Absorption of C14 Labeled Sucrose by Nectaries

Cecil Wright LeFevre
ABSORPTION OF C^{14} LABELED SUCROSE
BY NECTARIES
by
Cecil Wright LeFevre

A thesis submitted in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Agronomy

UTAH STATE UNIVERSITY
Logan, Utah
1958
ACKNOWLEDGMENT

My sincere appreciation is extended to Dr. Marion W. Pedersen for his help on formulating and directing this thesis work. I am also grateful to Dr. Herman H. Wiebe for his many valuable suggestions during the conduct of the research, to William P. Nye for his photographic work, and to Dr. Devere R. McAllister for his help and guidance during my stay at Utah State University.
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Review of literature</td>
<td>2</td>
</tr>
<tr>
<td>- Significance of nectar</td>
<td>2</td>
</tr>
<tr>
<td>- Structure of nectaries</td>
<td>5</td>
</tr>
<tr>
<td>- Physiological process of secretion</td>
<td>8</td>
</tr>
<tr>
<td>- Foliar absorption of sugar</td>
<td>12</td>
</tr>
<tr>
<td>Methods and procedure</td>
<td>13</td>
</tr>
<tr>
<td>Results</td>
<td>17</td>
</tr>
<tr>
<td>- Young growing alfalfa</td>
<td>17</td>
</tr>
<tr>
<td>- Alfalfa plants from flowering time to seed harvest</td>
<td>17</td>
</tr>
<tr>
<td>- Foliar application</td>
<td>17</td>
</tr>
<tr>
<td>- Time of absorption</td>
<td>17</td>
</tr>
<tr>
<td>- Other families</td>
<td>26</td>
</tr>
<tr>
<td>- Metabolic studies</td>
<td>26</td>
</tr>
<tr>
<td>Discussion</td>
<td>41</td>
</tr>
<tr>
<td>Summary and conclusions</td>
<td>42</td>
</tr>
<tr>
<td>Literature cited</td>
<td>43</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure                                                                 Page

1. Aparatus used to treat flowers with C\(^{14}\) labeled sucrose solution 14
2. Aparatus used to collect C\(^{14}\)O\(_2\) which was a product of respiration of plant inoculated with C\(^{14}\) labeled sucrose 16
3. Young alfalfa plant showing first flowers (photograph left, autoradiogram right) 18
4. Radioactivity in flowers of treated alfalfa plant (photograph left, autoradiogram right) 19
5. Radioactivity in seedpods and buds of an alfalfa plant treated through unpollinated flower (photographs left, autoradiograms right) 21
6. Alfalfa plant treated at near end of flowering period (photograph left, autoradiogram right) 22
7. Seeds from treated alfalfa plant (photograph left, autoradiogram right) 23
8. Alfalfa plant treated on leaf (photograph left, autoradiogram right) 24
9. Variation in activity time with alfalfa flowers (photograph top, autoradiogram bottom) 25
10. Birdsfoot trefoil, Lotus corniculatus, Family LEGUMINOSAE, (photograph left, autoradiogram right) 27
11. Columbine, Aquilegia formosa, Family RANUNCULACEAE (photograph top, autoradiogram bottom) 28
12. Sweet William, Dianthus barbatus, Family CARYOPHYLLACEAE (photograph left, autoradiogram right) 29
13. Chrysanthemum, Chrysanthemum, Family COMPOSITAE (photograph left, autoradiogram right) 30
14. Four O’Clock, Abronia, Family NYCTAGINACEAE (photograph left, autoradiogram right) 31
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.</td>
<td>Petunia, Petunia hybrida, Family SOLANACEAE (photographs top, autoradiograms bottom)</td>
<td>33</td>
</tr>
<tr>
<td>16.</td>
<td>Peach leaf bellflower, Campanula persicifolia, Family CAMPANULACEAE (photograph left, autoradiogram right)</td>
<td>34</td>
</tr>
<tr>
<td>17.</td>
<td>Apple, Malus pumila, Family ROSACEAE (photograph left, autoradiogram right)</td>
<td>35</td>
</tr>
<tr>
<td>18.</td>
<td>Horsechestnut, Aesculus hippocastanum, Family Hippocastanaceae (photograph left, autoradiogram right)</td>
<td>36</td>
</tr>
<tr>
<td>19.</td>
<td>Iris, Iris germanica, Family IRIIDACEAE (photographs left, autoradiograms right)</td>
<td>38</td>
</tr>
<tr>
<td>20.</td>
<td>BaCl\textsubscript{2} from Cl\textsubscript{14}O\textsubscript{2} which was a product of sucrose respiration of treated plant (photograph top, autoradiogram bottom)</td>
<td>39</td>
</tr>
<tr>
<td>21.</td>
<td>Chromatogram and autoradiogram of nectar from treated alfalfa plant (photograph top, autoradiogram bottom)</td>
<td>40</td>
</tr>
</tbody>
</table>
INTRODUCTION

Since the early Eighteenth Century the significance of nectar secretion has been debated. Some have reasoned that it is reabsorbed into the plant and used as energy for the developing embryo while others believe it is a waste product which attracts pollinating insects.

It has been suggested (Brink and Cooper, 1947) that the nutrient supply to developing ovules is a major factor in the development of seed. Also, alfalfa fields pollinated by pollen-collecting bees produce greater seed yields than those pollinated by nectar collectors (Bohart, Nye, and Levin, 1955). It is a well-known fact that more flowers are tripped by the pollen collectors, but it is debatable whether the nectar not taken by the pollinators has any influence on the quality or amount of seed set. It is then necessary to find whether the nectar not taken by bees is used by the plant before studies can be made to show its effect on seed and forage yields.

The object of this study was to treat flowers of several families with C14 labeled sucrose and determine by the use of autoradiograms if sugars can be absorbed by nectaries, where the sugars are translocated, and when absorption takes place.
REVIEW OF LITERATURE

Significance of nectar

Recent works on nectar significance are few; however, this subject has been debated since the early Eighteenth Century. Bonnier's review (1878) begins with Pontederia, who in 1720 first suggested that nectar accumulated for the purpose of nourishing the young embryo, and reviews the literature up to his time.

Bonnier (1878) discusses the early theory of Christian Conrad Sprengel that nectar existed to attract insects for the purpose of pollination. He also attacks Darwin's theory that nectar existed for the purpose of cross fertilization. Darwin (1910) reasoned that the plants excrete nectar to eliminate something injurious from the plant sap and in so doing attract insects to cross pollinate the plants. He reasoned that flowers with the largest nectar glands, producing the most nectar, would be most often crossed and thus give rise to more vigorous species.

Bonnier (1878) did not accept the theory that nectar was secreted only to attract bees for pollination or that color and odor of flowers was for insect pollinator attraction. He showed that bees visited flowers containing sugars, but which may not have an attractive flower or a sweet odor. He found that small flowers were visited just as much as large ones, and that bees would abandon the most perfumed and colored flowers to go to a saucer of syrup or honey which had no color or odor.

He stated that many species of bees can rob a flower of the nectar without fertilizing it. When aphid were present, bees were found to
gather honey dew. In long-tubed flowers bees were found to take the nectar by piercing the corolla tube at the base and not bothering the pollen or pollinating mechanism. He concluded that the popular theory of the role of nectaries was insufficient and conducted his own experiments (Bonnier, 1878).

Bonnier (1878) took nectar simultaneously from flowers of Mirabilis hybrida at different stages of flowering and found that the quantity was greatest at the moment of fertilization and then diminished until at the end of flowering it disappeared completely. By chemical analysis he found that the nectar contained a considerable proportion of non-volatile substances. He reasoned that if the water were simply to evaporate, there should be found on nectar-bearing tissue an abundant deposit of sugars. Bonnier could not even find sugar crystals with a microscope. He observed that when fertilization was prevented or where it could not occur, the nectar-bearing tissue entirely withered only when the sterile ovary also withered. He concluded that nectar-bearing tissue, whether floral or extra-floral, whether or not they emit a liquid to the outside, constitute special nutritive reserves in direct relation to the life of the plant.

Horwin (1933) observed that nectar became more concentrated the longer it remained in the flower until the second day. He observed that after nectar had been removed by pipettes from the flowers of the tulip tree (Liriodendron tulipifera), no more further visible secretion occurred. He does not state whether this plant has been fertilized or the stage of development of the flower. He did say that buds on moderate days opened with nectar while on hot days they did not and that buds which opened in the morning had more nectar than those opening in the
heat of the day.

Measurements of nectar content of flowers of different species which were carried out by Stapel and Lund (1945), Boetius (1948), and Pedersen and Todd (1949) all showed that the sugar concentration of nectar in flowers visited by bees was lower than in flowers protected from them. Raw's (1953) experiments with raspberries showed that total amounts of nectar and sugar were greater when nectar was removed more than once. Phillis and Mason (1936) in experiments with Sea Island cotton found dry material of the perianth to pass back into the plant before the flowers were shed. Their experiments were done by means of weighing and chemically analyzing many plants. Loss in dry weight of corollas removed from the plants was only half that of corollas left on the plant. They were unable to measure loss by respiration and could not make any conclusions on carbohydrates.

Maclachlan (1939) agrees that there might be some other use for nectar than attracting insects. He says that some plants have extra-floral nectaries. Many flowering plants yield no nectar while others yield much more than can be used by insects.

Boetius (1948) studied Echium and Rubus and classified the flowers into stages of nectar secretion. He divided the secretion process into 4 phases. The first was a producing stage where total quantity of sugar and nectar increased up to a maximum. The second was a dilution phase where sugar percentage decreased and total amount of nectar increased. In the third stage a decrease in quantity of dry substance could be observed, and in the final stage the nectar disappeared. Refractometer readings showed no sugar present in distilled water used to wash out flowers from which nectar had disappeared. He concluded that Bonnier's
observation—only inadequately taken into account in later investigations—regarding nectar being entirely resorbed towards the end of blooming can be absolutely confirmed.

Pankratova (1950) inserted a graduated capillary in the corolla tube of red clover to measure nectar flow. He used a microscope to measure rise and fall in the tube. He observed a negative rate of secretion in pollinated florets which could only be explained by re-absorption of nectar by the tissues of the floret. He quoted Bonnier's work but did not accept it because Bonnier in his treatise on nectar took up anti-Darwinian views, avoiding the question of the enormous adaptive evolutionary significance of nectar secretion and criticized the Darwinian conception of this process.

Agthe (1951) treated the phloem of Euphorbia with potassium florescein. Secreting nectaries became colored while those not secreting remained uncolored. When he replaced the nectar with a glucose solution colored with potassium florescein, the tissue of the young secreting nectaries remained uncolored while old shrunken nectaries became colored.

**Structure of nectaries**

The works of nectary structure are few. The first comprehensive discussion concerning them was by Linne in 1763 (Cook, 1923) under the title of "Nectar Florum." He called the organs nectaria and the sweet juice secreted nectar.

Bonnier (1878) attempted to classify nectar-bearing tissue according to structure but gave up when he found too much diversity in the morphological character of these organs. He observed considerable variation even in species. He discusses sugar-bearing tissue located in the following places: (1) cotyledons, (2) leaves, (3) stipules, (4) bracts,
between leaves and stems, (6) in sepals, (7) in petals, (8) between sepals and stamens, (9) in stamens, (10) between sepals, petals or stamens and the carpels, (11) in carpels, and (12) at the common base of all floral organs.

Fahn (1952) studied 100 species belonging to 50 families; of these he describes the following types: torus, perigonial, ovarian, and stylar. Correlation was found to exist between the structure of the nectaries and the shape of the filaments. The structure of the nectariferous tissue was essentially similar in the nectaries of all plants investigated. It consisted of cells filled with dense granular protoplasm. These cells were generally smaller than those surrounding them.

The structure of nectaries has also been studied in relation to secretion of nectar. Behrens (1879) published an important work on nectar secretion. He studied nectaries in terms of the secreting tissue. Examples of 5 types of nectar-secreting tissue were shown in a series of camera lucida drawings. They were described as follows:

Class No. 1—Secretion through non-cutinized thin walled epidermal cells. These cells join epidermal cells of cutinized stamens and sepals. The nectar cells contain a yellow metaplasm, but the epidermal cells are transparent. The secretion reaches the surface by diffusion through the epidermal cells.

Class No. 2—Secretion of nectar through thin walled epidermal papillae by process of diffusion. Some of the cells of the epidermal layer of this class are extended into large single celled papillae. The papillae contain a colorless and coarsely granular metaplasm. Dextrose and other carbohydrate bodies are present. Small amyloid bodies appear in the papillae and the secretion soon begins either at the apex or on
both sides. The drop enlarges and covers the entire papillae. Small bodies are noticed on the undersurface of the papilla, which act indifferently to reagents. The amyloid bodies disappear when the nectar appears on the outside and it is evident that nectar has passed through the walls, most possibly by means of a molecular change of the membrane.

Class No. 3—Secretion at the point of many-celled nectary papillae by means of a building up of collagen. The papillae in this class are many celled and are terminated by a single cell cap. Their outer walls are soft and possess no cuticle. The cap-cell of the young papillae has a cell wall as thick as that of the other cells, but gradually the wall at the top spreads so that one sees an outer and an inner part. The two parts are further separated until there is a half-moon shaped middle layer. This layer increases and the inner membrane or layer is pushed farther into the cell until the cavity of the cell is nearly filled up. Finally the outer layer of the wall breaks and the slime escapes.

Class No. 4—Secretion through the opening of stomata on the epidermal layer. In this class the nectar tissue is covered by an epidermis. In this epidermis are found stomata which are sometimes sunken. They look like ordinary stomata and have a stomatal chamber beneath them. The epidermal cells and guard cells of the stomata are cutinized. Secretion passes from the lower nectary tissue into the stomatal chambers and then out through the stomata.

Class No. 5—Secretion through sliming of the outer walls of the epidermis by means of the formation of collagen and destruction of the cuticle. In this class the outer walls of the epidermis are heavily cutinized. Three layers or bands in this wall can be seen, two of them firm and dark and a clear one between them. The secretion proceeds as
follows: The wall becomes slimy just under the cuticle and is split into tangential layers. As the formation of slime proceeds, certain high points are produced, raising up the cuticle, which is finally ruptured, thus allowing the slime formed by the nectar part of the wall to escape. Fahn's work (1952) showed similar results to that of Behrens.

Cook (1923) found the nectar gland of white clover to be seen as a little mound of tissue next to the ovary and internal to the free stamen. This gland was pyramidal in shape with the top being slightly oval. The secretion here was similar to Behren's class number one. He stated that it is the exception rather than the rule to find true stalked nectar glands.

In most all nectar glands studied vascular-bundles are found in close proximity to the nectar tissue (Cook, 1923; Fahn, 1952). They run either parallel or at right angles to the nectar cells. Morris (1941) found only in one specimen that a true bundle led into these cells and that bundle led beyond and supplied adjacent stamens also. Frey-Wyssling (1955) says that nectaries contain phloem elements in a similar way as hydathodes are provided with tracheids.

**Physiological process of secretion**

Maclachlan (1938) suggested that nectar is the result of an unbalance in the protein carbohydrate ratio. His thinking was that the nectary was a filter which would filter out protein and not sugars and that secretion of sugar would balance the ratio of the two substances. His theory is based on years of experience as a beekeeper, but it is accompanied with no experimental material.

Zimmerman (1953) discusses some of the early theories of the process of nectar secretion. He (Zimmerman, 1953) says that Bonnier was
unable to hold to his sugar reservoir hypothesis (Bonnier, 1878).

Zimmerman (1953) supported Wolf's hypothesis of nectar from the spur which held that floral nectaries had surplus nutrients to secrete during the periods of rapid assimilation to the time the flower drops. This coincides with Dunal (Bonnier, 1878) who in 1829 described nectar as an overflow of accumulated material.

Frey-Wyssling and Agthe (1952) traced the nectar with potassium florescein introduced into the phloem. They observed the movement of this material into the secreting nectaries. In immature nectaries not secreting, the material was traced into growing flowers and leaf organs. They concluded that nectar was secreted phloem sap.

Pankratova (1950) proposed the theory that secretion was the result of a differential capacity for secreting cells to hold sugars.

Pankratova (1950) quotes the works of the Russian, Lepehkin, in 1904 and 1907 and shows how he proposed that osmotic processes play a large part in nectar secretion. However, modern authors have found osmosis out of the question. Pankratova's (1950) experiments gave results opposite to that expected if osmosis were working. Beutler (1929) says that osmosis is not the solution to the problem because no liquid medium is present against which the exchange could occur. She says that root pressure cannot be considered because of the strong pressure needed for excretion of a concentrated sugar solution; however, Shuel (1956) found that dilute nectar can be secreted which would decrease the pressure needed. Shuel (1956) could not make accurate measurements of the nectar the moment it was secreted because of the changes taking place in the external solution. Shuel's (1956) work also suggested that osmotic work was not done. He, like Pankratova,
found concentration of nectar to be almost identical to that of the media in which the pedicle of the excised flower was placed.

Radtke (1926) suggested that the secretion of nectar was the result of metabolic activity of the nectary. He painted nectaries of different plants with a killing solution. The secretion of these nectaries permanently stopped while those washed with water continued to secrete as long as raw material was available in the nectar preparation. He concluded that secretion is a function of the protoplasm of the nectar tissue.

Salts are known to move from protoplasm into the vacuole sap in many cases. Stiles (1936) found that in order for plants to absorb salts they must be living and have the requirements for healthy existence. Stiles suggested that the problem of salt absorption is closely parallel to the problem of nectar secretion against an osmotic gradient. He suggested that the answer may be found in the study of the organic chemistry of the protoplasm.

Frey-Wyssling, Zimmerman, and Maurizio (1954) and Zimmerman (1954) found the phloem to contain mainly sucrose and to show very little invertase activity, but the moment nectar secretion began inversion could be detected and the nectar contained sucrose, fructose, glucose, and olegosaccharides of glucose and fructose. They concluded that the nectaries were not just valves from which phloem sap could be secreted, but genuine glands with a fixed active metabolism. They floated excised nectaries on media of glucose, sucrose, and fructose. The nectar from each of the flowers contained the same sugars, regardless of the media used. When pentose sugars were used, very little passed into the nectary. That which was secreted went through unchanged.
Shuel (1956) confirmed these results using several more sugars, including pentoses, hexoses, aldoses, ketoses, monosaccharides, disaccharides, and trisaccharides. This supported the theory that the nectarium is capable of forming not only invert sugars from complex sugars, but also complex sugars from simple sugars.

Ussing (1952) suggested that a substance may pass through a membrane in chemical combination with another diffusible substance and that the substance may be consumed or produced within the membrane.

Wilbrandt (1954) discussed how this may take place. He described what is called the membrane carrier theory. This mechanism consists of an enzyme on the outside of the membrane (he terms it the cis side and the other side the trans side) which catalyses the combination of the impermeable substrate with a carrier to make a permeable substrate carrier complex which goes through the membrane to the trans side where the complex undergoes hydrolysis to form the original compound. Most of these studies have been done using glucose to study transport through intestine, erythrocyte, and kidney membranes. LeFevre (1954) found ketose and aldose sugars to follow similar patterns. The coupling on the cis side is suggested to be an energy supplying reaction which is probably some sort of phosphorylation. Glucose-1-phosphate and glucose-6-phosphate have been tested and found not to penetrate the red cell membrane appreciably. Wilbrandt (1954) shows how Rosenberg in 1948 and 1950 suggested a metaphosphate ester but made no experimental test. In 1954 a method to test this still had not been worked out.

Sacher (1957) suggests that auxin effects selective permeability of cell membranes.
Foliar absorption of sugar

In 1883 Boehm (Went, 1948) demonstrated absorption of sugars by detached leaves floating on sucrose and dextrose solutions. Since that time this has been repeated with many variations. Detailed studies have been made of the uptake and distribution of urea tagged with $^{15}N$, $^{32}P$, $^{86}Rb$, radioactive sucrose, and others. All these materials absorbed by the leaf are distributed more or less throughout the plant (Burr, et al., 1956). Attempts to feed sugars to plants through roots have proved unsuccessful (Went, 1948). Burr, et al. (1956) fed $^{14}C$ to a sugarcane leaf. Eighteen hours later every part of the plant was strongly tagged. The fed leaf and growing tip were particularly rich. Kursanov (1956) got similar results.

Tukey, et al. (1956) found $^{32}P$ and $^{42}K$ applied to fruit trees just as the buds were commencing to swell to move through the bark and up through the branches and to concentrate near the buds, available for the flush of new growth. Loehwing (1942) states that food reserves previously mobilized in the perianth and accessory floral structures are commonly translocated to the sex organs with surprising rapidity just prior to anthesis.
METHODS AND PROCEDURE

Self-filling micro pipettes were made by sealing a capillary tube into a larger glass tube of 4 mm inside diameter with improved DeKhotinsky cement (figure 1). The larger tubes were drawn out and cut at a point where the capillary would fit snugly but not tight. A heated wire was placed on the cement stick until some material melted. A drop was then transferred to the joint to be sealed. These tubes were calibrated by weighing the empty tubes on an analytical balance and then weighing the same tubes filled with water. The capacity was determined by the difference of the 2 weights, assuming that 1 cc of water weighs 1 gram. Pipettes of .001 ml capacity were used.

With the use of this instrument micro amounts of 40 percent C14 labeled sucrose solution were applied to the nectaries of the flowers being treated. Each arrow on the photograph indicates the point of application of .001 ml of this solution unless otherwise specified. Five treatments of this type were made to alfalfa plants covering a considerable range in age. One test was made in which the application was made to a leaf. The treated spot was protected with a small insect cage held in place by a magnet on the leaf's underside. A time series test was also made on alfalfa. In this case the activity time (length of time between treatment and the excising and dissecting) was varied from one-half to 27 hours. Members of 10 plant families were used as indicated in the Results section. All plants were protected from pollinating insects either by the greenhouse or by a plastic screen cage in
Figure 1. Apparatus used to treat flowers with Cl\textsubscript{4} labeled sucrose solution. (a) rubber bulb, (b) rubber connection, (c) glass tube, (d) self-filling capillary tube.
the field. The length of time between treatment and dissection and other details are included in the legends which accompany the photographs.

The dissected plants were glued to blotter paper with mucilage and placed in a small plant press which was placed in an oven at 110° F until the plant material dried.

Autoradiograms were made using Super Panchro-Press type B film.

Six alfalfa blossoms on a raceme of an alfalfa plant were treated with C^{14} labeled sucrose. The plant was placed in a 20 x 12-inch bell jar and the air was bubbled through 10 percent potassium hydroxide solution for 5 days to collect C^{14} labeled carbon dioxide (KOH + C^{14}O_2 → KCl + CO_2) (figure 2). Barium chloride was used to precipitate barium carbonate (BaCl_2 + KCl + CO_2 → BaCO_3 + KCl + HCl). The barium carbonate was filtered out of solution and dried on a filter paper. The filter paper was glued to a blotter paper and an autoradiogram was made.

Nectar from an alfalfa flower on a treated plant was placed on an 11\frac{3}{4} x 11\frac{3}{4}-inch Whatman No. 1 filter paper with standard sugar checks. The paper was stapled in cylindrical shape and a chromatogram was made using ascending chromatography. A 12-inch jar with a parafilm lid was used for the container and a 4:2:1 solution of n-butyl alcohol, ethyl alcohol, and water was used as the solvent. The paper was sprayed with a 1:1 solution of 2 M. oxalic acid and 1.86 ml. aniline in 100 ml. ethyl alcohol and heated at 100° C for 10 minutes. It was sprayed again with a 0.05 percent resorcinol in 25 percent HCl solution and heated 1 minute at 75° C. An autoradiogram was made from the 5 x 7-inch section of the chromatogram showing the spots.
Figure 2. Apparatus used to collect $^{14}\text{H}_2\text{O}$ which was a product of respiration of plant inoculated with $^{14}\text{H}$ labeled sucrose. (a) vacuum, (b) 10 percent KOH solution, (c) paraffin seal.
RESULTS

Young growing alfalfa

The results of $^{14}C$ labeled sucrose application to a young growing alfalfa plant are shown in the autoradiogram of figure 3. Activity is shown in roots, stems, and leaves and is particularly strong in the treated flower, seed pods, and growing buds.

Alfalfa plants from flowering time to seed harvest

The results of alfalfa plants between flowering time and seed harvest are shown in figures 4 to 7. Activity was shown in buds, flowers, translocating stems, and nectar which had been produced since the treatment time. It was particularly strong in buds and in seed pods near the point of application. Figure 7 shows activity to be in pollen and mature seeds. Little or no activity was found in leaves which were fully developed at the time of application. Figure 5 shows the results of absorption by an unpollinated flower. The distribution of activity is similar to that of pollinated flowers.

Foliar application

The results of foliar application are shown in figure 8. The distribution of activity is similar to that of nectary absorption. It is found in the young developing regions. Activity is strong in the stems, both above and below the point of application; however, only the youngest leaves show the presence of much active material.

Time of absorption

The results of variation in activity time are shown in figure 9. Even in the shortest period of activity time (one-half hour), activity
Figure 3. Young alfalfa plant showing first flowers. (A) stem, (B) root, (C) control, (ca) calyx, (s) staminal column, (ov) ovary, (k) wind and keel petals, (st) standard petal. Arrow shows flower treated with 0.002 ml. 40 percent C\textsubscript{14} labeled sucrose solution. Activity time was 3 days. Racemes 1 and 2 were both pollinated. Treatment location was the greenhouse. The autoradiogram shows activity throughout the plant with the ovaries of the treated raceme and buds being particularly strong. Spots on right are contamination probably from pollen grains.
Radioactivity in flowers of treated alfalfa plant. (ca) calyx, (s) staminal column, (ov) ovary, (k) wing and keel petals, (st) standard petal. Raceme 2 was pollinated. Racemes 1 and 3 were not. Raceme 3 was in bloom when treated while raceme 1 was only a bud. Arrow shows treated flower. Leaves C were taken from the lower part of the plant. Nectar from raceme 1 was placed in circle X and that from raceme 3 was placed in circle O. Activity time was 5 days. Treatment location was the greenhouse. The autoradiogram shows activity in the nectar and younger plant parts. One flower which failed to become fertilized did not show radioactivity.
Figure 5. Radioactivity in seed pods and buds of an alfalfa plant treated through unpollinated flower (photographs left, autoradiograms right). (ca) calyx, (ov) ovary, (s) staminal column, (k) wing and keel petals, (st) standard petal. Arrow shows treated flower. This flower was not pollinated while the other flowers of racemes 1 and 2 were. No other flowers were pollinated. Activity time was 10 days. By this time the treated flower had died and dropped from the plant. Treatment location was the greenhouse. The autoradiogram shows activity in the developing buds and in the seed pods with higher concentration in the pods of the raceme containing the treated flower. Old leaves show little radioactivity.
Figure 5. Radioactivity in seed pods and buds of plant treated through unpollinated flower. Legend on facing page.
Figure 6. Alfalfa plant treated at near end of flowering period. Arrow shows treated flower. This was the last flower to bloom. The plant was 30 inches high and samples were taken at intervals down the entire stem. Treatment location was the field. Activity time was 42 days, from August 12 to frost. The autoradiogram shows radiation in burs nearest treated flower.
Figure 7. Seeds from treated alfalfa plant. (1) Spots where seeds from the 8 flowers treated with C14 labeled sucrose were placed. (2) Seed spots from raceme pollinated the same day. (3) Seed spots from raceme pollinated 5 days later. (4) Seed spots from raceme pollinated 9 days after treatment. (5) Seed spots from raceme pollinated 19 days after treatment. (6) Seed spots from raceme pollinated with radioactive pollen. (7) Filter papers used for pollination. Work was done in greenhouse. The autoradiogram shows 69 spots which coincides with number of seeds placed on paper minus those from raceme pollinated with radioactive pollen. The glue did not hold the seed in place so it is impossible to tell which spots on the autoradiogram match which seeds.
Figure 8. Alfalfa plant treated on leaf. Arrow shows the treated leaf. Circle on leaf is where insect cage was placed to protect radioactive sucrose. Leaves on the right (C) were at varying intervals down the stem. Activity time was 4 days. Treatment location was greenhouse. The autoradiogram shows activity in stems and blossoms with little in leaves.
Figure 9. Variation in activity time with alfalfa flowers (photograph top, autoradiogram bottom). (st) standard petal, (w) ovary wall, (ov) ovules, (s) staminal column, (k) wing and keel petals. Intervals were: (1) 1 hour, (2) one-half hour, (3) 3½ hours, (4) 8 hours, (5) 18 hours, and (6) 27 hours. The 18- and 27-hour treatment were subject to frost damage. Treatment was done in the field just at the time of the first frost. Top print is photograph; bottom is autoradiogram. The autoradiogram shows activity at one-half hour and intensity to vary with time.
is shown. As the activity time increases, the intensity of the autoradiograms increases. The 18- and 27-hour exposures did not respond accordingly because of frost damage.

Other families

In 10 families tested (figures 10-19) the results are similar. In all cases the autoradiograms show absorption of the radioactive sucrose and it is found where meristems have been active since the time of application and in tissues of stems, but not in fully developed leaves.

Metabolic studies

The precipitate (figure 20) from the treated plant shows radioactivity while that from the untreated plant does not.

The chromatogram (figure 21) shows glucose, fructose, and sucrose in nectar and the autoradiogram shows all three sugars to be products of $^{14}C$ labeled sucrose used to treat the plant.
Figure 10. Birdsfoot trefoil, Lotus corniculatus, Family LEGUMINOSAE. (ca) calyx, (st) standard petal, (k) wing and keel petals, (s) staminal column, (ov) ovary. (1) Treated raceme not dissected, (2) dissected raceme, (3) raceme not dissected. Arrow shows the treated flower. The activity time was 12 days. Treatment location was the greenhouse.
Figure 11. Columbine, Aquilegia formosa, Family RANUNCULACEAE. Arrow shows treated flower. Activity time was 9 days. Treatment location was H. W. Børsten's garden. (Photograph top, autoradiogram bottom).
Figure 12. Sweet William, *Dianthus barbatus*, Family *CAHYOPHYLLACEAE*. Arrow shows treated flower. Activity time was 11 days. Treatment location was M. W. Pedersen's garden.
Figure 13. *Crysanthemum*, *C. monogynum*, Family COMPOSITAE. Arrows show place of treatment. (1) and (3) flower and bud which grew little; (2) treated flower. Activity time was 11 days. Treatment location was the greenhouse. Flower was donated by Otto Reithman.
Figure 11. Four O'Clock, Abronia, Family NYCTAGINACEAE. Arrow shows incision in corola where flower was treated. (ca) calyx, (s) stamens, (co) corola, (ov) ovary. (1) Bud when treated. (2) Treated flower. (3) Fairly mature fruit when treated. Activity time was 7 days. Treatment location was the greenhouse.
Figure 15. Petunia, Petunia hybrida, Family SOLANACEAE. (photograph top, autoradiogram bottom). Arrow shows where flower was treated through incision in corola tube. (w) ovary wall, (p) placenta, (ov) ovules, (s) stamens, (sy) style, (co) corola, (ca) calyx. Stem 1 was excised from point 1 and stem 2 from point 2. C shows leaves taken from points down the stem. Treatment location was the greenhouse.
Figure 15. *Petunia Petunia hybrida*, Family SOLANACEAE. Top, photographs; bottom, autoradiograms. Legend on facing page.
Figure 16. Peach leaf bellflower, *Campanula persicifolia*, Family *COMANULACEAE*. Arrow shows treated flower (1). 2 is corola cut away to show essential pars. 3 and 4, whole untreated flowers. C, leaves from don the stem. Treatment location was M. H. Pedersen’s garden.
Figure 17. Apple, *Malus pumila*, family ROSACEAE. Stem excised from tree and treated in hot greenhouse while in a wet sand culture. Activity time was 3 days.
Figure 18. Horse chestnut, Aesculus hippocastanum, Family HIPPOCASTANACEAE. Stem excised from tree and treated while in wet sand in hot greenhouse. Activity time was 3 days.
Figure 19. *Iris, Iris germanica*, Family IRIDACEAE. (photographs left, autoradiograms right). First flower to open (not shown) was treated with .005 ml 40 percent Cl4 labeled sucrose solution. A, second from top flower showing split ovary. B, split stem showing inside and outside. C, leaf. (ov) split ovary showing ovules and placenta. (s) stamens, (sg) stigma. Treatment location was G. E. Bohart’s garden.
Figure 19. *Iris Iris germanica*, Family IRIDACEAE. Left, photographs; right, autoradiograms. Legend on facing page.
Figure 20. BaCl$^{14}$O$_3$ from C$^{14}$O$_2$ which was a product of sucrose respiration of treated plant (photograph top, autoradiogram bottom). (C$^{14}$) BaCl$^{14}$O$_3$ from treated plant. (C) BaCO$_3$ from control plant.
Figure 21. Chromatogram and autoradiogram of nectar from treated alfalfa plant (photograph top, autoradiogram bottom). (S) sucrose, (SFG) sucrose, glucose, and fructose mixture, (N-1) nectar from flower on treated raceme, (N-2) nectar from control flower, (G) glucose, (F) fructose.
The results of this study confirm the conclusion of Bonnier (1970) that nectar (as $^{14}C$ labeled sucrose) can be absorbed back into the plant. The accumulation of radioactive materials in the regions of meristimatic activity indicates that this material is used in the development of new tissues including the developing embryo.

The fact that absorption occurred very soon after treatment, that nectar produced on other flowers was radioactive, and that radioactivity absorbed from nectaries was found in young succulent regions indicate that absorbed nectar becomes a part of the nutrient supply of the plant.

The results of collecting $^{14}CO_2$ from the plant (figure 20) and the chromatogram of the autoradiogram of nectar from the treated plant (figure 21) show that absorbed sucrose enters into the metabolic system of the plant. These results support the newer theories of nectar secretion.
SUMMARY AND CONCLUSIONS

Carbon$^{14}$ labeled sucrose was applied to nectaries of alfalfa plants of varying ages and to plants in several other families using special self-filling micro-pipettes. Autoradiograms were made of plants to show the distribution of radioactivity. The time of absorption was indicated by varying the time between treatment and processing. Carbon$^{14}$ dioxide from the plant was collected in KOH and precipitated with BaCl$_2$. The resulting BaC$^{14}$O$_3$ was autoradiogramed to show that C$^{14}$O$_2$ was a product of respiration of the sucrose used in plant treatment. Autoradiograms were made from chromatograms of nectar to show which sugars contained the labeled carbon. It was concluded:

1. Sucrose placed on nectaries of many plants can be absorbed.

2. Absorption begins soon after time of application to alfalfa nectaries.

3. Absorbed sugars are translocated to young developing plant tissues.

4. Sucrose absorbed by nectaries is converted to other sugars and metabolic intermediates.
LITERATURE CITED


Bonnier, Gaston. 1878. Les nectaries. Étude critique, anatomique et physiologique. Annales des science naturelles, 6 série, Botanique. 8:5-212. (Trans. by E. F. Phillips.)


