THE ROOT-PARASITISM OF CASTILLEJA COCCINEA

by

William McLagan Malcolm II

AN ABSTRACT

Submitted to the College of Science and Arts,
Michigan State University of Agriculture and
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Castilleja coccinea (L.) Spreng., commonly called scarlet Indian paint-brush (Scrophulariaceae), is a species of flowering plants which extract materials from the vascular streams of the roots of nearby vascular plants. The attachment to a host plant is made by so-called haustoria, minute organs on the parasite roots. In each haustorium a vascular trace from the parasite root penetrates to the xylem of the host root. Eosin Y, fructose, and sulfate and phosphate ions are known to cross the haustorial connection, and only in the direction of the parasite. Presumably most or all of the substances in the vascular stream of the host can cross into the parasite.

Although the parasite is chlorophyllous and fully photosynthetic, it does not grow beyond the seedling stage unless it successfully penetrates foreign roots, a fact readily demonstrated by growing seedlings in pots without hosts. This host-requirement is not offset by artificial feeding of mineral nutrients or the more common vitamins, phytohormones, or respiratory substrates. Once host-contact is made, the foliage leaves of the seedling grow rapidly, producing in some four months a flat, leafy rosette about four centimeters in diameter. However, seedlings parasitic on even the same host plant have widely-varying rates of growth, perhaps a function of the number of their active haustoria. The rosettes bolt with or without cold treatment, and form the showy flowering shoots which give the parasite its common name of paint-brush. The parasitic attachment is not necessary for bolting——— mature rosettes pulled free of their hosts, washed free of foreign roots and debris, and potted separately will flower normally. Nonetheless, under natural conditions bolting rosettes enjoy extensive host contact.

The parasite attaches to a wide variety of host plants, including plants of species which do not occur in its range. In the greenhouse, some of these hosts support more vigorous growth of the parasite than others do. The hosts in a central Michigan community were ranked according to their ability to support the parasite. However, the distribution of the parasite in that community could not be related to the host ranking. That is, the parasite did not tend to occur more frequently around the hosts which support it best in the greenhouse.

Seeds of the parasite will germinate at the time of their dissemination, and they retain viability for at least two years when stored dry. The seeds will germinate at 4°C, but require light and moisture. Seeds stratified four weeks, then kept in a diurnal temperature range of 15°-38°C germinate at a level of 87%. The seedling hypocotyls are clothed abundantly with root hairs. The direction of root growth is not influenced by the presence of host roots. Induction of haustoria is not solely a result of physical contact with objects or with living tissue. Under natural conditions, the hosts suffer no obvious damage, but greenhouse-grown hosts supporting many parasites suffer twice the mortality of unattacked hosts.

castilleja

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A DISSERTATION

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plate 1

<u>Castilleja</u> <u>coccinea</u> flowering shoot (12X). The flowers and bracts of the inflorescence are tipped with scarlet, orange, or yellow, giving the plant its common name, paint-brush.

THE ROOT-PARASITISM OF CASTILLEJA COCCINEA

INTRODUCTION

Castilleja was named by Mutis in 1871 in honor of the Spanish botanist Domingo Castillejo (Fernald, 1950). The name usually is pronounced kas til LEE ya or kas til LAY ya, but sometimes the j is sounded as an English j rather than a y.

Castilleja is placed in the subfamily Rhinanthoideae of the figworts (Scrophulariaceae), a large and cosmopolitan family of flowering plants. The 250 or so species of the genus are mostly North American in distribution, and are particularly common in the western United States (Gleason, 1958). Castilleja is of little economic importance, but has attracted the attention of taxonomists for some years because of its complex species problems.

Castilleja coccinea (L.) Spreng.* (plate 1, frontispiece), commonly called scarlet Indian paint-brush, ranges from southern New England and Canada south to the northern borders of the Gulf states and west to Oklahoma (Pennell, 1935) (plate 2, page 7). The species in Michigan is scattered but common close to the shores of both peninsulas and in a few inland sites (plate 3, page 9). Plants of the species attack roots of nearby vascular plants and extract from them materials for their growth. The attack on the host plants is made by minute organs on the parasite roots, so-called haustoria, which adhere to the host roots and lyse or force access to the vascular elements (Solms-Laubach, 1867/1868) (plates

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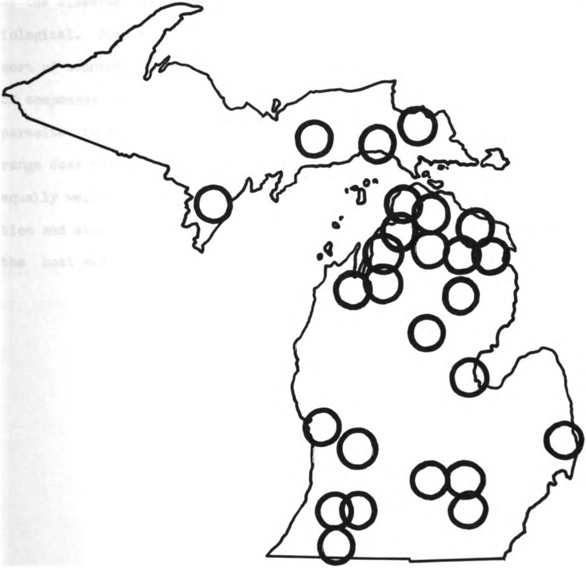
* the nomenclature in the dissertation is based on Fernald (1950)

plate 2

Approximate distribution of <u>Castilleja</u> <u>coccinea</u> (based on Pennell, 1935). (The outline map of North America is based on a Lambert azimuthal equal-area projection published by Rand McNally in Standard World Atlas, 1951.)

plate 3

Michigan collection sites, by county, of specimens of *Castilleja coccinea* entered in the herbaria of Michigan State University and the University of Michigan (state map from publication 49, Department of Conservation, Geological Survey Division, maps of the surface formations of the northern and southern peninsulas of Michigan).



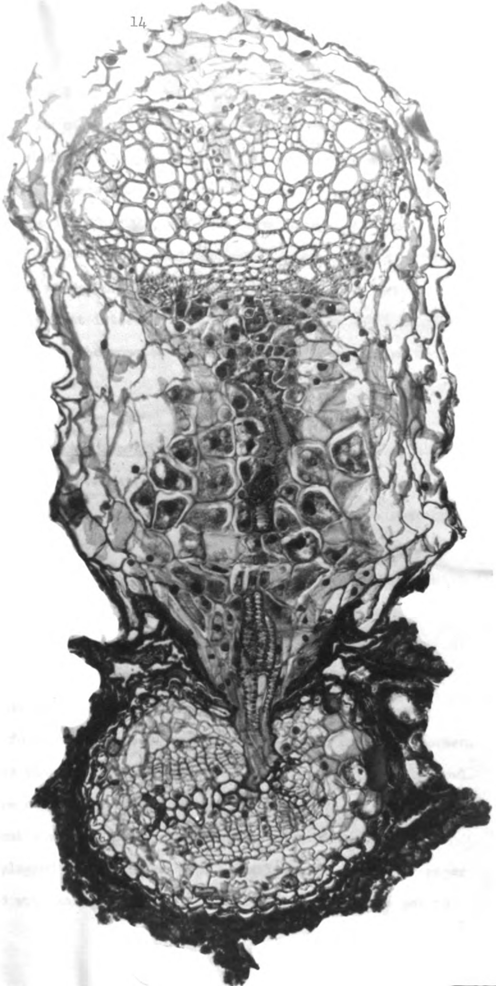
4 and 5, pages 12 and 14). Although the parasites are chlorophyllous and fully photosynthetic, to mature they must successfully penetrate foreign roots. This obligate root-parasitism of Castilleja coccinea is the topic of the dissertation. The approach to the problem is ecological and physiological. Some of the questions the study attempts to answer are 'What sort of connection does the parasite make with its hosts?', 'What kinds of compounds cross the haustoria?', 'Where in the life history of the parasite is the host-parasite interaction important?', 'How wide a host-range does the parasite have, and do various hosts support the parasite equally well?', 'Does the host-parasite interaction determine the distribution and abundance of the parasite in a community?', and 'What damage does the host suffer?'.

plate 4

Haustoria of _Castilleja_ _coccinea_ attached to host roots. The drawings were made from specimens ready for embedding and eventual sectioning (30X).

plate 5

Photomicrograph (270X) of a section through a haustorium of *Melampyrum lineare* parasitic on *Pinus banksiana*, jack-pine. Both the host root and the parasite root which produced the haustorium are in cross-section, but the vascular trace connecting the two is in longitudinal section. *Melampyrum lineare* is a green root-parasite closely related to *Castilleja coccinea*, and is common in jack-pine vegetation in Michigan.



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METHODS

parasite field growth data

A heavy population of Castilleja coccinea was selected early in 1960 for detailed field studies. The population lies south of Houghton Lake along both sides of old route US27 3.0 miles south of its junction with M55, in the NE¼ of section 33, T22N, R4W, Roscommon County, Michigan.

In April of 1961, 100 rosettes and seedlings of the Houghton population were marked with wooden or polystyrene pot labels. Their growth and maturation were recorded at intervals of about a month from April to September. The records included rosette diameter, height of flowering shoot, and degree of fruit ripeness.

pot culture

The studies of Castilleja coccinea nutrition, epicotyl growth, host-range, and host-parasite interaction required artificial culture of the parasite and its hosts under laboratory conditions. The culture technique used most often for these studies was simple pot-culture——given host plants to attack, Castilleja coccinea grows readily in flower pots in a greenhouse.

Preparation of the parasite seed and the hosts for pot-culture is time-consuming but demands no unusual equipment. Seed is collected by gathering ripe capsules in a polyethylene bag. The capsules later are broken open with forceps over a bowl covered with a piece of window screen. The screen excludes plant debris but allows the seeds to fall into the bowl. Potential hosts are dug up and potted in the field, brought into a greenhouse, and for about a week allowed to regenerate their damaged roots.
A 3½-inch pot is plugged at the bottom with a small square of blotter paper and nearly filled with sand. The sand is tamped lightly into the pot to

form a smooth surface. A host is washed free of soil and debris, and its roots are spread out on the surface of the sand. The roots then are covered with another quarter-inch of sand, and the pot is watered generously. Several hundred *Castilleja coccinea* seeds then are sprinkled evenly over the surface. The pot is placed in a greenhouse tray in half-inch-deep water. Germination begins in about four days, and the seedlings show signs of host-contact within two weeks.

For observation of the haustoria of pot-cultured parasites, the host and parasite simply are dumped from the pot and their tangled roots carefully washed free of sand in a bucket of water. The haustoria are seen best under a dissecting microscope because of their small size. If kept moist and handled carefully, a parasite and its host can be replanted successfully after being examined for haustoria.

germination

light requirement. (1) 500 *Castilleja coccinea* seeds were sprinkled into each of three 3½-inch pots covered with Petri dish bottoms wrapped in heavy aluminum foil. The foil of one Petri dish was left intact, but both of the others had a hole which allowed light to enter. One of the holes was 7 mm in diameter and the other was 45 mm. Both holes were cut close to the edge of the Petri dishes to accentuate any phototropic response of the seedlings. The set-ups are shown in plates 6-8 (pages 17-22).

(2) 100 *Castilleja coccinea* seeds were sprinkled into eight sand-filled 3½-inch pots with lids made from aluminum foil-covered plastic Petri dish tops. The pots were placed in standing water in a greenhouse and the per cent germination of the seeds recorded at two and four weeks. For controls, eight pots without lids were set up. Results are in table 2 (page 54).

[illegible]

plate 6

Demonstration of light requirement in the germination of the seeds of Castilleja coccinea. Five hundred seeds were sprinkled on the surface of the sand in the pot, and a foil-covered Petri dish bottom was placed over the pot to exclude all light. Only one seed had germinated after four weeks.


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[illegible]

plate 7

Effect of low light on the germination of seeds of _Castilleja coccinea_. A small hole cut in the foil of the pot-lid afforded a low level of light. In all, 15 out of 500 seeds germinated after four weeks, a germination level of 3%. All of the seedlings were markedly etiolated and showed a strong phototropic growth response.

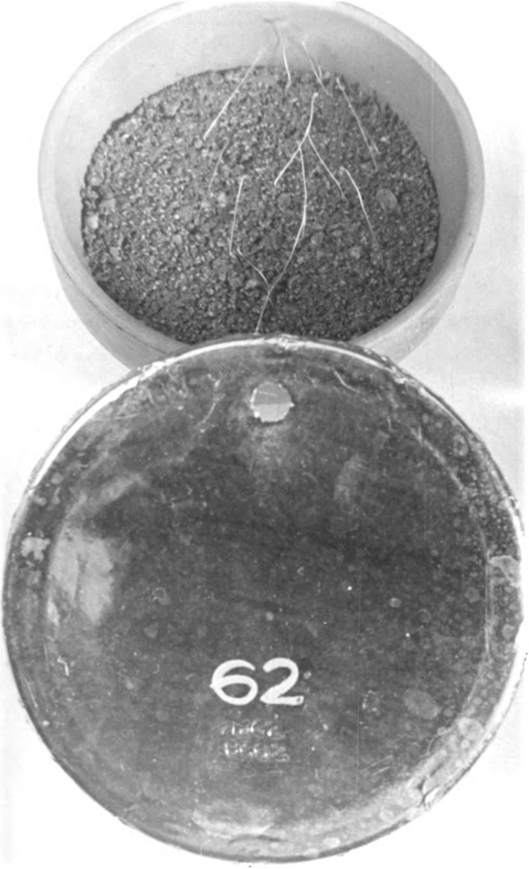
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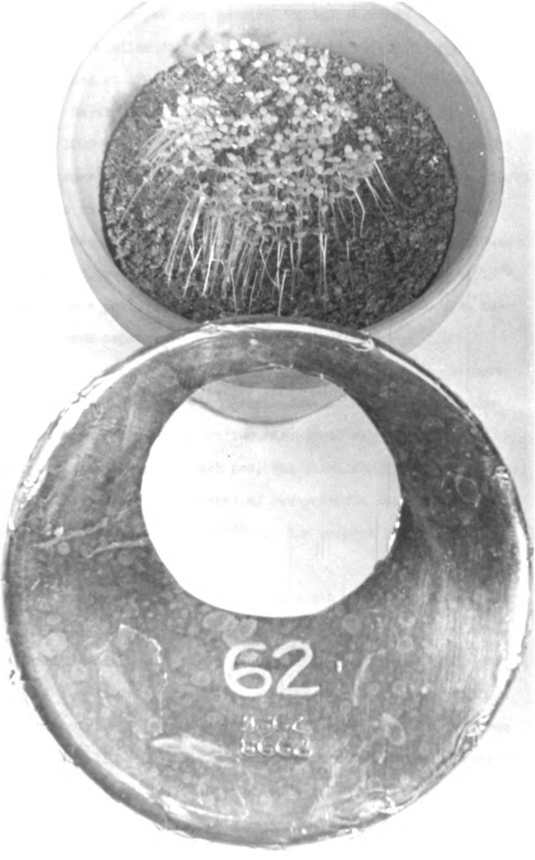
plate 8

Demonstration of normal germination level under a strong-light regime. The hole in the pot-lid was offset to accentuate the phototropic response of the seedlings. The germination level at four weeks was 65%.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(3) Five 100-seed lots were kept at 4°C on moist blotter paper in foil-covered Petri dishes. The per cent germination was recorded at two, four, and six weeks. Five similar dishes without the foil were set up as controls and illuminated at 4°C with a 40-watt incandescent bulb one foot from the dishes. Results are tabulated on page 54 (table 2).

stratification. 1000 Castilleja coccinea seeds were scattered on a moist disc of blotter paper in each of three Petri dishes. They then were placed for one month in a refrigerator held at 4°C. With the seeds still on it, each blotter disc was cut in half to make two lots of seeds. One lot was retained in the cold, but the other was placed in a cool greenhouse (15°-25°C). Plate 9 is a photograph of both lots of seeds from one dish two weeks after they were separated.

seed viability. Castilleja coccinea seeds of 0, 1, and 2 years age were sown on the surface of moist sand in 3½-inch pots in a greenhouse. The per cent germination was recorded at two, four, and eight weeks. 100 seeds of each age were tested in each pot, and three replicates were run. As well, five 100-seed lots were tested of two-year-old seedstock which had been stored at room temperature (20°C). The results of the trials are in table 2 (page 54).

hypocotyl growth and haustorium ontogeny

Plants of both Melampyrum lineare and Castilleja coccinea will parasitize host plants sandwiched between glass and blotter paper. Such a set-up is useful for studying the ontogeny of haustoria and the growth of the parasite hypocotyl. In plate 10 (page 27) is an exploded view of one of the sandwiches. All the materials in it are marketed in retail stores, and its construction is simple. A host which has been potted in the field

plate 9.

Demonstration of the effect of stratification on the germination level of seeds of <u>Castilleja coccinea</u>. A thousand seeds were placed on the blotter disc in the upper Petri dish. They were kept at 4°C for a month, then divided into two lots. The upper lot was retained in the cold but the lower was warmed to room temperature. Germination of the warmed seeds reached 87%, about a quarter again as high as the level of germination typical of seeds which have not been stratified. Germination does occur even at 4°C if light and moisture are sufficient, but the rate of germination is low —— about six weeks are required for the level of germination to reach 60%.


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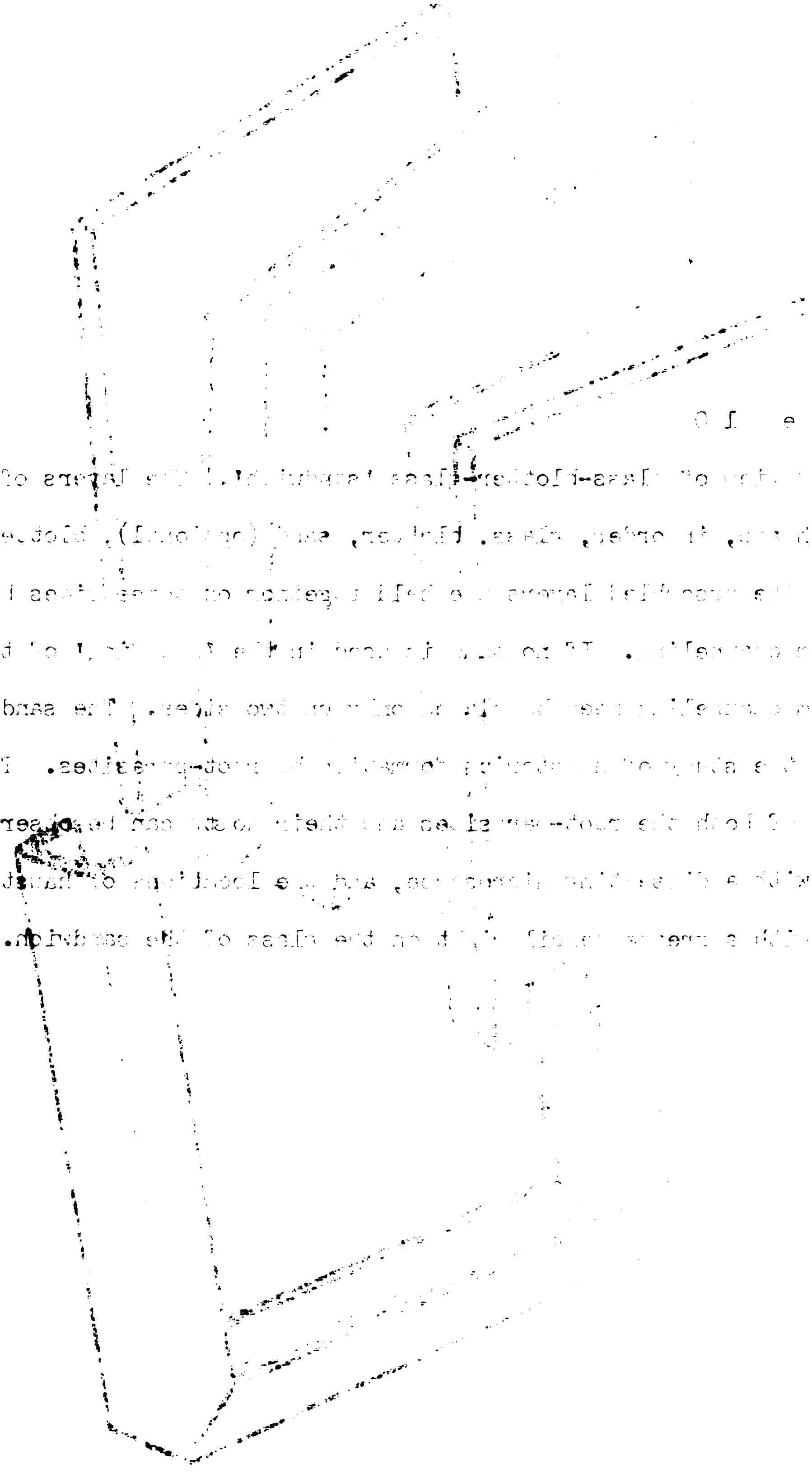
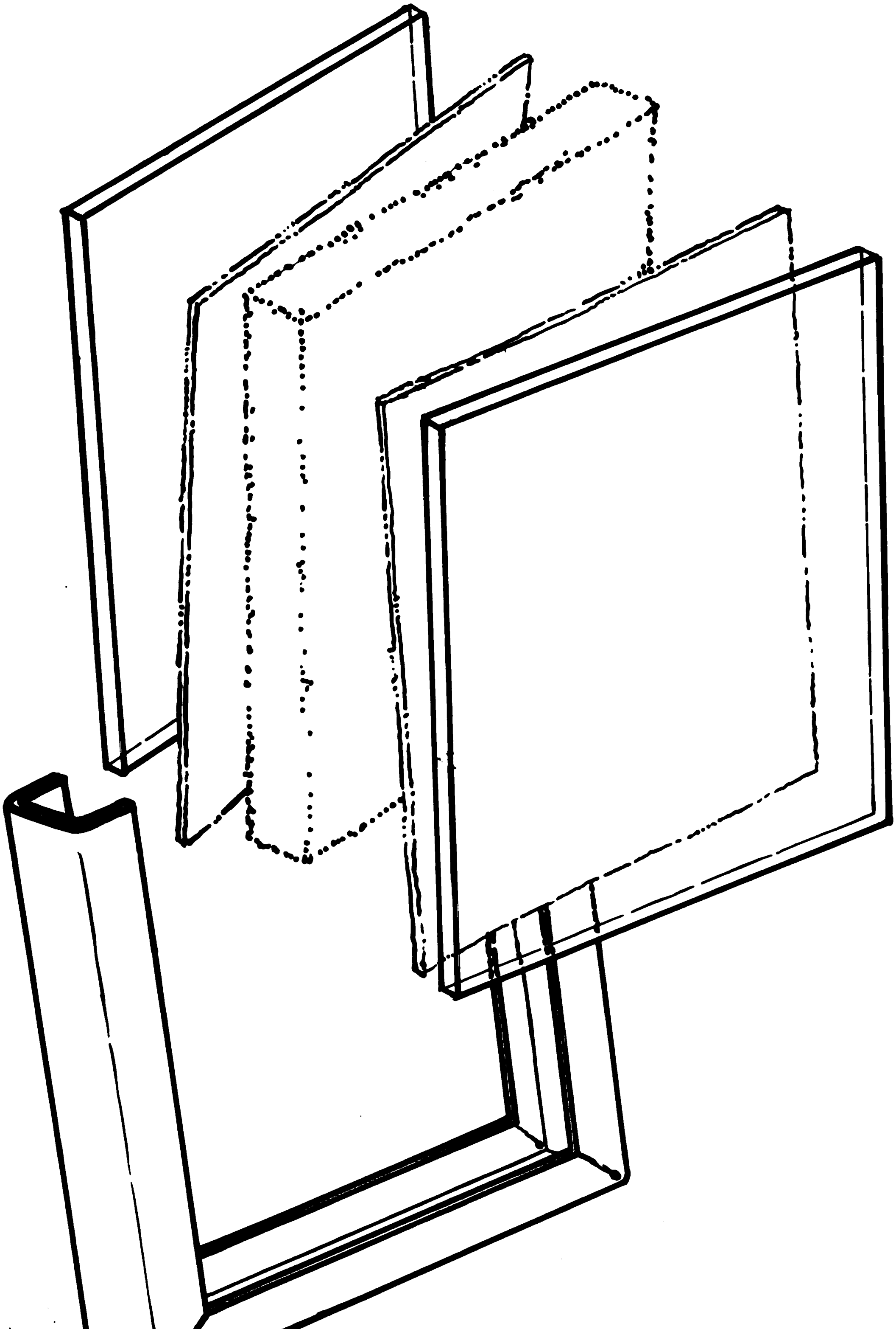


plate 10

Exploded view of glass-blotter-glass 'sandwich'. The layers of the sandwich are, in order, glass, blotter, sand (optional), blotter, and glass. The assembled layers are held together on three sides by aluminum channeling. If no sand is used in the 'stuffing' of the sandwich, aluminum channeling need be placed only on two sides. The sandwich is used in the study of haustorium formation by root-parasites. The root systems of both the root-parasites and their hosts can be observed easily with a dissecting microscope, and the locations of haustoria marked with a grease pencil right on the glass of the sandwich.



and allowed to regenerate its roots for a week is washed free of soil and debris, and its roots are flattened out on a four-inch square of blotter paper. Several of the roots are positioned along the top inch of the blotter. A four-inch square of double-weight glass then is placed over the roots to flatten them and hold them in position against the blotter. A second piece of glass is placed behind the blotter to form a sandwich with 'bread' of glass and 'stuffing' of the blotter and host. The sandwich is held together along its sides and bottom by half-inch-wide aluminum U-channeling (plate 11, page 30). The assembled sandwich must be about as thick as the interior width of the channeling. This width is made up by placing sand or more squares of blotter paper between the two pieces of glass. Large hosts are best accommodated in larger sandwiches, 6 or 8 inches on a side. The completed sandwich is placed upright in a tray of water in a greenhouse. After a few days, when the host roots are 'trained' to stay in position, the front piece of glass is removed and seeds of *Castilleja coccinea* sown as close as possible to the top of the blotter. The parasite seedlings must emerge from the top of the sandwich, which they can not do if they are placed far down on the blotter or if the sandwich is too tightly pressed together. After the seedlings are established, any disassembling of the sandwich must be done carefully, since the haustoria are minute and fragile. Also, debris-eating maggots often invade the sandwiches, and, in times of food stress, destroy the parasite seedlings. Such pests can be eliminated by 25% Malathion or some other insecticide.

epicotyl growth and host-range

The growth of individual rosettes was followed for several months by

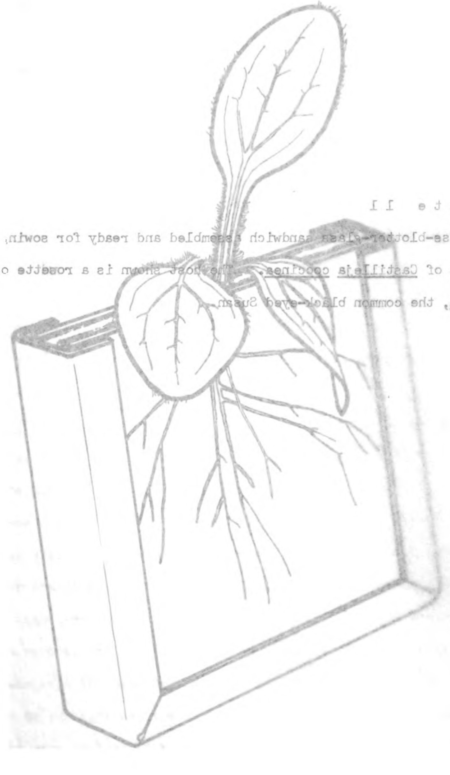
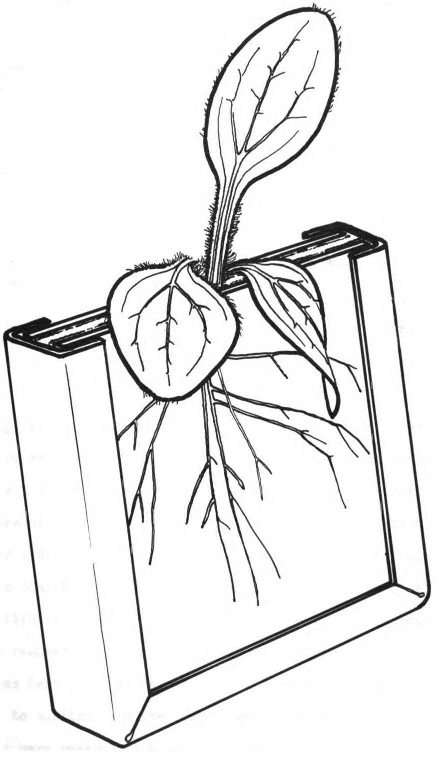


plate 11

A glass-blotter-glass sandwich assembled and ready for sowing with seeds of Castilleja coccinea. The host shown is a rosette of Rudbeckia hirta, the common black-eyed Susan.



labelling selected rosettes grown in pot-culture with various hosts. The rosettes were labelled with paper discs marked with a number and glued to the heads of insect pins pushed into the sand of the host-pot (plate 12, page 33). In all, 350 rosettes were studied in this way. Their growth was measured as centimeters of rosette diameter and number of foliage leaves. Later, height of the flowering shoot was recorded in centimeters.

physiology

<u>starch test for photosynthesis</u>. Leaves of <u>Castilleja</u> <u>coccinea</u> to be tested for their starch content were killed in boiling water and leached of their chlorophyll in boiling ethanol. They were then immersed in boiling water again, and finally soaked in iodine-potassium iodide solution for five minutes. The test-solution was made up of 15 g of potassium iodide in a liter of distilled water, to which 5 g of crystalline iodine was added. A positive reaction was the formation of an intense blue complex of starch and iodine.

<u>starch synthesis assay</u>. All the leaves used in the tests for starch synthesis were excised and floated on distilled water in darkness for 24 hours to deplete their food reserves. At the end of this quarantine period, the leaves were divided into four lots of ten leaves each. The first lot was tested for starch immediately. The second lot was placed in sunlight and tested for starch after six hours. The third was placed in a small beaker inside a tightly-closed jar containing a half-inch of 10% potassium hydroxide which removed the carbon dioxide from the atmosphere inside the jar. The jar was kept in total darkness for six hours, then exposed for another six hours to sunlight and the leaves tested for starch. The leaves of the fourth lot were smeared with petroleum jelly, refloated on distilled water

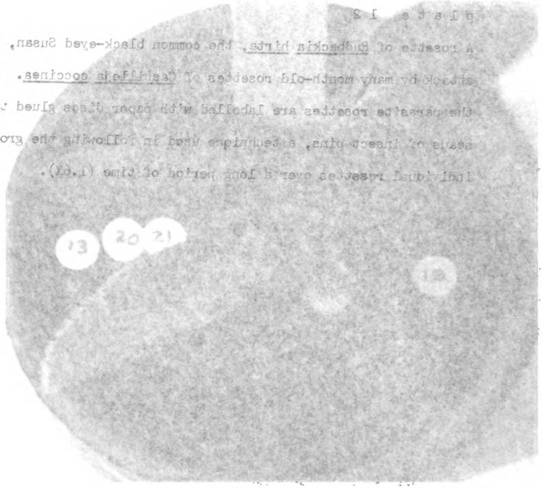
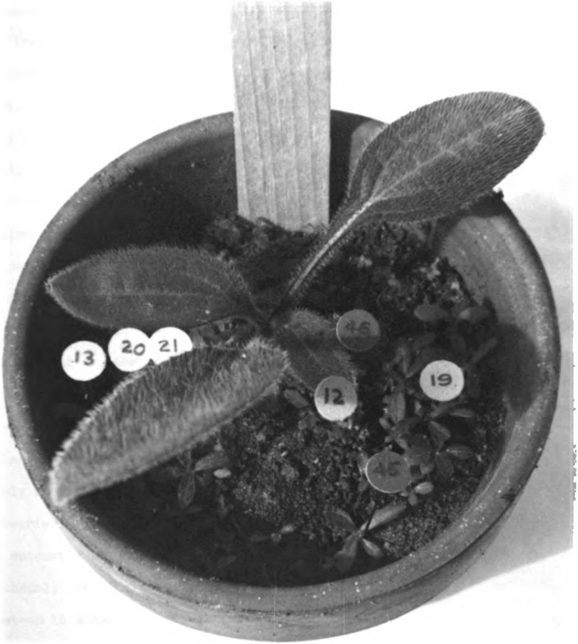

13
20
21
10

plate 12

A rosette of Rudbeckia hirta, the common black-eyed Susan, under attack by many month-old rosettes of Castilleja coccinea. Some of the parasite rosettes are labelled with paper discs glued to the heads of insect pins, a technique used in following the growth of individual rosettes over a long period of time (1.6X).



13
20
21
46
12
19
45

in a Petri dish, left in sunlight for six hours, and then tested for their starch content.

carbon fixation assay. One set of experiments was designed to test the ability of host-free Castilleja coccinea rosettes to fix carbon dioxide when illuminated. Castilleja coccinea rosettes of various ages were pulled free of their hosts, separated into four groups, and placed on moist blotter in four plastic Petri dishes. They were kept in darkness for twelve hours. All four groups then were given $C^{14}O_2$ for thirty minutes, but two groups were lighted and two retained in the dark. They then were weighed, killed, macerated, each pipetted as three aliquote into planchets, dried, and their activity estimated with a 1000-count.

Also an estimation was made of the level of photosynthesis of rosettes still parasitic on host plants. The several rosettes selected were parasitic on Lactuca canadensis in two pots. The bottom from a clear plastic Petri dish was placed over each pot, isolating the rosettes and their host inside a small volume of air. In the pot with the plants was a vial of $NaHC^{14}O_3$. An excess of acid was added to the bicarbonate with a medicine dropper stuck through a hole in the Petri dish bottom. Cellophane tape was placed quickly over the hole after the acid was added, and the whole assembly was placed in a hood under strong illumination behind a water heat-barrier. After 30 minutes, the host and the parasites were weighed in an automatic balance, killed in boiling distilled water, macerated in 95% ethanol, and their activity sampled with dried aliquots in planchets. The set-up is shown in plate 13 (page 36).

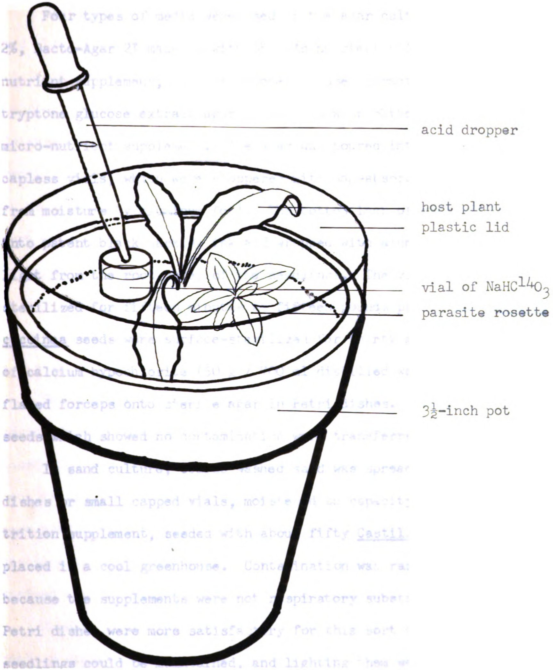
[illegible]

[illegible]

[illegible]

plate 13

Diagram of experimental set-up for assay of photosynthesis by *Castilleja coccinea* and its host, *Lactuca canadensis*.


acid dropper
host plant
plastic lid
vial of $NaHC^{14}O_3$
parasite rosette
3½-inch pot

nutrition supplements. The two techniques used for growing Castilleja coccinea in nutrition supplement experiments were aseptic agar culture and sand culture.

Four types of media were used in the agar culture ——— Bacto-Agar 2%, Bacto-Agar 2% made up with Shive's nutrient solution and a micro-nutrient supplement, Bacto tryptone glucose extract agar 2%, and Bacto tryptone glucose extract agar 2% made up with Shive's solution and a micro-nutrient supplement. The agar was poured into inch-by-three-inch capless vials, which were stoppered with non-absorbent cotton protected from moisture by aluminum foil. The bottom inch of each vial was dipped into patent black masking ink and wrapped with aluminum foil to exclude light from the root zones of the seedlings. The vials and agar then were sterilized for fifteen minutes at fifteen pounds pressure. Castilleja coccinea seeds were surface-sterilized for thirty minutes in a solution of calcium hypochlorite (50 g / 700 ml distilled water), and placed with flamed forceps onto sterile agar in Petri dishes. After four days, those seeds which showed no contamination were transferred to the vials.

In sand culture, coarse washed sand was spread into either Petri dishes or small capped vials, moistened to capacity with the proper nutrition supplement, seeded with about fifty Castilleja coccinea seeds, and placed in a cool greenhouse. Contamination was rarely a problem, simply because the supplements were not respiratory substrates. In general, the Petri dishes were more satisfactory for this sort of study, because many seedlings could be maintained, and lighting them was simplified. However, in sterile culture, Petri dishes were found to be useless, since contaminants invariably got in, apparently through the film of water which condensed on the top of the dish during the daytime. In the case of sand culture with distilled water, the sand used was acid-leached marine white sand.

host-range

Castilleja coccinea was grown from seed in pots with single hosts, and its success noted for each of the hosts. All the hosts were collected in the Houghton Lake study area, transported in 3½-inch pots to East Lansing, and placed in standing water in a greenhouse at Michigan State University. After a week, the host plants which did not survive potting were discarded, and the remaining hosts repotted in strained, washed sand in new 3½-inch pots plugged with blotter paper. Ten pots of each host were made up in this way, except in the cases of Lactuca canadensis, Fragaria virginiana, and Chrysanthemum leucanthemum, of which respectively 50, 20, and 20 pots were made up. In each of the host pots were sown over 500 seeds.

haustorium anatomy and ontogeny

The anatomy and ontogeny of haustoria, either field-collected or harvested from glass-blotter sandwiches, were studied by sectioning. Each haustorium was killed and fixed still attached to a portion of the host root it had attacked. The fixing solution used was FAA, and the specimens were readied for embedding by immersion in the standard tertiary butanol histologic solutions. The specimens were sectioned in rubber paraffin, stained with safranin and fast green, and mounted serially in balsam.

Haustoria of known age were harvested by growing the parasite and its host in glass-blotter sandwiches (see page 27), locating the incipient haustoria with a dissecting microscope, noting the location and date right on the glass of the sandwich with a grease pencil, and later disassembling the sandwich to collect haustoria of various ages.

host-requirement

The host-requirement in epicotyl growth of Castilleja coccinea was established in two ways ——— by growing hundreds of seedlings in pots with and without host plants, and by divorcing already-established rosettes from their hosts.

In the first technique, all the pots used in the experiments were new, and the sand in them was strained and washed to exclude all plant debris.

In the second technique, both greenhouse-grown and field-collected rosettes were used. In both cases, the rosettes were teased away from their hosts gently, and repotted alone in strained, washed sand in new pots.

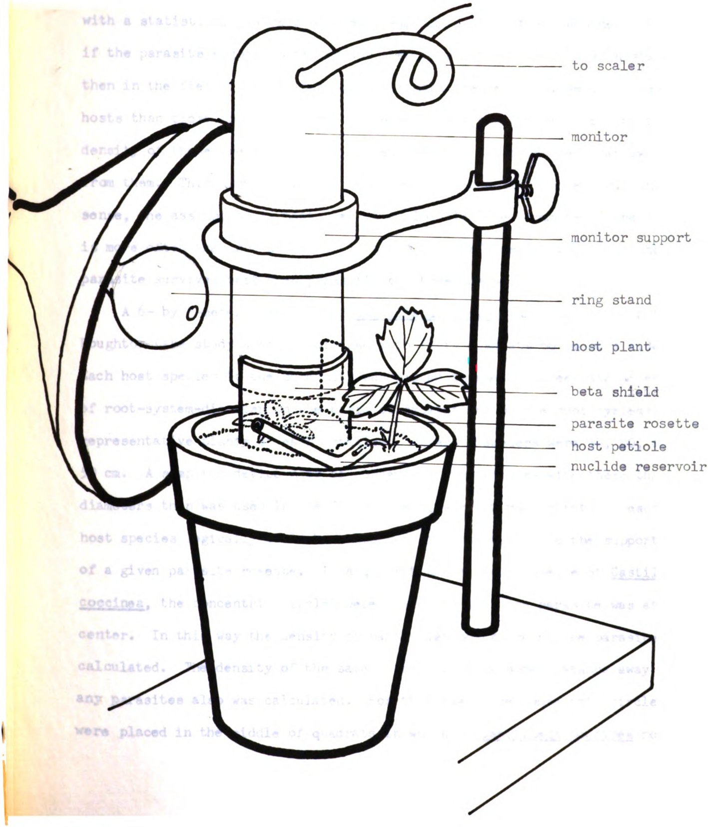
haustorial uptake

Transfer of eosin Y and labelled phosphate, sulfate, and fructose across the haustoria was done with potted specimens grown from seed in the greenhouse. The hosts were either Fragaria virginiana or Antennaria neglecta. In all the transfer experiments, the substance to be tested was introduced into the host or parasite simply by dipping a cut leaf blade or petiole into a solution of the substance for about a half hour. Transfer was detected by a color change in the case of eosin Y, and by beta emission in the case of the labelled compounds. In transfer of the labelled compounds, because the source of nuclide was in the same pot with the plant being monitored, a particle shield was set up around the plant and the Geiger-Müller tube. The experimental arrangement is diagrammed on page 41 (plate 14).

[illegible]

plate 14

Drawing of experimental set-up for the transfer of labelled compounds between *Castilleja coccinea* and its hosts.


to scaler
monitor
monitor support
ring stand
host plant
beta shield
parasite rosette
host petiole
nuclide reservoir

parasite micro-distribution

The correlation between the various hosts' ability to support Castilleja coccinea and the field distribution of Castilleja coccinea was tested with a statistical analysis of field sampling data. It was assumed that if the parasite enjoys better growth when parasitic on particular hosts, then in the field it would tend to occur more frequently close to those hosts than close to any others. Conversely, one could expect to find the density of those preferred hosts higher close to the parasites than away from them. This assumption was the base of the sampling procedure. In a sense, the assumption is that the act of locating the parasite in the field is more often than not an act of locating the preferred hosts, since the parasite survives best when parasitic on those hosts.

A 6- by 3-meter community of Castilleja coccinea and its hosts in the Houghton Lake study area was divided into fifty quadrats 60 cm on a side. Each host species in the community was placed in one of three categories of root-system-diameter on the basis of excavations of the root systems of representative plants of each species. These diameters were 30, 40, and 50 cm. A sampling device made of concentric wire circles with these three diameters then was used in the field to estimate how many plants of each host species logically could be suspected of contributing to the support of a given parasite rosette. If a quadrat contained a rosette of Castilleja coccinea, the concentric circles were placed so that the parasite was at the center. In this way the density of particular hosts around the parasite was calculated. The density of the same hosts occurring some distance away from any parasites also was calculated. For this task, the concentric circles were placed in the middle of quadrats in which no Castilleja coccinea rosette occurred. The statistical analysis involved testing for a significant difference between the density of each host near a rosette and its density away from a rosette. All of the fifty quadrats were used. 22 had no Castilleja coccinea rosettes, while 28 did. The test used was based on the t distribution. A sample calculation is given on page 44.

STATISTICAL COMPARISON OF DENSITY OF *LOBELIA SPICATA* CLOSE TO AND DISTANT FROM *CASTILLEJA COCCINEA*.

Number of *Lobelia* plants present in each of 28 quadrats in which *Castilleja coccinea* also was present ——— 12, 5, 3, 3, 6, 4, 5, 7, 8, 3, 2, 4, 3, 3, 1, 1, 6, 3, 6, 0, 0, 0, 0, 0, 0, 0, 0, 0. Number of *Lobelia* plants present in each of 22 quadrats in which *Castilleja coccinea* was absent ——— 1, 4, 8, 1, 1, 5, 3, 1, 7, 1, 6, 5, 4, 1, 0, 0, 0, 0, 0, 0, 0, 0.

X_1 *Castilleja* present		X_2 *Castilleja* absent	
ΣX	$= 85$	ΣX	$= 48$
ΣX^2	$= 507$	ΣX^2	$= 246$
$(\Sigma X)^2 / n$	$= 258.0357$	$(\Sigma X)^2 / n$	$= 104.7272$
$\bar{X}$	$= 3.0357$	$\bar{X}$	$= 2.1818$

$$s_x^2 = (507 - 258.0357 + 246 - 104.7272) \ / \ (48) = 8.1299$$

$$s^2_{\bar{x}_1 - \bar{x}_2} = 8.1299 \ (1/28 + 1/22) = 0.6599$$

$$s_{\bar{x}_1 - \bar{x}_2} = \sqrt{0.6599} = 0.8123$$

$$t = (3.0357 - 2.1818) \ / \ (0.8123) = 1.0512$$

$$t_{97.5(d.f.48)} = 2.011 \text{ (Hald, 1952)}$$

$2.011 > 1.0512$, therefore the density of *Lobelia* close to *Castilleja* is not significantly different from what it is distant from *Castilleja*.

RESULTS

THE PARASITE

ontogeny

An ovary of a Castilleja coccinea plant produces up to 300 seeds, each less than 1 mm in length (plates 15 and 16, pages 47 and 49). The fruits of the adult plant are two-loculed capsules, from which the seeds are shaken by wind or passing animals. The seeds are light, averaging 0.056 mg in weight, and they scatter readily.

Castilleja coccinea in the Houghton Lake area typically is biennial in its development. In the laboratory the seeds will germinate at the time of capsule dehiscence, but in the field the bulk of germination occurs in the spring. Maturation of the rosettes usually is complete by the end of the summer. The rosettes bolt the following spring, often very early. In greenhouse culture, they do not require a period of cold to bolt, which suggests that in the field they may behave occasionally as annuals and bloom in their first season of growth. Tabulated on pages 50-52 (table 1) are the results of a field study of 100 rosettes and seedlings in the Houghton Lake area. The data include rosette diameter, height of flowering shoot, and degree of fruit ripeness.

germination

Germination of the seeds requires moisture and light. Seeds placed on the well-lighted surface of a water-retentive material will germinate in four to seven days at room temperature. Thus for adequate storage, the seeds must be kept dry.

Germination is precluded in darkness, but the light requirement is met with low light intensity. However, seedlings kept in low light are

plate 15

Seeds of _Castilleja_ _coccinea_ (90X).

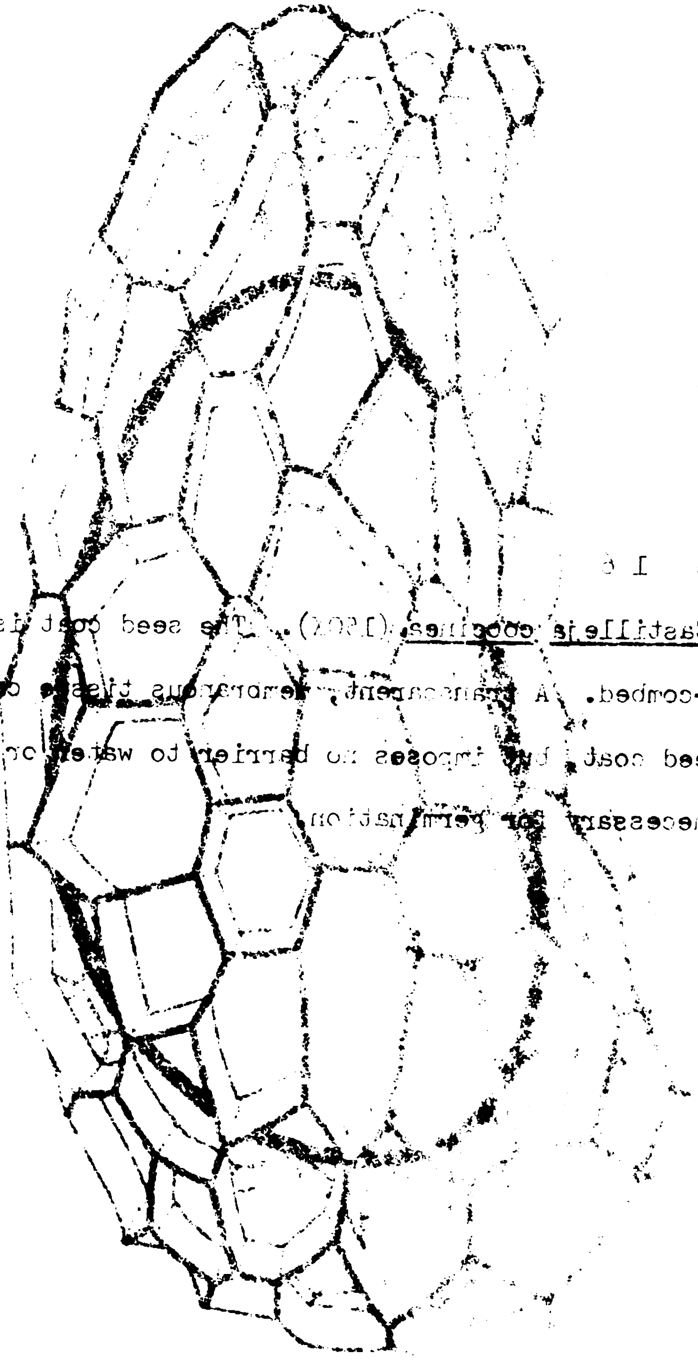


plate 16

Seed of Castilleja coccinea (150X). The seed coat is cellular-favose, or honey-combed. A transparent, membranous tissue covers the 'windows' of the seed coat, but imposes no barrier to water or light, two of the factors necessary for germination.

table 1

GROWTH DATA OF A *CASTILLEJA COCCINEA* POPULATION IN THE HOUGHTON LAKE AREA

s ——— seedling (epicotyl spread less than 0.5 cm)

r ——— rosette (0.5 cm or more in diameter)

e ——— bolting rosette

f ——— flowering or fruiting

The numbers after r are rosette diameters in centimeters, and after e are heights of flowering shoots in centimeters. The numbers 1-3 after f indicate (1) flowers largely immature, but at least one open, (2) about half the inflorescence has ripening capsules, but some corollas are still intact, (3) all the corollas are withering, and most of the capsules are ripe.

date tag	5.20.61	6.17.61	7.3.61	7.18.61	8.3.61	9.6.61
1	e3	e20f2	e21f3	f3	f3	f3
2	e8	e20f3	e19f3	f3	f3	f3
3	e1	e25f3	e25f3	f3	f3	f3
4	r2	r2	r2	r2	r2	r2
5	e2	missing	dead r2	dead r2	dead r2	dead r2
6	e7	e21f3	e20f3	f3	f3	f3
7	e4	e16f3	e16f3	f3	f3	f3
8	e2	e10f1	e12f2	f3	f3	f3
9	e3	e14f1	f3	f3	f3	f3
10	r1	e5	e11f1	e12f2	f3	f3
11	r3	r3	r4	r5	r5	r6
12	r2	r3	r4	r4	r4	r4
13	s	r½	r1	missing	missing	missing
14	e2	e9f1	e9f3	f3	f3	f3
15	e7	e18f3	e18f3	f3	f3	f3
16	e6	e22f3	e22f3	f3	f3	f3
17	e4	e19f3	e19f3	f3	f3	f3
18	s	r1	r1	missing	missing	missing
19	e9	e25f2	e25f3	f3	f3	f3
20	s	r½	r1	r1	r1	r1
21	s	s	r½	dead	missing	missing
22	s	r1	r2	r2	r2	r2
23	s	missing	missing	missing	missing	missing
24	e6	e8f3	f3	f3	f3	f3
25	s	s	r½	r½	r½	missing

table 1 (continued)

date tag	5.20.61	6.17.61	7.3.61	7.18.61	8.3.61	9.6.61
26	e5	e17f1	e17f3	f3	f3	f3
27	e3	e12f1	e12f3	f3	f3	f3
28	e8	e12f2	e15f3	f3	f3	f3
29	r1	r1	r2	r2	r2	r2
30	e3	e3	e4f1	f3	missing	missing
31	r½	r½	r1	r1	r1	r1
32	e2	e4f1	e5f2	e5f2	missing	missing
33	e2	e10	e10f1	f3	f3	f3
34	s	r½	r½	missing	missing	missing
35	s	r1	r2	r2	r2	r3
36	r2	r2	r2	r2	r2	r2
37	s	r1	r2	r1	r1	r2
38	s	r½	r1	r1	dead r1	missing
39	s	r½	r1	r1	r1	r1
40	s	s	s	missing	missing	missing
41	r1	e1	e1	e1	dead e2	missing
42	r3	r3	r3	r3	r3	missing
43	e4	e12f1	e13f3	f3	f3	f3
44	e6	e23f1	e25f3	f3	f3	f3
45	e3	e11f1	e10f3	f3	f3	f3
46	e4	e14f1	e20f2	e20f3	f3	f3
47	e4	e19f1	e25f2	f3	f3	f3
48	e12	e28f2	e28f3	e27f3	f3	f3
49	e4	e9	e17	broken	f3	f3
50	e6	e18f2	e18f3	f3	f3	f3
51	e3	e14f2	e13f3	f3	f3	f3
52	e3	e16f1	e16f3	f3	f3	f3
53	e4	e16f3	e16f3	f3	f3	f3
54	e1	e1	e1	necrotic	missing	missing
55	e8	e26f1	e27f2	e33f1	f2	f3
56	e6	e28f1	e35f2	e32f2	f3	f3
57	e10	e25f3	e28f2	e32f2	f3	f3
58	e4	e15	e27f1	e30f2	f3	f3
59	e10	e24f3	e24f3	f3	f3	f3
60	r1	missing	missing	missing	missing	missing
61	e4	e13f2	e12f3	f3	f3	f3
62	e10	e22f1	e22f1	e24f2	f3	f3
63	e4	e21f1	e22f3	f3	f3	f3
64	e2	e11f1	e11f2	f3	f3	f3
65	e5	e22f2	e22f3	f3	f3	f3

table 1 (concluded)

date tag	5.20.61	6.17.61	7.3.61	7.18.61	8.3.61	9.6.61
66	e5	e24f2	e25f3	f3	f3	f3
67	e8	e24f2	missing	missing	missing	missing
68	e3	e17f1	e17f1	f3	f3	f3
69	e6	e17f3	e18f3	f3	f3	f3
70	e4	e16f2	e17f3	f3	f3	f3
71	e4	e17f2	e17f3	f3	f3	f3
72	r2	r2	r2	r2	r2	r2
73	e1	e1	e2	r2	r2	r2
74	r1	r2	r3	r3	r3	dead
75	r2	r2	r3	r3	r3	r3
76	r4	e7	e20f1	e21f2	f3	f3
77	r3	r4	r5	r5	r5	r5
78	r3	r3	r4	r5	r5	r5
79	r1	r1	r2	r2	r2	r2
80	r3	r3	r3	r3	r3	dead
81	s	r½	missing	missing	missing	missing
82	s	r½	r1	missing	missing	missing
83	s	r½	r1	missing	missing	missing
84	s	s	missing	missing	missing	missing
85	r2	r2	r2	missing	missing	missing
86	r2	r2	r3	r3	r3	r3
87	r2	e8	e20f1	e22f2	f3	f3
88	r3	e3	e4	missing	missing	missing
89	r2	e2	e2	dying	dead e2	dead e2
90	r2	r2	r2	r2	r2	r2
91	r1	r1	r1	r1	r1	r2
92	r2	r2	r2	r2	r3	r3
93	r1	r1	r1	missing	missing	missing
94	e2	e8f1	e5	f3	missing	missing
95	e2	e8f1	e10f2	f3	f3	f3
96	e1	e8f1	e11f2	f3	f3	f3
97	e3	e12f3	e20f1	f3	f3	f3
98	e3	e13f1	e15f3	f3	f3	f3
99	e3	e12f2	e13f3	f3	f3	f3
100	e10	e17f3	e17f3	f3	f3	f3

markedly etiolated. In table 2 (page 54) are compared the germination trials of illuminated and darkened seeds. The set-ups are shown in plates 6 -8 (pages 17-22).

Lighted and moistened seeds will germinate at 4°C, but only at a low rate. However, seeds chilled moist for one month and then warmed and lighted germinate both more quickly and at a higher per cent than do seeds which have not been so stratified. However, it must be emphasized that seeds collected in mid-summer germinate at a 70% level without cold treatment, and therefore it can not be said that stratification is a germination requirement, even though it does increase both the rate and the level of germination.

Tabulated on page 54 (table 2) are the results of germination trials in the greenhouse of seeds of 0-2 years age stored dry at 4°C and of two-year-old seeds stored dry at room temperature. Seeds kept dry at either temperature suffer little decline in viability over a period of two years.

table 2

RESULTS OF GREENHOUSE TRIALS OF SEED-GERMINATION AND SEEDLING-SURVIVAL

seed age	date collected	storage	treatment	seeds / pot	replicates	average per cent germination			
						2 weeks	4 weeks	6 weeks	8 weeks
2 davs	13 July 1962	4°C	greenhouse**	100	3	61	63		57
345 days	3 August 1961	4°C	greenhouse	100	3	32	46		38
738 days	3 July 1960	4°C	greenhouse	100	3	70	65		51
738 days	3 July 1960	20°C	greenhouse	100	5	62	55		42
2 days	13 July 1962	20°C	darkened	100	1	0	0.3		
2 days	13 July 1962	20°C	greenhouse	100	1	50	70		
24 days	13 July 1962	20°C	darkened	100	8	0	1.3		
24 days	13 July 1962	20°C	greenhouse	100	8	59	65		
3, 31 days*	13 July 1962	20°C	darkened, moist, 4°C	100	5*	0	0	0.7	
3, 31 days*	13 July 1962	20°C	lighted, moist, 4°C	100	5*	0.5	12	59	
31 days	13 July 1962	20°C	darkened, moist, 4°C for 4 weeks, then lighted and warmed to 20°C	1000	3	0	0	87	

* the replicates were run at different times

** 15°-38°C diurnal temperature range, approximately a 16-hour day

hypocotyl growth

Three or four days after Castilleja coccinea seeds are moistened, lighted, and warmed, the cotyledons turn green and can be seen easily through the seed coat. On the fourth or fifth day, the elongating radicle of the embryo pushes through the seed coat, usually bending in a strong negative geotropic response (plate 17, page 57).

The seedling hypocotyl continues to elongate steadily after germination. Roots of greenhouse seedlings grown under glass often reach a length of 5 cm. The root system of these seedlings consists of a very few slender roots sparsely branched and growing almost vertically downward. Under greenhouse conditions, growth continues for up to four months without benefit of host-contact.

The apex of the radicle of the germinating embryo is conical and subtended by a prominent collar of root hairs up to 0.5 mm long (plate 17, page 57). As the radicle elongates, root hairs are produced behind its apex (plate 18, page 59), but not so copiously as on the roots of many autotrophic plants. These subapical root hairs are seen readily on seedlings grown behind glass. After extensive contact with a host has been made, the root apices die back to the most distal haustorium, and many root hairs are lost.

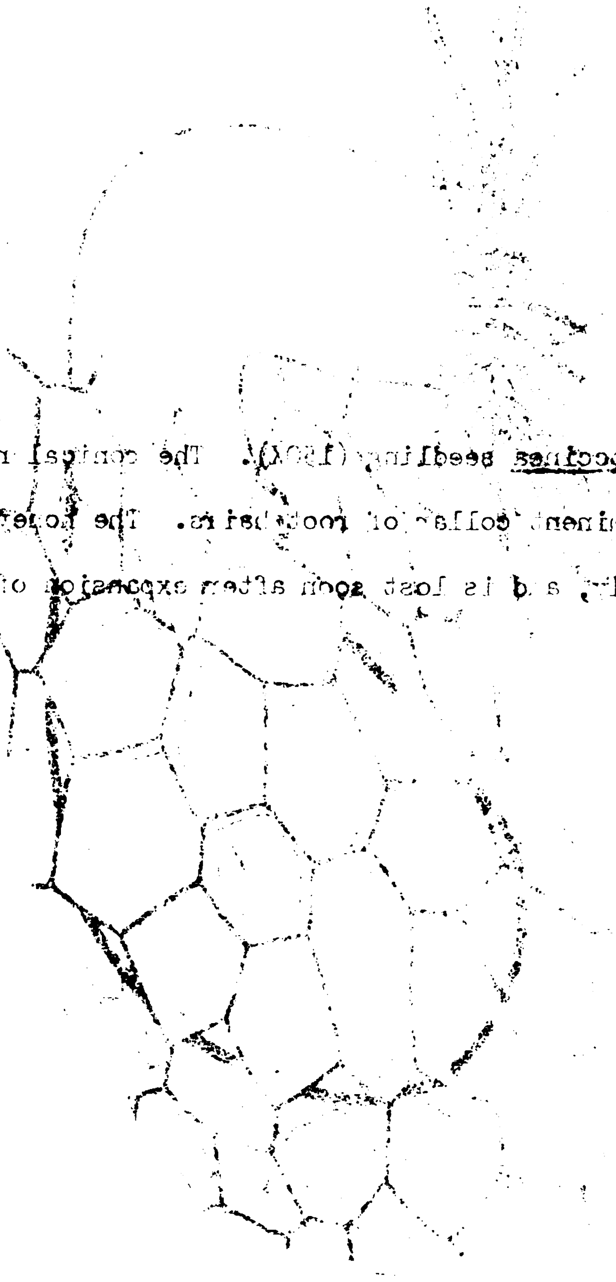


plate 17

Young <u>Castilleja</u> <u>coccinea</u> seedling (150X). The conical radicle is subtended by a prominent collar of root hairs. The honey-comb seed coat ruptures easily, and is lost soon after expansion of the cotyledons.

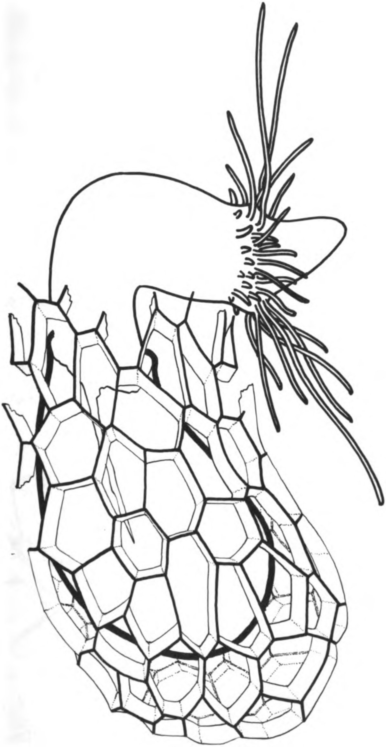
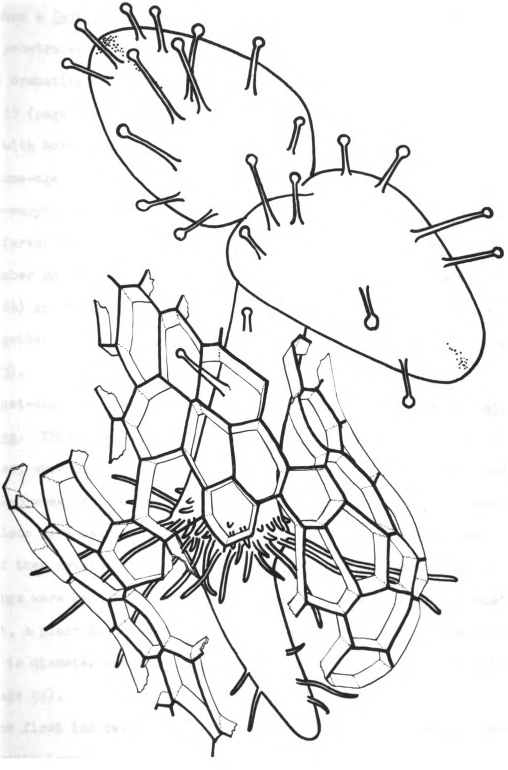


plate 18

Castilleja coccinea seedling with expanded cotyledons (150X). The glandular multicellular trichomes abundantly clothe the cotyledons, leaves, stem, and flowers of the plants. Root hairs are produced initially in a collar on the hypocotyl at the 'soil-line', but later are produced just behind the apex of each root tip.



epicotyl growth

When a Castilleja coccinea seedling contacts a host root and successfully penetrates to the vascular stream, the foliage leaves of the seedling expand dramatically, often doubling in length in 24 hours. Graphed in plate 19 (page 62) are the growth curves of three rosettes grown behind glass with host plants.

Same-age rosettes, even those parasitic on the same host plant, have widely-varying growth rates. The growth curves of three rosettes parasitic on different hosts are drawn in plate 20 (page 63), with time graphed against the number and the length of the foliage leaves. As well, drawn in plate 21 (page 64) are the growth curves of three groups of rosettes, each group growing together in one pot and parasitic on only one host plant (see plate 12, page 33).

Host-contact is essential for maturation of the rosettes of Castilleja coccinea. This assertion is based on two lines of evidence. (1) Immature rosettes, when divorced from their hosts, fail to mature further, and die after 2-3 weeks of apparent inactivity. (2) Some four thousand seedlings in 12 lots have been kept without hosts on a variety of substrates, and none of them developed more than five foliage leaves. About 500 of the seedlings were kept host-free for as long as four months. Given host-contact, a plant in those four months normally would have formed a rosette 2-3 cm in diameter and made up of 10-15 leaves about 15 mm in length (plate 29 , page 95).

The first indication of bolting is the production of mitten leaves. The rosette leaves are always entire, while those of the flowering shoot are single or double mittens, often deeply lobed (plate 22, page 66).

Greenhouse-grown rosettes readily flower without cold-treatment, usually by the sixth month after sowing. Thus cold-treatment is not an important factor in triggering bolting of mature rosettes.

The bolting shoot soon produces flowers and the showy colored bracts of the inflorescence (plates 23 and 1, page 68 and frontispiece). The inflorescence is indeterminate in its growth pattern, and 5-20 flowers are produced on a typical shoot. Flowering in the Houghton Lake area begins in early May, and continues into September, with the peak in late June. The capsules ripen from June through September, with the peak in mid-July. However, dehiscence of the abaxial locule of the capsule is delayed as much as a month, a mechanism which disseminates seeds throughout the growing season.

The parasitic attachment is not required for bolting and flowering once the rosette has reached maturity. Tabulated on page 69 (table 3) are the heights of flowering shoots of 30 mature rosettes, half of them teased free of their hosts and repotted, and half of them left undisturbed in the small blocks of vegetation they were collected in. The difference in mean height between the two lots of rosettes is no greater than that expected by chance, as tested with the t distribution.

Although the rosettes can flower detached from their hosts once they reach maturity, there is no evidence that they are detached or otherwise isolated from their hosts during their normal flowering period. Eosin transfer experiments in the greenhouse are as successful with bolting rosettes as with young rosettes, and in histologic section the haustoria of adult plants in the field appear functional.

plate 19

Growth of Castilleja coccinea rosettes in response to host-contact

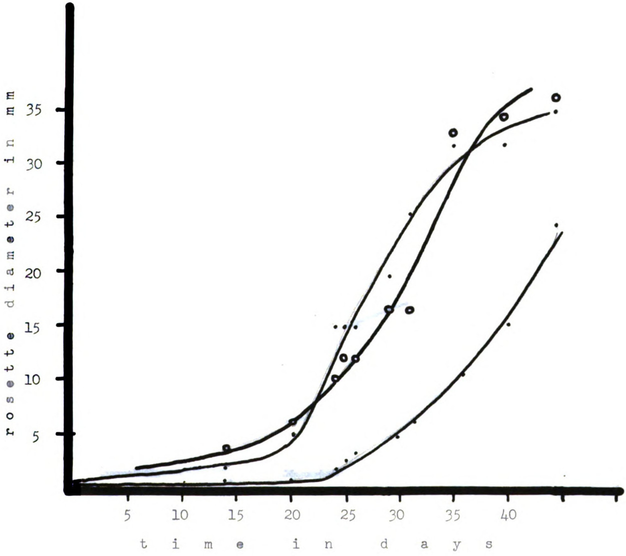
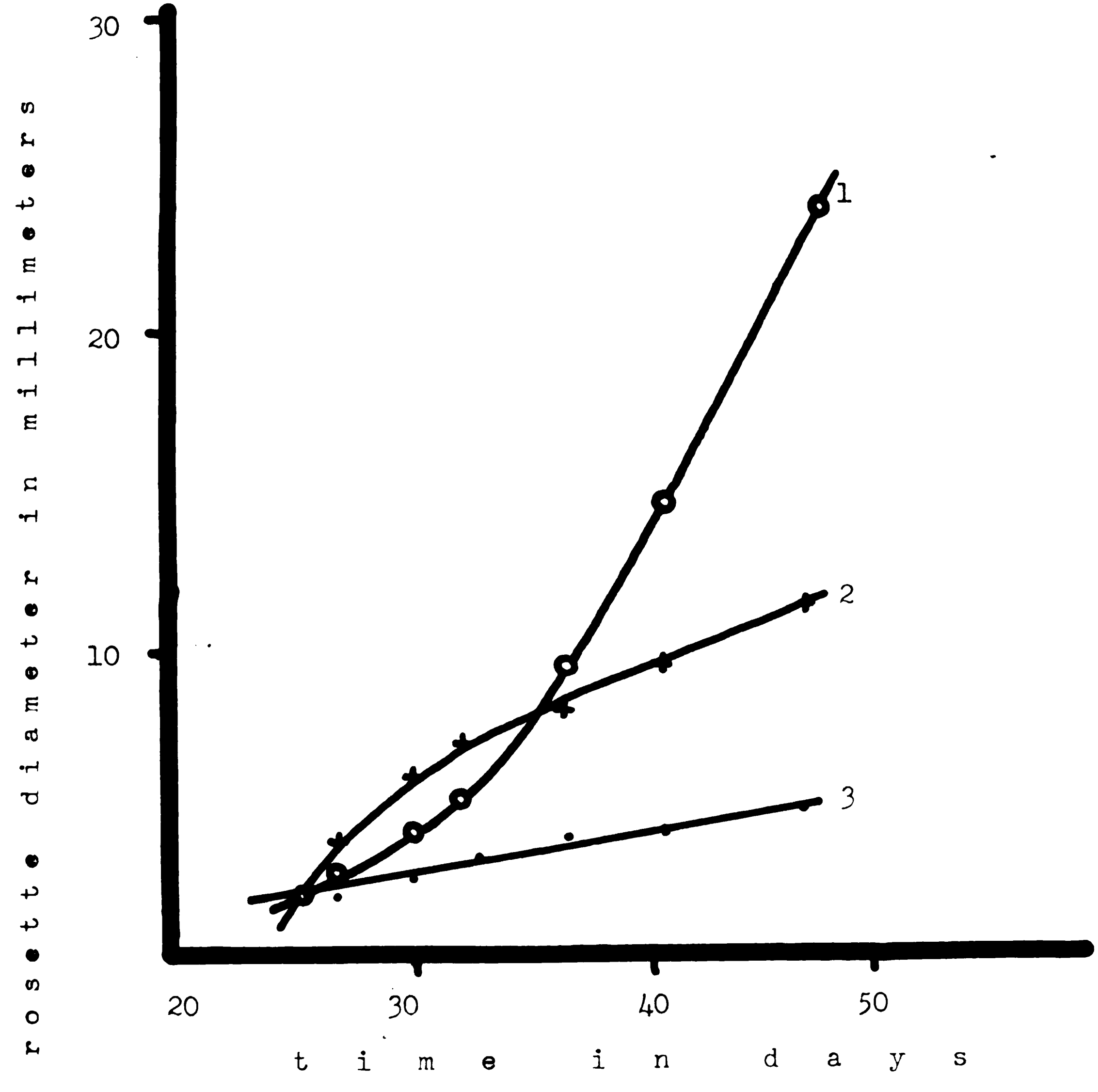


plate 20

Growth curves of three greenhouse-grown Castilleja coccinea rosettes parasitic on different hosts.



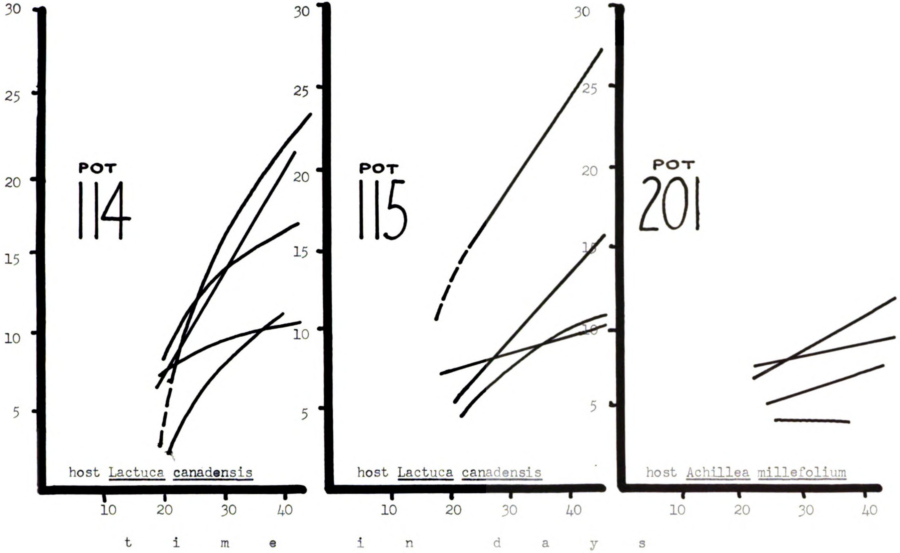
1 parasitic on Hieracium aurantiacum

2 parasitic on Lactuca canadensis

3 parasitic on Antennaria neglecta

p l a t e 21

Growth curves of three groups of rosettes parasitic on single hosts.



[illegible]

plate 22

Five _Castilleja_ _coccinea_ rosettes parasitic on _Krigia_ _biflora_, which has died back to only two leaves in the center of the pot. The largest rosette is bolting and producing the lobed 'mitten' leaves of the flowering shoot. All the rosettes are the same age, about six months, but are of markedly different size. Initially 600 seeds were sown in the host-pot, and over 100 seedlings survived their first month. Those which failed to attack the host eventually died (1.8X).

124

[illegible]

plate 23

Bolting rosette of _Castilleja_ _coccinea_. The same rosette two weeks younger is pictured in plate 22 (page 66).


124
37

table 3

HEIGHTS OF FLOWERING SHOOTS OF NORMAL AND HOST-DIVORCED ROSETTES IN CM

normal	host-free
20	12
20	12
25	10
21	23
16	11
10	14
14	19
9	28
18	9
22	18
19	14
25	16
8	16
17	26
12	27

normal	host-free
$n = 15$	$n = 15$
$\Sigma X = 256$	$\Sigma X = 255$
$\Sigma X^2 = 4390$	$\Sigma X^2 = 4397$
$(\Sigma X)^2/n = 4369.0667$	$(\Sigma X)^2/n$ 4335.0000
$\bar{X} = 17.0667$	$\bar{X} = 17.0000$

$s_x^2 = 0.1452$

$s^2_{\bar{x}_1 - \bar{x}_2} = 0.01936$

$s_{\bar{x}_1 - \bar{x}_2} = 0.1391$

$t = 0.4794$

$t_{97.5(d.f.28)} = 2.048$ (Hald, 1952)

$0.4794 < 2.048$

difference in means not significant at 5% level

physiology

photosynthesis

Castilleja coccinea is a species of wet meadow and savannah vegetation. It rarely occurs in the shade of other plants. Its leaf mesophyll is undifferentiated and highly lacunar (plate 24, page 72). The epidermal cells of the leaf lie in a single layer, are conical, and lack a cuticle. Glandular, multicellular trichomes are abundant on the stem and on both leaf surfaces. Stomata also are plentiful——————their density averages about 29,700 per square centimeter (10 counts) on the lower (abaxial) leaf surface, and 4950 per square centimeter (10 counts) on the upper (adaxial) leaf surface.

On the basis of experimental evidence, Castilleja coccinea is photosynthetic. In one set of experiments, presence of starch in leaf tissue was considered a demonstration of photosynthetic activity. The evidence is that (1) starch is formed by seedlings and excised rosette leaves under strong illumination, but not in darkness, (2) seedlings and excised leaves illuminated in an atmosphere free of carbon dioxide synthesize no starch, and (3) illuminated seedlings and excised leaves synthesize no starch when their stomata are plugged with petroleum jelly (table 4, page 73).

Another set of experiments was designed to test the ability of host-free Castilleja coccinea rosettes to fix carbon dioxide when lighted. Castilleja coccinea rosettes of various ages were pulled from their host-pots, separated into four groups, and placed on moist blotter discs in plastic Petri dishes. They were kept in darkness for twelve hours. All four groups then were given $C^{14}O_2$ for 30 minutes during which two of the groups were lighted and two were retained in the dark. They then were killed and prepared for radioactive assay. The results are in table 5 (page 74). The darkened rosettes showed almost no increase over background, while the lighted rosettes showed substantial levels of radioactivity.

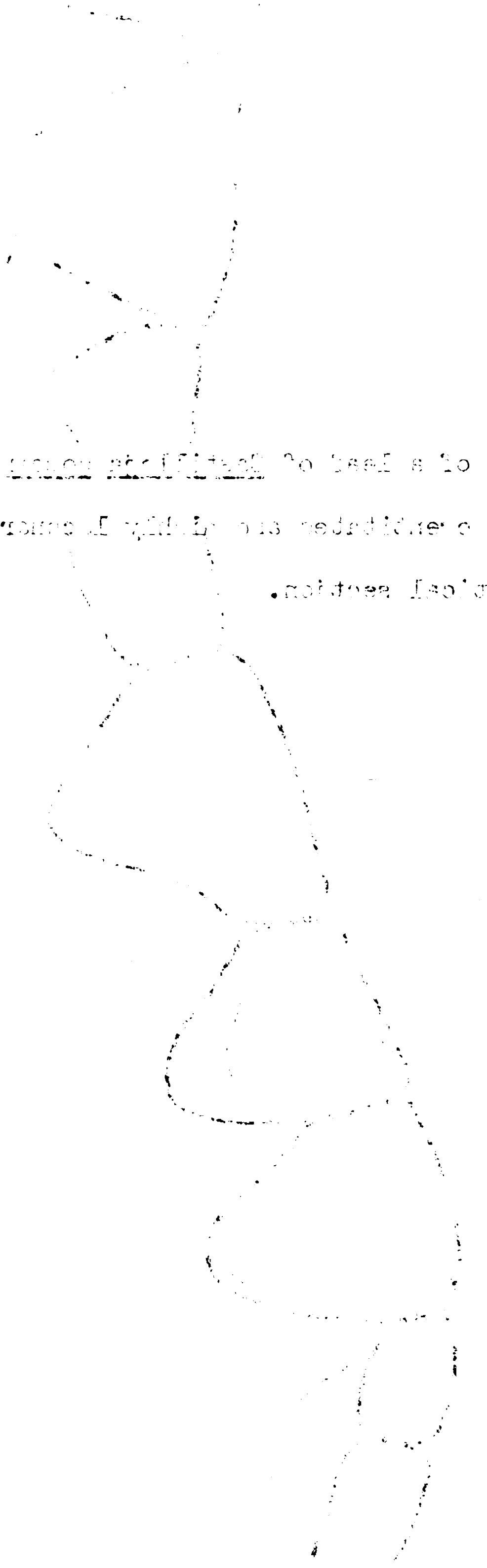


plate 24

Photomicrograph of a cross-section of a leaf of Castilleja coccinea (500X). The mesophyll of the leaf is undifferentitated and highly lacunar. The epidermal cells are conical in vertical section.

table 4

RESULTS OF TESTS FOR STARCH IN EXCISED LEAVES AND HOST-LESS SEEDLINGS OF CASTILLEJA COCCINEA

+ starch present

— starch absent

light regime	treatment	excised leaves	seedlings
daylight	none*	+	+
darkness	none	—	—
daylight	CO_2 excluded	—	—
daylight	stomata plugged	—	—

*all leaves were excised and floated on distilled water in Petri dishes in the dark for 24 hours before treatment.

t a b l e 5

Comparison of carbon-14 fixation by illuminated and darkened excised leaves of *Castilleja coccinea* rosettes.

group	light regime	weight	time* (three aliquots)			average	corrected**
1	darkness	0.09214 g	38.52	39.13	37.72	39.46	39.30
2	darkness	0.09150	36.60	36.42	36.25	36.42	36.53
3	light	0.09177	0.98	0.97	1.05	1.00	1.00
4	light	0.08843	0.83	0.87	0.83	0.83	1.04

* Each time figure is the time in minutes required to record 1000 counts.

** This correction allows for the differences in weight among the parasite leaves sampled. Thus, the figures in the corrected column are times for equal weights of tissue to yield 1000 counts.

An estimation also was made of the level of photosynthesis of rosettes still parasitic on host plants. $C^{14}O_2$ was given to Castilleja coccinea rosettes and their hosts for 30 minutes. The host and the parasites then were weighed, killed, macerated, and their activity sampled. The results are in table 6 (page 76). The level of CO_2 fixation by the parasites was only slightly higher than that of excised leaves. However, the host plant fixed about five times as much carbon per gram of tissue as the parasite did.

table 6

Comparison of carbon-14 fixation by *Lactuca canadensis* and *Castilleja coccinea* in a parasitic couple.

pair	weight	time* (three aliquots)			average	corrected**
Lactuca canadensis	0.12217 g	0.11	0.15	0.16	0.14	0.14
Castilleja coccinea	0.09255	0.99	1.10	0.69	0.93	0.70
Lactuca canadensis	0.10631	0.15	0.10	0.11	0.12	0.12
Castilleja coccinea	0.07544	0.74	0.78	0.81	0.78	0.56

* Each time figure is the average time in minutes (of 10 trials) required to record 1000 counts.

** This correction allows for the difference in weight between the parasite leaf sample and the host leaf sample. Thus, the figures in the corrected column are times for equal weights of host and parasite tissue to yield 1000 counts.

nature of the parasitism

In view of the facts that Castilleja coccinea is photosynthetic and yet requires host-contact for maturation, and responds quickly to host-contact, it seems likely that some micro-metabolite is one of the substances the parasite uses from its hosts. For this reason, host-less parasite seedlings grown in sand or agar were irrigated with a few of the best-known plant vitamins and hormones. However, none of the treatments simulated the effects of host-contact. A list and description of the treatments is in table 7 (page 78). In general, the concentrations of the compounds were chosen to include the range of concentrations reported effective for each compound.

table 7

List of micro-metabolite treatments given *Castilleja coccinea* in sand and agar culture.

compound	method	concentration
thiamine	sand culture	100, 10, 1, 1/10, and 1/100 ppm
niacin (nicotinic acid)	sand culture	100, 10, 1, 1/10, and 1/100 ppm
biotin	sand culture	100, 10, 1, 1/10, and 1/100 ppm
inositol	sand culture	100, 10, 1, 1/10, and 1/100 ppm
ascorbic acid	sand culture	100, 10, 1, 1/10, and 1/100 ppm
pyridoxine hydrochloride	sand culture	100, 10, 1, 1/10, and 1/100 ppm
indoleacetic acid	sand culture	10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, 10^{-11}, and 10^{-13} M
naphthaleneacetic acid	sand culture	10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, 10^{-11}, and 10^{-13} M
kinetin	sand culture	16, 8, 3, and 2 ppm
gibberellic acid	sand culture	1000, 300, 40, 8, and 2 ppm
nutrient solution (Shive, 1915)	aseptic agar culture	
Bacto tryptone glucose extract agar	aseptic agar culture	
water extract of *Lactuca canadensis*	sand culture	
distilled water	sand culture	

THE HOSTS

The host-lists for various root-parasites have been compiled largely by excavating the parasite roots and simply noting the plants the haustoria are attached to. Unfortunately, some if not all root-parasites also form haustoria on materials which are nutritionally inert. Castilleja coccinea, for example, will form haustoria on pebbles, grains of sand, aluminum foil, and leached pith. The suspicion arises, therefore, that not all the hosts a parasite makes root connections with are functional hosts. This difficulty can be avoided by growing the parasite in pots with single hosts and noting its success on each one (plate 12, page 33).

In this way Castilleja coccinea was tested for its ability to parasitize successfully 17 vascular species common in an area of central Michigan where Castilleja coccinea itself is abundant. The host species used in the study were Antennaria neglecta (plate 26, page 83), Fragaria virginiana, Chrysanthemum leucanthemum, Achillea millefolium, Populus deltoides, Lobelia spicata, Alnus rugosa, Hieracium aurantiacum, Lactuca canadensis (plate 29, page 95), Krigia biflora (plate 22, page 66), Danthonia spicata, Rudbeckia hirta (plate 12, page 33), Rubus hispidus, Panicum sphaeroides, and three Solidago species, S. juncea (plate 25, page 81), S. graminifolia, and S. rugosa. Specimens of each species are filed in the Michigan State University herbarium.

All of the 17 hosts supported the parasite's growth, but the rosettes parasitic on Populus deltoides, Alnus rugosa, and Rubus hispidus never matured (table 8, page 84).

Under natural conditions a Castilleja coccinea rosette usually parasitizes several hosts around it, and these rarely are of the same species

[illegible]

[illegible]

[illegible]

plate 25

A mature rosette of *Castilleja coccinea* parasitic on a rosette of *Solidago juncea*.

192

[illegible]

[illegible]

[illegible]

plate 26

Two rosettes of *Castilleja coccinea* parasitic on a vegetative shoot of *Antennaria neglecta*.

136

table 8

LIST OF HOST SPECIES IN GREENHOUSE CULTURE OF *CASTILLEJA COCCINEA*

- *Achillea millefolium*
- * *Alnus rugosa*
- *Antennaria neglecta*
- *Chrysanthemum leucanthemum*
- † *Coleus blumei*
- *Danthonia spicata*
- *Fragaria virginiana*
- † *Hieracium aurantiacum*
- † *Kalanchoë verticillata*
- *Krigia biflora*
- *Lactuca canadensis*
- *Lobelia spicata*
- *Panicum sphaeroides*
- * *Populus deltoides*
- * *Rubus hispidus*
- *Rudbeckia hirta*
- *Solidago graminifolia*
- *S. juncea*
- *S. rugosa*
- † *Tragopogon pratensis*

* supported only early growth of the parasite

† species not native to the range of *Castilleja coccinea*

total genera represented 18

families represented 7

monocotyledons 2

unless the host dominates the area. This multiple host-contact is demonstrated readily by excavating and tracing the roots of the parasite. However, it is difficult to trace more than a few of the connections, since in the task of tracing each one, many haustoria, host roots, and parasite roots are destroyed.

Castilleja coccinea successfully parasitizes hosts of a variety of species, but usually in greenhouse culture a few host species appear to support its growth better than others do. In table 9 (page 86), several hosts are ranked according to the number of parasites they support in greenhouse culture. This ranking may reflect nothing more than the density of their root systems, but nonetheless in greenhouse culture there is a striking difference in the vigor and numbers of the parasite on different hosts. The ranking of each host species is based on the average number of parasite rosettes supported by that host in 10 different pots.

table 9

RANKING OF SELECTED HOSTS ACCORDING TO THE AVERAGE NUMBER OF *CASTILLEJA COCCINEA* ROSETTES THEY SUPPORT IN GREENHOUSE CULTURE *

	at 4 weeks	at 8 weeks	bolting
Lactuca canadensis	28.4	12.1	1.3
Fragaria virginiana	21.5	10.4	0.9
Chrysanthemum leucanthemum	18.0	10.5	0.8
Krigia biflora	22.9	9.2	0.6
Rudbeckia hirta	19.7	8.2	0.6
Lobelia spicata	10.7	9.2	0.5
Antennaria neglecta	4.3	9.5	0.5
Solidago juncea	8.3	7.9	0.5
Hieracium aurantiacum	4.7	7.7	0.3
Solidago rugosa	1.7	1.4	0.3
S. graminifolia	2.1	0.9	0.2
Danthonia spicata	7.0	1.2	0.1
Panicum sphaeroides	1.0	0.7	0.1
Populus deltoides	0.3	0.4	0
Rubus hispidus	0.5	0.3	0
Alnus rugosa	0.2	0.2	0
control	0	0	0

* averages based on a total of 10 pots per host species

THE HOST-PARASITE CONNECTION

The mature haustorium of Castilleja coccinea consists of four distinct regions (plate 27, page 89) ——— (1) a central vascular trace which connects the stele of the host root with the stele of the parasite root, (2) a mass of densely-staining parenchyma surrounding the vascular trace, (3) an outer rind or cortex of vacuolate parenchyma, and (4) a pad of columnar cells next to the host root.

The rate and course of haustorium development in Castilleja coccinea and Melampyrum lineare were studied by harvesting and sectioning haustoria of known age. Several typical haustoria studied in this way are diagrammed in plate 28 (page 91) in successive stages of development. These stages include (1) the formation of papillae on the parasite root at the point of eventual haustorium formation, (2) the lysing or softening of the host root apparently by secretions of the columnar cells, (4) the penetration of the host root by an elongating wedge of parenchyma tissue originating in the interior of the haustorium, (5) the differentiation of vascular elements in the center of the parenchymatous wedge, and (6) the differentiation of a mass of densely-staining parenchyma around the central vascular trace. The internal cells of the host root rarely are distorted next to the haustorium, suggesting that they are not forced aside or compressed by the penetrating haustorial tissue.

The formation of haustoria apparently is not induced by living tissue alone. To be sure, most of the haustoria of Melampyrum lineare and Castilleja coccinea plants are found attached to host roots, but plants of both species also form haustoria on sand grains, pebbles, and organic debris. In the laboratory Melampyrum lineare attacks its own discarded seed coat, and both it and Castilleja coccinea produce haustoria on the cut surface of

plate 27

Photomicrograph of haustroium of <u>Castilleja coccinea</u>, about 400X. Only part of the vascular trace connecting the stele of the host with that of the parasite is visible, since the trace meanders somewhat in the body of the haustorium.

plate 28

Stages in the development of haustoria by *Castilleja coccinea*.

moist, leached elder pith. Moreover, *Castilleja coccinea* and *Melampyrum lineare* plants grown behind the glass of glass-blotter sandwiches form haustoria at only a few of the numerous sites where parasite and host roots touch. Thus, physical contact alone does not induce haustorium formation. It also appears that the roots of these two root-parasites do not seek out host roots chemotactically. The roots of over 100 *Melampyrum lineare* seedlings and 200 *Castilleja coccinea* seedlings grown in 38 separate glass-blotter sandwiches were not visibly attracted to host roots.

THE HOST-PARASITE INTERACTION

Four aspects of the interaction of <u>Castilleja</u> <u>coccinea</u> and its hosts were studied ——— the host-requirement of the parasite, transfer of materials across the haustoria, damage to the host in the interaction, and the effect of the interaction on the distribution of the parasite in a community.

<u>host-requirement</u>. Contact with a host is essential for maturation of the rosettes of <u>Castilleja</u> <u>coccinea</u>. This assertion is based on two lines of evidence. (1) About four thousand seedlings have been kept without hosts on a variery of substrates, including sand, loam, blotter paper, elder pith, agar, agar with mineral nutrient supplements, and agar with full nutrient supplement. None of the seedlings developed more than five foliage leaves, and none of the leaves was longer than six millimeters. About 500 seedlings were kept for as long as four months. Given host-contact, a plant in those four months normally would have formed a rosette 2-3 cm in diameter made up of 10-15 leaves about 15 mm in length (plate 29, page 95). (2) Immature rosettes, when divorced from their hosts, fail to mature further, and die after 2-3 weeks of apparent inactivity. Death usually involves necrosis of the apical region. Since mature rosettes can be pulled free of their hosts without ill effect, it is unlikely that physical injury alone causes the death of the young rosettes.

<u>haustorial uptake</u>. Haustorial transfer of materials was tested with four compounds, two of them common ions (sulfate and phosphate), one the hexose fructose, and one eosin, a mildly toxic dye. All four compounds will traverse the haustorial connection in the direction of the parasite but apparently not in the direction of the host. The eosin Y was introduced into the

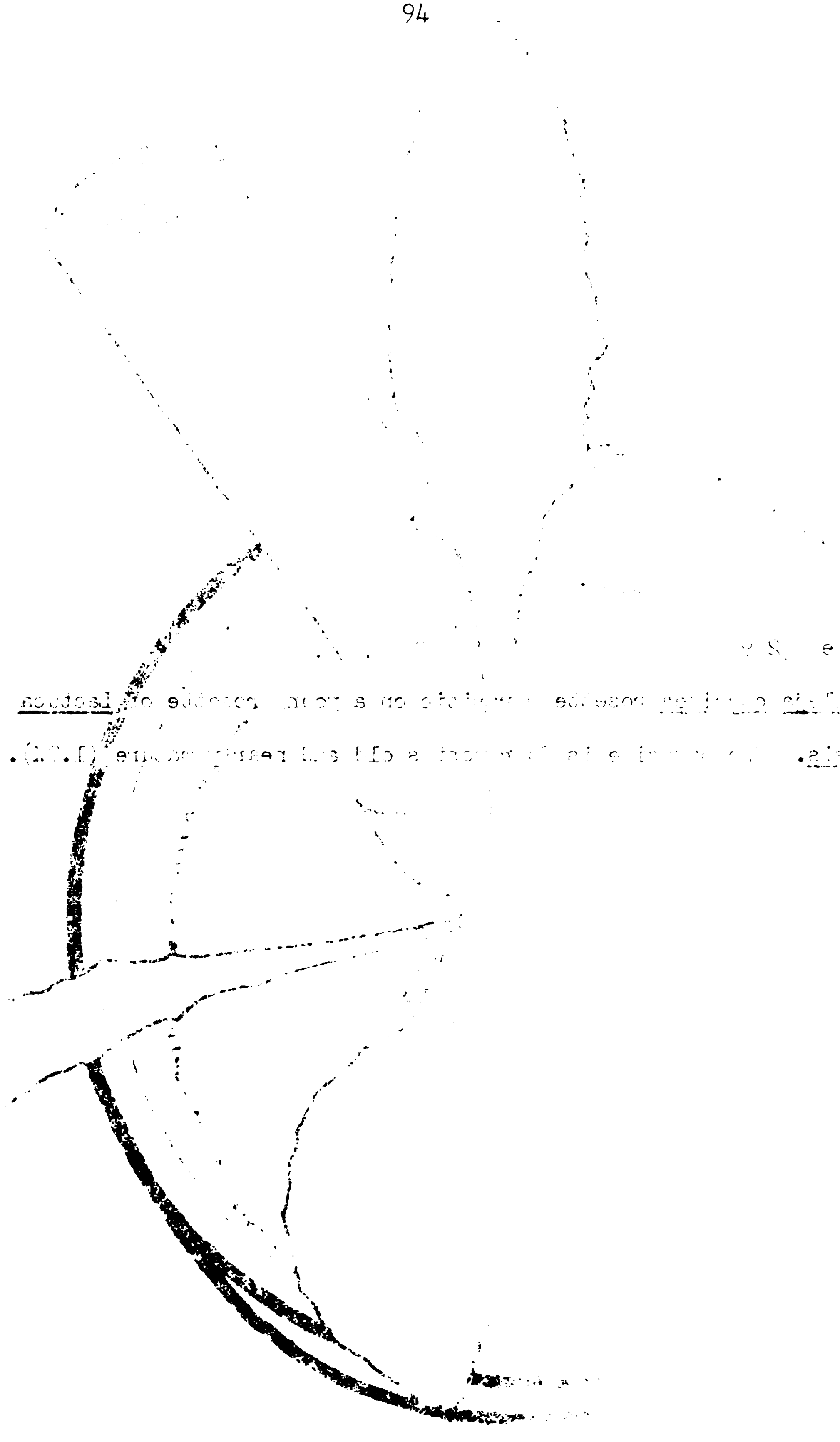
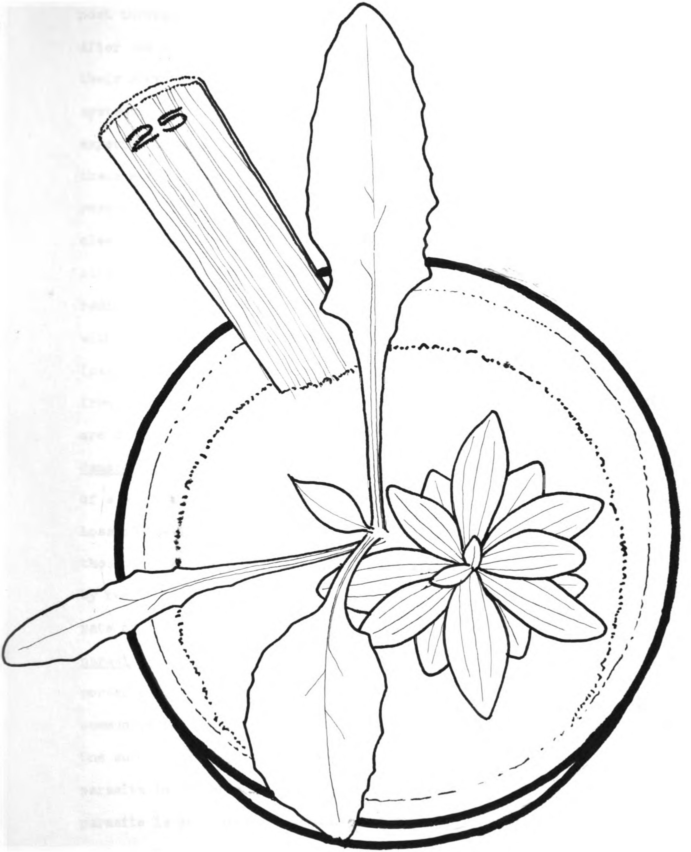


plate 29

A *Castilleja coccinea* rosette parasitic on a young rosette of *Lactuca canadensis*. The parasite is four months old and nearly mature (1.7X).


25

host through a cut petiole, and was detected in the parasite by its color. After the transfer, the parasite and its host were dumped from their pot, their roots washed, and the path of the eosin traced through the two root systems. Only some of the host roots had carried the dye, but earlier experiments with eosin transfer had suggested this would be the case. In these earlier transfers, pots were used which contained several parasite rosettes. Introduction of the dye into only one of the several host petioles resulted in accumulation of the dye in only one or a few of the parasites. Transfer of the fructose and the two anions was studied by using radio-isotopes and monitoring their accumulation in the parasite tissue with a Geiger-Müller tube connected to a scaler and timer. In plate 30 (page 97) are the data from a transfer of C^{14}-fructose to the parasite from Fragaria virginiana. Similar graphs for transfer of $S^{35}O_4$ and $P^{32}O_4$ are on pages 31 and 32 (plates 98 and 99).

damage to the host. Only a crude analysis has been made of the performance of a plant under attack by Castilleja coccinea. The rates of attrition and loss of heavily-attacked Lactuca canadensis are noticeably different from those of parasite-free hosts. Of 22 potted Lactuca canadensis hosts attacked by twenty of more rosettes for 4-8 weeks, only 5 survived. In contrast, the rate of die-off of unattacked controls was 7 out of 40.

parasite micro-distribution. Castilleja coccinea in the greenhouse is supported better by some hosts than by others. Thus, the hosts in a given community can be ranked according to their ability to support the parasite. One such community was studied to determine if the distribution of the parasite in it reflects the ranking of hosts. It was presumed that the parasite is more abundant around the hosts which support its growth best.

plate 30

Graph of fructose ($C_6^{14}H_{12}O_6$) transfer from *Fragaria virginiana* to *Castilleja coccinea*.

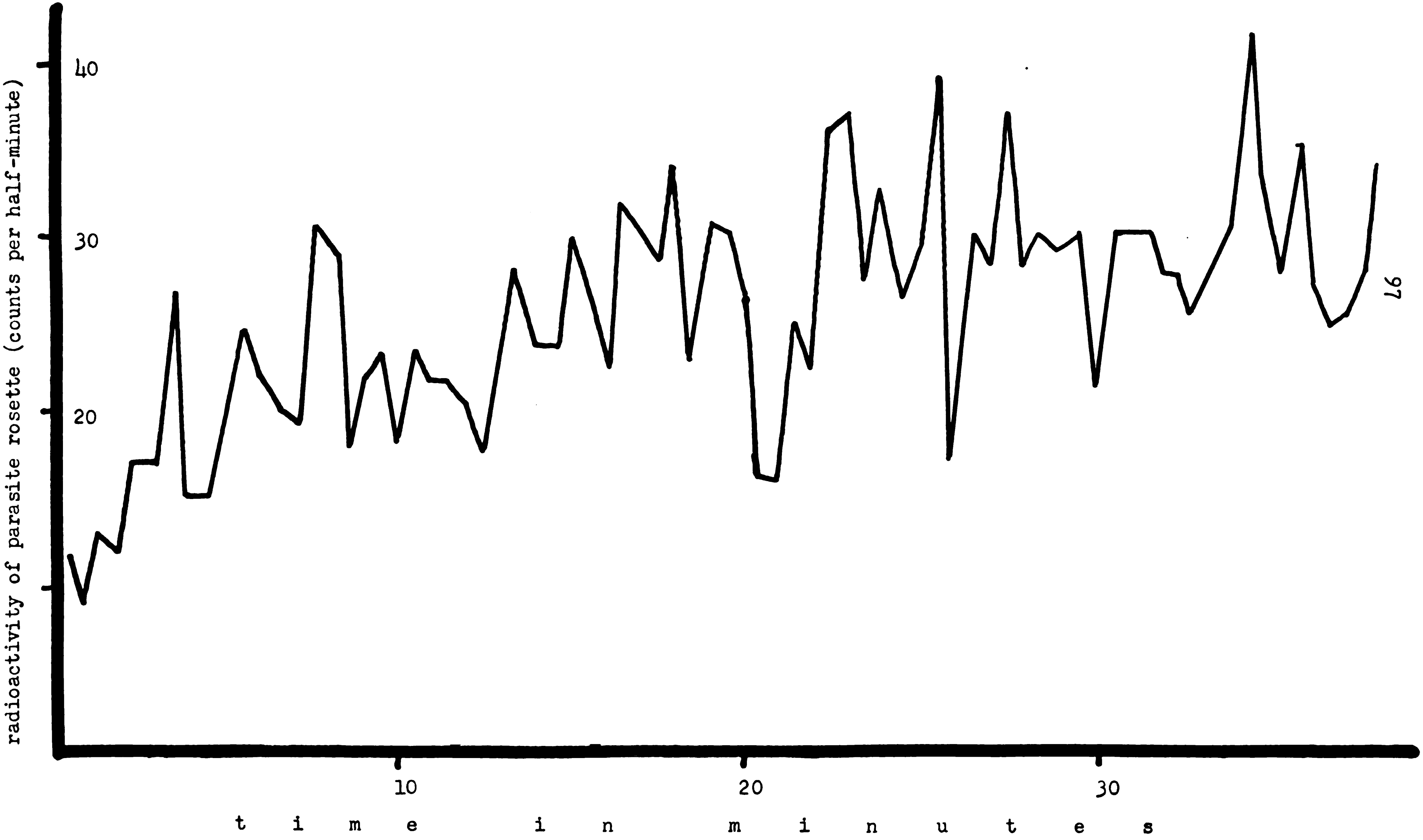


plate 31

Graph of a $P^{32}O_4$ transfer from *Fragaria virginiana* to *Castilleja coccinea*.

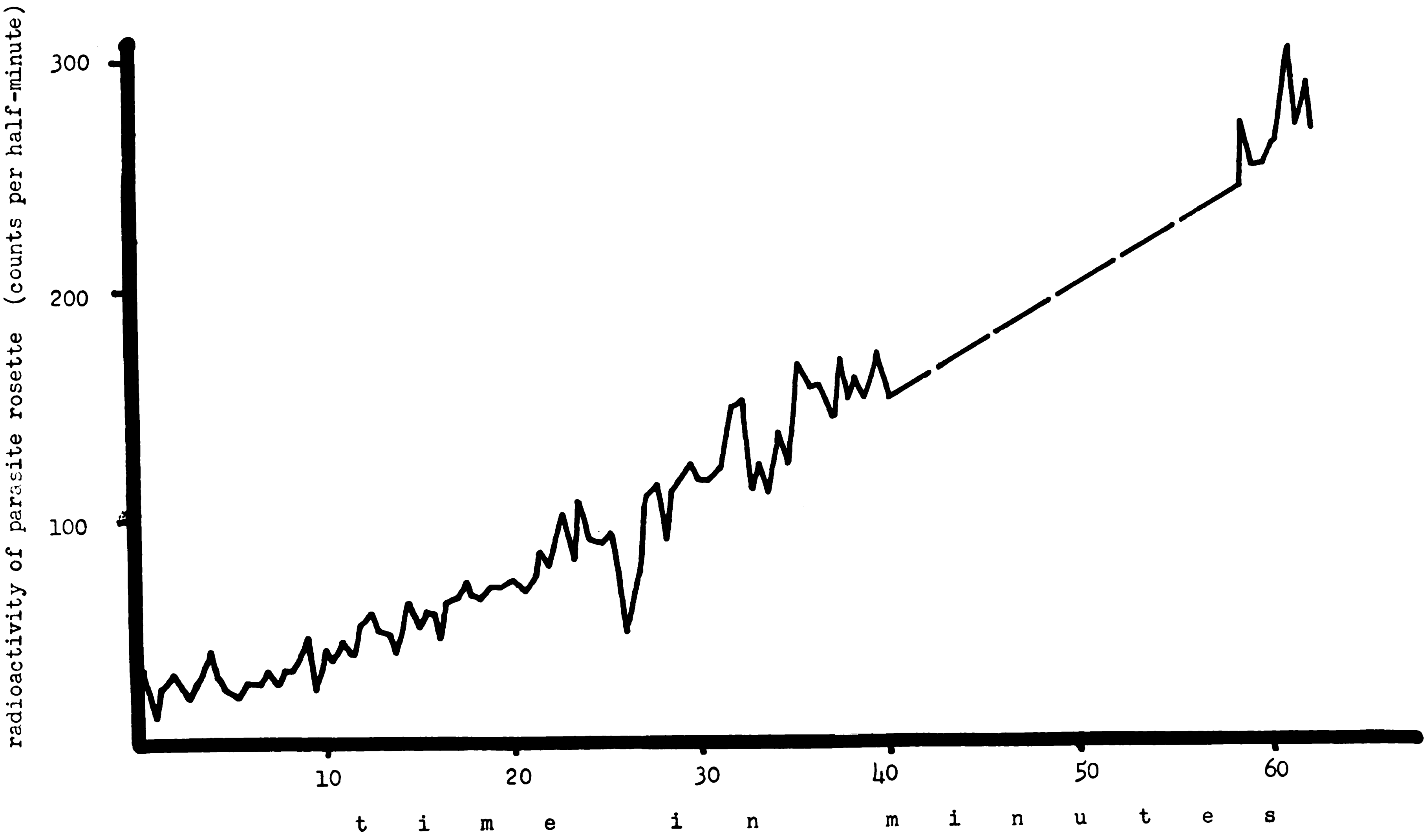
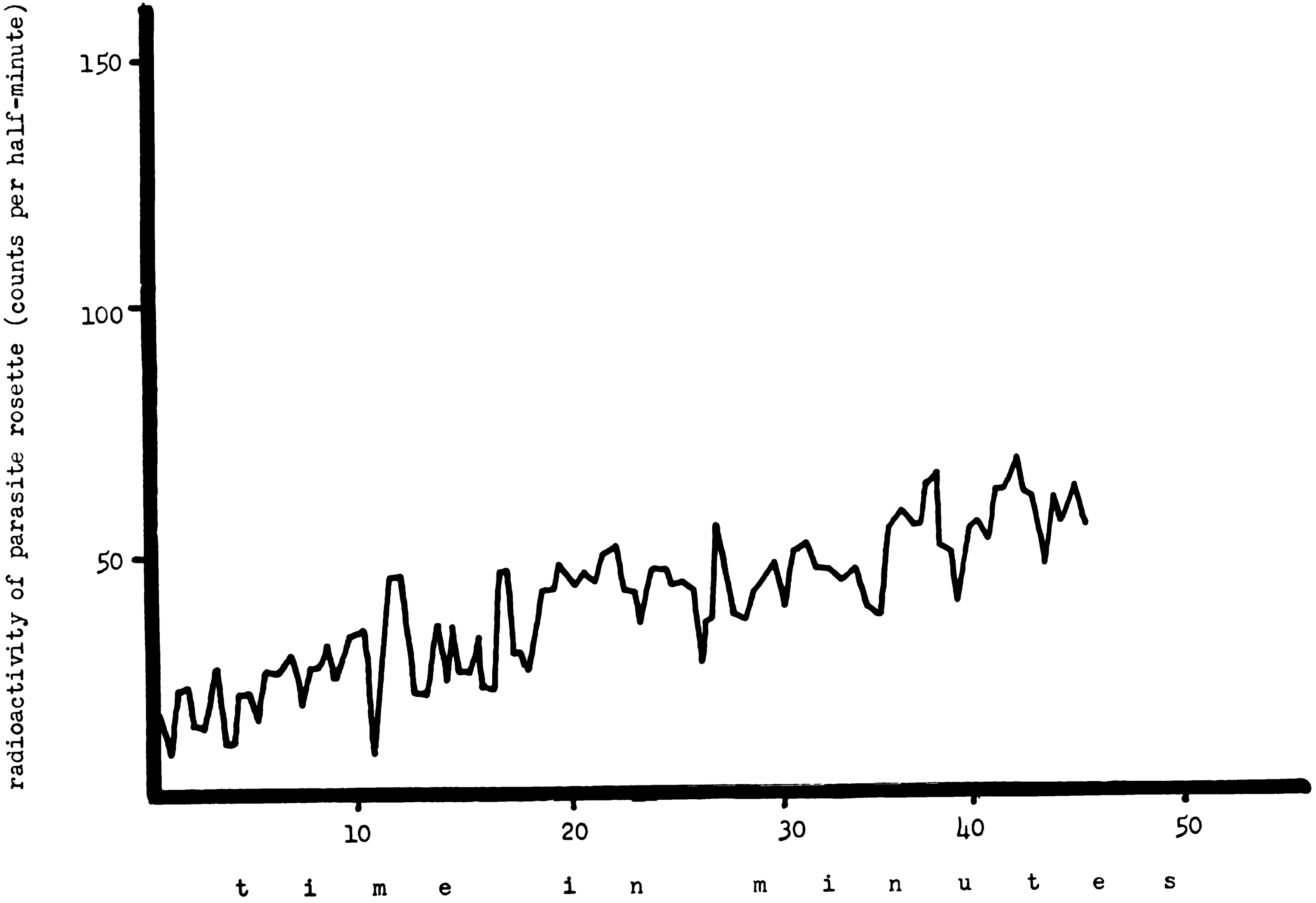


plate 32

Graph of a $S^{35}O_4$ transfer from <u>Fragaria virginiana</u> to <u>Castilleja coccinea</u>.



Thus, the study was set up to compare the density of each host away from the parasite and close to it. However, no significant difference was found between the densities of hosts close to the parasite and the densities of the same hosts some distance away from the parasite. The sampling data are tabulated on pages 101 and 102 (table 8). The numbers indicate how many individuals of the host species occurred in a quadrat, but where the individuals were too numerous to count or were clonal, as many grasses are, presence is noted simply by the letter _e_, and no attempt is made to record the number of individuals present. Results of the statistical analysis of the data are on page 103(table 9). Only some of the host species were tested statistically. Those that were not tested either turned up in too few quadrats (fewer than 5), or else they were clonal and individuals could not be counted accurately.

table 8

Castilleja coccinea micro-distribution sampling data

o *Castilleja coccinea* present in quadrat

x *Castilleja coccinea* absent from quadrat

host	quadrat number																								
	o	o	o	x	o	o	o	x	x	x	o	o	x	o	o	o	o	o	x	x	x	o	o	x	x
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Achillea millefolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alnus rugosa	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0
Antennaria neglecta	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	0	e
Carex aurea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carex granularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysanthemum leucanthemum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Danthonia spicata	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Eleocharis compressa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Equisetum arvense	0	0	0	1	0	0	3	0	0	1	0	1	0	0	0	0	0	0	0	6	1	5	0	0	0
Fragaria virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Habenaria lacera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium aurantiacum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juncus effusus	0	0	2	0	0	0	0	0	0	2	1	1	0	0	3	0	0	0	0	0	0	0	1	0	0
Krigia biflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactuca canadensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liatris aspera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobelia spicata	0	0	0	0	0	2	0	0	0	1	4	3	4	3	1	0	0	0	0	0	0	1	6	8	1
Oxalis violacea	1	2	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	2	3	1	3	1	1	1
Panicum sphaerocarpon	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Pinus strobus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa compressa	e	e	e	0	e	e	e	e	0	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Polygala sanguinea	3	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	3	3	1	3	1	1	1
Rudbeckia hirta	1	2	0	1	0	0	0	1	0	2	0	0	0	1	0	1	0	0	0	0	1	0	0	1	2
Rumex acetosella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scirpus lineatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sisyrinchium montanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiraea alba	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0
Solidago graminifolia	0	0	0	0	0	0	0	1	1	0	4	0	0	0	0	0	0	1	0	0	0	0	2	1	0
Solidago juncea	1	1	7	9	8	4	2	4	2	1	0	5	0	6	2	4	3	1	4	5	6	3	1	4	0
Solidago rugosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

table 8 (concluded)

Castilleja coccinea micro-distribution sampling data

host	quadrat number																								
	x	o	o	o	x	x	x	x	o	o	o	o	x	o	o	o	o	x	x	o	x	x	x	x	o
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Achillea millefolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alnus rugosa	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Antennaria neglecta	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	o	e	e	e	e	e	e	e	e
Carex aurea	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Carex granularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysanthemum leucanthemum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Danthonia spicata	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Eleocharis compressa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Equisetum arvense	0	0	0	0	0	0	0	2	4	3	0	0	0	0	0	1	0	0	0	6	2	4	0	0	1
Fragaria virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0
Habenaria lacera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hieracium aurantiacum	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Juncus effusus	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
Krigia biflora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactuca canadensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liatris aspera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobelia spicata	1	3	6	12	5	0	3	0	5	3	3	6	1	4	5	7	8	7	1	3	6	5	1	4	0
Oxalis violacea	1	2	0	0	0	0	1	2	2	2	3	0	1	0	0	0	0	1	1	0	1	1	0	1	0
Panicum sphaerocarpon	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Pinus strobus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poa compressa	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e	e
Polygala sanguinea	2	1	1	2	4	1	6	3	3	6	1	2	3	2	1	2	1	1	2	0	5	2	2	2	0
Rudbeckia hirta	2	0	0	0	0	0	1	0	2	0	1	0	1	1	0	0	0	0	1	1	0	0	0	1	2
Rumex acetosella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scirpus lineatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sisyrinchium montanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiraea alba	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	1	0	1	2	0
Solidago graminifolia	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Solidago juncea	0	1	1	1	3	5	2	4	7	0	4	0	0	4	5	3	5	2	0	1	0	1	1	1	3
Solidago rugosa	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

table 9

COMPARISON OF DENSITY OF HOSTS CLOSE TO AND DISTANT FROM *CASTILLEJA COCCINEA*

values of t computed for selected hosts of *Castilleja coccinea*

Alnus rugosa	1.4384
Equisetum arvense	0.1819
Juncus effusus	1.0907
Lobelia spicata	1.0512
Polygala sanguinea	1.9137
Rudbeckia hirta	0.3265
Spiraea alba	0.0616
Solidago graminifolia	0.1070
S. juncea	0.7595

theoretical $t_{97.5\ (d.f.48)} = 2.011$ (Hald, 1952)

All the t values computed for the hosts are less than 2.011. Thus, for any one of the hosts tested, there is no reason to believe that its density close to the parasite is different from its density some distance away from the parasite. Stated another way, the ability of each host to support the parasite in the greenhouse is not reflected in the distribution of the parasite in the community. A similar analysis was made for *Lobelia spicata*, *Rudbeckia hirta*, and *Solidago juncea* taken as a group. Of the hosts which occurred often in the sampling data, these three supported the parasite best in greenhouse culture. As separate species, they were not significantly denser around the parasite than away from it, but they were tested as a group to increase the sensitivity of the analysis. However, even as a group they showed no significant tendency to occur more frequently close to the parasite ——— $t = 0.9797$, which is less than $t_{(d.f.148)\ 97.5} = 1.978$ (Hald, 1952).

DISCUSSION AND LITERATURE REVIEW

vascular parasite classification

Vascular plant parasites are classified according to various schemes, but most often they are divided into two large groups, those with chlorophyll and those without. The chlorophyllous parasites are variously termed hemi-parasites, half-parasites, or hydro-parasites, since they are presumed photosynthetic and dependent on their hosts for only water and mineral nutrients. Unfortunately, the classification of parasites by their color is artificial ——— some genera contain both green and non-green plants, and in fact some parasites produce chlorophyll as adults but not at all in their first year or two or growth.

Two additional characters used in classifying vascular parasites are the production of haustoria and the location of the haustoria on the host. Most of the vascular parasites produce haustoria, and most haustoria are formed on the roots of hosts. However, the exceptions are important ones ——— the mistletoes and dodders produce their haustoria above-ground on the leaves and stems of their hosts. Such exceptions complicate attempts at classifying vascular plant parasites. The difficulty can be resolved fully only with an elaborate, and cumbersome, classification system. One scheme is outlined on page 105. It is artificial but compatible with what is known of heterotrophy in higher plants. <u>Castilleja</u> <u>coccinea</u> and other species of green root-parasites belong to the category marked with an asterisk.

- VASCULAR PLANTS
    - chlorophyllous
        - autotrophic
        - root-grafting
        - haustoriate
            - * haustoria below-ground (hypogeal)
            - haustoria above-ground (epigeal)
    - achlorophyllous
        - saprophytic
        - parasitic
            - not haustoriate
            - haustoriate
                - haustoria hypogeal
                - haustoria epigeal

A <u>root-parasite</u> is any vascular plant which parasitizes the roots of other vascular plants by means of haustoria, or, more simply, a root-parasite is any vascular plant forming root haustoria. The definition is a loose one, but must remain so until root-parasitism is better understood —— as yet little is known of what compounds the parasites obtain from their hosts, and what they do with those compounds. The phrase <u>green root-parasite</u> refers to vascular plants which are green and which produce root haustoria —— <u>Castilleja</u> falls in this group. <u>Full parasite</u> or <u>holoparasite</u> applies to (1) any non-green vascular plant, (2) the young unpigmented stages of <u>Tozzia</u> and <u>Striga</u>, which are chlorophyllous as adults, and (3) root-grafting or haustoriate vascular plants which produce non-functional chlorophyll or which produce chlorophyll only during food stress. <u>Vascular parasite</u> is taken to mean a vascular plant which requires interaction with a host at some time during its life cycle. This definition, however, can be applied rigorously only to individual plants, since plants of even the same species vary in their host requirement as a result of either their genetic make-up or the variables in their environment. For example, thick stands of the green root-parasite <u>Euphrasia</u> <u>minima</u> grown without hosts often include a few flowering individuals, but plants grown singly rarely flower

(Heinricher, 1917). Apparently the parasites attack one another, and some get more than they give up, thereby gaining an edge over their neighbors. Chance, or else genetic variability among the parasites, determines which individuals prosper. Nonetheless, regardless that individual root-parasites can be induced under artificial conditions to mature without host-contact, probably root-parasites as populations do require hosts. Under natural conditions and taken as populations, it is likely that no species of root-parasites can survive without hosts.

the green root-parasites

The green root-parasites belong for the most part to the figwort and sandalwood families, respectively the Scrophulariaceae and Santalaceae. To the Scrophulariaceae alone belong almost 500 green root-parasitic species (Cannon, 1909). The eastern North American genera of parasitic Scrophulariaceae are *Pedicularis*, *Gerardia*, *Melampyrum*, *Euphrasia*, *Odontites*, *Rhinanthus*, *Orthocarpus*, and *Castilleja*. Only *Comandra* of the Santalaceae ranges into our area.

As a group, the green root-parasites include herbs, shrubs, and trees, but most of the species native to North America are herbaceous. They rarely look any different from autotrophic plants, and the parasitism of some of them went unsuspected for as long as 60 years after publication of the first account of root-parasitism.

As might be suspected, root-parasites often are noxious agricultural and silvicultural pests, and surprisingly, this is true of both green and non-green root-parasites. In this country, a green root-parasite introduced from Africa (witchweed, *Striga asiatica*) seriously reduces corn production in the Carolinas. In turn-about, however, plants of at least one genus of green root-parasites are exploited commercially — the aromatic wood of *Santalum album*, the Indian sandal of commerce, is prized in the cabinet and perfume trades (Bailey, 1951).

THE PARASITE

ontogeny

The tolerance of *Castilleja coccinea* seedstock to two-year desiccation is not shared by the seeds of *Melampyrum*, a genus of root-parasites in the same subfamily as *Castilleja*. Gislen in 1949 and Gautier many years earlier (1908) noted that *Melampyrum* seeds quickly lose viability when allowed to dry out for even a few days. Seedstock of *Melampyrum arvense* dried for ten days germinates at only a low per cent, and seeds kept dry for twenty days die (Gislen, 1949, and Heinricher, 1909). Using this fact, Gislen was able to assert that *Melampyrum* did not reach Sweden as a contaminant of wheat in the early days of oceanic shipping, since the seeds surely would have died in the weeks required for ocean transport.

The high per cent germination of unchilled *Castilleja coccinea* seeds is in marked contrast to some other root-parasites, both native and Old World. The seeds of *Melampyrum lineare*, a green root-parasite common in Michigan jack-pine vegetation, require 80-100 days of moist storage at 4°C to break what is really a double dormancy — about 40 days for radicle dormancy and an additional 40 days for epicotyl dormancy (Cantlon *et al.*, 1962). Moreover, only a third of each season's seed crop germinates after the four months of cold-treatment. Under natural conditions the remaining two-thirds of the seed crop presumably germinates in the second or third years. In the laboratory these ungerminated seeds can be stimulated to germinate by treatment with gibberellic acid, but not by leaching, scarification, or treatment with various growth supplements (Curtis and Cantlon, 1962).

Presence of a host is not required for germination of the seeds of *Castilleja coccinea*, but seeds of several other root-parasites do have such a requirement. Absence of a host precludes germination of *Striga lutea* (Brown and Edwards, 1944), *S. hermonthica* (Brown *et al.*, 1949), *Orobanche minor* (Brown *et al.*, 1951), *O. speciosa* (Chabrolin, 1934), *O. cumana* (Bartcinskii, 1934), *Alectra vogelii* (Botha, 1948), and several other species. *Striga asiatica*, a native of Africa and since 1956 a serious threat to corn yield in the Carolina states, also is a member of this group. Using *Striga lutea* and *Striga hermonthica*, Brown and his associates in England worked for several years first to substantiate the requirement and then to discover what compound is involved in the germination stimulation. Unfortunately, the stimulant has never been identified, but its characteristics are well-established (Brown, 1946). (1) It is active in hormonal concentrations of 10^{-3} to 10^{-6} grams / liter. (2) The parasite seeds react to the stimulant with as little as 30 seconds exposure——60 seconds exposure induces a 70% germination level in *Orobanche*, compared with 0.1% germination with no exposure. (3) The substances are not simple inorganic compounds, nor are trace elements involved. (4) Probably a variety of activators in nature stimulate the germination of the various species of parasites. There is no single universal stimulant. (5) No known vitamin or phytohormone is involved. (6) The source of the activator is not unique——different tissues from various species produce it.

Although the natural stimulant still is not isolated and identified, some compounds have been found which have the same effects. These include

D-xyloketose, thiourea, kinetin (6-(2-furfuryl) amino purine), and certain other 6-substituted aminopurines (Brown et al., 1949, and Worsham et al., 1959). The last of these, the purine derivatives, have an effect beyond germination stimulation. They substitute at least in part for host-contact as well. Striga seedlings left in the natural stimulant germinate, but never produce cotyledons and their shoot apices do not elongate, but those kept in purine solutions do (Worsham et al., 1959).

Brown et al. (1949b) studied the physiological effects of some of the substances which induce Striga germination. Both the natural stimulant and D-xyloketose produce up to 60% increases in the volume of root segments of pea and corn. The volume increase in a result of cell extension, not division. The concentration of D-xyloketose used was less than 10 ml / 1, and according to Brown is '...the first record...of a stimulating effect on the growth of plant tissues...[by] a simple sugar in...[so low a concentration]'.

Root-parasites which require a host-secretion for germination are thought simply to have lost the ability to produce by themselves enough of some compound vital in germination. The evidence for this comes from work on Striga and Orobanche. Brown and his colleagues (1946, 1951) noted that moistening the seeds of Orobanche and Striga before treating them with the host stimulant enhances their germination. The per cent germination increases steadily the longer the seeds are given this pre-treatment, until, after 14 days for Orobanche and 21 for Striga, there is no appreciable increase in the per cent. After this optimum length of pre-treatment, the germination percentage of Orobanche seeds remains almost constant for at least a year, but that of Striga falls off noticeably. Brown and his co-workers suggested that the seeds, once moistened, begin to produce by themselves a stimulant which is or is similar to the stimulant secreted from host roots. As this natural stimulant builds up, less and less host stimulant is needed to trigger germination. But eventually, for some reason, the parasite seed stops accumulating its own stimulant. In Orobanche, after 14 days the synthesis of the stimulant equals its breakdown, or else synthesis stops and the stimulant already produced is protected from destruction. In Striga, on the other hand, after 21 days the breakdown of the stimulant occurs faster than its synthesis.

Brown's idea suggests a manner in which root-parasitism may have evolved. A plant which produces haustoria has available for its use everything its host translocates in conductive tissue. It is thus no longer under selective pressure to maintain a working autotrophic metabolic system. By genetic accident it can lose some functions of a normal autotroph and suffer not at all. Even mutations that are ordinarily lethal might be perpetuated. In this process, however, the root-parasite becomes more or less dependent on its host. Orobanche and Striga apparently have lost only partly their ability to produce a compound essential in germination. This loss makes them host-dependent but not strictly parasitic, since what they require is a root secretion which the host normally 'discards'. They have suffered other metabolic losses as well, however, losses which do make them parasitic. Quite aside from their germination stimulation, haustorial contact with a host is necessary for their growth and maturation after germination. Castilleja coccinea requires no germination stimulant. Evidently in this area of metabolism it is autonomous, and only in its maturation processes is it host-dependent.

Root-parasites tend to be similar in their morphology. Systematists recognize this, and have grouped the bulk of them into only a dozen families.

This morphological kinship of root-parasites makes sense if root-parasitism does arise by genetic accident in root-grafting or haustorium-forming plants. Genetic systems which are alike enough to produce plants of similar morphology doubtless are alike enough to suffer similar genetic accidents.

For that matter, it may be that the genetic system of any plant would lose what *Striga*, *Castilleja*, or other root-parasites have lost, given the chance ——— that is, given the ability to produce haustoria. To be sure, root-parasites are characterized by more than the ability to produce haustoria ———their transpiration and guttation volume is large (Seeger, 1910), the osmotic concentration of their tissues is high (Solomon, 1952), they often lack root hairs (Heinricher, 1917), and many of them germinate only when stimulated by a host secretion (Brown, 1946). However, when root-parasites first evolved, they may not have enjoyed these mechanisms, most of which make them more effective root-parasites. Instead, they may have acquired such features after their root-parasitism was firmly established genetically. This seems very likely in the case of host stimulation of germination——— without hosts the parasites can not survive beyond germination, and therefore selection doubtless favors mechanisms for ensuring host-contact. *Striga* and *Orobanche* have one such mechanism, the stimulation of germination by substances diffusing from host roots. Host-specificity is the next step in this selection process. Apparently some hosts afford better parasite growth than do other hosts, and therefore selection favors mechanisms for 'recognizing' these hosts, such as a response to their characteristic root-secretions.

This, incidentally, illustrates the axiom that a mutation or new gene combination varies in its selective value with the particular needs of the plant that produced it. Probably seeds of many plants, not just root-parasites, by mutation have become dependent on root-secretions for their germination. But, whereas such a dependence is of definite survival value to a root-parasite, it has no survival value or even is detrimental to autotrophic plants. Accordingly, selection would increase rapidly the frequency of such a mechanism in populations of root-parasites but not at all in populations of autotrophs.

If root-parasites have indeed lost some of the functions of autotrophs, there is the possibility that a portion of their genetic code is not used. That is, since a root-parasite has an external supply of vital compounds, then its gene loci involved in the synthesis of such compounds can be altered by mutations. Eventually, such loci might serve useful functions, although to be sure it is naïve to think that many of such freely-mutating loci would be anything but detrimental. At any rate, the more a root-parasite becomes dependent upon its host, the more loci are freed for coding new genetic information, at least some of which could be useful to the parasite. This might also explain why Castilleja varies widely in its morphology.

The ability to produce haustoria is the most striking feature of root-parasites. The first step in the evolution of haustoria likely was a tendency to form root-grafts. This speculation is supported by two observations. First, a tendency to root-graft is common among plants. The roots of some pines growing in stands graft freely, and, in fact, the living root systems of otherwise dead trees are kept alive by photosynthate contributed by other trees in the stand in a sort of host-parasite exchange (Bormann, 1957). Presumably the advantage to the contributor-trees is the supply of nutrients and water absorbed by the parasitic root systems. Moreover, some trees in such stands dominate the bulk of their root-grafts with their neighbors, and thus receive an inordinate supply of nutrients and water. As a result, they enjoy better growth. This helps explain how a few individuals in a stand of genetically-identical trees can mature faster than their neighbors.

Second, man's extensive success with grafting suggests that there are few barriers to the intimate union of unrelated plants. In this regard, it is significant that all of the host-specific root-parasites exploit hosts unrelated to them, and the root-parasites with a wide host range attack members of many different families. For that matter, the interaction of scion and stock of grafted plants often is a sort of parasitism. A flower bud of one plant grafted into a twig of another contributes nothing but its genetic information, while both the support and the raw materials for its flowering and fruiting are contributed by the stock plant.

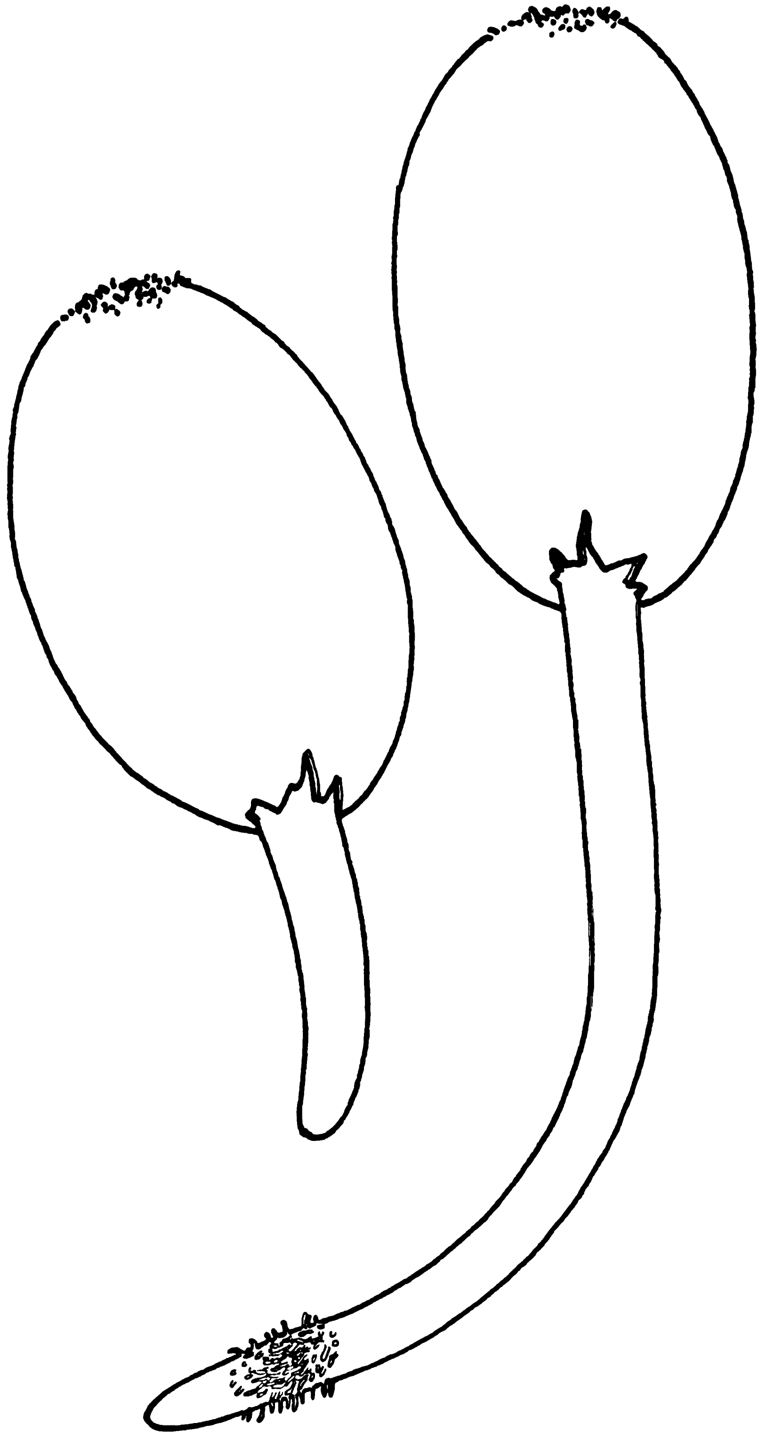
Granted that there is a widespread grafting capacity in plants, there are three mechanisms for effecting the grafts (MacDougal and Cannon, 1910)—(1) grafting of two roots growing close together, (2) grafting of one shoot, or its adventitious roots, with another shoot, and (3) grafting of a stem with the roots of a seedling lodged in its bark or in a wound. Root-grafting doubtless is the most likely of the three mechanisms. Roots grow entangled in the soil, often touching one another, and rarely are disturbed. In contrast, aerial contact is infrequent, and is subject to disturbance by environmental forces such as wind. Successful aerial parasites either twine about their hosts as dodder does, or produce adhesives for ensuring firm host contact, as do the seeds of the *Arceuthobium* mistletoes (Peirce, 1905). This suggests that most higher plant parasites are root-parasites simply because root-grafting is both more common and more stable than other natural grafting mechanisms.

The selective advantage of vertical growth of the roots of *Castilleja coccinea* seedlings lies in early contact with host roots. The sandy soil that *Castilleja coccinea* typically grows in dries out to a depth of 1-2 cm in the summer months. As a result, few roots grow close to the surface, and, for a parasite seedling to attack a host, it must first reach the root zone below the dry surface soil. In contrast to *Castilleja coccinea*, *Melampyrum lineare* is not strongly geotropic in its early growth. And, significantly, the site of germination of the seeds of *Melampyrum lineare* is moist jack-pine litter. The fine rootlets of jack-pine and other hosts are protected from desiccation by the forest litter, and thus grow near the surface of the soil well within reach of the elongating *Melampyrum* radicles.

The production of root hairs by *Castilleja coccinea* apparently is in contrast to some European and Asian root-parasites (Heinricher, 1917, and Stephens, 1912), which lack root hairs altogether. *Melampyrum* produces minute, cylindric appendages somewhat like root hairs (Sablon, 1887) but called papillae or trichomes (plate 33, page 116). Similar structures have been described for *Lathraea* (Heinricher, 1895), *Cuscuta* (Peirce, 1893), *Santalum* (Barber, 1906), and several other genera. Their function is not known, although Kusano went so far as to call them hair-tendrils in *Aeginetia* (1908/1909) because '...[They]seem to attach...firmly to the...[host root] and then to coil or contract throughout...[their] whole length, whereby the seedling is drawn closer to the host...'. Kusano noted the tendril action only in *Aeginetia*, however, and speculated that the papillae or trichomes of *Melampyrum*, *Santalum*, and *Lathraea* serve simply to attach the parasite root to the host root. It does seem that on *Melampyrum lineare*

plate 33

Germinating seeds of Melampyrum lineare. The root hair-like trichomes are thought to secrete a substance which attacks the roots of nearby vascular plants in the early stages of haustorium penetration (Heinricher, 1909).



seedlings they are most abundant at points on the root where haustoria are developing. Melampyrum lineare seedlings placed on moist elder pith produce a number of haustoria on the pith, and the papillae are observed easily with a dissecting microscope or hand lens. As well, in histologic sections of Melampyrum lineare haustoria, the papillae show up as long vacuolate cells. Their walls are continuous with the walls of the host cells they touch. It thus appears that they secrete either a cementing substance which effects firm contact with the host root, or a lysing substance which acts on the host root during penetration. Castilleja coccinea haustoria in section also show papillae-like cells in addition to root hairs, but the papillae are not obvious in living material. However, this might be a result of the small size of both the papillae and the haustoria of Castilleja coccinea.

When root-parasites first attracted attention at about the turn of the century, lack of root hairs was considered good evidence that the hosts afforded the parasites only water and mineral salts. Most of the root-parasites are chlorophyllous, and the only gross morphological differences between them and autotrophs are the absence of root hairs and the presence of haustoria. Thus, reasonably enough, they were presumed to be autotrophs which had lost root hairs, the usual organs of absorption, and instead produced haustoria to rob other plants of water and mineral nutrients (Koch, 1889, Kostytschew, 1924, and Heinricher, 1917). Recent evidence (Härtel, 1959) suggests that the parasites do extract water and ions from their hosts, but they do not stop there, and take food substances and phytohormones as well (Nelson and Rogers, 1959).

physiology

The demonstration of starch in illuminated leaves and seedlings of Castilleja coccinea suggests that the parasite is photosynthetic. The starch test for photosynthetic activity doubtless is a valid one, but results of such tests on root-parasites must be interpreted with caution, since the glucose or glucose-1-phosphate utilized by the parasite in starch synthesis may be provided by host plants. To avoid this possibility, every leaf used in the experiments was excised and floated on distilled water in darkness for 24 hours to deplete its food reserves. Nonetheless, the starch detected in the parasite leaves perhaps was derived from host-provided reserves of sugar or its phosphorylated derivatives.

The conclusion from the photosynthesis experiments is that Castilleja coccinea has its own photosynthetic machinery and uses it. However, since haustorial transfer of fructose has been demonstrated, there is good reason to suspect that the parasite, by plugging into the vascular streams of other plants, obtains food materials it does not itself synthesize. That is, Castilleja coccinea is photosynthetic, but it doubtless receives host photosynthate as well. Whether or not the parasite ever uses the metabolites it pirates is not yet established. There are three possibilities in this regard ——— (1) the parasite does not metabolize the food materials it receives from its host, (2) the parasite's nutrition is supported by photosynthate from both itself and its host, and (3) the parasite does not metabolize its own photosynthate.

Heinricher (1917) thought that green root-parasites do not use the food substances pirated from their hosts, but he did not support this presumption. When root-parasites first came under serious scrutiny, it was possible to demonstrate transfer of only dyes across the haustoria. As a result, it was speculated on purely anatomical grounds what the parasite normally obtains from its hosts. Those parasites that have both phloem and xylem connections in their haustoria were thought to be dependent on their hosts for food, water, and mineral nutrients, but those with only xylem connections were thought dependent only for water and mineral nutrients. For example, Peirce (1893) presumed that the mistletoe species *Viscum album*, because it has no sieve tubes in its haustoria, is a 'water-parasite', and that '...its host performs for it only the functions of a root——————absorption, conduction, and mechanical support'. On this basis, it would be concluded that *Striga* and *Castilleja* are not dependent on their hosts for food materials, since neither has phloem elements in its haustoria or phloem connections with its hosts (Stephens, 1912). However, fructose readily crosses the haustoria of *Castilleja*, and according to Rogers and Nelson (1959) 'sugar' crosses the haustoria of *Striga*. Nonetheless, these experimental sugar transfers do not demonstrate that the parasites normally obtain sugar from their hosts. The details of the *Striga* transfer have not yet been published, but in at least the *Castilleja* transfer the labelled sugar was introduced into the host through a cut petiole. As a result, the fructose surely had access to xylem tissue as well as to phloem, and could have entered into the parasite across the wholly xylem connections of the haustoria. Thus, it still is not known if *Castilleja coccinea* takes food substrates from its hosts. The problem could be solved by (1) demonstrating the presence of photosynthesis products in the xylem of normal hosts, or by (2) introducing labelled sugar into only the phloem of the host and noting whether transfer occurs. Of course, even if host photosynthate is found to enter the parasite, there remains the question of whether the parasite uses that photosynthate.

THE HOSTS

All the hosts of Castilleja coccinea tested are perennial or biennial. However, since the parasite will attack the common radish, Raphanus sativus, and corn, Zea mays, it is at least likely that annuals serve as suitable hosts. Also, the hosts need not be mature ——— seedlings of Tragopogon pratensis, Lactuca canadensis, Danthonia spicata, and Kalanchoë verticillata are attacked readily.

Melampyrum in Europe (Heinricher, 1909) apparently only rarely attacks annuals in artificial culture. In contrast, however, Orthocarpus purpurascens, a root-parasite indigenous to the American deserts, parasitizes mostly annuals (Cannon, 1909). The parasite itself is a desert annual, and germinates and matures only when rain is sufficient. The many other desert annuals with the same rain-dependence are the most abundant hosts in the area, and thus are the most often attacked.

In the greenhouse Castilleja coccinea successfully parasitizes a variety of hosts, but a few hosts appear to support its growth better than others do. The reason for this is not yet clear, although it is suspected that the best hosts are simply those with the densest root systems. For example, Lactuca canadensis doubtless is the best host of the twenty of so tested, and it produces the most massive and branched root system. Whatever the reason for the parasite's varying growth on different hosts, almost all the plants tested supported the parasite to maturity. Thus, it seems reasonable to assert that Castilleja coccinea can parasitize almost any higher plant it normally occurs with. Moreover, the parasite attacks plants which never occur in the same area with it. For example, Castilleja coccinea will parasitize Kalanchoë verticillata, a succulent greenhouse herb native to South Africa, and Coleus blumei, a native of Java.

THE HOST-PARASITE CONNECTION

The mechanism by which root-parasites extract host materials is not fully understood. There are at least three possibilities——host root pressure, osmotic absorption by haustoria, and transpiration pull (Härtel, 1959). Each has been championed as the sole mechanism for the extraction process, but seeming exceptions inevitably have been cited. Nonetheless, (1) most plants maintain positive root pressure, (2) the tissues of a root-parasite typically have a higher osmotic concentration than that of its hosts, and (3) the transpiration volume of many root-parasites is unusually high.

osmotic absorption. MacDougal and Cannon (1910) studied in an unusual way the osmotics of host-parasite pairs. They pierced the succulent stems of cacti and slipped in cuttings of various autotrophs to serve as 'parasites'. None of the 'parasitic' plants maintained itself against a host of higher osmotic concentration. In contrast, those with a favorable osmotic differential usually were successful parasites, and some of them survived for two years.

Solomon (1952) studied the osmotics of an actual root-parasite and its hosts. He used Striga parasitic on sorghum, which was grown in sand and watered with various concentrations of nutrient solutions. As expected, he found that the osmotic value of the sorghum increases as the osmotic value of its culture solution increases. But, in contrast, that of the parasite is high to start with, and increases only slightly at very high nutrient concentrations. At the highest concentration the osmotic values of both parasite and host are about equal at 9 atmospheres. But at the lowest concentrations, the osmotic value of the parasite exceeds that of the host by nearly 8 atmospheres. And, interestingly, the yield of the sorghum at the highest concentration is about the same attacked and unattacked by the parasite, but at lower concentrations, the yield drops off in the attacked plants. Solomon reasoned that, at the low nutrient concentrations, because there is an 8-atmosphere difference in osmotic pressure between the host and the parasite, the parasite is able to deprive the host of large quantities of water and thus reduce its yield. In contrast, at the high concentrations of nutrient solution, when the osmotic pressures of host and parasite are practically the same, the parasite can extract almost nothing from the host, and in fact leads a precarious existence. The sorghum plants are not always attacked under such conditions, and the _Striga_ plants which do attack die early, often without flowering.

transpiration pull. Härtel (1941) has studied what he calls the stress on the water economy of the host caused by the parasite's transpiration pull. He measured in the field the daily transpiration patterns of parasites (_Pedicularis foliosa_) and of both attacked and unattacked hosts (_Avena caucasica_). In graphing the results, he noted no marked difference in the transpiration patterns of the parasites and the unattacked hosts, but he did note a difference between those of the attacked and the unattacked hosts. The daily transpiration level of the parasitized host drops markedly in the afternoons. This drop in transpiration Härtel attributed to the effect of the parasite's transpiration pull. He asserted that the parasite pumps enough water out of the host to cause a passive closure of the host stomates, drastically reducing the transpiration volume of the host. As a result of the stomate closure the host suffers a decrease in gas exchange and a corresponding decrease in photosynthesis. But, supposedly, the host compensates for this loss of photosynthate by an increase in photosynthesis during the early-morning hours of each day, before the parasite can draw off enough water to close the host's stomates.

Seeger (1910) compared the transpiration levels of two root-parasites and six autotrophs. According to his data, both root-parasites transpire more heavily than any of the autotrophs tested———

> '...a piece of leaf of *Euphrasia rostkoviana* or *Odontites verna* transpires five-fold that of *Nuphar luteum* (emergent leaf), six to seven times more than *Gentiana*, *Callisia*, *Lamium*, and other mesophytes, forty times more than *Rhododendron* (xerophyte). And, *Veronica chamaedrys*, also a member of the Scrophulariaceae, loses only one-third as much water in transpiration'. [German translation]

The functions of the four portions of the haustorium of *Castilleja coccinea* and other root-parasites are only partly understood. The central vascular trace doubtless conducts host materials into the parasite, but the function of the densely-staining parenchyma around the vascular trace is not known. The cortical rind presumably protects the haustorial connection. The pad of columnar cells, at least in the haustoria of *Cuscuta*, was thought by Peirce (1893) to '...exude...a solvent which attacks and dissolves the walls and contents...of the cortical and epidermal cells... [of the host]'. Similar structures occur in the haustoria of most root-parasites. In *Castilleja coccinea* and *Melampyrum lineare* haustoria the walls of the pad cells appear bonded to the host (plate 8, page 14), which does suggest lysing or softening of the host cells. The cells of the host root rarely are distorted next to the haustorium, suggesting that they are not forced aside or compressed by the penetrating haustorial tissue. This is the best evidence that the haustoria lyse rather than force access to the stele of the host. Apparently the elongating wedge of haustorium tissue merely fills a cavity in the host root formed by the breakdown of the tissue lying in its path.

The factors inducing the formation of haustoria by root-parasites are largely unknown. Heinricher (1897, 1931) and Sablon (1887) asserted that the parasites produce haustoria only in the presence of living roots. Other workers have suggested that physical contact alone induces haustorium formation. Barber (1907) found a sandal tree (*Santalum album*) root which '...had made five attempts to penetrate a refractory particle of quartz'. Dodder (*Cuscuta*) haustoria can be induced experimentally by pressing the dodder shoots in tinfoil (Knapp, 1954). Peirce as early as 1894 induced dodder haustoria simply by locally irritating the shoots. But, he noted that a haustorium formed in this way does not mature unless it soon obtains nutrients from whatever it penetrates. The developing haustorium is not sustained by food transported from some other portion of the dodder plant. Such a mechanism has obvious selective advantage. Only those haustoria which contribute to the parasite's nutrition ever mature, '...an economy of materials and of energy' (Peirce, 1894).

The roots of *Castilleja coccinea* plants grown behind glass in glass-blotter sandwiches do not visibly grow toward nearby host roots. The lack of a chemotactic mechanism for locating host roots would seem disadvantageous to root-parasites, but the density of roots is high in many soils. No chemotactic mechanism has been demonstrated for even the host-specific root-parasites. In fact, *Orobanche* is stimulated to germinate by a host substance diffusing as far as a centimeter from its host, and yet the seedling radicle can elongate only a scant two millimeters (Sunderland, 1960). As a result, many seedlings die because they are unable to elongate enough to attack the very root which stimulated their germination. But even though root-parasites do not locate host roots chemotactically, it is significant that most parasites with a wide host-range germinate independently, while host-specific parasites usually require a host secretion for germination.

THE HOST-PARASITE INTERACTION

host-requirement

The inability of *Castilleja coccinea* to grow beyond the seedling stage without host-contact is shared by only some root-parasites. Others, including a few *Melampyrum* species, are able to mature in the absence of a host (Heinricher, 1909). Usually such plants are stunted, discolored, and sensitive to drought, but they nonetheless flower and set seed. Thus, it is obvious that some green root-parasites are less dependent on their hosts than *Castilleja coccinea* is.

The results of divorcing *Castilleja coccinea* from its hosts demonstrate that the parasitic attachment is not required for bolting once the rosette has reached maturity. However, flowering in rosette-forming plants such as *Castilleja coccinea* involves two processes, the bolting of the flowering shoot and the formation of floral primordia. It is not yet known if the host is required for initiation of floral primordia, but it is likely since rosettes which are divorced from their hosts before they are mature typically cease apical growth, and eventually suffer apical necrosis.

Although the rosettes can flower detached from their hosts once they are mature, there is no evidence that they are detached in the wild during their flowering period. To be sure, some of the haustoria of a rosette likely plug up during the winter months, and some host roots or even whole hosts die, and as well some parasite roots die. Thus, unless new haustoria are formed in the second season of growth, a year-old rosette has less host-contact than does a rosette about four months old. Nonetheless, bolting rosettes doubtless enjoy extensive host-contact.

The selective advantage of the ability to bolt without a host is not clear. Of course, if the hosts were all annuals, such a mechanism would be essential for the survival of a biennial parasite such as *Castilleja coccinea*. But, the fact is, few of the hosts of *Castilleja coccinea*, at least in the Houghton Lake area, are annuals. *Polygala sanguinea* and *Trifolium agrarium* are the only abundant annuals in the vegetation. None of the four or five dominants of the vegetation is an annual. However, it is likely that some of the roots of the perennial hosts die back during the winter months. Any advantage the parasite gains in being independent during its second year may derive from this die-back of host roots and the resulting loss of functional haustoria.

It would be interesting in this regard to study the perennial *Castilleja* species so common in boreal regions and western North America. For three reasons it is unlikely that these perennial *Castilleja* are independent of their hosts even after they reach maturity (assuming that they are parasitic). (1) The adult plants of *Pedicularis* and *Gerardia*, two genera of root-parasites related to *Castilleja*, maintain many haustoria which in microscopic section appear functional. (2) The haustoria of *Comandra* and *Gerardia* attain a diameter of 1-2 cm, suggesting that they grow and function for several years. (3) Mature perennial *Castilleja* is notoriously hard to transplant, even into pots of its native soil. If perennial *Castilleja* are indeed host-dependent, they likely maintain host-contact either by a spring burst of root-growth and haustorium production or by the yearly enlargement of existing haustoria in pace with the growth of host roots.

ion uptake

Beath *et al.* (1941) asserted that at least one species of *Castilleja*, *Castilleja chromosa*, accumulates minerals independently of its host plants. Beath and his colleagues were interested in *Castilleja* as an indicator plant in seleniferous areas in the western states. They assumed that all *Castilleja* plants are root-parasitic, and could contain only as much selenium as their hosts. They found, however, that their samples of *Castilleja chromosa* parasitic on *Chrysothamnus pumilis* contained 258 ppm of selenium but the *Chrysothamnus* only 1 ppm. Moreover, the converse was true—they got a low selenium count for *Castilleja chromosa* parasitic on *Astragalus bisulcatus*, normally a selenium-accumulating species. Their conclusion was that '...*Castilleja chromosa* can absorb selenium from a raw seleniferous shale regardless of the kind of host plant with which it is associated'.

Beath and his co-workers assumed that *Castilleja* can not selectively accumulate selenium from its hosts. However, this is only an assumption. Probably the haustoria impose no permeability barrier to most substances in the vascular system of the host, since the connection between host and parasite in histologic section appears to be a xylem-to-xylem contact. Nonetheless, the parasite tissue may be selective in its use of the host materials which reach it. Thus the lack of correspondence in selenium content of host and parasite may indicate (1) as Beath suggests, independent absorption by *Castilleja* or (2) selective use by the parasite of whatever host substances reach it through its haustoria. At any rate, Beath has shown that the selenium content (not necessarily the selenium-absorbing capacity) of Castilleja chromosa plants is independent of whatever hosts they attack.

damage to the host

In any discussion of parasitism, there arises the question of damage to the host. It seems only logical that a plant suffers a lower rate of growth when supporting a parasite. In theory, the parasitism does no damage only if the host has more than it needs of whatever the parasite takes. Certainly it would be to the advantage of the parasite not to kill its hosts or even to impair their vigor, especially if the parasite is a perennial.

Some plants enjoy 'luxury uptake' or mineral nutrients, and often have apparent 'excesses' of compounds such as free amino acids. A root-parasite likely would not damage its hosts by extracting such materials. If root-parasites do pirate only 'excess' host compounds, they are exploiting a source of energy and materials not available to other higher plants except as root secretions. However, it could be argued that such excess host compounds are available only at certain times in the host's life cycle. Unless the parasite restricts its extraction to those periods, it must extract more than just 'excess' host compounds.

The question of host damage will be answered satisfactorily only when it is discovered what the parasite takes from its hosts, but the problem can be studied in other ways until that information is obtained. What is needed is a statistical analysis of the performance of pairs of host plants, one plant of each pair attacked and the other not. A significant difference in, say, plant height or number of leaves between the attacked and unattacked hosts would indicate that the parasite does lower the vigor of its hosts. It would be ideal, of course, to use genetically-identical hosts for such a study, and, conveniently, this is possible ——— Castilleja coccinea will parasitize Kalanchoë verticillata, a greenhouse herb which asexually produces hundreds of genetically-identical plantlets along the margins of its leaves. This host has another advantage in that Castilleja coccinea will parasitize the plantlets when they are not much larger than the parasite itself, and thus any drain the parasite imposes on them will be obvious. An analysis of host-damage using Kalanchoë and Lactuca canadensis as hosts will be made in growth-control chambers during the winter of 1962-1963.

A crude analysis has been made of the attrition and loss of Lactuca canadensis under heavy attack by Castilleja coccinea. The results suggest that the host suffers damage only when the parasites are abundant. However, such a heavy attack by Castilleja coccinea rarely occurs naturally. Not only are the rosettes scattered in the field, but also each rosette typically attacks several plants around it ——— this is demonstrated readily by excavating the rosette's root system. Thus, the attack of a rosette is divided among several hosts, and presumably no one host suffers unduly. The results of artificial host-damage experiments must be evaluated with this in mind. It is likely that given a very heavy parasite attack, any host plant would suffer a lower rate of growth.

Although Castilleja coccinea typically does not subject its hosts to heavy attack, some root-parasites do, and inflict major losses in the yield of crops. An alarmed California farmer wrote to J. Burtt Davy in 1898

> 'You will find enclosed a branch of a weed [Orthocarpus pusillus Benth.] which has lately made its appearance in our valley lands, and it appears to take possession, and wherever it comes the grass

> disappears, but still it does not seem to grow thick enough to choke out the grass... Many of our fields that have always been very productive of clover and also rye-grass are being covered with this, and at a distance it looks like moss...'.

Alectra vogelii causes 'serious damage to leguminous crops...of the Transvaal and Southern Rodesia' (Botha, 1948). Striga wherever it becomes established reduces considerably the yield of its corn and sorghum hosts. Härtel (1941) studied the carbohydrate content of alpine grasses attacked and unattacked by Pedicularis. He found no difference in carbohydrate content of attacked and unattacked plants. The raw fiber content of the hosts similarly does not change during parasitic attack. Härtel did note, however, that the attacked plants wither earlier in the season than unattacked plants do.

In short, the evidence indicates that hosts of root-parasites, including Castilleja coccinea, accrue no unusual damage unless the parasites are abundant. But, unfortunately, quite a few species of root-parasites do build up heavy populations and become pests, especially on agricultural land.

parasite micro-distribution

The environment of any plant is at least partly biotic, but the environment of a root-parasite is largely biotic ——— the parasite's vascular stream is continuous with the vascular streams of host plants. To be sure, any environment can be defined in physical and chemical terms, and the distinction erased between biotic and abiotic. Thus it is immaterial to the plant whether a given environmental factor is biotic or abiotic. But, taken as a whole, the environment of a root-parasite, because it is largely biotic, surely is different from that of an autotroph. Thus, the variables of a root-parasite's environment are not all the same as those of an autotroph's environment. For example, water uptake by a root-parasite is affected not only by soil moisture, but also by the uptake capacity of host plants. In short, the host is a major factor in the environment of any root-parasite.

The distribution and abundance of a plant species are results of the interactions of the genetic make-up of the individuals of that species and the many variables of the environment. Where the rigor of a few or even one environmental variable exceeds the species' genetically-fixed capacities, the species is not successful. This is as true for micro-distribution and abundance as it is for, say, continental distribution and abundance, although environmental variables tend to vary less over small surfaces. As yet, no plant is known so well that its success can be predicted under simulated environments. In fact, few are well known to predict their performance under varying regimes of the most important environmental factors of light, moisture, and temperature. Moreover, the detailed pattern of these factors in the environment through time and in space is poorly known.

Because of this ignorance of what factors in the environment really control a species' field performance, and how those factors are distributed, it is worthwhile to study the micro-distribution of a root-parasite such as Castilleja coccinea. Castilleja coccinea is known to require in its environment a host or hosts, and the location of those hosts can be plotted accurately. Thus here is a plant, one of whose environmental requirements is clear-cut and also has an obvious pattern in the environment.

In greenhouse culture the plants of some host species support the growth of Castilleja coccinea better than others do. Thus, the hosts in a given community can be ranked according to their ability to support the parasite. Then, on this basis, one can try to explain the field distribution of Castilleja coccinea in that community. However, the micro-distribution of Castilleja coccinea in one such community in the Houghton Lake area did not reflect this host ranking. The reasons for the failure might be several —— (1) compared with other factors which influence the distribution of Castilleja coccinea, host-suitability might be insignificant, (2) the sample size may have been too small, (3) the estimations of root-spread of the hosts, an assumption made in the study, may have been in error, and (4) at least some flowering (second-year) parasites were included in the study, and the hosts recorded for them in reality may not have been hosts, since bolting rosettes do not require host-contact. In this last case, the hosts which nourished the parasite might have died previous to the study. If other, non-host plants took their place, spurious data were collected in the study, and even if nothing took the place of the real host, that host nonetheless went undetected. Further studies will be made of this question of the effect of hosts on parasite micro-distribution. One approach will be experimental —— the parasite will be grown in controlled environments, and its survival noted for various combinations of hosts and various host densities.

CONCLUSIONS

1. *Castilleja coccinea* is biennial in its development, at least in the Houghton Lake area of Michigan. Seed germination and maturation of the rosettes occur during the first season, and bolting of the rosettes, flowering, and fruiting occur in the second season.

2. Seed germination requires light, moisture, and moderate temperatures. Presence of a host is not a germination requisite, nor do host roots influence the direction of growth of the parasite roots.

3. Production of haustoria by parasite roots is not solely a result of physical contact with an object or with living tissues.

4. The xylem of host and parasite are connected through the vascular trace in each haustorium. Eosin Y, C^{14}-fructose, $S^{35}O_4$, and $P^{32}O_4$ will cross the haustorial connection into the parasite, and presumably most or all the substances transported in the host xylem cross as well.

5. The parasite apparently has its own photosynthetic machinery and uses it.

6. Nonetheless, successful penetration of host roots is necessary for growth of the parasite beyond the seedling stage.

7. Thus, it is likely that the host compound (or compounds) most vital to the parasite is a phytohormone or enzyme prosthetic group rather than a respiratory substrate. This compound is not one of the more common vitamins or auxins.

8. The parasitic attachment is not necessary for bolting of the rosette, but under natural conditions bolting rosettes do enjoy extensive host-contact.

9. The parasite has a wide host-range, and will attack plants which do

not occur in its natural range.

10. However, the hosts do not support the parasite equally well, and on the basis of greenhouse studies the hosts in a given community can be ranked according to their ability to support the parasite.

11. This ranking of hosts is not reflected in the field micro-distribution of the parasite in the community.

12. The hosts accrue little damage in the host-parasite interaction except when under heavy parasite attack.

LITERATURE CITED

Bailey, L. H., 1951, Manual of Cultivated Plants, The MacMillan Company, New York.

Barber, C. A., 1906, Studies in root-parasitism, the haustorium of *Santalum album*, part 1. early stages, up to penetration, Mem. Dept. Ag. India, Bot. Ser. 1(1)I:1-30.

————————, 1906, Studies in root-parasitism, the haustorium of *Santalum album*, part 2. the structure of the mature haustorium and the inter-relations between host and parasite, Mem. Dept. Ag. India, Bot. Ser. 1(1)II:1-58.

————————, 1907, Parasitic trees in southern India, Proc. Cam. Phil. Soc. 14:246-256.

Bartcinskii, R., 1934, C. R. Acad. Sci. U.R.S.S. 1:343 (cited in Knapp, R., 1954).

Beath, O. A., C. S. Gilbert, and H. F. Eppson, 1941, The use of indicator plants in locating seleniferous areas in western U.S.A. IV. progress report, Amer. J. Bot. 28(10):887-900.

Bormann, F. H., 1957, Moisture transfer between plants through intertwined root systems, Plant Phys. 32:48-55.

Botha, P. J., 1948, The parasitism of *Alectra vogelii* Benth. with special reference to the germination of its seeds, South Afr. J. Sci. 44:119.

Brown, R., and M. Edwards, 1944, The germination of the seed of *Striga lutea*, I. host influence and the progress of germination, Ann. Bot. N.S. 8:131-148.

Brown, R., 1946, Biological stimulation in germination, Nature (London) 157(3977):64-68.

Brown, R., A. W. Johnson, E. Robinson, and A. R. Todd, 1949, The stimulant involved in the germination of Striga hermonthica, Proc. Roy. Soc. London B 136:1-12.

Brown, R., E. Robinson, and A. W. Johnson, 1949b, The effects of D-xyloketose and certain root exudates in extension growth, Proc. Roy. Soc. London B 136:577-591.

Brown, R., A. D. Greenwood, A. W. Long, and G. J. Tyler, 1951, The stimulant involved in the germination of Orobanche minor Sm. 2. Chromatographic purification of crude concentrates, Biochem. Jour. 48(5):564-568.

Cantlon, J. E., E. J. C. Curtis, and W. M. Malcolm, 1962, manuscript submitted to Ecology.

Chabrolin, C., 1934, La germination des graines d'Orobanche, Compt. Rend. Acad. Sci., Paris 198:2275-2277.

Curtis, E. J. C., and J. E. Cantlon, 1962, manuscript.

Davy, J. B., 1898, Parasitism of Orthocarpus pusillus, Erythea 6:93.

Fernald, M. L., 1950, Gray's Manual of Botany, Eighth Edition, American Book Company, New York.

Gautier, L., 1908, Sur le parasitisme du Melampyrum pratense, Rev. gen. Bot. 20:67-84.

Gislen, Torsten, 1949, Problems concerning the occurrence of Melampyrum arvense in Sweden, Oikos 1:208-234.

Gleason, H. A., 1958 (second printing), The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada, New York Botanical Garden.

Hald, A., 1958 (second printing), Statistical Tables and Formulas, John Wiley and Sons, New York.

Härtel, O., 1941, Über die Ökologie einiger Halbparasiten und ihrer Wirtspflanzen, Ber. dtsch. bot. Ges. 59:136-148.

————————, 1956, Der Wasserhaushalt der Parasiten, Handb. d. Pflanzenphys. 3 (Pflanze und Wasser):951-960.

————————, 1959, Der Erwerb von Wasser und Mineralstoffen bei Hemiparasiten, Handb. d. Pflanzenphys. 11 (Heterotrophie):33-45.

Heinricher, E., 1897, Die grünen Halbschmarotzer I. *Odontites*, *Euphrasia*, und *Orthantha*, Jahrb. f. wiss. Bot. 31:77-124.

————————, 1909, Die grünen Halbschmarotzer V. *Melampyrum*, Jahrb. f. wiss. Bot. 46:273-376.

————————, 1917, Zur Physiologie der Schmarotzenden Rhinantheen, besonders der Halbparasitischen, Naturwissenschaften 5:113-119.

————————, 1931, *Monographie der Gattung Lathraea*, Jena.

Knapp, R., 1954, Experimentelle Soziologie der höheren Pflanzen, Ulmer, Stuttgart.

Koch, L. von, 1889, Zur Entwicklungsgeschichte der Rhinanthaceen (*Rhinanthus minor* Ehrh.), Jahrb. f. wiss. Bot. 20:1-37.

Kostytschew, S., 1924, Untersuchungen über die Ernährung der grünen Halbschmarotzer, Beih. bot. Abl., Abt. 1, 40:351-373.

Kusano, S., 1908/1909, Further studies on *Aeginetia indica*, Bull. Coll. Ag., Tokyo Imp. Univ. Japan 8:59-77.

MacDougal, D. T., and W. A. Cannon, 1910, The conditions of parasitism in plants, Car. Inst. Wash. Pub. 129.

Peirce, G. J., 1893, On the structure of the haustoria of some phanerogamic parasites, Ann. Bot. 7:291-328.

Peirce, G. J., 1894, A contribution to the physiology of the genus *Cuscuta*, Ann. Bot. 8:53-118.

—————, 1905, Dissemination and germination of *Arceuthobium occidentale*, Eng., Ann. Bot. 19:99-114.

Pennell, F. W., 1935, The Scrophulariaceae of eastern temperate North America, Monog. Acad. Nat. Sci. Phila. 1:1-650.

Rogers, W. E., and R. R. Nelson, 1959, Studies on the translocation of minerals and carbon compounds between corn and *Striga asiatica*, Jour. Elisha Mitch. Sci. Soc. 75(2):67 (abstract).

Sablon, Leclerc du, 1887, Recherches sur les Organes d'Absorption des Plantes Parasites (Rhinanthacees et Santalacees), Ann. Sci. Nat. Bot. Ser. 7(6):90-117.

Shive, J., 1915, A three-salt nutrient solution for plants, Amer. Jour. Bot. 2:157-160.

Seeger, R., 1910, Versuche über die Assimilation von *Euphrasia* (s.l.) und über die Transpiration der Rhinantheen, Sitzber. Kais. Akad. Wiss., Math.-Nat. Klasse 119:987-1004.

Solms-Laubach, H. Grafen zu, 1867-1868, Ueber den Bau und die Entwicklung der Ernährungsorgane parasitischer Phanerogamen, Jahrb. f. wiss. Bot. 6:509-638.

Solomon, S., 1952, Studies in the physiology of phanerogamic parasitism with special reference to *Striga lutea* Lour. and *S. densiflora* Benth. on *Andropogon sorghum* Hack. 1. the osmotic pressure of the host and parasite in relation to the nutrition of the host, Proc. Indian Acad. Sci., Sect. B 35:122-131.

Stephens, E. L., 1912, The structure and development of the haustorium of *Striga lutea*, Ann. Bot. 26:1067-1076.

Sunderland, N., 1960, Germination of the seeds of angiospermous root parasites, *The Biology of Weeds*, a symposium of the British Ecological Society, Blackwell Scientific Publications, Oxford.

Vallance, K. B., 1951, Germination of seeds of *Bartsia odontites*, Nature 167:732.

Worsham, A. D., D. E. Moreland, and G. C. Klingman, 1959, Stimulation of *Striga asiatica* (witchweed) seed germination by 6-substituted purines, Science 130(3389):1654-1656.

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