SOME FACTORS AFFECTING NECTAR SECRETION IN RED CLOVER

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(WITH THREE FIGURES)

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Introduction

In many cross-pollinated crop plants, pollination is dependent upon bee activity which is influenced in part by nectar availability and quality. If some means could be found to increase the amount of nectar secreted by the floral nectaries of these plants, beekeepers would be encouraged to keep more colonies of bees in the vicinity of the crops, and pollination and fruit set would be improved. The problem of nectar secretion, therefore, is of more than academic interest to the agronomist.

The first comprehensive work published on nectar secretion was that of BONNIER (3) who made physiological, anatomical, and microchemical studies in a large number of plant species. Although considerable work has been done since Bonnier's time, no essentially new lines of investigation have been followed. Studies have been made of the influence on nectar secretion of temperature (5, 9, 11), rainfall (3, 10), soil water content (2), light (9), latitude and altitude (3), soil type (8), and atmospheric humidity (3, 12, 14, 15, 19, 20). Unfortunately, much of this work has been done with small samples and without adequate experimental control. As a result, much disagreement exists in the literature.

Excellent anatomical studies of the passage of nectar from the nectary cells to the exterior have been made by DAUMANN (4) and RADTKE (13). Considerable interspecies variation in the visible mechanics of the process was noted.

Little is known about the basic mechanism of nectar secretion. Two general theories are extant: 1. That sugar secretion and water secretion are distinct phases, water secretion being analogous to guttation and dependent on root pressure (18). 2. That secretion occurs as one phase, nectar being secreted in its final form as the result of a specific cellular activity (2, 13). BONNIER (3) has presented evidence for a positive relationship between secretion and root pressure. RADTKE (13), on the other hand, has shown that secretion can occur in the absence of root pressure in isolated flowers floated on sucrose solutions.

Recently ARENS (1) has advanced an hypothesis in which secretion, absorption, and transport of solutes are treated as closely related phenomena.
dependent on electro-osmotic currents at the surface of the tonoplast. The energy necessary for the maintenance of oxidation-reduction potentials across the membrane is assumed to be supplied by respiration.

The present work represents the initial part of an investigation of the influence of several factors on nectar secretion in red clover (*Trifolium pratense* L.). It concerns the effects of temperature, solar radiation, atmospheric humidity, and genotype.

**Materials and methods**

All red clover plants used in the study were of the Kenland variety and were cultured in the greenhouse. Samples for nectar assay were taken from three sources: transplants to the greenhouse from the field in October or November, seedlings, and plants propagated asexually. Plants were maintained in 10-inch or 12-inch pots in a fertile garden soil. All plants used in a specific experiment were given pre-treatment conditions of illumination, temperature, fertility, and available water, as similar as possible. Transplants collected in mid-November and seedlings started at the same time were in bloom by the middle of February. After January 10, the natural photoperiod was extended to 14 hours with illumination from General Electric projector flood lamps spaced at intervals of 28 inches. From a height of three feet above the plants they provided illumination of 250 to 300 foot-candles at the level of the plant tops.

The Gubin centrifuge method, as modified for direct volumetric measurements (17), was used to take yield measurements. Inflorescences were inverted in a special centrifuge flask and centrifuged for six minutes at an average force of approximately 470 gravity, the centrifugate being collected in a calibrated capillary well. The outer 4 to 6 mm. portion of the florets was clipped off prior to centrifugation. A binocular microscope (7×) equipped with an ocular scale was used to measure the height of the nectar in the capillary well. The refractive index of the nectar was measured with an Abbe refractometer, and from this the percentage of total sugar (chiefly sucrose, glucose, and fructose) was calculated by referring to sucrose tables. The total error of volumetric measurements was of the order of ± 3%. The precision of the method was earlier studied in detail by Swanson and Shuel (17).

**Basis for calculation of nectar yields**

Because of the wide variation existing in the number of florets per inflorescence, it was necessary to find some basis for comparing nectar yields other than that of yield per head. A weight basis was therefore adopted. Weight of the inflorescence was found to be closely correlated with floret number in red clover growing in the greenhouse (17). The validity of the weight basis was further tested by calculating the regression of nectar yield per unit weight of inflorescence on weight of inflorescence. In a sample of 100 heads the regression coefficient was nonsignificant, indicating that yield per unit of head mass was independent of head size.
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Results

The influence of night temperature on nectar secretion

Wide deviations between day and night temperatures have been thought to be more favorable for rapid nectar secretion than relatively constant temperatures (10, 11). This belief has had its basis in hive records rather than in data from controlled experiments. In order to test directly the effects of various night temperatures on nectar secretion in red clover, the following treatments were assigned to four groups of plants chosen at random: Group I with night temperatures of 78 ± 1° F, Group II with night temperatures of 70 ± 1° F, Group III with night temperatures of 60 ± 1° F, Group IV with night temperatures of 50 ± 1° F. The temperature range of 50 to 78° F was chosen because it encompasses the night temperatures usually encountered in the field during the season when red clover is in bloom. All groups of plants were kept under photoperiods of 14 hours (8 A.M. to 10 P.M.), the last four or five hours of which consisted of artificial illumination of 250 to 300 foot-candles. Groups II and III, which had to be moved some distance to special controlled-temperature rooms, were kept on trucks. Group IV was moved each night to a force-ventilated dark cabinet (4 x 4 x 4 ft.). The 78° F room was equipped with a 1300-watt thermostatically operated fan-forced heater. In the 60° F room no such provision was necessary, as the ambient temperature was always higher than 60° F. Plants of Group I were kept on a greenhouse bench where thermostatting gave excellent control of night temperature at 70° F. No control was attempted over day temperatures, which fluctuated, with daily illumination, between 65 and 85° F.

In the period between February 8, and March 14, 1950, nectar was extracted on each of 24 days on which samples commensurate with the size of each plant group could be obtained. The first samples were taken on the morning after the first night under controlled temperatures. Data were analyzed statistically by calculating means and variances of nectar volume-yield, nectar sugar weight-yield, and nectar sugar concentration for each group of plants. Groups were compared by the t test. The experimental results are presented in table I.

The lack of significance in differences between mean nectar volume yields and mean nectar-sugar weight yields indicates that, if real differences did exist, they were too small to be detected with the technique and sample size used. Mean differences in nectar sugar concentration were of statistical significance. Because of an important effect of atmospheric vapor pressure on post-secretion changes in nectar concentration, which will be discussed in a later section, too much importance should not be attached to the concentration data. No provision was possible for keeping the plants under conditions of constant humidity; and it was evident, from an examination of hygrothermograph records, that vapor pressure varied from station to station. Differences in nectar concentration may be attributable, at least in part, to vapor pressure differentials. Weight yields, derived from the prod-
TABLE I
NECTAR PRODUCTION IN RED CLOVER AT DIFFERENT NIGHT TEMPERATURES.

<table>
<thead>
<tr>
<th>Group</th>
<th>Night temperature</th>
<th>Number in sample</th>
<th>Mean nectar volume µL/g. in-</th>
<th>Mean sugar yield mg/g. in-</th>
<th>Mean sugar concentration Wt. % sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>florescence</td>
<td>florescence</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>78° F</td>
<td>65</td>
<td>17.1 ± 0.9†</td>
<td>16.1 ± 1.0</td>
<td>69.4 ± 0.4</td>
</tr>
<tr>
<td>II</td>
<td>70° F</td>
<td>28</td>
<td>18.3 ± 1.6</td>
<td>17.1 ± 1.6</td>
<td>67.7 ± 0.6</td>
</tr>
<tr>
<td>III</td>
<td>60° F</td>
<td>54</td>
<td>15.8 ± 1.1</td>
<td>14.3 ± 1.0</td>
<td>68.6 ± 0.4</td>
</tr>
<tr>
<td>IV</td>
<td>50° F</td>
<td>33</td>
<td>15.1 ± 1.2</td>
<td>13.5 ± 1.2</td>
<td>66.5 ± 0.5</td>
</tr>
</tbody>
</table>

Significant differences between means
None None

*Significant at 5% level.
**Significant at 1% level.
†Standard error of a mean.

In a subsequent experiment, the influence of night temperature in the range 60 to 70° F was further investigated. Plants were kept under fluorescent illumination, without supplemental Mazda lighting, of about 1000 foot-candles at the plant tops, throughout the course of the experiment. Both day and night temperatures were controlled. Twenty-four red clover plants were paired on the basis of size and age, and one member of each pair was assigned at random to one of two rooms. The night temperature in one room was kept at 70 ± 1.0° F, the night temperature in the other room at 60 ± 1.5° F. During the 13-hour photoperiod both rooms were thermostatted for 77° F. Operation of the refrigeration compressors was regulated by Minneapolis-Honeywell Chronotherms. Only the cooling phase of temperature control was automatic; there was no heating element for raising the temperature rapidly at the beginning of the photoperiod. Temperature elevation was dependent upon the heat emitted by the fluorescent lamps, and consequently room temperatures did not reach 77° F until several hours after the beginning of the photoperiod. A slightly longer time was required for the attainment of this temperature in the room with the 60° F night temperature. Except for this temperature differential of about two degrees during the first four hours, temperature conditions were similar in the two rooms during the photoperiod. The experiment was begun on April 22. Samples for nectar assay were collected on April 24 and daily thereafter through May 5, with the exception of May 4. The low vigor of the plants at this time rendered further attempts at sampling useless.
Yield values, expressed as mg. sugar per unit weight of inflorescence, were analyzed by variance analysis. Total variance was divided into several components, each assignable to a specific source of variation. Variance due to days included the effects of daily fluctuations in environment and physiological changes in the plants due to low light intensity. Table II contains the results of the analysis of variance.

**TABLE II**

ANALYSIS OF VARIANCE IN NECTAR YIELD (MG. SUGAR/G. INFLORESCENCE) IN RED CLOVER KEPT AT NIGHT TEMPERATURES OF 60° AND 70° F. UNDER ARTIFICIAL ILLUMINATION.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>10</td>
<td>2608.7</td>
<td>260.7</td>
<td>2.9*</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>40.1</td>
<td>40.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Days x temperature</td>
<td>10</td>
<td>215.4</td>
<td>21.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Sampling error</td>
<td>110</td>
<td>10029.7</td>
<td>91.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 1% level.

As in the previous experiment, mean yields of nectar sugar in plants kept at night temperatures of 60 and 70° F did not differ significantly. The significant variation in daily yields can be attributed chiefly to the progressive decline in plant vigor under limiting light intensity. This decline was reflected in inflorescence size; the average inflorescence weight decreased from 970 mg. on the first day to 500 mg. on the twelfth day. In neither of the two temperature experiments was there evidence of a favorable effect on nectar secretion of wide diurnal temperature variation.

**THE INFLUENCE OF SOLAR RADIATION**

As nectar contains the products of photosynthesis, nectar production must ultimately be dependent upon illumination. How direct the association is between secretion and illumination is not known. The present section relates to an attempt to measure the relationship between the quantity of sugar secreted as nectar in red clover blossoms and the amount of solar radiation reaching the plant.

A continuous record of sunlight was obtained with a Leeds and Northrup Micromax connected to an Eppley pyrheliometer. The pyrheliometer was mounted immediately under the glass roof above the greenhouse bench on which the plants were disposed. Thirty-six samples, collected daily from 23 plants and comprising a total of 320 inflorescences, were assayed for nectar. The values obtained for daily mean yields and sugar concentrations of nectar were correlated with daily records of solar radiation. The latter values, which were the integration of intensity and duration of illumination, were obtained by measuring the area under the Micromax curve.
with a planimeter and converting the measurement to gram calorie hours. A portion of the time-series curve for daily mean weight yields and daily amounts of sunlight recorded is shown in figure 1. Table III contains the correlation coefficients for yield and illumination and for concentration and illumination.

The amount of nectar sugar in the inflorescence on a particular day is apparently related to the photosynthetic activity of the plant during the previous day. The coefficient \( r \) for this correlation is 0.424, a value which is highly significant statistically, although not so high as might be expected in view of the fact that all nectar sugar is derived from photosynthesis.

![Graph](https://example.com/graph.png)

**Fig. 1.** Time-series curve in illustration of the relationship between the weight of sugar excreted in nectar and the total solar radiation incident upon the red clover plant expressed as gram calorie hours.

The explanation for the failure to find a higher value of \( r \) may lie in one or more of the following considerations: 1. Weight of nectar sugar collected on a particular day was a function of solar radiation on the second day previous to harvesting, as well as the day previous. This relationship is indicated by the significant correlation coefficient between weight of nectar sugar and solar radiation during the previous two days. 2. Factors other than solar radiation affect the amount of sugar excreted in nectar. The wide deviation of nectar sugar values from a linear relationship with irradiation on the previous day may be due to the regression of sugar weight on these factors. 3. It is possible that nectar secretion takes place only after
a threshold level of sugar is reached in the plant. The regression of weight of nectar; sugar on illumination would in that case be non-linear over the lower part of the irradiation range.

The value of $r$ for illumination and volume yield was of a slightly lower order than the value for illumination and sugar weight yield, and there was no apparent association between illumination and nectar concentration, i.e., the sugar–water mole fractions.

**THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON NECTAR CONCENTRATION AND VOLUME**

Lacking exact knowledge of the physical condition of nectar as it passes through the membrane of the nectary epidermal cells, one can postulate two ways in which atmospheric humidity might influence the water concentration of nectar: 1. Indirectly, through its effect on transpiration and cell turgidity; the relative amounts of water and sugar in nectar during secretion may be influenced by the hydrostatic system of the plant. 2. Directly, through hygroscopic changes in the nectar after secretion.

Inverse correlations between nectar sugar content and relative humidity have been found by several investigators (12, 14, 15, 19). Effects of temperature and water vapor have not always been separated. Relative humidity can have no effect on any process per se, but only through the action of one or both of its components, temperature and water vapor concentration. It is with the effect of the water vapor component that the present section is connected. To measure that effect, temperature must be controlled.

A hygrothermograph record of relative humidity in the greenhouse section housing the clover plants was kept during the month of April, 1950. At night the temperature was maintained at 70 ± 1° F. Fluctuations in the record were due, then, to changes in vapor pressure. The mean vapor pressure for the period 8 p.m. to 8 a.m. of each night was correlated with the mean nectar concentration and volume yield in flowers collected for nectar.
assay in the morning between 8 A.M. and 9 A.M. Samples were taken on each of 26 days and comprised a total of 260 inflorescences.

The coefficient of correlation $r$ with vapor pressure was $-0.83$ for sugar concentration and $+0.54$ for nectar volume. For the size of sample used, 26, any value greater than ± 0.49 is highly significant. The variation of nectar sugar concentration and volume with vapor pressure is illustrated in figure 2. It appears, from the high value of $r$ for vapor pressure and nectar concentration, that atmospheric vapor pressure is a major factor in determining the percentage of water in the nectar at the time of its extraction. At this time the vapor pressure of the nectar has reached, or is approaching, an equilibrium with the vapor pressure of the surrounding atmosphere. The lower value of $r$ for volume was due, presumably, to the lower degree of precision of volume measurements.

The data contain no evidence as to whether the influence of vapor pressure is only a simple hygroscopic one or is exerted in part through the medium of the plant. Post-secretion changes in nectar concentration were next investigated in excised inflorescences. Red clover heads were split lengthwise and the two halves were placed in atmospheres of different vapor pressure. One member of each pair was kept in a humidity chamber over water, the other over a sulphuric acid–water mixture. Both groups were kept at a temperature of 52° F. Vapor pressures in the two chambers were 9.9 mm. Hg and 4.4 mm. Hg, respectively. At the end of eight hours the
half-heads were centrifuged and the refractive indices of the nectar read. The mean nectar sugar concentration was 10.8% lower in half-heads maintained under a vapor pressure of 9.9 mm. Hg than in those under a vapor pressure of 4.4 mm. Hg, as shown in table IV. The difference between means was highly significant statistically.

**TABLE IV**

**SUGAR CONCENTRATIONS OF NECTAR IN HALF-HEADS OF RED CLOVER KEPT AT DIFFERENT VAPOR PRESSURES FOR EIGHT HOURS.**

<table>
<thead>
<tr>
<th>Pair number</th>
<th>A (4.4 mm. Hg)</th>
<th>B (9.9 mm. Hg)</th>
<th>A-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62.5</td>
<td>51.7</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>65.2</td>
<td>54.2</td>
<td>11.0</td>
</tr>
<tr>
<td>3</td>
<td>67.6</td>
<td>53.3</td>
<td>14.3</td>
</tr>
<tr>
<td>4</td>
<td>69.9</td>
<td>57.0</td>
<td>12.9</td>
</tr>
<tr>
<td>5</td>
<td>69.4</td>
<td>56.4</td>
<td>13.0</td>
</tr>
<tr>
<td>6</td>
<td>70.1</td>
<td>61.5</td>
<td>8.6</td>
</tr>
<tr>
<td>7</td>
<td>67.8</td>
<td>57.2</td>
<td>10.6</td>
</tr>
<tr>
<td>8</td>
<td>67.0</td>
<td>56.2</td>
<td>10.8</td>
</tr>
<tr>
<td>9</td>
<td>68.5</td>
<td>58.4</td>
<td>10.1</td>
</tr>
<tr>
<td>10</td>
<td>62.4</td>
<td>55.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Mean</td>
<td>67.0</td>
<td>56.2</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Value of t**

15.4

**Minimal value of t for significance at 1% level**

3.3

**Rate of approach to equilibrium between nectar and the surrounding atmosphere**

Rate of approach to equilibrium was next studied. In order to ascertain the importance of hygroscopic changes in the attainment of equilibrium, the study was conducted in both attached inflorescences in the greenhouse and excised inflorescences in controlled-humidity chambers. Theoretically, the establishment of equilibrium in attached inflorescences could proceed via both hygroscopic and secretory routes; in isolated flowers, the possible influence of the hydrostatic system of the plant is removed.

Atmospheric vapor pressures were calculated from greenhouse hygrothermograph records for a four-day period when vapor pressure changed gradually from 15.2 mm. Hg to 8.2 mm. Hg. Vapor pressures over specific sulphuric acid–water mixtures in humidity chambers were known for any given temperature. Direct vapor pressure measurements were not readily obtainable for nectar and so estimated values of theoretical vapor pressure, based on chemical analyses, were used instead. To estimate the theoretical vapor pressure, both the total sugar content and the molal ratio of mono-
saccharides to disaccharides must be known. Weight percentages of total sugars were obtained from refractive indices; weight percentages of reducing sugars were determined by the SOMOGYI method (18). From these data molal ratios of invert sugar to sucrose were calculated.

Representative samples of nectar were taken from both freshly harvested inflorescences and inflorescences stored for 20 hours under a vapor pressure of 7.6 mm. Hg and a temperature of 50° F. Under these conditions deterioration of stored blossoms was not serious.

In duplicate analyses on two composite samples from each source the following molal ratios of invert sugar to sucrose were found: Nectar from freshly harvested heads—2.5 : 1, 2.0 : 1; nectar from stored heads—1.7 : 1, 1.3 : 1. For subsequent calculations of vapor pressure of nectar from freshly harvested heads, the mean molal ratio of 2.25 : 1 was used; for nectar from stored heads, the mean ratio of 1.5 : 1.

Theoretical vapor pressures of nectar from freshly harvested inflorescences were calculated from mean daily sugar concentration values. Excised heads were stored at a vapor pressure of 3.9 mm. Hg and a temperature of 50° F for 12 hours prior to taking the initial concentration readings. This was done to ensure that the initial vapor pressure of the nectar would differ appreciably from that of the atmosphere in which it was to be placed for the experiment. All excised heads were then split lengthwise and one member of each pair of half-heads was centrifuged and assayed for nectar concentration. From the mean concentration value found at this time the initial vapor pressure of the nectar was calculated. The remaining half-heads were transferred to an atmosphere of vapor pressure 7.7 mm. Hg and temperature 50° F. Centrifuging of these half-heads was done in duplicate at two-hour intervals until the refractive indices of the extracted nectar had reached an apparently constant value, and the theoretical vapor pressure of the nectar at this value was calculated.

For the calculation of vapor pressures, vapor pressure depression curves were constructed from data in the INTERNATIONAL CRITICAL TABLES (7). Data for mixtures of sugars were not available, however, and data for glucose were available only for solutions of molality less than one, in which range the relative vapor pressure depression curve for glucose parallels that of sucrose but is about 12% lower. Any values for vapor pressure of nectar taken from these curves were therefore subject to serious uncertainties. In order to define approximately the limits for vapor pressure values, calculations were made by two methods: 1. Both the sucrose curve and the extrapolated glucose curve were used. Vapor pressure depressions of the individual sugars were calculated independently of one another and added together, the effect of a high concentration of one sugar upon vapor pressure lowering by another being disregarded. 2. The sucrose curve alone was used, the molality of invert sugar being added to that of sucrose and the vapor pressure depression calculated for the sum. The fact that relative
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vapor pressure lowering per mol of sucrose was greater than the relative vapor pressure lowering per mol of a monosaccharide was disregarded.

Method 1 would give too low a value for vapor pressure depression and method 2 too high a value. Rates of change in vapor pressure, which are of more interest than absolute values, are similar for the two methods of calculation. Curves derived from values obtained with both methods are presented in figure 3.

![Figure 3](image_url)

Fig. 3. Changes in vapor pressure of nectar with changing atmospheric vapor pressure in attached red clover inflorescences in the greenhouse and in excised inflorescences in controlled-humidity chambers.

Over a 72-hour period vapor pressure in nectar in the greenhouse changed by 4.4 mm. Hg. Over a 12-hour period the vapor pressure of nectar in the humidity chamber changed by about 0.7 mm. Hg. While on the one hand the higher initial vapor pressure gradient in the humidity chamber would at first favor a more rapid approach to equilibrium there, on the other hand the higher greenhouse temperature (70° F as compared with 50° F) would at least partially compensate for the differential due to unequal gradients. With due consideration for these uncontrolled variables, it would appear that rates of change in vapor pressure were of a similar order in attached
and excised inflorescences. If this be so, changes in nectar concentration can in the main be accounted for by direct interchange of water molecules between the nectar and the atmosphere.

**NECTAR STUDIES IN CLONES**

In order to study the relationship of nectar yield to genotype, clones were established from three red clover plants of the Kenland variety. Initial propagation was by crown division, further propagation by a method of stem cuttings (6). Cuttings were taken from actively growing plants and inserted in moist Vermiculite. Cuts were made about one fourth inch below the second node from the stem apex. The cuttings were kept in reduced light under the greenhouse bench for three to four weeks, then transferred to sand in five-inch pots. After six weeks in sand they were re-potted in a 4:1 mixture of garden soil and sand. At this time the substratum was inoculated with a culture of *Rhizobium trifolii*. Plants were in flower six months after the cuttings were made.

**TABLE V**

**MEAN NECTAR YIELDS AND SUGAR CONCENTRATIONS IN THREE CLONES OF KENLAND VARIETY RED CLOVER.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number in sample</th>
<th>Mean nectar volume (μl./g. inflorescence)</th>
<th>Mean concentration (% sugar by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone 23</td>
<td>49</td>
<td>44.4 ± 1.6*</td>
<td>63.9 ± 1.0</td>
</tr>
<tr>
<td>Clone 10</td>
<td>53</td>
<td>39.0 ± 1.9</td>
<td>63.9 ± 0.9</td>
</tr>
<tr>
<td>Clone 4</td>
<td>48</td>
<td>39.7 ± 1.5</td>
<td>63.7 ± 1.2</td>
</tr>
</tbody>
</table>

Least mean difference necessary for significance at 5% level:

Clone 23/Clone 10—5.0 μl.
Clone 23/Clone 4—4.3 μl.

*Standard error of a mean.*

A summary of nectar yields and concentrations for the clones, sampled over a five-week period in the spring of 1950, is given in table V. The apparent superiority of Clone 23 over Clones 4 and 10 with respect to volume yield indicates that selection of plants for high nectar yield is possible.

**GENETIC AND ENVIRONMENTAL SOURCES OF VARIATION IN NECTAR YIELD AND SUGAR CONTENT**

The contributions of genetic and environmental sources to total variation were compared in analyses of variance on samples taken from Clones 23 and 10 on each of eight days. Tables VI and VII contain the results of these analyses. Variance was divided into components attributable to heredity (clones), to environment (days), and to the interaction between the two. In the analysis of yield, unit variance was of the same order of magnitude for clones and days. In the analysis of concentration, unit variance for clones was only 4.2 as compared with 323.4 for days. Moreover, the unit error variance, representing variability within individual samples,
TABLE VI
ANALYSIS OF VARIANCE IN NECTAR YIELD (MG. SUGAR/G. INFLORESCENCE) IN CLONES 23 AND 10 ON EIGHT DAYS.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clones</td>
<td>1</td>
<td>595.3</td>
<td>595.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Days</td>
<td>7</td>
<td>3258.0</td>
<td>465.4</td>
<td>3.1*</td>
</tr>
<tr>
<td>Days x clones</td>
<td>7</td>
<td>826.3</td>
<td>118.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Sampling error</td>
<td>48</td>
<td>7305.4</td>
<td>152.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 5% level.

was only 6.8. In a similar analysis of variance in concentration in which Clones 10 and 4 were compared, the unit variance for days was 393.6, that for clones 0, and the unit error variance 12.9.

From these data the inference may be drawn that, in the three clones tested, concentration of nectar at the time of secretion was a relatively constant value within a genotype (and almost identical for the three genotypes) and that the major source of variation was environmental. No significant difference between yield means in Clones 23 and 10 appeared when the variance analysis form was followed (cf. tables V, VI). This probably resulted from the reduction made in sample number in order that sample size might be the same on each day. Only 64 inflorescences could be used in the analysis of variance, whereas 102 were used in the analysis reported in table V.

Discussion and conclusions

Knowledge of the influence of various factors on nectar secretion is of interest not only because of its application to pollination problems, but also because of its bearing on the mechanism of secretion. The effects of temperature, solar radiation, atmospheric humidity, and genotype were discussed in previous sections of this paper. The combined effects of these factors, and the bearing which the results of the present experiments have on the mechanism of secretion, remain to be considered. To attempt to explain such a complex process as secretion on the basis of a few of the many factors involved would of course be abortive. Nevertheless, a few salient

TABLE VII
ANALYSIS OF VARIANCE IN NECTAR CONCENTRATION (PER CENT. SUGAR BY WEIGHT) IN CLONES 23 AND 10 ON EIGHT DAYS.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Variance</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clones</td>
<td>1</td>
<td>4.2</td>
<td>4.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Days</td>
<td>7</td>
<td>2263.7</td>
<td>323.4</td>
<td>47.9*</td>
</tr>
<tr>
<td>Days x Clones</td>
<td>7</td>
<td>74.5</td>
<td>10.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Sampling error</td>
<td>48</td>
<td>324.0</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 1% level.
facts have emerged from these studies which are relevant to any hypothesis of nectar secretion: 1. There was a highly significant direct correlation between the amount of solar radiation reaching the plant and the amount of sugar secreted as nectar. Quantity of illumination had no apparent effect on the sugar concentration of the nectar, i.e., the sugar–water mole fractions. 2. There was a high inverse correlation between the sugar concentration of the nectar at the time of its extraction and the average vapor pressure of the atmosphere surrounding the plants during the 12-hour period preceding extraction. The concentration–vapor pressure correlation was reflected in a lower, but highly significant direct correlation between vapor pressure and nectar volume. A condition of equilibrium, or an approach to equilibrium, between the nectar and the atmosphere, is indicated. Whether the approach to equilibrium was through post-secretion interchange of water molecules alone, or partly through the secretion of nectar of varying concentration cannot be stated with certainty; however, it appears, from a comparison of rates of change in nectar vapor pressure in attached and excised inflorescences, that hygroscopic action is sufficiently rapid to account for observed changes in concentration. 3. In an analysis of variation of nectar concentration in three clonal populations, environmental factors (responsible for day-to-day variation) were found to contribute far more to the total variation than genotype. In one such analysis (table VII) the error variance, the measure of variation between individual inflorescences, was 6.8. This variance contained, in addition to variation among inflorescences from the same plant, the effects of differences among plants within a clone arising from slight dissimilarities in environmental history. It seems reasonable to conclude, therefore, that in the genotypes studied the sugar concentration of the nectar during secretion was fixed within relatively narrow limits by the heredity of the plant. These limits cannot be stated exactly; however, if the standard deviation of a single determination be taken as a measure of dispersion, about two thirds of the nectar concentration values should fall within ± 2.6% of the mean.

The following hypothesis is offered in explanation of the facts listed above: The concentration at which nectar is secreted is determined by the heredity of the plant within limits of perhaps ± 3% of the mean value. The total amount of sugar secreted is dependent upon the sugar metabolism of the plant which is in turn influenced by the quantity of illumination received. Since the amount of sugar available for secretion and the concentration of the nectar at the time of its secretion have been defined within limits, the volume of nectar secreted is, ipso facto, delimited. Following secretion the nectar in the corolla tube approaches an equilibrium with the water vapor pressure of the surrounding atmosphere through an interchange of water molecules.

This hypothesis is in accordance with the views of BEUTLER (2) and RADTKE (13) that nectar is secreted in its final form, in other words, that sugar and water are secreted as a solution.
Summary

The influence on nectar secretion in red clover of night temperature, solar radiation, atmospheric humidity, and genotype was studied. The Gubin centrifuge method, as modified for direct volume measurement, was used to measure volume yield. Refractive indices, from which sugar concentrations were derived, were measured with the Abbe refractometer.

The influence of night temperatures in the range of 50 to 78° F was studied under conditions of both natural and artificial illumination. Under neither condition could any effect of temperature on the amount of nectar secreted be detected.

Over a period of two months, a quantitative relationship between weight of sugar secreted in nectar and the amount of solar radiation incident upon the plant was observed. Quantity of irradiation had no apparent effect on the concentration of sugar in the nectar.

From studies of the influence of atmospheric humidity it appears that nectar in the corolla tube approaches a state of equilibrium with the vapor pressure of the surrounding atmosphere. Vapor pressure probably has the greatest influence of all environmental factors on the sugar concentration of the nectar at the time of its extraction. Dilution or concentration of nectar with changing vapor pressure appears to be achieved mainly through post-secretion hygroscopic changes rather than through the medium of the hydrostatic system of the plant.

An investigation of the relation of genotype to nectar secretion was conducted in three clonal populations. The apparent superiority of one clone over the others with respect to yield suggests the possibility of selection for high-yielding strains.

In analyses of variance in which total variation was assignable to hereditary and environmental sources, environment was found to contribute by far the major portion of the total variation in sugar concentration of nectar in the three genotypes studied.

An hypothesis is advanced to account for the observed effects on nectar secretion of the several factors studied.

The work reported here is the result of co-operative research between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration of the U. S. Dept. of Agriculture, and the Department of Botany and Plant Pathology, Ohio State University, Columbus, Ohio. The author is indebted to Dr. C. A. Swanson, Department of Botany and Plant Pathology, Ohio State University, and to Dr. E. A. Hollowell and Mr. J. G. Dean, Jr., B.P.I.S.A.E., U. S. Dept. of Agriculture.

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1. ARENS, KARL. The active membrane, an hypothesis to explain the transfer of water and solutes in plants, as depending upon respiration. Rev. Can. de Biol. 8: 157-172. 1949.


ERRATA

Volume 26:

Page 521, line 3, liberation of organic phosphate should read liberation of inorganic phosphate.

Page 598, line 29, component may be expressed should read component (solvent or constituent solute) may be expressed.

Page 598, line 30, molal free energy difference per liter of component flux should read molal free energy difference divided by the partial molal volume (at its reference state) of the constituent component subject to flux consideration.

Page 599, lines 23 and 24, \( \bar{v} \) in liters should read \( \bar{v} \) in liters per mole.

Page 599, lines 27 and 28, \( \bar{v} \) is the partial molal volume of the constituent solute in solution, in liters should read \( \bar{v}^* \) is the partial molal volume of the constituent solute in solution, in liters per mole.

Pages 599 to 608, factor \( v \) should read \( V^0 \).

Page 600, line 9, delete of solute flux.

Page 603, line 16, equation (27) should read \( \text{REE} = \frac{m}{L^k_x} \times \frac{m}{L^t_x} \times \frac{L^s_x}{m} = \frac{mL^s_x}{4} \times \frac{1}{t} \).

Page 603, line 18, The ratio \( \bar{v}/m \) should read The factor \( A \times \bar{v}^* \).

Page 603, line 19, delete of solute flux.

Page 603, line 20, RNI is in grams should read RNI is in grams per square centimeter per second.

Pages 655 to 672, factor \( V \) should read \( V^0 \).

Page 657, line 45, delete In other words, flux intensities are not forces per unit area, but rather are forces concerned with the ordered movement of a unit volume of a constituent component of solution through a unit of distance.

Pages 699, 701, 703, 705, and 707, title, Acetaldehyde should read Aldehydes.

Page 787, line 37, in the 5/P and 2/P groups should read in the 5P and 2P groups.

Volume 27:

Page 104, lines 38 and 42, and Page 105, lines 3 and 4, Method 1 should read Method a and Method 2 should read Method b to correspond with designations in figure 3.

Page 109, line 12, affect should read effect.

Page 140, paragraph 2, line 5, non-reducing sugars in non-treated lots should read non-reducing sugars as was in non-treated lots.

Page 463, line 23, K, the constant, should read K, the constants.

Page 463, line 25, \( V^1 \) should read \( V^1 \).

Page 466, legend for figure 5, interference of Na and Rb uptake should read interference of Na with Rb uptake.

Page 469, Table I, Figure 5 should read Figure 3.

Page 469, Table I, Figure 6 (left) should read Figure 4 (left).

Page 469, Table I, Figure 11 should read Figure 8.

Page 470, line 10, which the un-competitively affected should read which are un-competitively affected.

Page 471, Table II, Figure 6 (right) should read Figure 4 (right).

Page 471, Table II, Figure 7 should read Figure 5.

Page 530, line 5, although specifically should read although not specifically.

Page 530, line 15, adenosine triphosphate should read adenosinephosphatase.