalfa as one of the state's two most valuable legumes (Stennet, Mattern and Fuller 1963). In areas of the United States where trefoil is grown, it has become the most frequently used plant in association with soil and water conservation projects (Stennet, Mattern and Fuller 1963). Trefoil is increasingly visible along road right-of-ways, on mine spoils and even in suburban lawns in Canada and the Northeastern United States. It flourishes in these diverse ecological habitats because of several outstanding characteristics. These include: suitability to low fertility soils, resistance to most crown and root diseases, winter hardiness, tolerance to drought and flood conditions (Seany and Henson 1970), and tolerance to salt (Crane 1984).

Despite these outstanding survival characteristics the use of birdsfoot trefoil as a bee forage is limited due to its moderate nectar potential (0.19 to 0.55 mg/floret/day; Crane 1984). Several studies have indicated that increasing nectar production in legumes through phenotypic recurrent selection is promising. Murrell et al. (1982) found

sufficient genetic variation in nectar volume among 8 birdsfoot trefoil cultivars to make selecting for high nectar production feasible. In addition, results from inheritance studies indicated that nectar production in birdsfoot trefoil is a highly heritable trait and may be amenable to genetic manipulation. Teuber & Barnes (1979a) reported similar inheritance patterns for nectar production in alfalfa, a closely related legume, and were able to increase nectar volume after two generations utilizing phenotypic recurrent selection.

Sampling procedures necessary for screening plant populations for nectar production are both time consuming and tedious. The development of a preliminary screening technique for rapidly assessing a floret's nectar potential would reduce the time required for screening nectar production. Nectar production has been shown to be related to several floral characteristics. Murrell et al. (1982) found that aroma strength, petal area and phloem supply were correlated with nectar production in birdsfoot trefoil. High nectar producing alfalfa clones were characterized by having large receptacle reservoirs and many stomata (Teuber et al. 1980).

Increased nectar production should result in improved seed yields and perhaps in higher honey returns as well. The effect on honey production would be dependent upon the effects of pollination on nectar production. Collison (1973)

and Pankiw & Bolton (1965) found that pollination terminated nectar secretion in cucumbers and alfalfa respectively. Several researchers have examined the effects of repeated nectar sampling on nectar production where nectar was removed but probably was not accompanied by pollination (Southwick 1983, Willson et al. 1979 and Fahn 1949). Willson et al. (1979) found that nectar production in Asclepias was stimulated by repeated sampling. Multiple bee visits have been observed for birdsfoot trefoil (De Grandi-Hoffman 1982) indicating that nectar secretion and possibly production continue after pollination.

The original goal of this study was to select for high nectar carbohydrate production in the genus Lotus. The time involved in using the customary method of screening for nectar production prohibited examination of all the plant material available. As a result, the potential for using the size of a floret's receptacle as an indicator of nectar carbohydrate production during preliminary screenings was investigated.

As these studies progressed, it became clear that bees were attracted to florets for several days following anthesis, which suggested that nectar secretion and possibly production continued after pollination. This phenomenon is referred to as "nectar replenishment", with the hope that the implications will contribute to accurately projecting the total amount of carbohydrates produced by a given floret, and

when floral densities are determined, the honey potential per unit area.

The research reported in this thesis unfolded as 3 investigations. The first entailed selecting for high nectar carbohydrate producing plants from a large and diverse Lotus population. The second, was designed to determine the value of a floret's receptacle size in projecting it's nectar potential. The final study examined the interaction of carbohydrate production type and pollination on nectar production throughout a floret's life.

Literature Review

Nectar Production

Birdsfoot trefoil (Lotus corniculatus L.) is a long day perennial plant in the family Fabaceae (Leguminosae). It is recognized for both its nutritional value to livestock (Hughes 1965) and its potential for production of surplus honey (Pellet 1976).

Nectar is a sugary substance produced by plants and is exchanged for indispensable pollination services that the bee performs at the time of removal. Bees are able to manufacture honey from nectar through two distinct processes. One brings about a chemical change in the sugar and the other results in a physical change whereby surplus water is eliminated (Shuel 1979).

In birdsfoot trefoil, the nectary is located at the base of the hypanthium, abaxial to the ovary. Nectar is secreted from stomates which are arranged in a band, midway up the nectary (Murrell et al. 1982).

Bees are the only insects that pollinate the trefoils to an appreciable degree (Badger & Anderson 1962). The process of nectar removal and potentially, pollination, begin when a bee alights on a banner petal and probes her probocis down into the nectary. This pressure causes the thickened staminal filaments to push a quantity of pollen out of the keel opening. As the pressure continues, the stigma also protrudes, thus causing the stigmatic membrane to rupture with a resultant release of fluids that provide a medium for

pollen tube growth. At this point, either self- or cross-pollination can occur (Seany and Henson 1970). Florets that are not visited by bees remain open and have fertilizable ovules for 8-10 days, whereas florets that are visited by bees last less than four days (McGregor 1976).

The Influence of Flower Number on Nectar Production

The number of flowers/unit area is an important factor in nectar production and potential seed yield. High levels of flowering are needed to attract and retain pollinators in birdsfoot trefoil (De Grandi-Hoffman 1980). Eriksson (1979) found that the number of bees visiting different cultivars of red clover was dependent on the amount of nectar produced and the number of flower heads per unit area. In order to optimize a forager's response, a plant should produce large amounts of nectar and/or large numbers of florets.

Variability of Nectar Production

Several studies have indicated that attractiveness is a function of color (Clement 1965; Kauffeld and Sorensen 1971), aroma (Clement 1965) and nectar sugar (Pedersen 1953). Nectar is the primary attractant followed by color and aroma, which are secondary attractants. Selection for one or more of these factors should result in increased floret attractiveness and therefore an improvement in seed yield.

Recently, several researchers have examined the variability associated with nectar production in birdsfoot

trefoil. A fivefold range in nectar volume was observed when individual plants of 'Carroll', 'Leo', 'Maitland' and 'Wallace' were screened for nectar production (Murrell et al. 1982). Similar results have been reported for alfalfa (Medicago sativa) where sufficient genetic variability existed in nectar volume, concentration and total sugar among 82 cultivars to make breeding for nectar production feasible (Barnes & Furgala 1978).

Szabo (1982) examined nectar production in rapeseed as an additional plant breeding objective for the improvement of rape as an oil crop. A twofold difference in nectar production was found between the two species he examined. B. napus secreted twice as much nectar as B. campestris and possessed significantly larger flowers than the latter. Substantial differences for nectar production have also been reported for other plants, including: red clover (Trifolium pratense L.), white clover (Trifolium repens) (as reported by Shuel 1975), cucumbers (Cucumis sativus) (Collison 1973) and blueberries (Brewer & Dobson 1969).

Floral Characteristics as Indicators of Nectar Yield

A relationship between flower size and nectar production has been reported for several plant species. Szabo (1982) found that large flowers produced more nectar than small flowers in the genus Brassica. For alfalfa, both flower size and visual nectar quantity were found to be associated with nectar volume (Barnes & Furgala 1978).

A significant correlation ($r=0.358$, $P<0.05$) between petal area and nectar volume was found for 8 varieties of birdsfoot trefoil (Murrell et al. 1982). In addition, a good phloem supply was common to high nectar producers and large flower stature in itself was not a reliable indicator of nectar potential (Murrell et al. 1982).

Floret receptacle diameter as an indicator of nectar yield has been examined for alfalfa. Teuber and Barnes (1979a) successfully identified high and low nectar producers in an alfalfa population by using the single phenotypic trait, receptacle diameter as a preliminary screening tool. High nectar producing clones were characterized by having large nectar reservoirs and many stomata.

In a later study, phenotypic recurrent selection was successfully used in selection programs designed to manipulate nectar volume or receptacle diameter (Teuber et al. 1983).

Inheritance of Nectar Production

The increase of nectar production in flowering plants through genetic manipulation has been the focus of several studies in recent years. Murrell et al. (1982) found a highly significant correlation ($r=0.96$) between the progeny and their mid parent values of a diallele cross of 2 high and 2 low nectar producing birdsfoot trefoil cultivars. A high GCA (General Combining Ability) effect and a non-significant SCA (Specific Combining Ability) effect were obtained. From

these results, they concluded that nectar production in birdsfoot trefoil is governed by additive gene effects. This in conjunction with the high parent-offspring correlation for nectar yield, indicated that nectar production in birdsfoot trefoil is a highly heritable trait and may be amenable to genetic manipulation.

Pedersen (1953) studied the inheritance of nectar production in alfalfa and found a significant parent-offspring correlation in nectar yield from a polycross progeny test. He concluded that nectar production in alfalfa was inherited quantitatively.

In a quantitative inheritance study (Teuber & Barnes 1979a) found that clones with the largest additive effects produced the most nectar. Crosses between high nectar producing plants produced the progeny with the highest nectar production. These results indicated that nectar volume in alfalfa should respond to selection systems designed to utilize additive genetic variance.

Methods for Analyzing Nectar Production

Various methods have been developed for quantifying nectar and nectar sugar production. Microcapillary pipettes are used to both extract and measure the volume of nectar produced by a floret. Pressure is applied to the floral calyx and the resultant bead of nectar is drawn into the microcapillary pipette either by capillary action or through suction. Pressure is continued until the available nectar is

depleted. The volume of nectar is determined by measuring the length of the nectar column usually in microliters (De Grandi-Hoffman 1980). If determination of sugar concentration is desired, most often this is analyzed with a refractometer. The chief disadvantages of this method are: (1) failure to remove all of the nectar from the floret; (2) failure to withdraw highly viscous nectar (more than 60% solids); (3) difficulty in removing all of the nectar from flowers with inaccessible or small nectaries; and (4) a refractometer is needed to determine the sugar concentration of the nectar (Roberts 1978). Inouye (1980) found that the non-sugar constituents of nectar can potentially contribute to the refractive index and result in an under- or overestimate of nectar sugar concentration. Fahn (1949) & Willson et al. (1979) found that the act of extracting nectar, may in itself damage nectiferous tissue and affect nectar production. The chief advantages of this method are: (1) the low cost and availability of pipettes; and (2) the flower is not destroyed by the process of nectar extraction thereby leaving it intact for further analysis.

A centrifugal method was developed by Swanson and Shuel (1949) to assay the average quantity of sugar produced by a floret. Florets are secured and suspended by a split cork during the centrifuging process. The microliters of nectar are determined from the height of the liquid in the calibrated well of the centrifuge tube. Sugar concentration is then determined with a refractometer in the same manner as

described above. The chief disadvantages of this method are: (1) the nectar analysis is very time consuming; (2) several pieces of equipment are required for the analysis; (3) the analysis must be performed shortly after the florets are picked, thereby reducing the numbers of samples that can be collected and analyzed; and (4) the nectar sugar concentration may be under-or overestimated because of the possible additive effects of the non-sugar constituents to the refractive index as mentioned above.

Calibrated syringes are also used to extract the volume of nectar secreted by a floret. The chief advantages of this method are the low cost and availability of the syringes. The chief disadvantages are: (1) a refractometer is needed to determine nectar sugar concentration; and (2) complete removal of all of the nectar from the floret is unlikely.

A fourth method, water extraction, relies on the diffusion of nectar sugars into a known amount of distilled water. Florets are collected and flower parts that might interfere with the nectar flow, are removed. The florets are allowed to remain in water for some period of time (usually 60 minutes) to allow the available nectar to diffuse into the solution. After removing the florets, the rinsate can be stored in the freezer for later analysis. Analysis consists of adding several reagents to the rinsate that develops a colored product with simple sugars. After color development, the carbohydrate content is quantified spectrophotometrically with the aid of a standard curve. The

chief disadvantage of this method is that only data for total sugar is obtained while information about nectar concentration and volume are lost. The advantages are: (1) rapid collection of nectar samples in the field; (2) rapid analysis of samples; (3) good accuracy is obtained with this method if nectar flow from the florets into the solution is not obstructed; and (4) the analysis can be performed long after the collection (Roberts 1979).

The Effects of Flower Age, Nectar Removal and Pollination on Nectar Production

Several studies indicate that the age of a floret is an important factor in nectar production. Beutler (1953) found that bagged Aesculus flowers produced uniform amounts of nectar for six days before a decline in nectar carbohydrate production was observed. In bagged Asclepias flowers, sugar production peaked at fifty hours after anthesis and thereafter steadily declined (Southwick & Southwick 1983).

Pollination is another important factor in nectar production. In certain species of plants, pollination is known to terminate nectar production (Pankiu & Bolton 1965; and Collison 1973). In other species, nectar is actively secreted after pollination (DeGrandi-Hoffman 1980).

The effects of nectar removal on nectar production, without pollination, have also been examined (Southwick 1983; Willson et al. 1979 and Fahn 1949). Southwick (1983) found

no significant differences in accumulated nectar sugar between bagged Asclepias syriaca flowers that had been sampled every hour, every two hours (both over a 24 hour period) and once in 48 hours. However, highly significant differences were found in nectar volume between these categories. Similar results were obtained by Plowright (1981) where repeated sampling had no effect on nectar sugar production in Clintonia flowers. No significant differences were found in total lifetime sugar production between florets in which nectar was removed at daily intervals and those in which nectar had been removed only once in the life of a floret. Several researchers however, have found nectar production to be stimulated by repeated sampling. Raw (1953) obtained significantly higher nectar yields from multiple collections of nectar from bagged raspberries than he did from single collections. Similar results were reported by Willson et al. (1979). The rate of nectar production in Asclepias verticillata decreased over time for bagged florets sampled only once, but in repeatedly sampled florets, nectar sugar production appeared to be stimulated.

SELECTION FOR HIGH NECTAR CARBOHYDRATE PRODUCTION IN
BIRDSFOOT TREFOIL

INTRODUCTION

Birdsfoot trefoil (L. corniculatus) is a perennial forage legume recognized for both its nutritional value to livestock (Hughes 1965) and its potential for production of surplus honey (Pellet 1976). Due to its soil and water conservation properties, it is grown along road right-of-ways, on mine spoils and on hilly, marginal and poorly drained soils where alfalfa (Medicago sativa L.) and other legumes will not persist (Murrell et al. 1982).

The improvement of seed yield in birdsfoot trefoil resulting from increasing nectar production has received the attention of several researchers in recent years (Murrell et al. 1982, DeGrandi-Hoffman 1982). Murrell et al. (1982) determined that nectar production plays a major role in attractiveness to pollinators and potential seed yields. They also studied the inheritance of nectar production in eight cultivars of birdsfoot trefoil and found an almost two-fold range between the highest and lowest nectar producers. They determined that nectar production is a highly heritable trait and that it is inherited quantitatively.

Teuber and Barnes (1979a) reported similar inheritance patterns for nectar production in alfalfa. They were able to increase nectar volume in all cultivars after two generations utilizing phenotypic recurrent selection.

Although birdsfoot trefoil is credited with only a moderate nectar potential (0.19 mg./floret/day to

0.55mg./floret/day; Eva Crane 1984), it was chosen for this study because of its numerous agronomic characteristics that enable self maintenance in many ecological habitats where other legumes will not succeed (Murrell et al. 1982). It was also chosen because previous studies (Murrell et al. 1982 & Teuber & Barnes 1979a) indicated the potential for increasing nectar production in trefoil and alfalfa through phenotypic recurrent selection is very promising.

Development of a high carbohydrate producing plant that is self maintainable would serve the dual roles of producing surplus honey as well as in reclaiming lands that might otherwise remain valueless. The objectives of this study were to select for high nectar carbohydrates from a large and diverse population of the genus Lotus and make further increases in nectar carbohydrates through breeding.

MATERIALS AND METHODS

During the Summer of 1984, ten plants each of 236 Lotus accessions (obtained from the Northeast Regional Plant Introduction Station) were transplanted from the greenhouse as 8 inch plants, into a uniform sandy clay loam on the Michigan State University Campus. This planting represented 7 different species of Lotus (See Table 1.1). All accessions had winter hardy ratings of five or above and were from diverse geographical locations. The plants were allowed to mature during 1984 without evaluation for nectar production.

First Selection for Nectar Carbohydrate Production

Of the 236 accessions, 190 were evaluated for nectar carbohydrates during 1985. The remaining accessions did not survive the first winter and therefore could not be used. As the plants approached 1/10 bloom of their first flowering peaks, they were cut back and then allowed to flower again. This was done to both stimulate and synchronize flowering. Because of the large number of plants, they were divided into fourteen groups based on flowering periods. Hereafter, these groups of plants are referred to as cohorts. All plants within a cohort were sampled during one sampling period. Similar climatic conditions (sunny, calm and low relative humidity) existed during the sampling periods for all cohorts.

Three plants from each accession, selected on the basis

of flowering vigor, were sampled for total nectar carbohydrates. Five florets were collected from each plant, the keel petal was removed to insure maximum nectar extraction, and the remainder of the flower was immersed in distilled water. The florets remained in water for sixty minutes to allow the available nectar to diffuse into the solution. After removing the florets, the solution was frozen. Total carbohydrate content was determined by the spectrophotometric method of Roberts (1979).

Environmental influences on carbohydrate production were controlled by comparing total carbohydrate content only within a cohort. Climatic influences were further reduced by always collecting samples between 12:00 and 16:00 (EDST), based on the results of a preliminary study which indicated that carbohydrate content was highest and the most stable at this time.

To select for high carbohydrate production, each accession was rated as a low, moderate or high nectar carbohydrate producer based on the scale in Table 1.2. The top 10% of each cohort (a total of 67 accessions) were selected for further evaluation.

Second Selection

On August 8, 1985 the plants from the first selection, were cut back again to both stimulate and synchronize flowering. Twenty-six of the 67 accessions from the first

Table 1.1. Species included in study.

-
- 1) L. caucasicus
 - 2) L. corniculatus
 - 3) L. decumbens
 - 4) L. denticulatus
 - 5) L. pedunculatus
 - 6) L. strictus
 - 7) L. tenuis
-

Table 1.2. Scale used for rating carbohydrate content of accessions in 1985 and 1986.

Rating	Carbohydrates mg./floret
low	0.01- 0.019
moderate	0.020-0.039
high	0.040-

selection bloomed for a third time. Since the number of flowering peaks per season and carbohydrate production per floret are both important components of nectar production, only the plants that bloomed again were evaluated further. Twenty-five florets from each of the twenty-six accessions were sampled again for total nectar carbohydrates during a single sampling period.

Breeding Program

During the Fall of 1985, three high, two moderate nectar carbohydrate producers and one low nectar carbohydrate producer were selected for breeding purposes from the twenty-six accessions selected for final evaluation. The selected plants were cut back to 4 inches to minimize possible damage from transplanting and to stimulate flowering. The plants were transferred to and grown in 12 inch pots in vermiculite, and received 16 hours of daylight from high pressure sodium lamps. During peak bloom, the plants were hand pollinated without emasculation in a diallel cross. Five crosses were made from each pairing combination. The seed was collected from the crosses and the progeny were grown in the greenhouse under the same conditions as the parents.

Field Evaluation for Stability of Carbohydrate Production

During the Spring of 1986, the six accessions used in the breeding program were evaluated for phenotypic stability of carbohydrate production in the field. Two standard birdsfoot trefoil cultivars, 'Viking' and 'Dawn', were

included in this evaluation. Each plant was separated at the crown into 15 clones. The plants were arranged in a randomized complete block design at three locations: the Michigan State University Campus, at E. Lansing, MI; the Trevor Nichols Experiment Station at Fennville, MI; and the Kellogg Biological Station at Hickory Corners, MI. The plants in the plot at the Trevor Nichols Experimental Station was eaten by a woodchuck and were eliminated from this study. Nectar samples were collected from the remaining locations on each of six sampling dates. Sampling dates were treated as replications.

Analysis of variance was performed to determine if significant differences existed in carbohydrate production between accessions and locations.

RESULTS

A fourteen-fold difference was found in total carbohydrate production between the highest and lowest nectar carbohydrate producer (0.07 to 0.005 mg./floret). The highest nectar producing plant was from Argentina and was erect in growth habit. The lowest nectar producing plant was from Spain and was prostrate. Both of these plants were among those examined for phenotypic stability of carbohydrate production as well as those selected for breeding to increase nectar carbohydrates.

Results from Breeding

From 157 hand pollinations, 18 seed pods developed (with an average of 5 seeds/pod) and from these, 11 matured. Progeny from the 11 were reared under greenhouse conditions but none survived 5 months or reached reproductive maturity.

Phenotypic Stability for Carbohydrate Production

Analysis of variance of the phenotypic stability data showed significant differences among the plants selected for nectar carbohydrates. Location did not have a significant effect on carbohydrate production. Also, there was no significant interaction between locations and accessions.

Significant differences ($P < 0.05$) in total nectar carbohydrates existed between one of the high nectar producers and the standard cultivar 'Dawn', versus all of the other plants evaluated (Table 1.3). A trend toward a

Table 1.3. Average carbohydrate production across locations of plants selected for phenotypic stability and breeding during 1985 and 1986.

Geographic Origin ----- Accession #	Rating for 1985 +	Ave. Carbohydrate (mg./floret) (n=2) (n=8-11) *		Rating for 1986 +
Italy ----- 228233	high	0.050	0.068±0.006a	high
USA/ 'Dawn' ----- G19669		----	0.066±0.007a	high
Canada ----- 472010	high	0.060	0.040±0.009b	high
Argentina ----- 331177	high	0.040	0.046±0.007b	high
Sweden ----- 235101	moderate	0.020	0.029±0.010b	moderate
Spain ----- 246732	moderate	0.020	0.038±0.003b	moderate
Yugoslavia ----- 251423	low	0.010	0.034±0.005b	moderate
USA/ 'Viking' ----- G13511		----	0.033±0.005b	moderate

* Means followed by the same letter are not significantly different at the 0.05 significance level as determined by Duncan's new multiple range test.

+ Ratings were determined by the scale in Table 1.2.

++ Seed for these and all the accessions used in this study were obtained from the Northeast Regional Plant Introduction Station-Geneva, New York.

decrease in carbohydrate production with each successive sampling date was observed. These results imply a relationship between total carbohydrate production and the flowering peak in which samples were taken (Table 1.4). The three high and the two moderate carbohydrate producing plants produced more during the first flowering peak, and less during the second. The low carbohydrate producing plant produced more carbohydrates during the second peak, and the least during the first.

DISCUSSION

With one exception, all of the plants' carbohydrate production were stable for both field locations for 1986 (Table 1.3). The exception was from an accession (#251423) which rated as a low carbohydrate producer in 1985, but rated as a moderate producer in 1986.

Significant differences in nectar volume due to environmental influences in the field have been reported (Teuber & Barnes 1979). However, Walker et. al (1974) found that environmental influences did not significantly affect total carbohydrates in the field and proposed that field screening for carbohydrate production should be effective.

Results from this study indicate that screening for carbohydrate production in the field based on two sampling dates is a feasible way of selecting for high carbohydrate

Table. 1.4. Average carbohydrate production across locations in the first and second flowering peaks for the accessions during 1986,

Accession Number	Flowering Peak		Nectar Production Rating +
	1st (mg.carbohydrate/floret)	2nd	
228233	0.091	0.058	high
'Dawn'	0.090	0.062	standard
331177	0.065	0.041	high
472010	0.066	0.031	high
246732	0.045	0.034	moderate
251423	0.034	0.039	low
'Viking'	0.040	0.031	standard
235101	0.038	0.021	moderate

+ Rating indicates the carbohydrate production of the selected accessions as compared to all of the accessions in the second selection for nectar production in 1985.

production in birdsfoot trefoil. We successfully identified high carbohydrate producers using this approach and recommend it for evaluating large plant populations in the field.

A trend was observed of a decrease in carbohydrates with each successive sampling date, which was consistent for all of the accessions. Therefore, the time of sampling within a flowering season should not greatly influence the success in selecting for nectar carbohydrate production.

AN EVALUATION OF FLORET RECEPTACLE SIZE AS AN INDICATOR OF
CARBOHYDRATE PRODUCTION IN SELECTED SPECIES OF LOTUS

INTRODUCTION

Plant scientists over the last four decades have shown a correlation between flower size and the amount of nectar produced. More nectar was collected from plants that had large flowers than those with small flowers in the genus Brassica (Szabo 1982). Fahn (1949) reported a correlation between the quantity of nectar produced and the volume of nectar producing tissue in 11 species of the tribe Citreae.

Teuber & Barnes (1979a) successfully identified high and low nectar producing alfalfa plants by using the single phenotypic trait, floret receptacle diameter. High nectar producing clones were characterized by having large nectar reservoirs and many stomata. Selection for nectar volume resulted in an increase of both nectar volume and receptacle diameter, whereas selection for receptacle diameter resulted only in an increase of receptacle diameter. Based on these results, they suggest the following procedure when selecting for nectar production: initial screening of populations using receptacle diameter, followed by nectar analysis from the plants with the largest receptacles.

Flower size has been evaluated as a potential indicator of nectar volume in birdsfoot trefoil. Murrell et al. (1979a) found a significant correlation ($r=0.358$, $P<0.05$) between petal area and nectar volume. Although significant,

they concluded that the r-value was too low to show any real promise for using flower size as an indicator of nectar yield.

In both alfalfa and trefoil, the nectaries are located within the receptacle. In birdsfoot trefoil, the nectaries are situated at the base of the hypanthium abaxial to the ovary and secretory stomates are in a band about midway up the nectary (Murrell et al. 1982). The nectary of alfalfa is similarly located at the base of the staminal column on the abaxial side of the ovary (Teuber & Barnes 1979a). Both in trefoil and alfalfa the nectary has been described as an annular toral or discoid nectary (Murrell et al. 1982 and Teuber & Barnes 1979a) respectively.

The objective of this study was to evaluate floret receptacle size as a potential indicator of carbohydrate production in three species of Lotus. The relationship between carbohydrate production and three indices of receptacle size (diameter, height and volume) are examined.

MATERIALS AND METHODS

During the summer of 1986, the relationship between receptacle size and nectar carbohydrate production was examined in 3 species of Lotus (L. corniculatus, L. tenuis & L. caucasicus) during their first and second flowering peaks. Twenty-two to twenty-eight plants from each species, were chosen on the basis of flowering vigor from an established Lotus population planted in 1984 on the Michigan State University Campus, East Lansing MI.

The receptacle diameter and height (Figure 2.1) of 10 florets per plant, each from different positions on the plants, were measured with a hand held gauge. The gauge had 13 size categories for both receptacle diameter and height measurements which ranged from 1.25 to 3.75 mm. for receptacle diameters and 2.275 to 5.0 mm. for receptacle heights (Table 2.1). After recording the measurements, total nectar carbohydrates were extracted from the florets. The keel petals were removed (to insure maximum nectar extraction) and the remainder of the flower was immersed in distilled water. The florets remained in water for 60 minutes to allow the available nectar carbohydrates to diffuse into the solution. After removing the florets, the solution was frozen and then analyzed at a later date by the spectrophotometric method of Roberts (1979).

Table 2.1. Receptacle diameter and height categories as determined from gauge measurements.

Size Category	Receptacle Diameter Range (mm)	Receptacle Height Range (mm)
1	1.25- 1.49	2.275-2.99
2	1.5 - 1.74	3.0 -3.124
3	1.75- 1.99	3.125-3.24
4	2.0 - 2.124	3.25 -3.374
5	2.125-2.24	3.375-3.49
6	2.25 -2.374	3.5 -3.74
7	2.375-2.49	3.75 -3.99
8	2.5 -2.74	4.0 -4.124
9	2.75 -2.99	4.125-4.24
10	3.0 -3.124	4.25 -4.374
11	3.125-3.24	4.375-4.49
12	3.25 -3.49	4.5 -4.74
13	3.5 -3.75	4.75 -5.0

To estimate receptacle volume, the floret receptacle was treated as a cylinder for which volume calculations were made from each set of receptacle diameter and height measurements taken. Receptacle diameter, height and volume were regressed against total carbohydrates to determine which parameter, if any, is a good indicator of carbohydrate production within the three species of Lotus studied.

RESULTS

The size of the floret's receptacle appeared to be associated with total nectar carbohydrates. Fairly high r -values were obtained when any of the three receptacle parameters (diameter, height or volume) were regressed against total carbohydrates (Table 2.2). Receptacle volume was the best or equivalent to the best indicator of total carbohydrates for L. caucasicus ($r=0.526$) and L. tenuis ($r=0.630$). Receptacle height was the best indicator of carbohydrate production for L. corniculatus ($r=0.642$) (Figures 2 to 4).

The r -values obtained from the regressions were higher for the first flowering peak than for the second for all three species.

DISCUSSION

All of the parameters (diameter, height and volume) of the floret receptacle tested were indicators of nectar carbohydrate production in birdsfoot trefoil. These results do not support Murrell et al. (1982) findings that a single easily applied criterion does not exist to screen trefoil populations for nectar production. The discrepancies between the two studies may be due in part to using receptacle size

Table 2.2 Results from linear regressions of nectar carbohydrates on receptacle size parameters in three species of Lotus.

Species	Flowering Peak	Receptacle Indices	R-Value	Intercept	Slope
<u>corniculatus</u>	1	diameter	0.509	0.125	0.0952
<u>corniculatus</u>	2	diameter	0.360	0.029	0.0424
		Ave.	0.435		
<u>corniculatus</u>	1	height	0.772	0.896	0.298
<u>corniculatus</u>	2	height	0.512	0.274	0.100
		Ave.	0.642		
<u>corniculatus</u>	1	volume	0.537	-0.041	0.010
<u>corniculatus</u>	2	volume	0.424	0.003	0.005
		Ave.	0.481		
<u>tenuis</u>	1	diameter	0.692	0.042	0.045
<u>tenuis</u>	2	diameter	0.568	0.063	0.054
		Ave.	0.630		
<u>tenuis</u>	1	height	0.441	0.042	0.021
<u>tenuis</u>	2	height	0.360	0.146	0.053
		Ave.	0.400		
<u>tenuis</u>	1	volume	0.659	-0.005	0.004
<u>tenuis</u>	2	volume	0.601	-0.017	0.007
		Ave.	0.630		
<u>caucasicus</u>	1	diameter	0.634	0.147	0.103
<u>caucasicus</u>	2	diameter	0.345	0.040	0.041
		Ave.	0.489		
<u>caucasicus</u>	1	height	0.566	0.430	0.145
<u>caucasicus</u>	2	height	0.448	0.176	0.060
		Ave.	0.507		
<u>caucasicus</u>	1	volume	0.675	-0.043	0.010
<u>caucasicus</u>	2	volume	0.377	-0.003	0.004
		Ave.	0.526		

as an indicator of carbohydrate potential as opposed to petal area. Furthermore, considerably more plant material (62 accessions) were evaluated in this study as compared to the 8 varieties examined by Murrell et al.

Although higher r -values than obtained in this study would have been desirable, the apparent rarity of a floret with a large receptacle and low carbohydrate production is of value when screening plant populations for high nectar production. Only florets with large receptacles would need to be evaluated with carbohydrate analysis, thereby greatly reducing the amount of time spent on screening plants of little or no value. These results are similar to the findings of Teuber et al (1983) that initial screening of alfalfa populations using visual estimates of receptacle size, followed by actual nectar analysis of plants with the largest receptacles, is an effective and rapid method of screening for high nectar production.

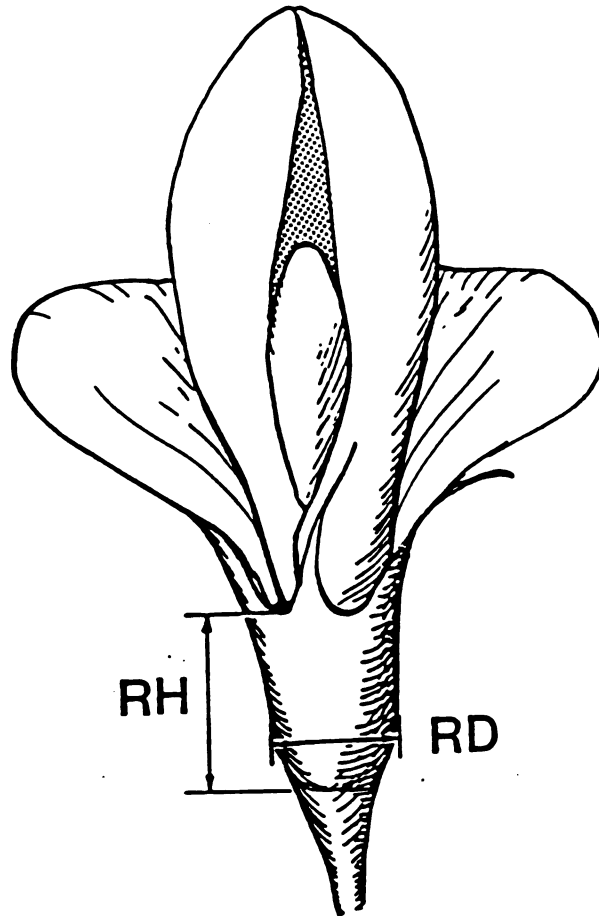


Figure 2.1. Generalized diagram of a Lotus flower showing the receptacle's diameter and height.

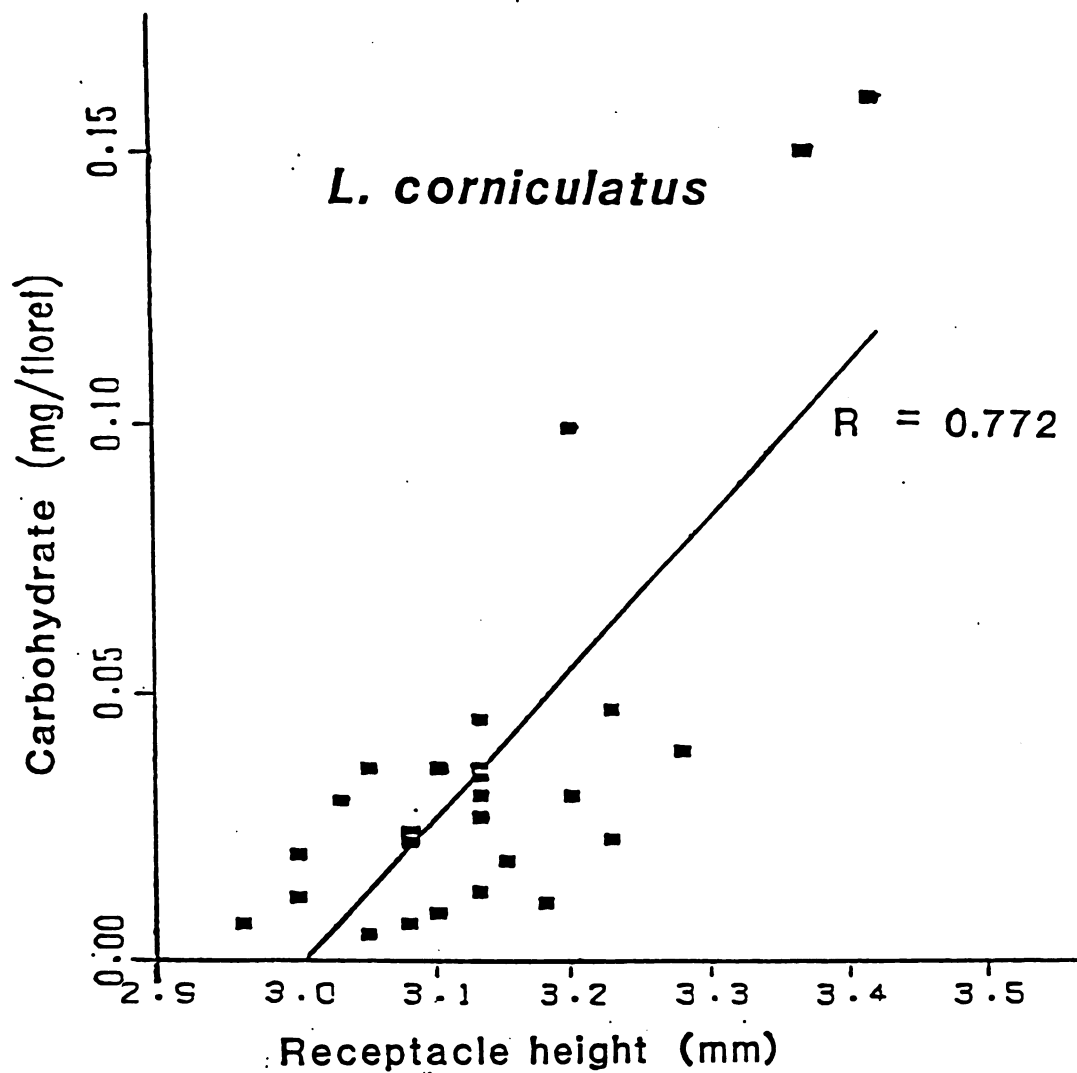


Figure 2.2. Linear regression of total nectar carbohydrates on receptacle height in the first flowering peak of *L. corniculatus*.

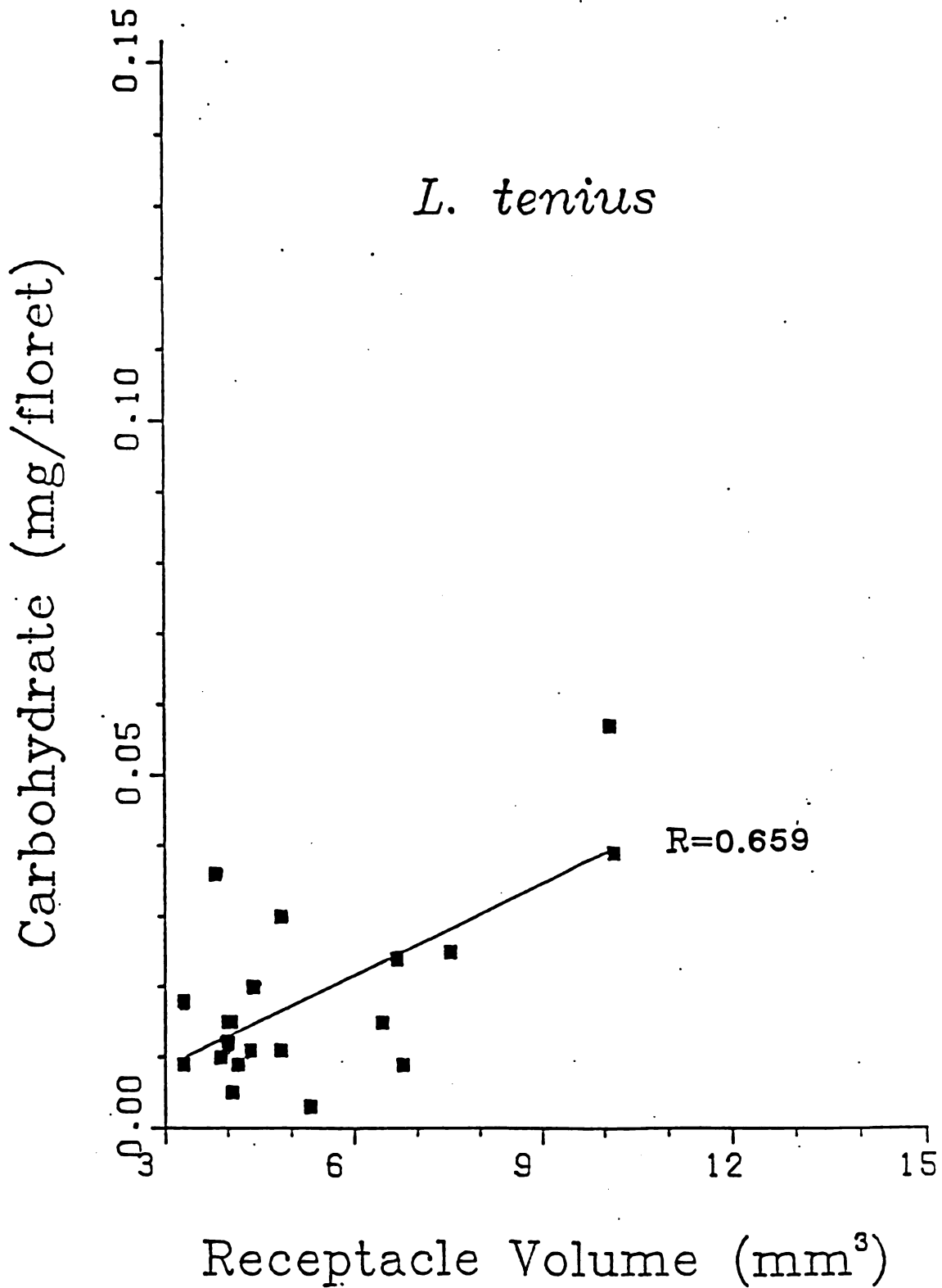


Figure 2.3. Linear regression of total nectar carbohydrates on receptacle volume in the first flowering peak of *L. tenuis*.

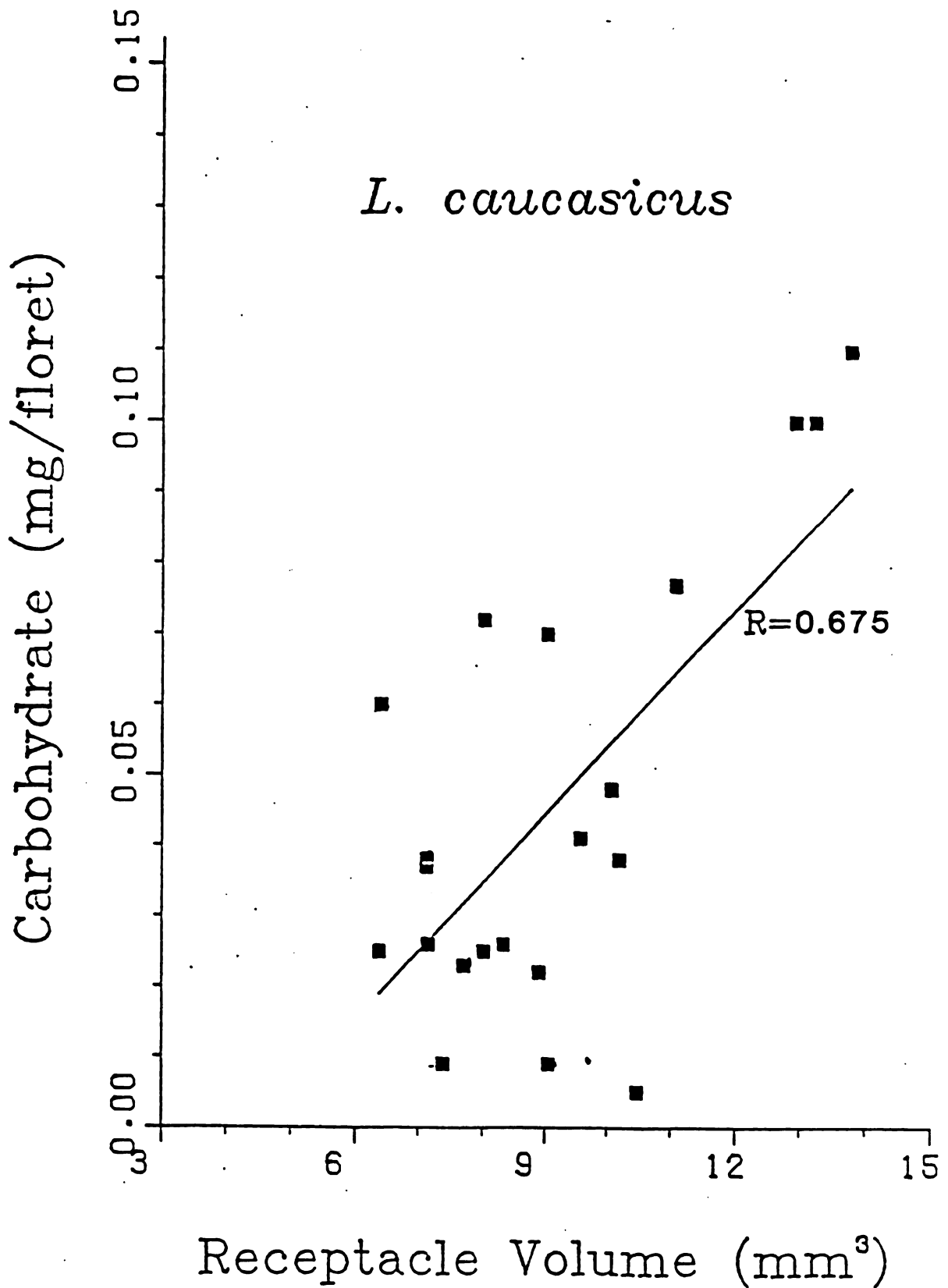


Figure 2.4. Linear regression of total nectar carbohydrates on receptacle volume in the first flowering peak of *L. caucasicus*.

THE EFFECTS OF HONEYBEE VISITATION ON THREE CARBOHYDRATE
PRODUCTION TYPES OF BIRDSFOOT TREFOIL

INTRODUCTION

Murrell (1980) reported that nectar production plays a major role in cultivar attractiveness and potential seed yields in birdsfoot trefoil (Lotus corniculatus). Several studies have indicated that floret age is an important factor in nectar production; in Asclepias, Southwick & Southwick 1983; and Willson et al. (1979) found that nectar concentration, volume and amount of sugar varied with age.

Pollination is another important factor in nectar production. In certain species of plants, pollination is known to terminate nectar production (Pankiw & Bolton 1965; Collison 1973) while in other species, nectar is actively secreted after pollination (De Grandi-Hoffman 1980; Wilson et al. 1979 and Fahn 1949). Several researchers have examined the effects of repeated sampling on nectar production in which nectar was removed from florets but where pollinations probably did not occur (Southwick 1983; Willson et al. 1979; Raw 1953 and Fahn 1949). Willson et al. (1979) found that nectar production in Asclepias was stimulated by repeated sampling. Raw (1953) obtained similar results for nectar production in raspberries that were repeatedly sampled. In contrast, Plowright (1979) found that total lifetime sugar production in Clintonia flowers was not significantly affected by daily nectar removals.

Cruden & Hermann (1983) suggest that a complete

evaluation of a plant's nectar production includes evaluating daily patterns of secretion, the volume of nectar secreted and an analysis of its sugars and other constituents. Several aspects of nectar production in birdsfoot trefoil have been examined. Nectar volume and its influence in cultivar attractiveness and potential seed yields was studied by (De Grandi-Hoffman 1980). The inheritance of nectar production was examined by (Murrell et al 1982).

Multiple bee visits have been observed for birdsfoot trefoil (De Grandi-Hoffman 1982) indicating that nectar secretion and possibly production continue after pollination.

MATERIALS & METHODS

The nectar secretion cycles of six birdsfoot trefoil accessions were examined during late August and early September of 1986. The accessions were selected from a third year Lotus planting located on the Michigan State University campus. Two accessions were chosen from each of three previously selected groups: high, moderate and low nectar producers (Chapter I). The cultivar 'Viking' was also included in this study for comparison.

Nectar samples were taken from three categories of florets: 1) florets that were always excluded from pollinators 2) florets that were always exposed to pollinators and 3) florets that were exposed to pollinators except during the 24 hours prior to sampling. These categories will be referred to hereafter respectively as 'always covered', 'always open' and 'covered 24 hours'.

Florets in the 'always covered' and 'covered 24 hours' categories were covered with fiberglass screening (18 x 16 mesh) to exclude pollinators and nectar robbers. A frame under the screening provided support and allowed air circulation throughout the planting. Approximately 300 buds were tagged in the "balloon stage" for all accessions. Nectar samples from 'Viking' were taken from 'always covered' and 'always open' florets on the day of anthesis and

thereafter from all categories for the rest of the floret's life (3 days). Nectar samples from the high, moderate and low carbohydrate production types, were collected as described above, except, the number of collections was reduced to two, due to lack of florets. For each accession, 20 to 40 florets per day were sampled in each category. Florets were collected from each plant, the keel petal removed to insure maximum nectar extraction and the remainder of the floret was immersed in distilled water. The florets remained in the water for sixty minutes to allow available nectar to diffuse into the solution. After removing the florets, the solution was frozen. Total carbohydrates were determined by the spectrophotometric method developed by Roberts (1979).

A bee hive was placed approximately 500 ft. from the planting to insure adequate bee visitation. Bee visits were observed daily for 5 florets in the category, 'always open' for the 3 carbohydrate production types and 'Viking'. Nectar was then immediately extracted and analyzed from these florets. The data obtained from these analyses were used to determine whether 'always open' florets were foraged sufficiently to serve as a basis for estimating daily nectar carbohydrate productions. Because the carbohydrate levels of 'always open' florets and 'always open just visited' florets were similar, the carbohydrate content of 'always open' florets is a good indicant of the amount of nectar that is

left behind by a foraging population. This value served as the base carbohydrate level for computing net carbohydrates produced over a 24 hour period for previously visited florets. This production figure was calculated by subtracting the base value for a given day from the carbohydrate level on the following day in 'covered 24 hr' florets of the same age and production type. Similar 24 hr production values were computed for florets that were not visited by bees, 'always covered'. This production figure was calculated by subtracting the carbohydrate level of 'always covered' florets from similar values of the same production type on the following day. Daily carbohydrate production values were summed over time to give cumulative productions.

Data, where appropriate, were subjected to analysis of variance followed by Duncan's multiple range test.

RESULTS

The most commonly seen insects visiting the plantings were honeybees (Apis mellifera). Cabbage butterflies (Pieris rapae L.) and bumblebees (Bombus spp.) were also observed occasionally on the flowers.

'Viking' flowers, that were open to pollinators on each of four days, lasted 4 days and produced nectar throughout that period. This conclusion is based on the results from nectar sampling (Table 3.1), as well as observations of bee visits throughout this time period.

The average amount of carbohydrates for 'always open' florets was relatively consistent for all three carbohydrate production types and for 'Viking' over time. These results indicate that a certain amount of nectar is produced by a floret that is inaccessible to bees and remains in the florets after a bee visitation. No significant differences in carbohydrate content were found between florets in which bee visits were observed and those in which bee visitations were assumed. These results validated the assumption that florets open to pollinators received sufficient bee visitations to lower nectar carbohydrates to levels similar to those found in florets in which bee visits were observed (Table 3.2).

No significant differences in daily carbohydrate content

Table 3.1. Daily net and total carbohydrate production for low, moderate and high carbohydrate producing plants.

Day	Always Covered				Total	Covered 24 hours				Total
	1	2	3	4		Net	1	2	3	
Low	0.019	0.041	0.012	-----	0.072	0.019	0.049	0.048	-----	0.116
Med	0.030	0.053	0.008	-----	0.091	0.034	0.050	0.056	-----	0.140
High	0.069	0.027	0.018	-----	0.114	0.069	0.080	0.091	-----	0.240
Vik +	0.025	0.073	0.013	0.052	0.163	0.025	0.065	0.093	0.069	0.252

+ Vik = Viking

* Blank values result from insufficient flowers to continue study for four days.

Table 3.2 Daily average levels of carbohydrate (mg/floret) for 'always open' florets and for florets' in which bee visits were observed immediately prior to sampling.

Day	'always open' (mg. carbohydrate) /floret	observed
1	0.0113±0.0009	0.0120±0.0008
2	0.0150±0.0007	0.0182±0.0017
3	0.0197±0.0013	0.0205±0.0005

There were no significant differences in carbohydrate content for florets in which bee visitations were observed and those in which bee visitations were assumed at the ($P < 0.05$) significance level as determined by an one-way analysis of variance.

were found between florets 'always covered' and 'covered 24 hours' (Table 3.3). However, differences in daily, net carbohydrate production occurred between the two groups (Table 3.1). For florets 'always covered', net carbohydrate production increased from day 1 to 2 and then decreased on day 3 for low and moderate nectar producers. High nectar producers' net carbohydrates steadily increased over a 3 day period. For florets 'covered 24 hours', net carbohydrate production increased over three days for the three production types. Also, there was a 1.6 to 2-fold difference in total carbohydrate production between 'covered 24 hours' and 'always covered' florets for a three day period.

For 'Viking', net carbohydrate production for 'always covered' florets, increased over 4 days. In contrast, the net carbohydrate production for 'covered 24 hours' florets, increased over the first 3 days and decreased on the fourth.

Similar results were obtained for total carbohydrate production for 'Viking' as for the three production types, where there was 1.6 difference in total carbohydrate production between 'covered 24 hours' and 'always covered' florets for a 4 day period.

Table 3.3. Average carbohydrate content for florets in designated categories over three days.

Day	Sampling Category	Ave.Carbohydrates (mg./floret) *
3	covered 24 hours	0.0898 $\pm$ 0.0050 a
3	always covered	0.0878 $\pm$ 0.0051 a
2	always covered	0.0795 $\pm$ 0.0038 b
2	covered 24 hours	0.0742 $\pm$ 0.0029 b
1	always covered	0.0358 $\pm$ 0.0037 c
3	always open	0.0201 $\pm$ 0.0010 d
2	always open	0.0158 $\pm$ 0.0006 d
1	always open	0.0119 $\pm$ 0.0006 d

* Means followed by the same letter are not significantly different at the ($P < 0.05$) significance level as determined by Duncan's new multiple range test.

DISCUSSION

The results of this study indicate that nectar removal with probable pollination, stimulates nectar production in birdsfoot trefoil. These results are similar to the findings of (Raw 1953 & Willson et al. 1979) for milkweed and raspberries, respectively in that nectar production was stimulated by nectar removal. However, the patterns of carbohydrate production found in this study were different from those found by these researchers in that they did not find the well defined pattern of increasing net nectar production with time as was found in this study. These differences are most likely due to the different techniques used for nectar extraction and the different plant species examined. Willson et al. (1979) and Fahn (1949) found that the act of extracting nectar may in itself injure nectiferous tissue and reduce nectar production. This may account at least in part for these differences. In this study, the bees were allowed to remove the nectar, therefore the potential of damaging nectar producing tissue was reduced.

Even though daily, carbohydrate content was the same for 'always covered' and 'covered 24 hours', daily, net carbohydrate production differed between these two groups. Several hypotheses explain these results. Lotus may continue producing carbohydrates which is reabsorbed when it reaches a

certain level if it has not been removed in some manner. Carbohydrate production is related to a floret receptacle size (Chapter II & Teuber et al 1983), suggesting that this reabsorption of nectar may occur when the receptacle reservoir has reached its holding capacity. When nectar removal does not occur, the florets may reabsorb the sugar and channel it into other physiological processes. Willson & Bertin (1979) found that reabsorption occurred in A. syrica florets that were not repeatedly sampled. This study suggests that when nectar removal occurs, the Lotus floret is stimulated to replace the nectar; hence, the total carbohydrate production of repeatedly sampled florets surpasses that of florets pollinated only once. Wilson et al. (1979) found that florets that were repeatedly sampled replaced the amount of carbohydrates removed. An alternative hypothesis that may explain this phenomenon is that carbohydrate production in a Lotus flower may simply turn off when it reaches a certain level.

The findings that net carbohydrate production for 'Viking' increased over three days and then decreased on the fourth, may indicate that physiologically, the plant has recognized the occurrence of some change such as fertilization that was initiated with pollination. Shuel (1984) suggested that fertilization seems to activate a feedback mechanism which switches off nectar secretion. Miller (1969) noted that some crosses between trefoil clones

set very few seeds due to pollen incompatibility and more than one bee visit was sometimes required. Also, Bubar (1958) found that ovary development may vary considerably for the length of time it remains fertile. Fertilization usually occurs 24 to 48 hours after pollination (Giles 1949). The occurrence of nectar replenishment over several days may to maintain floret attractiveness to pollinators until the plant has recognized the occurrence of ovule fertilization.

Patterns of carbohydrate production were consistent for all three production types, however the amount of carbohydrates produced differed. This similarity probably indicates that the pollination strategies of these three groups are also similar. The pattern of carbohydrate production for days 1-3 exhibited by the 3 carbohydrate production types is likewise exhibited by 'Viking'. It is probable therefore that the 3 carbohydrate production types would have experienced a decrease in net carbohydrate production on the fourth day, similar to that of 'Viking' had sufficient flowers been available for a fourth day of sampling in these plants.

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