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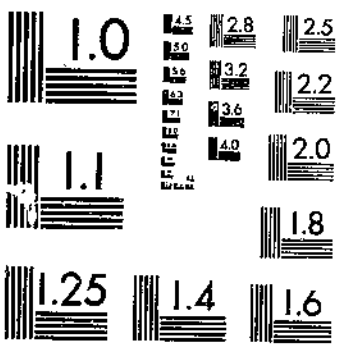

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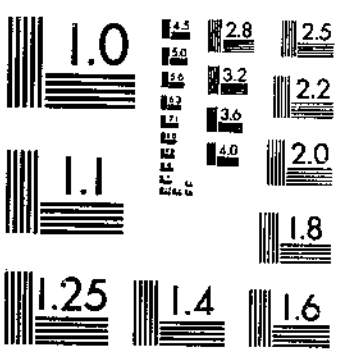

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NATIONAL BUREAU OF STANDARDS-1963-A

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Relationship of Insects to the Spread of Azalea Flower Spot [1]

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INTRODUCTION

A serious spot disease and blight was first reported in April 1931 near Charleston, S. C., as attacking the flowers of cultivated azalea at the height of bloom. A systematic investigation of the disease, caused by *Ovulinia azaleae* Weiss, was begun in 1933, a preliminary report of which appeared in 1935 and more complete accounts in 1940.[3]

[1] Received for publication May 26, 1941.

[2] At the time of the investigation reported on herein, the junior author was senior pathologist in the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

This bulletin is a report on one phase of the investigations on the azalea flower spot disease conducted by the United States Department of Agriculture largely at the Magnolia Gardens, Charleston, S. C., but also at other gardens and estates, as mentioned in the bulletin. The kindness of C. Norwood Hastie, owner of Magnolia Gardens, and of the owners and superintendents of the other gardens who opened their estates for the investigations is deeply appreciated.

The following persons aided in the field studies: Howard Carraway in 1936, R. H. Nelson and C. B. Reid in 1937, and J. W. Carraway in 1938. The writers are grateful to these men and also to the late Miss Grace Sandhouse and other specialists in the Division of Insect Identification of the Bureau of Entomology and Plant Quarantine for determining the large numbers of insects used in experimental tests.

[3] WEISS, FREEMAN. A FUNGUS SPOT OF AZALEA FLOWERS. (Abstract) Phytopathology 25: 35. 1935.

——— OVULINIA, A NEW GENERIC SEGREGATE FROM SCLEROTINIA. Phytopathology 30: 236-244, illus. 1940.

——— and SMITH, FLOYD F. A FLOWER-SPOT DISEASE OF CULTIVATED AZALEAS. U. S. Dept. Agr. Cir. 556, 28 pp., illus. 1940.

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Azalea flower spot is characterized by the appearance of small specks (fleck stage) in the flower parts, which rapidly enlarge and become blotches with irregular margins. These blotches spread to include one or more petals. The affected tissue softens and collapses, a stage known as limp blight. The course of the disease from the first visible fleck to limp blight requires about 3 days, and when all the flowers of plants in full bloom are affected a most unsightly effect is produced (fig. 1, *B*). The sudden appearance and rapid spread of the disease were at first believed to involve some agent of dissemination other than wind or rain, and as large numbers of insects were observed to visit azalea flowers they were suspected of being vectors. It was also thought that insects might be responsible for the seasonal initiation of the disease in cultivated plantings, since the first infections were found after insect activity on the flowers had been observed for several days or weeks at the beginning of the period of bloom.

Entomological investigations, which followed a survey in 1934, were conducted from 1935 to 1938, inclusive. They included observations on the habits of the insects visiting azalea flowers and experiments to determine the relationship of insects to: 1, The scratchlike abrasions at the entrance to the tubular part of the flowers and to the primary infections of flower spot; 2, the secondary spread of the disease within a given planting and to distant plantings, and 3, the introduction of the disease into the garden each year either from some wild host or from overwintering quarters of the insect. An abstract[4] on the preliminary results of the experiments was published in 1938.

The general conclusions resulting from the investigations are included in circular 556, referred to above, which is intended as a source of general information on the whole subject of this serious disease. The present bulletin relates in greater detail the experimental procedure and the results obtained in the entomological studies which formed a basis for conclusions drawn in that circular.

INSECTS VISITING AZALEAS AND OBSERVATIONS ON THEIR HABITS

The most conspicuous insects observed on cultivated azaleas include bumblebees (*Bombus* spp.), carpenter bees (*Xylocopa* spp.), the ground-nesting bee *Emphoropsis floridana* (Smith), the honeybee (*Apis mellifera* L.), and other Hymenoptera, thrips, small numbers of various Diptera, Coleoptera, Lepidoptera, and Heteroptera, and several species of spiders. Collections of insects were made between February 14 and April 19 in the several years. The observations are recorded in detail both for their possible bearing on the dissemination of the disease by insects and for their value on the habits of the insects in azalea gardens.

Bumblebees

All individuals of the five species of *Bombus* observed on azalea flowers were queens except for a few workers and one male of *B. bimaculatus* Cresson and workers of *B. griseocollis* (Deg.). An occasional specimen of *B. americanorum* F. was observed early in the season, but this species did not become general on azalea until late

[4] Weiss, Freeman, and Smith, Floyd F. present status of azalea flower spot. (Abstract) Phytopathology 28: 21. 1938.

in the flowering season. It was not common in any of the gardens except Middleton Place, where about 75 or 100 could be observed at one time on a group of azalea plants. Even when very abundant,

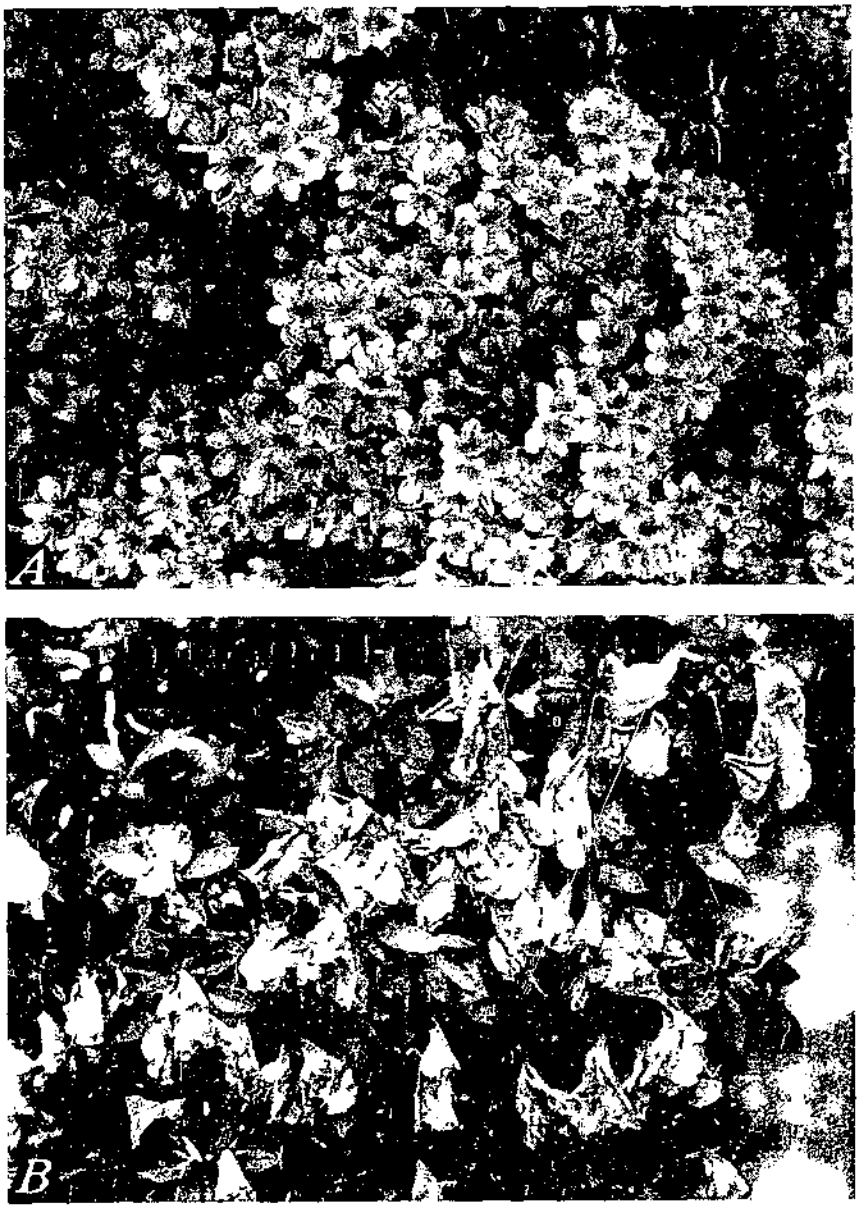

FIGURE 1.—*A*, Azalea plant in full bloom with flowers undamaged; *B*, plant with flowers in the limp blight stage

this species suddenly disappeared from the gardens at about 1 p. m., whereas the other species fed until dusk.

Bombus bimaculatus, *B. impatiens* Cresson, and *B. griseocollis* appeared relatively early in the flowering season but were not observed

before the first azaleas bloomed and the investigations had been under way for 10 days or more. Owners of gardens state, however, that an occasional bumblebee is seen on warm days throughout the winter.

These species become gradually more numerous as the flowering season advances. *Bombus bimaculatus* was more abundant in 1936 and 1937 than the other two species, whereas *B. impatiens* was the dominant one in 1938. *B. griseocollis* has been found in only small numbers throughout the season. Some of *B. impatiens* were seen on cool days early in the spring before the other species were present. This species was often observed feeding on limp-blighted azalea flowers, on fallen flowers, and on the receptacle about the base of the ovary after the corolla had fallen. Only a few of *B. fraternus* Sm. were collected and these appeared rather late in the season.

The observations on species of bumblebees indicated that they apparently have no marked host preferences, since individuals were observed feeding successively on azalea, *Magnolia liliflora*, rose, wisteria, spirea, and clover. They are extremely roving in their habits, and, after feeding on a few flowers in a given locality, may suddenly fly away in a nearly straight line, sometimes through woods or even across a river and marsh over a mile in width. Evidence that they travel considerable distances was obtained when bumblebees marked in one garden were recovered the same day in other gardens from 0.4 to 0.8 mile away, and the recovery of one *Bombus bimaculatus* in a garden 5 miles distant on the eighth day after it was marked. At times the bumblebees appeared to be travelling in rather definite routes. In one garden (Drayton Hall) the bumblebees approached the end of an azalea hedge, fed on a few flowers, and passed on through the woods without changing their general course or visiting other azaleas in the hedge.

Carpenter Bees

Males of *Xylocopa micans* Lepeletier were observed to hover about azalea and other flowers rather early in the season, but they did not alight to feed. Females appeared in moderate numbers on wisteria and later went to azalea. They were abundant on a few azalea plants in one section of Magnolia Gardens, but in other parts of this garden and in other plantings they were always rather scarce. The extremely active females rapidly visited one flower after another, landed forcibly on each one, and entered the corolla with vigorous leg movements.

Males of *Xylocopa virginica* Drury punctured the corolla tube near the base and fed from the outside of the azalea flower instead of entering the flower as did all other observed species. Only a few males were observed in azalea gardens in February 1936, but they became very abundant a month later on wisteria as it came into flower. In 1934 to 1936, inclusive, they occurred in large numbers on azalea in April, then suddenly disappeared. In other years they were not found in large numbers on azalea, and only a few females were ever captured.

This species and also *Xylocopa micans* seemed to be attracted to certain azalea plants or groups of plants where large numbers of both species were present. Both species appeared to be attracted by diseased flowers in the limp-blight stage, over and into which they crawled, all parts of their bodies coming into contact with the spore-bearing surface of the flowers.

Ground-Nesting Bees

The solitary ground-nesting bee *Emphoropsis floridana* appeared on warm days with the first bumblebees and in 1934 and early in 1935 was the dominant species until the various species of *Bombus* became more abundant. In 1935 and later years this species was very scarce during midseason, and many dead adults were found along the paths at Magnolia Gardens. It was supposed, but not demonstrated, that the fungicidal dusts and sprays applied there were toxic to this species. This insect continued to be the dominant species during most of each season at Middleton Place, where no dust was applied.

Males appeared before the females and were very active and wary of capture while feeding within the flowers. The females were less active and were more easily captured. It is believed that tests with females gave a fair index of the relationship of this species to the spread of the disease, since they fed more regularly than did the males and in their movements came into more intimate contact with the flowers.

Honeybees

Early in the season honeybees were the most conspicuous flying insects in the gardens, where they usually fed in camellia flowers although occasionally visiting azaleas. They seemed to be attracted to limp flowers on azalea that had been killed by frost, and on certain days later in the season fed in moderate numbers on healthy azalea flowers. They occurred in large numbers on crab apple, on photinia, and especially on holly, when these plants were in bloom. Azaleas were apparently not a favored food plant and were visited by honeybees only when other flowers were not available.

Thrips

Among nine collections of thrips made from azalea flowers in April 1934, four species, *Frankliniella tritici* (Fitch), *F. fusca* (Hinds), *Heterothrips azaleae* Hood, and *Leptothrips mali* (Fitch), were recognized. Most of the specimens were of the two species *F. tritici* and *H. azaleae*. The latter occurred in greatest numbers at Middleton Place. During the course of field studies in April 1935 these thrips were again observed to be abundant on cultivated azalea, but during the same period in 1936 they were very scarce. The flowering period of cultivated azaleas in 1937 and 1938 was much earlier than in previous years, and only an occasional adult of *F. tritici* was found. Adults of *H. azaleae* were abundant in all years on the late-appearing flowers of *Rhododendron nudiflorum* (L.) Torr. in nearby woods. This species apparently caused an inconspicuous russeting of the stamens of cultivated azaleas. *F. tritici* was found deep in the corolla of azalea and caused no obvious destruction of color or other injury.

Ants

Ants of several species in the genera *Crematogaster*, *Dorymyrmex*, *Formica*, *Pheidole*, *Ponera*, and *Prenolepis* were abundant on the soil and mulch among the azalea plants. They were often observed on stems and leaves of azaleas, where they were apparently attending scales (*Pseudaonidia paeoniae* (Ckll.)) and whiteflies. In repeated

examinations only an occasional ant was found on the flowers, except in one instance where infected flowers were hanging in limp masses and a few ants were crawling over them. Numerous ants were seen caught in the sticky exudate from sepals below the flowers, which appears to form an effective barrier against them. When the ants in transmission tests were shaken onto flowers they often became entangled in the exudate.

Flies

Adults of the conspicuous bee fly *Bombylius azaleae* Shannon occurred in small numbers on azalea throughout the season. They were exceedingly active on the wing and very difficult to capture, so few were obtained for transmission tests. Observations on the dainty movements of this insect in feeding indicated, however, that it would become contaminated with spores or disseminate them less readily than would other species. This fly approached the flower, came to rest very lightly on the stamens, then, after having fed deeply in the corolla with its long proboscis, departed, scarcely having touched the corolla.

Miscellaneous flies were observed in small numbers on warm days in 1937 and 1938 on early azalea and camellia flowers, where in most cases they appeared to be sunning themselves. They seemed to disappear from azalea later in the season.

ACTIVITY OF BEES IN VISITING FLOWERS

Information on the relative activity of the various species of bees as manifested by the number of flowers visited in a given time seemed of possible importance in relation to the spread of the disease. Observations on the number of flowers visited in 1 minute by individuals of five species of bees were made on April 10, 1936, at a temperature of 75° F. and on April 16 at a temperature of 68°, and the results are given in table 1.

Table 1.—*Observations on number of azalea flowers visited by certain species of bees per minute, at two different temperatures, Magnolia Gardens, Charleston, S. C., 1936*

Temperature and species	Bees observed	Flowers visited in 1 minute		
		Maximum	Minimum	Average
68° F.:	*Number*	*Number*	*Number*	*Number*
Bombus americanorum	5	12	8	10.4
B. impatiens	4	11	4	9.0
Emphoropsis floridana	6	9	3	5.5
Xylocopa micans	1	4	4	4.0
X. virginica	1	7	7	7.0
75° F.:				
B. impatiens	4	13	9	11.2
E. floridana	5	10	7	8.6

The 2 species *Bombus impatiens* and *Emphoropsis floridana* were observed to visit a maximum of 13 and 10 flowers per minute, respectively, at 75° F. At 68° F. the greatest number of visits per minute was made by *B. americanorum*.

CAUSE OF INSECT ABRASIONS AND THEIR RELATION TO FLOWER SPOT INFECTION

Early in the study of the factors influencing flower spot infection circumstantial evidence was obtained that the sharp claws and spines on the legs of bees caused abrasions (fig. 2) on the corolla as the insects pushed their way into the flower.

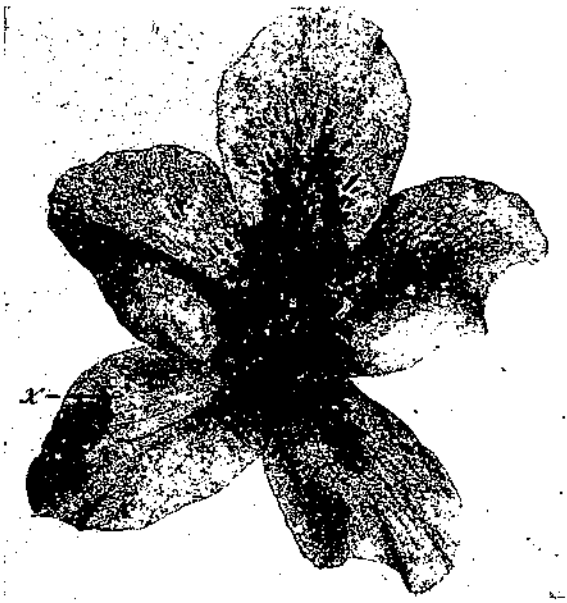

FIGURE 2.—Abrasions on the lower left petal *(x)* and in the throat of an azalea flower attributed to insect injury and sometimes associated with early flower spot infection.

These abrasions were most evident early in the flowering period. Often the first flower spot infections appeared on these abraded flowers and sometimes appeared to originate in the scratches. This led to the suspicion that insects visiting the flowers dropped spores, thus causing initial flower spot infections. Although it was known that the flower spot pathogen (*Ovulinia azaleae*) was capable of infecting flowers without mechanical injury, the importance of insect visits in the cases of naturally occurring flower injuries and early infections demanded elucidation.

It was observed that during the first week of April 1936, at Magnolia Gardens, flower spot from secondary infections became increasingly more prominent and overshadowed the characteristic scratchlike injuries attributed to insect activity. After a wave of heavy infection from April 6 to 12, no rain fell for a week, and there was comparatively little spread of the disease to the later developing flowers, but the abrasions became very prominent on flowers exposed to full sunlight. Since the injuries developed during a period of drought and high temperature, without an apparent increase in the number of insects, it appeared that certain weather conditions might favor

or retard their development. A similar flower injury (fig. 3, *A*) became prominent at this time in field cages into which insects were introduced, and also in the laboratory inoculation chambers.

An experiment was then conducted to determine whether insects could cause flower abrasions similar to those observed in nature and whether humidity affected their development.

An individual of each of four species of bees was held with tweezers by one hind leg and allowed to walk over, scratch, and bite six groups of unblemished flowers of the same variety and age. Three of the groups were atomized with water before the test, whereas the flowers

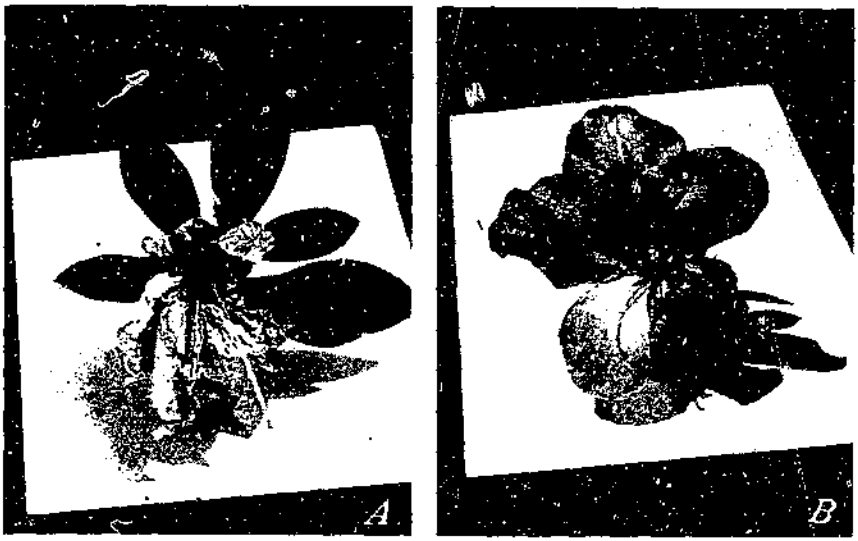

FIGURE 3.—Two types of injury to azalea flowers: *A*, Extensive injury to a late-season flower after numerous visits by bees, showing the tissue clotted and purple and collapsed; *B*, white margins developing in a flower kept in a moist atmosphere after it had been mechanically wounded by insects.

in the other three groups were injured while dry. Visible scratches and small punctures were made by the insects in the flower petals in all groups. A group each of moistened and of dry flowers was then handled as follows in an attempt to incubate any infection in them under (1) very moist, (2) moderately moist, and (3) dry conditions:

In the first case the flowers were thoroughly sprayed with an atomizer and placed in a celluloid cage in a bottle of water to be maintained in saturated atmosphere. They were sprayed twice daily to keep them moist.

In the second the flowers were placed in a cage without their being sprayed. Moderately high humidity was maintained.

In the third case the flowers, in bottles of water as before, were left uncovered in the laboratory under drier conditions than in the preceding case.

The white lines of scratches and their whitened margins, most prominent in the case of those under high humidity (table 2), were evident on the day after the wounding of the tissue, and the margins

TABLE 2.—*Summary of subsequent development of injuries after a mechanical wounding of azalea flowers by bees, Charleston, S. C., 1936*

Species of bee in test	Variety of azalea	Degree and type of subsequent flower injury in—					
		Saturated atmosphere of atomized cages		Moderately high humidity in cages not atomized		Dry-room atmosphere	
		Moistened before injury	Dry when injured, moistened after injury	Moistened before injury	Dry when injured, not moistened later	Moistened before injury	Dry when injured, not moistened later
Bombus americanorum.	Coccinea	Broad, white margins to 2 mm. width along scratches.	Narrow, white margins.	Narrow, white margins, in some cases absent.	Broad, purple margins of collapsed tissue.	Broad purple margins.	Broad purple margins except for a few whitened scratches.
B. bimaculatus.	Phoenicia	White scratches without margins.	White scratches without margins or with narrow, white margins.	Broad, transparent purple margins.	Narrow, transparent purple margins.	Broad clotted purple margins to 2 mm. width, not transparent.	Do.
B. impatiens	Formosa	White scratches with or without indistinct narrow white margins.	do	Distinct, narrow, white margins, others broad to purple.	Broad, purple margins.	Broad purple margins to 3 mm. width.	As in the preceding, but with narrow purple margins.
Emphoropsis floridana.	Formosa	do	do	White scratches with white or purple margins.	Narrow, dark-purple margins.	do	Do.

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very slowly broadened during the following 3 days (fig. 3, *B*). The whitened tissue did not shrink or collapse, and it appeared that a leaching out or oxidation of the coloring matter caused the bleached effect. The bleached areas, if allowed to dry, became thin and nearly transparent and collapsed if the whole petal was involved. In such cases the margins broadened and appeared purple as discussed later.

In the moderately moist and in the dry tests, the margins of the injured spots were purple or clotted in appearance, except for a few instances in which the tissue was white, as in the very humid condition. The tissue in the purple areas at first collapsed, then gradually dried and became thin and papery and nearly transparent except for the retained purple coloration. The development of this injury was a gradual process, continuing up to the close of the experiment. Apparently under the moderately humid and rather dry-room conditions the tissue adjacent to wounds dried out and shriveled, but with the color retained in the cells. This is further indicated by the fact that if injured flowers with white margins that had developed under very humid conditions are placed in a drier atmosphere, the margins continue to extend but retain the purplish color.

Examination of flowers in the gardens showed white scratches in some cases, particularly in the early part of the season. Usually, however, the margins of the injuries were purple and collapsed at this season, whereas later in the season broad purple margins developed around injuries as occurred in laboratory tests. If these types of injury are compared with those in the laboratory tests, it would appear that the conditions prevailing in the garden, particularly as the season advances, were drier and more favorable for the development of the purple type of injury. The conditions of high humidity necessary for prominent development of white lines and margins occurred rarely or not at all in the gardens.

In the laboratory tests greater contrasts in both types of injury were evident on the dark-red variety Coccinea than on either of the lavender varieties, Formosa or Phoenicia. The pink varieties in the garden showed less color and greater transparency in the injured areas. As judged by the laboratory tests, the characteristic abrasions on the flowers could well be caused by bees. Bruises and other injuries could result from wind or hard-blown dust particles.

The following observations and records are presented to show a lack of relationship between the occurrence of flower spot infections and insect abrasions. In the 320 transmission experiments with living insects in 1936, visible abrasions caused by the leg spines or by the chewing mouth parts were evident on 1,124 petals of 447 flowers. In these same tests, 231 petals of 142 flowers developed the flower spot disease, but on only 18 petals of 12 flowers were the infections associated with the mechanical injuries. Since 92.2 percent of the infections developed in the absence of evident mechanical injuries, it appeared that the latter, as observed on flowers in nature, were not an essential aid to the fungus in entering the plant tissue, and the occurrence of both infection and mechanical injury on the same flowers was therefore merely coincidental.

OCCURRENCE ON INSECTS OF CONIDIA OF THE ORGANISM CAUSING AZALEA FLOWER SPOT

On March 27 to 29, 1935, bees were captured in paper cylinders from infected flowers and chloroformed. The tarsi were removed

and examined in lactic acid-phenol under the microscope for the presence of the characteristic spores of flower spot. The results from an examination of seven insects are given in table 3.

TABLE 3.—*Occurrence of conidia of Ovulinia azalea on bees*

Insect No.	Species	Part of insect examined	Spores found
1	*Emphoropsis floridana*	Tarsi and tibiae of forelegs	1 viable spore.
2	*Bombus griseocollis*	Tarsi of three legs on one side	Do.
3	do	do	1 spore germinating.
4	*Emphoropsis floridana*	do	None found.
5	*Bombus americanorum*	Tarsi of forelegs	2 viable spores.
6	do	do	1 viable spore.
7	*Xylocopa micans*	Tarsi of fore and hind legs	4 viable spores.

From these limited observations it is evident that the four species of bees taken from diseased flowers generally bear spores. It is believed that many more spores were present on the parts examined but that because of the dense covering of hairs on the legs they were hidden from view.

DISEASE TRANSMISSION BY INSECTS

The dissemination by insects of the organism causing flower spot was investigated from 1934 to 1938, inclusive. The studies during the first two seasons were conducted under conditions that did not prevent accidental contamination of the flowers inoculated with insects. The studies in 1936 were conducted under laboratory conditions that eliminated the unfavorable factors but gave information only on the relationship of the insects to the disease late in the flowering season. During the last 2 years investigations were conducted throughout the flowering season, and these gave a more complete picture of the relationship of insects to the disease organism and its hosts.

PRELIMINARY STUDIES, 1934 AND 1935

A brief survey of azalea gardens was made in 1934 to investigate the relationship of insects to the spread of the disease. In a preliminary series of tests 14 bees, including *Bombus americanorum*, *B. bimaculatus*, *B. griseocollis*, *Xylocopa micans*, and *X. virginica*, apparently initiated infection.

Field studies were conducted in 1935 between March 26 and April 13, during which period the insects attained their greatest seasonal abundance on azalea, which was then in the latter part of its flowering season. In a series of 93 tests with the above-mentioned insects and, in addition, *Bombus impatiens* and *Emphoropsis floridana*, the results again indicated that insects were responsible for disease spread, but these results were considered unreliable for the following reasons. The flowers to be used for testing insects were selected on plants in the open and were enclosed with the insects in a transparent bag (fig. 4). The flowers were often blemished by mechanical injuries or incipient spot infection that later developed into more advanced stages of the disease, both on checks and on insect-infested flowers. In addition some insects cut their way out of the bags and escaped. Because later work was more extensive and more definite as to results, these preliminary investigations are not included herein.

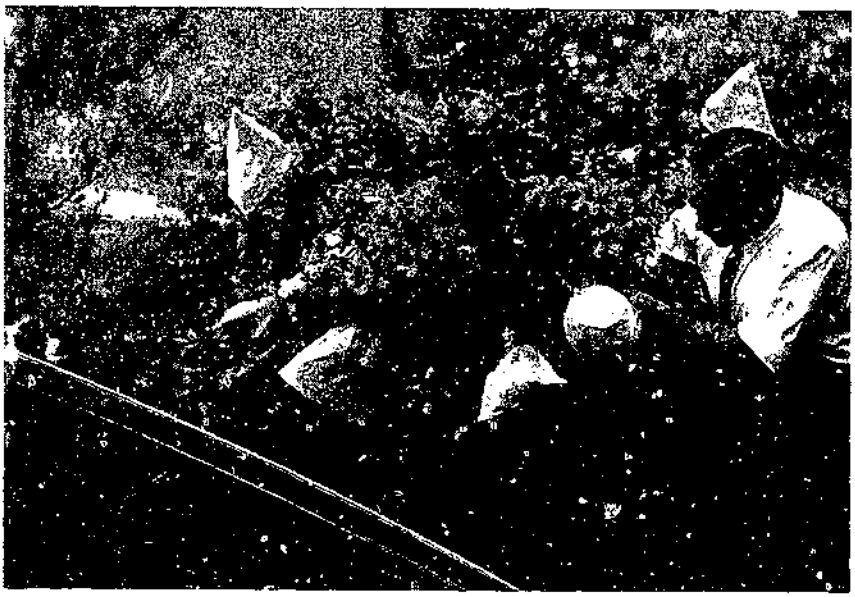

FIGURE 4.—Transparent bags used for confining insects in transmission tests during 1931 and 1935.

IMPROVED METHODS FOR COLLECTING INSECTS AND TESTING THEIR INFECTIVITY

Capturing bees and flies from azaleas with a net was objectionable because of damage to the delicate flowers in the garden. It was unsatisfactory because of additional chances for contaminating the insect by contact with infected flowers that might be knocked off by the sweep of the net. The objective was to capture the insect by a method that would avoid accidental contamination either through the collecting apparatus or by changing the insect's habitual procedure in visiting flowers to collect nectar or pollen. At the time of capture the insect may have become naturally contaminated with spores on that particular diseased flower, or be already contaminated with spores from previous visits to other flowers and be dislodging the same on the flower now being visited, and thereby cause infection.

After preliminary successful tests of capturing insects in paper tubes, the following apparatus and procedure were adopted in the capture of all large insects: Open-end glass vials, 1 inch in diameter and 6 inches long, were marked to distinguish the two ends and were provided with a cheesecloth-covered cotton plug for each end. After an insect had alighted on a flower and had pushed its way into the corolla, or had begun to feed on the outside of the corolla tube in the case of *Xylocopa virginica*, the plug was removed from one end of a vial and the opening was placed over the insect but not touching the flower tissue. After the insect had finished feeding, it would back out of the corolla into the tube. As soon as the insect discovered that its body was in contact with a foreign object it usually quickly turned about to escape and in doing so went farther into the vial, whereupon the cotton plug was replaced.

Two other pieces of apparatus were on hand in the garden to complete the test without delay. Incubation cans were made either from 1-pound coffee cans or from tin cracker cans [5] of approximately the same size (fig. 5). A 2-dram homeopathic vial was fastened to one

FIGURE 5.—Inoculating azalea flowers with an insect inside a portable inoculating chamber, showing incubation can and collecting vial and the sleeves through which the hands were inserted.

side of the can either with a strip of adhesive tape or with a loop of wire with ends projecting through two nail holes and twisted together

[5] WEISS, FREEMAN, and SMITH, FLOYD F. AN INCUBATING CAN FOR LABORATORY OR FIELD USE. Phytopathology 2: 117-119, illus. 1912.

outside. Each can was then balanced at an angle and high-melting paraffin was poured in to fill the recesses between vial and can to permit ease in cleaning and in removal of the insects, and a piece of paper towel was placed in the bottom of each can to absorb any excess moisture, in which the insects might drown. A stem usually bearing two azalea flowers from disease-free plants was inserted in the vial, which had previously been filled with water. The vial held the flowers in an upright position and away from the side of the can so that any infection could develop only in the normal manner. The flowers were moistened by being atomized with water to encourage development of infection by any spores dislodged from the insect during the process, and the lid was replaced on the can until it was time to make the inoculation. This type of incubation can was generally more satisfactory than the celluloid cages (fig. 8, *A*) used in earlier tests.

The inoculation chamber (fig. 6) was a rectangular box 14 by 14 by 18 inches with a floor of wood, a door in one side, celluloid on the other three sides and on top, and circular armholes with sleeves placed in opposite sides through which to insert the hands for manipulating the insect and making the inoculation. Folding legs on the box made the inoculation chamber portable so that it could be readily carried with the other equipment to the various gardens and nurseries. It was thus possible to make the inoculation test within 1 minute after an insect was captured. This reduced the chances for the test insect to dislodge any spores from its body between the time of capture and the exposure of the azalea flowers in the incubation can. If the insects had to be taken to the laboratory for the inoculations to be made, from 10 minutes to 8 hours would have elapsed after they had been captured in the various gardens.

When an inoculation test for determining infectivity of an insect was made, the insect was removed from the opposite end of the vial by means of tweezers gripping a middle leg or a wing (fig. 5) within the inoculation chamber (fig. 6). The insect was allowed to scratch, bite, and buzz over the test flowers for approximately one-fourth to one-half of a minute, depending upon its activity, and then was released in the incubation can and the cover replaced.

The following precautions were taken to avoid accidental contamination of test insects: The insect was removed from the end of the vial opposite to that by which it was captured, to avoid chance contamination with the edge of the vial that might have touched the azalea flower. Vials were washed in warm, soapy water, and tweezers were immersed in 95-percent ethyl alcohol after each test. In most instances the insects were captured by one person, and another made the actual inoculations of flowers in the chamber; otherwise the hands were washed after the capture of the insects and before the inoculations were made.

Thrips were collected from the flowers with a suction apparatus and, after they had been anesthetized with chloroform, were shaken onto a clean paper and several transferred by a sterile brush to each flower in a test. After they revived they crawled about among the flower parts and appeared to behave normally.

A few ants were collected with a suction apparatus from azalea plants but the great majority used in the present investigations were taken at paper cups containing sugar-water placed beneath azalea

bushes in areas where the disease had been serious in previous years. In preliminary tests the ants were stupefied as were the thrips, then transferred by brush to the azalea flowers, but this procedure was

FIGURE 6.—Portable chamber within which azalea flowers were inoculated with insects.

unsatisfactory because all the ants did not recover and, in addition, the treated ants secreted formic acid that spotted the flower petals (fig. 7, A) and caused injury very much like that by flower spot in its

speck stage of development. The injury by the insect secretion could be distinguished by the even margins of areas and the fact that they did not increase with further incubation, whereas the margins of flower spot lesions were uneven and enlarged rapidly (fig. 7, *B*).

In subsequent tests the ants were shaken to the bottom of the vial, which was then unstopped and quickly set in an incubation can, where the ants soon crawled out and over the flowers.

A modified Berlese funnel (fig. 8, *B*) that was used for extracting insects and other animals from samples of soil consisted of a square extracting compartment in which four soil samples enclosed in wire mesh were supported on stiff wires run through holes in the side of the

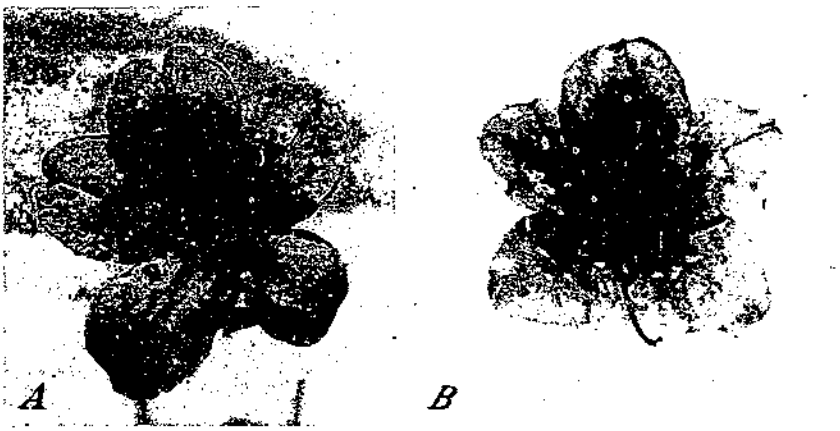

FIGURE 7.—*A*, Flower of azalea, variety Brilliant, showing decolorized areas apparently caused by formic acid secreted by ants; *B*, flower of variety Comptess de Nieuport, showing decolorized spots of flower spot infection in the speck stage.

compartment (fig. 8, *B* and *C*). The funnel below ended in a narrow opening 1½ inches in diameter. A coffee-can cover with a hole in the center was soldered to the funnel about 4 inches above the opening. An incubation can with flowers was then slipped up into the cover and held in place so that dirt falling from above would land on the bottom of the can and not on the flowers that were located to one side and above the funnel opening. Insects and other animals from the soil samples while crawling about in the can came in contact with the flowers. A new set of flowers was exposed daily, and 3 or 4 days were required to drive out the inhabitants of one set of soil samples.

The inoculated flowers were held in the cans for 2 to 4 days at a temperature of 68° to 72° F. in a thermostatically controlled incubation chamber in the laboratory. The flowers were then examined for the presence of disease infection.

After the conclusion of a test the cans were cleaned of all plant material and paper, then washed in warm soapy water and rinsed, after which they were ready for use again. This procedure for cleaning utensils and equipment had been previously found adequate for eliminating any spores or conidia of *Orulinia*, the causal organism.

All insects not positively identified at the time the test was closed were preserved for examination by specialists.

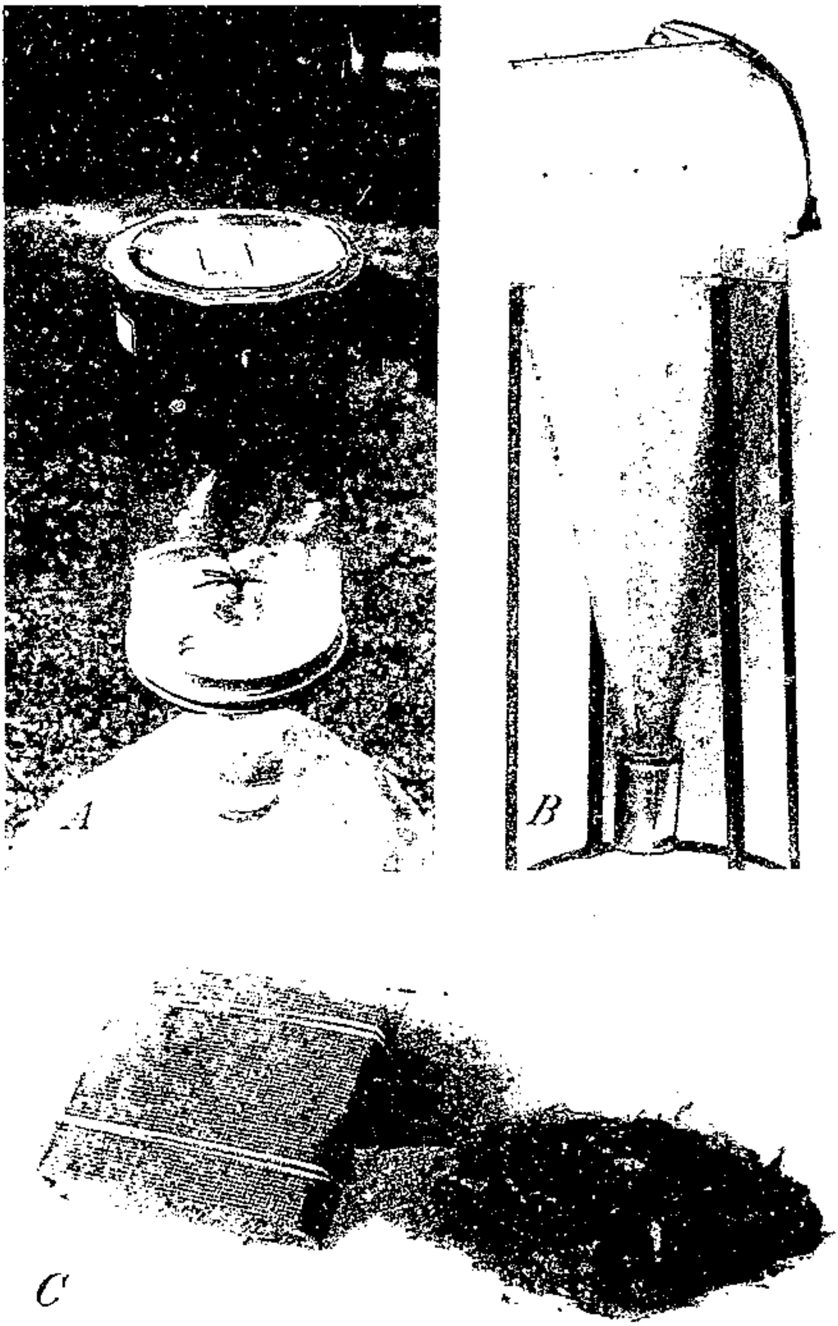

FIGURE 8.—Apparatus [illegible]. A, Cell [illegible] incubation cage [illegible]. B, [illegible] Berlese funnel for extracting soil [illegible] C, [illegible] sample of soil and mulch [illegible]

The species of insects used in tests depended on their seasonal abundance, the limitations of personnel for making collections, and the laboratory facilities for making the tests. Some species were absent early in the season but appeared at various times on azaleas later in the flowering period. Long periods were spent in searching for insects early in the season, whereas later only a short time was required to capture sufficient numbers for tests in all available incubation cans.

Factors that affected the abundance of insects on azaleas were temperature and hosts. There was little insect activity below 60° F. Honeybees preferred other flowering plants to azalea and fed in large numbers on such plants as camellia and holly when these were in flower, and appeared to feed on azalea only when no other plants were available. Carpenter bees appeared to prefer wisteria to azalea. They gradually appeared in increasing numbers from winter quarters and fed on wisteria until its flowers matured, then suddenly transferred to azalea late in its flowering season.

In 1937 and 1938 the azalea flowers were taken from plants in a nursery or a greenhouse where no disease occurred, but as an additional precaution all flowers were incubated for possible development of accidental infection before being used for inoculation tests. Records were kept of the variety of azalea used in each test, but since there was no evident difference in susceptibility under the conditions of these experiments these records are not included.

Studies in 1936

The entomological investigations in 1936 were conducted between March 25 and April 22 and included 21 series of transmission tests with flower-frequenting insects. The laboratory procedure for collecting insects as previously described under methods and equipment was adopted, and this, it is believed, insured against accidental contamination.

Three hundred and twenty transmission tests were made with 15 species of living insects (table 4). Positive cases of transmission of the infection were obtained with honeybees, 4 species of bumblebees, 2 solitary bees, 2 carpenter bees, and 1 thrips. In single tests no infections were obtained with *Bombylius azaleae*, *Euphoria sepulchralis* (F.), *Osmia lignaria* Say, or *Vespula maculifrons* (Buyss.). Only 1 infection developed on flowers exposed to 14 honeybees. In the 13 tests with 110 adults of *Frankliniella tritici* no infection occurred, although the thrips were taken from diseased flowers along with *Heterothrips azaleae*, 1.8 percent of which caused infection on flowers. The species of *Bombus* and of *Xylocopa* showed higher percentages of infective insects than the others for the entire season, ranging from 28.5 to 72.7 percent for 6 species in the 2 genera. The data as presented in table 4, however, should not be taken to indicate the relative efficiency of the insects in causing infection, since they were collected in different parts of Magnolia Gardens and at different times in the season.

TABLE 4.—*Summary of transmission tests of azalea flower spot by insects, Magnolia Gardens, Charleston, S. C., 1936*

Species of insect	Tests	Insects in tests	Infective insects	Infections (average for all individuals)
	Number	*Number*	*Percent*	*Number*
Apis mellifera	14	14	7.1	0.07
Bombus americanorum	28	28	39.3	.89
B. bimaculatus	21	21	28.5	.62
B. griseocollis	12	12	33.3	.67
B. impatiens	129	129	39.5	.85
Bombylius azaleae	1	1	0	0
Emphoropsis floridana	62	62	19.4	.5
Euphoria sepulchralis	1	1	0	0
Frankliniella tritici	13	110	0	0
Heterathrips azaleae	15	168	1.8	.024
Osmia lignaria	1	1	0	0
Tetralonia sp.	4	4	25.0	.5
Vespula maculifrons	1	1	0	0
Xylocopa micans	7	7	71.4	1.43
X. virginica	11	11	72.7	2.45

Reference to table 5, however, indicates that considerable variation in the number of cases of infection occurred in the several series. In general, the highest percentage of infection occurred during or just after the period in which limp-blighted flowers were most abundant. In the tests with *Bombus impatiens*, however, infections were obtained most consistently. This species was the most common species of bumblebee occurring in the gardens in 1936.

TABLE 5.—*Seasonal variation in occurrence of infectivity among individuals of insect species that transmitted azalea flower spot in Charleston, S. C., in 1936*

Date of collection	*Apis mellifera*		*Bombus americanorum*		*B. bimaculatus*		*B. impatiens*		*B. griseocollis*		*Emphoropsis floridana*		*Heterothrips azaleae*		*Tetralonia* sp.		*Xylocopa micans*		*X. virginica*	
	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Mar. 26	----	----	----	----	3	1	1	0	----	----	4	0	----	----	2	0	----	----	----	----
Mar. 27	2	0	----	----	3	0	6	0	1	0	6	1	----	----	----	----	1	0	----	----
Mar. 28	----	----	1	0	----	----	8	2	----	----	2	0	----	----	----	----	----	----	----	----
Mar. 29	1	0	----	----	2	1	11	3	----	----	1	0	----	----	----	----	----	----	1	0
Mar. 30	----	----	----	----	3	2	11	4	1	1	2	1	----	----	----	----	----	----	1	1
Apr. 1	----	----	----	----	1	1	13	8	----	----	----	----	----	----	----	----	----	----	----	----
Apr. 2	----	----	1	0	1	0	4	1	----	----	----	----	----	----	----	----	----	----	----	----
Apr. 6	----	----	----	----	----	----	17	1	----	----	5	0	----	----	----	----	----	----	----	----
Apr. 8	----	----	1	1	2	0	6	4	1	0	----	----	----	----	----	----	----	----	----	----
Apr. 9	11	1	----	----	3	1	27	10	3	2	----	----	----	----	----	----	----	----	----	----
Apr. 11	----	----	----	----	1	0	12	10	----	----	3	2	----	----	----	----	----	----	4	4
Apr. 14	----	----	6	5	----	----	3	3	----	----	11	2	----	----	2	1	----	----	1	1
Apr. 15	----	----	----	----	----	----	1	1	----	----	11	4	----	----	----	----	6	5	3	2
Apr. 16	----	----	----	----	----	----	----	----	----	----	----	----	100	3	----	----	----	----	----	----
Apr. 17	----	----	7	1	1	0	5	2	1	0	13	0	68	0	----	----	----	----	1	0
Apr. 19	----	----	12	4	1	0	4	2	5	1	4	2	----	----	----	----	----	----	----	----
Total for season	14	1	28	11	21	0	129	51	12	4	62	12	168	3	4	1	7	5	11	8

In the 320 transmission tests 20.49 percent of 693 exposed flowers became infected and showed 231 separate infections, or 0.33 per flower, while 0.82 percent of 364 unexposed check flowers showed only 3 infections, or 0.0082 infection per flower.

Transmission of Flower Spot on Heads or Legs or on Pollen from Insects

In a series of inoculation tests made in 1936 with insect parts, captured bees were anesthetized with chloroform and the head and legs of each bee were severed from the body and gently wiped over the petals of different flowers and then placed in the corollas of those same flowers. After the flowers and insect parts had been atomized with water and held for incubation, observations were made on the development of infection (table 6).

Table 6.—*Summary of tests with heads, legs, and pollen from insects in azalea flower spot transmission, Magnolia Gardens, Charleston, S. C., 1936*

Species of vector	Insects tested	Infective insects by—			Infections (average for all individuals)	Tests with pollen
		Heads	Legs	Heads or legs		
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Number*
Apis mellifera	22	9.1	9.1	9.1	0.18	1
Bombus americanorum	16	12.5	6.3	12.5	.19	3
B. bimaculatus	12	0	8.3	8.3	.08	4
B. griseocollis	6	0	0	0	0	0
B. impatiens	130	5.4	3.8	7.7	.09	31
Bombylius azaleae	1	0	0	0	0	0
Emphoropsis floridana	21	19.0	9.5	23.8	.29	4
Osmia lignaria	1	100.0	100.0	100.0	1.00	0
Xylocopa micans	12	16.7	16.7	16.7	.33	0
X. virginica	23	8.7	13.0	13.0	.22	0

These tests were made with insects collected at the same time as were those discussed in table 4. A lower percentage of infection was obtained in the tests with heads and legs of insects than with living ones, except with *Emphoropsis floridana*. Infection was obtained with both head and legs of *Osmia lignaria* in a single test by this method whereas no infection developed with 1 living adult of this species. In the 244 tests with parts of 10 species of insects a total of 37 infections developed on 433 flowers, 20 with the heads and 17 with the legs. None of the 121 unexposed check flowers developed infection.

In these tests the pollen from 43 individual bees was moistened with water and placed on petals of a third flower accompanying those inoculated with head and legs, respectively. In none of these tests with pollen was a positive reaction obtained, even with the pollen taken from 4 bees whose head or legs, or both, initiated infection. In 1937, similar tests with pollen taken from 122 bees, all of which caused infection in transmission tests, did not result in a single infection. Spores picked up on the body could conceivably be combed into the pollen baskets and some were identified in an examination of pollen from *Bombus impatiens* and *Emphoropsis floridana*, but for some reason the spores failed to cause flower infection.

Transmission Tests in 1937 and 1938

SEASONAL VARIATION IN INFECTIVITY OF INSECTS FROM DIFFERENT GARDENS

The experiments in 1936 demonstrated that insects were capable of transmitting azalea flower spot during the season coinciding with natural secondary infections. The objectives in 1937 and 1938 were

to determine the overwintering condition of the organism and whether insects were involved in the establishment of primary infections on the early appearing azalea flowers.

In 1937, collections of insects were made at Magnolia Gardens at frequent and regular intervals throughout the season, and less frequent collections were made at 7 other localities. These collections showed that most of the previously discussed species of *Bombus*, *Emphoropsis*, and *Xylocopa* were present in all localities and that a varying number of other species occurred, most of which did not transmit the disease. Table 7 and the accompanying discussion include the details of 2,831 inoculation tests with 8,071 insects and spiders collected from or near azalea during these 2 years.

TABLE 7.—*Summary of transmission tests with insects collected in various localities during 1937 and 1938*

WOODS NURSERY AT MAGNOLIA GARDENS, CHARLESTON, S. C.

Species of insect	Year of test	Tests	Insects in tests	Insects causing infections	Infections	
					Maximum by one insect	Average for all individuals
		Number	*Number*	*Percent*	*Number*	*Number*
Apis mellifera	1937	7	21	4.8	1	0.19
	1938	2	6	0	0	0
Bombus americanorum	1937	1	1	0	0	0
	1938	1	1	25.0	1	.25
B. bimaculatus	1937	38	38	15.8	13	.71
	1938	7	7	14.3	9	1.28
B. griseocollis	1937	1	1	0	0	0
	1938	1	1	0	0	0
B. impatiens	1937	12	12	8.3	3	0.42
	1938	15	15	20.0	2	0.4
Emphoropsis floridana	1937	162	164	15.2	3	.37
	1938	20	26	7.7	2	.11
Leptocera sp	1938	1	2	0	0	0
Leucophenga sp	1938	1	1	0	0	0
Megaselia sp	1938	1	2	0	0	0
Pellenes sp	1938	1	1	0	0	0
Phytophaga sp	1938	3	21	0	0	0
Pseudochelaria sp	1938	1	1	0	0	0
Pheidole dentata Mayr	1938	8	183	0	0	0
P. dentigula M. R. Smith	1938	1	20	0	0	0
P. morrisi var. *vancouverensis* For	1938	9	153	1.3	1	.01
Prenolepis sp	1938	3	42	0	0	0
P. imparis (Say)	1938	7	109	0	0	0
Xylocopa micans	1937	2	2	0	0	0
X. virginica	1937	1	2	0	0	0
	1938	2	4	50.0	1	.5

DRAYTON HALL, CHARLESTON, S. C.

Apis mellifera	1937	16	48	4.2	2	.08
	1938	80	229	2.2	3	.03
Bombus americanorum	1938	5	5	0	0	0
B. bimaculatus	1937	18	18	33.3	2	.61
	1938	14	14	14.4	2	.21
B. griseocollis	1937	1	1	0	0	0
	1938	3	3	33.3	20	9.67
B. impatiens	1937	18	18	5.5	1	.06
	1938	109	109	35.8	70	3.16
Colletes rufithorax	1938	1	1	0	0	0
Emphoropsis floridana	1937	66	67	5.9	4	.13
	1938	29	30	40.0	15	1.83
Eristalis transversus Wied	1938	1	1	0	0	0
Syrphus ribesii (L.)	1938	1	1	0	0	0
Xylocopa micans	1938	11	11	0	0	0
X. virginica	1937	6	6	33.3	2	.67
	1938	8	8	25.0	6	1.1

TABLE 7.—*Summary of transmission tests with insects collected in various localities during 1937 and 1938*—Continued

MIDDLETON PLACE GARDENS, CHARLESTON, S. C.

Species of insect	Year of test	Tests	Insects in tests	Insects causing infections	Infections	
					Maximum by one insect	Average for all individuals
		Number	*Number*	*Percent*	*Number*	*Number*
Apis mellifera	1937	39	119	0	0	0
Bombus americanorum	1937	1	1	0	0	0
B. bimaculatus	1937	140	140	5.0	4	.11
B. griseocollis	1937	4	4	0	0	0
B. impatiens	1937	16	16	6.3	2	.13
Emphoropsis floridana	1937	99	99	2.8	2	.04
Leptoglossus oppositus (Say)	1937	1	1	0	0	0

THE TEA FARM, SUMMERVILLE, S. C.

Andrena sp. (near *mandibularis* Robt.)	1938	7	7	0	0	0
Apis mellifera	1938	32	91	3.3	3	.77
Bombus americanorum	1938	12	12	66.7	11	4.67
B. bimaculatus	1938	22	22	60.0	8	1.86
B. griseocollis	1938	4	4	100.0	4	2.25
B. impatiens	1938	84	84	48.8	17	3.67
Cyrtopogon sp. (Asilidae)	1938	1	1	0	0	0
Emphoropsis floridana	1938	61	61	18.0	7	.64
Eristalis dimidiatus (Macq.)	1938	2	2	0	0	0
Halictus smitacinae Robt.	1938	1	1	0	0	0
Polistes canadensis var. *annularis* (L.)	1938	1	1	0	0	0
Prenolepis imparis	1938	27	505	0.71	0	.41
Syrphus ribesii	1938	1	1	0	0	0
Tetralonia atriventris Smith	1938	1	1	0	0	0
T. fulvolincta Cress	1938	1	1	0	0	0
T. rosae Robt.	1938	1	1	0	0	0
Xylocopa micans	1938	3	3	0	0	0
X. virginica	1938	15	15	6.7	1	.02

CYPRESS GARDENS, CHARLESTON, S. C.

Apis mellifera	1937	20	60	3.3	3	.06
	1938	1	3	0	0	0
Bombus bimaculatus	1937	39	39	17.9	4	.41
	1938	11	11	36.4	2	.45
B. griseocollis	1938	1	1	100.0	7	7.0
B. impatiens	1937	15	15	26.9	2	.4
	1938	28	28	50.6	19	2.96
Emphoropsis floridana	1937	14	14	0	0	0
	1938	19	19	47.4	8	1.63
Xylocopa micans	1938	13	13	46.2	10	2.0
X. virginica	1937	1	1	0	0	0
	1938	13	13	7.7	42	10.92

BELLE ISLE GARDENS, GEORGETOWN, S. C.

Anthomyiinae (gen. and sp. unknown)	1937	1	1	0	0	0
Apis mellifera	1937	29	87	0	0	0
Bombus americanorum	1937	1	1	0	0	0
B. bimaculatus	1937	18	18	22.2	7	1.06
B. fraternus	1937	3	3	66.7	6	2.67
B. griseocollis	1937	6	6	33.3	2	.5
B. impatiens	1937	2	2	0	0	0
Emphoropsis floridana	1937	82	86	10.5	12	.37
Hylemya sp.	1937	8	8	12.5	3	.37
Sarcophaga cimbicis	1937	1	1	0	0	0
Sarcophaga sp.	1937	1	1	0	0	0
Xylocopa micans	1937	5	5	40.0	4	1.4
X. virginica	1937	30	30	53.5	14	2.93

TABLE 7.—*Summary of transmission tests with insects collected in various localities during 1937 and 1938*—Continued

ORTON PLANTATION, WILMINGTON, N. C.

Species of insect	Year of test	Tests	Insects in tests	Insects causing infections	Infections	
					Maximum by one insect	Average for all individuals
		Number	*Number*	*Percent*	*Number*	*Number*
Apis mellifera	1937	3	10	0	0	0
Bombus bimaculatus	1937	11	11	0	0	0
B. griseocollis	1937	1	1	0	0	0
B. impatiens	1937	3	3	0	0	0
Emphoropsis floridana	1937	6	6	0	0	0
Xylocopa virginica	1937	4	4	0	0	0

MAGNOLIA GARDENS, CHARLESTON, S. C.

Andrena sp.	1937	1	1	0	0	0
Anthomylinae	1937	1	1	0	0	0
Anyphaena sp.	1937	1	1	0	0	0
Apis mellifera	1937	251	739	0	0	0
	1938	98	283	1.06	4	.03
Bombus americanorum	1936	19	19	0	0	0
B. bimaculatus	1937	109	109	32.1	8	.99
	1938	16	16	12.5	6	.44
B. fraternus	1937	1	1	0	0	0
B. griseocollis	1937	3	3	0	0	0
	1938	11	11	36.4	5	.91
B. impatiens	1937	47	47	27.7	14	1.27
	1938	229	229	15.3	8	.31
Calliphora sp.	1937	1	1	0	0	0
Coleoptera sp.	1937	1	4	0	0	0
Dictya sp.	1937	1	1	0	0	0
Dorymyrmex sp.	1937	1	4	0	0	0
Emphoropsis floridana	1937	103	105	18.1	5	.32
	1938	52	52	1.9	1	.02
Erigone sp.	1937	1	1	0	0	0
Eristalis transversus	1937	2	2	0	0	0
Formica pallidefulva Latr.	1937	1	4	0	0	0
Homalodisca triquetra (F.)	1937	1	1	0	0	0
Hylemya sp.	1937	6	6	0	0	0
Lonchaea sp.	1937	1	1	0	0	0
Mesogramma geminatum (Say)	1937	1	1	0	0	0
Myospila meditabunda (F.)	1937	1	1	0	0	0
Nezara viridula (L.)	1937	2	2	0	0	0
Phaonia sp.	1937	1	1	0	0	0
Pheidole sp.	1937	2	19	0	0	0
Polistes canadensis var. *annularis* (L.)	1937	1	1	0	0	0
Prenolepis imparis	1937	67	3,000	0	0	0
Prenolepis sp.	1937	1	9	0	0	0
Prochyliza xanthaspoma Walk.	1937	1	1	0	0	0
Sarcophaga singularis Ald.	1937	1	1	0	0	0
Sarcophaga sp.	1937	1	1	0	0	0
Tachinidae (indet.)	1937	1	1	0	0	0
Thanaos horatius Scud. and Burg.	1937	1	1	0	0	0
Vespula maculifrons	1937	2	2	0	0	0
Xylocopa micans	1937	8	8	25.0	8	1.4
	1938	38	38	5.3	2	.08
X. virginica	1937	86	86	68.6	10	2.43
	1938	3	3	0	0	0

WOOD'S NURSERY

The Wood's Nursery at Magnolia Gardens is in a clearing located about one-fourth mile from the exhibition gardens, and contains azaleas of many types on which the disease became very severe each season. Investigations here were valuable for comparison with those obtained in the nearby garden where a program for control of the disease was under way. The first artificial infection was obtained here on March 9, 1938, but not until 6 days later was one obtained at Magnolia Gardens. The proportion of infective insects was gener-

ally higher in collections from the Wood's Nursery (table 7) than from the gardens proper.

Among the five species of ants collected here two infections were obtained with *Pheidole morrisi* var. *vanceae*.

DRAYTON HALL

Azaleas are allowed to grow in a natural state at Drayton Hall, about 0.8 mile from Magnolia Gardens. Flower spot appeared here at about the same time as at Magnolia Gardens but progressed more rapidly to the limp-blight stage. The first infection in 1938 was obtained here on March 9 and the greatest number of infections (70) on one set of flowers with a single insect was obtained here. Infection with insects was more consistently obtained here (table 7) in 1937 and 1938 than at Magnolia Gardens.

MIDDLETON PLACE GARDENS

Middleton Place Gardens are located on a rather high bank of the Ashley River, and the azaleas, growing in open courts and with good air circulation, include chiefly varieties that begin to flower somewhat later than in other gardens. During the course of these investigations flower spot was less severe here than in other gardens. Ten collections of insects (table 7) were made here in 1937 between February 15 and March 18. Although this disease was present on a few plants on February 15, the first insect that caused infection was collected February 25. The proportion of infective insects in all collections at these gardens was low throughout the season.

TEA FARM

The azaleas at the Tea Farm are growing in a glade surrounded by tall trees that protect them from cool spring breezes, and both flowers and insects appear somewhat earlier than in other gardens.

Limp-blighted flowers were scattered throughout the farm on March 1, 1938, and the number gradually increased until about March 17 to 20, when most of the flowers were in the limp-blight stage, and these dried during the next few days. A relatively high percentage of infection was obtained with insects at this farm, particularly late in the season. Eleven collections of insects were made here in 1938, and insects were infective (table 7) beginning with the first collection, March 1. A large number of ants were collected at this farm, but infection was not obtained with them until on March 14, when many flowers were in the limp-blight state. The first of three infections with honeybees from this farm did not occur until March 12.

CYPRESS GARDENS

Azaleas at Cypress Gardens are growing on the banks of bodies of water beneath a high canopy of trees. At the time the collections were made in these gardens in 1937 and 1938 the incidence of disease was rather high, and the percentage of infective insects was also high (table 7). In 1938, collections were made at the nursery planting of these gardens located on a bluff where the disease was much less prevalent. Fewer infections with insects were obtained here than with those from the main planting.

BELLE ISLE GARDENS

Belle Isle Gardens are located in a cove on Georgetown Bay where the azaleas are protected by large trees in open glades, and they

appear to flower earlier here than in some other gardens. The azaleas are permitted to grow with a natural accumulation of mulch from tree leaves. Four collections were made at this garden in 1937 between February 14 and March 21 (table 7). No disease was observed on flowers when the first collection was made, but the disease in limp-blight stage was conspicuous on February 22. Insects captured on this date caused infections in tests. The disease had not spread markedly to later flowering azaleas on March 12, but on March 21 many flowers had passed through the limp-blight stage of the disease and were drying. A considerably higher proportion of insects captured on this date caused infection than had caused it on preceding dates. One new vector, *Hylemya* sp., was collected at this garden.

ORTON PLANTATION

Azaleas at Orton Plantation are growing in an open garden sloping down to a marsh. Practically all the flowers had been destroyed by a series of frosts in 1937, and at the time the 1 collection (March 13) was made at the garden only a few late varieties were in flower. Although a few limp-blighted flowers were present none of the 28 insects of 6 species caused infection (table 7).

HAMPTON PARK

Azaleas in Hampton Park are planted along drives or are growing in nursery beds. No disease was found in these plantings until late in the season of 1937. In 1 collection made on March 23 from azaleas in this park no infections were obtained with 18 *Apis mellifera*, 1 *Bombus impatiens*, 6 *Emphoropsis floridana*, 1 *Xylocopa micans*, and 1 *X. virginica*.

MAGNOLIA GARDENS

Studies of insects occurring on azaleas were conducted more intensively at Magnolia than at any of the other gardens in 1937 and 1938. Conditions here differed from those in the other gardens because efforts were made to control or retard the progress of the disease by hand picking of infected flowers supplemented by dusting in 1937 and the use of sprays in 1938. Although the primary infections of the disease were present on the earliest appearing flowers on January 20 in 1937 and on February 14 in 1938, and although insects were tested for infectivity beginning January 29 and March 1, respectively, disease transmission was not obtained until February 19 and March 15, respectively, in the two years. No infections developed in tests with honeybees in 1937 and not until March 30 in 1938. A large number of species of insects belonging to several orders, but chiefly Diptera, were used in tests, as they were found on azaleas early in the season of 1937, but none were found to be infective (table 7). Large numbers of ants were also collected in 1937 at sirup baits, but none caused infection in tests.

Infection with the common vectors was generally low throughout the season in both years except in 1937, when it increased markedly near the end. A seasonal comparison of the rate of infection in all gardens is evident in table 8, where tests with all individuals from all gardens of species causing infection in 1937 are tabulated according to the date of collection. It will be noted that the first infections in any garden occurred on February 19 in 1937 and gradually increased to the end of the season late in March.

TABLE 8.—*Seasonal variation in occurrence of infectivity among individuals of insect species collected in various localities that transmitted azalea flower spot, 1937–38*

COLLECTIONS MADE FROM AZALEA IN 8 LOCALITIES IN NORTH CAROLINA AND SOUTH CAROLINA

Date of collection	*Apis mellifera*		*Bombus americanorum*		*B. bimaculatus*		*B. fraternus*		*B. griseocollis*		*B. impatiens*		*Emphoropsis floridana*		*Hylemya* sp.		*Pheidole morrisi* var. *vanceae*		*Prenolepis imparis*		*Xylocopa micans*		*X. virginica*	
	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective
1937	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Jan. 29	9	0																						
Jan. 30	12	0																						
Jan. 31	55	0																						
Feb. 2	25	0													2	0								
Feb. 3	37	0													1	0								
Feb. 5	19	0													2	0								
Feb. 6	234	0																						
Feb. 8	132	0																						
Feb. 9	6	0																						
Feb. 12	108	0																						
Feb. 14	72	0							1	0	1	0	9	0	1	0								
Feb. 15	119	0			4	0					1	0	2	0										
Feb. 16	3	0									2	0												
Feb. 17	6	0			1	0					9	0			1	0								
Feb. 18	33	0			24	0			1	0	9	0	5	0										
Feb. 19					10	1					1	1												
Feb. 20	9	0			1	0																		
Feb. 22	21	0			12	2			1	1	2	0	4	1	7	1								
Feb. 23	6	0			36	0							11	0										
Feb. 24	60	2			11	2					6	0											1	0
Feb. 25	64	2			29	1			2	0	8	0	11	1									1	0
Feb. 26					3	0																		
Mar. 3	18	0			4	0					2	0												
Mar. 4					8	0							10	0										
Mar. 5	6	0			1	0					1	0	11	0										
Mar. 8	3	1			9	3					12	1	81	7									1	0
Mar. 9					30	2					1	1	32	1										
Mar. 10					28	5					9	4	14	0										
Mar. 11	5	0			7	0					10	0	47	1										
Mar. 12	12	0			8	0			4	1	1	0	58	3									1	0
Mar. 13	10	0			11	0			1	0	3	0	6	0									4	0
Mar. 14					10	4					2	2	17	2										
Mar. 15					5	3					2	1												

Mar. 16					7	2					1	0												
Mar. 17					24	2			3	0	4	2	80	15									2	1
Mar. 18					46	8			3	0	9	1	61	3									5	2
Mar. 19					19	10					4	2	18	8									12	7
Mar. 21					3	3	3	2					15	5							1	0	29	16
Mar. 22					12	11					6	3	27	2							5	2	13	8
Mar. 23	18	0			4	2					3	2	10	3							2	0	16	11
Mar. 24					2	1					3	0	10	3							1	0	30	24
Mar. 25											2	0	8	4							5	2	14	8
Total for season	1,102	5			369	62	3	2	16	2	114	20	547	59	14	1					14	4	129	77

COLLECTIONS MADE FROM 5 GARDENS IN CHARLESTON, S. C.[1]

1938																								
Feb. 25													1	0										
Mar. 1	9	0																						
	12	0											10	2										
Mar. 2	27	0																						
	45	0			1	0					1	0	19	0										
Mar. 3	21	0																						
	2	0			3	0					1	0											13	1
Mar. 5	6	0																						
	30	0											3	0							1	0		
Mar. 7	12	0																						
	26	0	1	0							8	1	4	0					19	0				
Mar. 8	21	0																						
Mar. 9	46	0	2	0							3	0												
	64	0	1	0	1	0			1	0	9	0	3	1			153	2			3	0	1	0
Mar. 10	15	0																						
	9	0																	208	0				
Mar. 11	6	0																						
	36	0			2	1					2	0							165	0				
Mar. 12	18	0									6	0												
	42	0	1	0	5	0					17	5	1	0					61	0	1	0	3	0
Mar. 14	52	0			2	0					16	0	1	0									1	0
	42	2			1	0					21	5	3	1					57	1	4	0	1	1
Mar. 15	23	0	2	0	2	0			1	0	20	2	1	0							7	0		
	3	0			3	0					2	0	5	0							2	0		
Mar. 16			2	0	2	0					31	1	1	0										
Mar. 17			1	0	1	0					14	0												
	12	1	1	0	3	1					16	4	15	1					94	0	1	0		
Mar. 18			2	0							6	0									1	0		
	3	0	1	0	9	0			1	0	36	4	3	0							2	0	2	0
Mar. 19			1	0	3	1			1	0	17	4	3	0										
			1	1	1	0					14	7												

[1] Numbers in upper line opposite each date refer to tests with insects from Magnolia Gardens; those in the second line refer to those from other gardens.

TABLE 8.—*Seasonal variation in occurrence of infectivity among individuals of insect species collected in various localities that transmitted azalea flower spot, 1937–38*—Continued

COLLECTIONS MADE FROM AZALEA IN 8 LOCALITIES IN NORTH CAROLINA AND SOUTH CAROLINA

Date of collection	*Apis mellifera*		*Bombus americanorum*		*B. bimaculatus*		*B. fraternus*		*B. griseocollis*		*B. impatiens*		*Emphoropsis floridana*		*Hylemya* sp.		*Pheidole morrisi* var. *vanceae*		*Prenolepis imparis*		*Xylocopa micans*		*X. virginica*	
	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective	Total	Infective
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Mar. 21	------	------	2	2	4	4	------	------	------	------	14	14	13	7	------	------	------	------	70	3	1	0	------	------
Mar. 22	------	------	3	1	5	3	------	------	2	1	22	3	4	0	------	------	------	------	------	------	14	0	------	------
Mar. 23	------	------	------	------	------	------	------	------	1	0	43	20	26	4	------	------	------	------	------	------	------	------	4	0
Mar. 24	3	0	------	------	11	4	------	------	1	1	28	14	19	9	------	------	------	------	------	------	13	6	13	10
	4	0	3	0	3	0	------	------	4	1	44	12	18	1	------	------	------	------	------	------	11	2	------	------
Mar. 25	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
	------	------	1	0	------	------	------	------	1	1	10	9	11	9	------	------	------	------	------	------	------	------	1	1
Mar. 26	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
	------	------	7	5	5	5	------	------	------	------	14	12	------	------	------	------	------	------	------	------	1	0	------	------
Mar. 30	21	3	2	0	2	1	------	------	2	1	29	8	8	0	------	------	------	------	------	------	1	0	2	0
Mar. 31	2	0	4	0	1	0	------	------	1	1	21	5	16	0	------	------	------	------	------	------	4	0	------	------
	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
Total for season	283	3	19	0	16	2	------	------	11	4	229	35	52	1	------	------	------	------	------	------	38	2	3	0
	329	3	19	9	54	18	------	------	5	2	236	95	136	34	------	------	153	2	674	4	29	6	38	13

In the tests of 1938 the seasonal collections of infective species are tabulated separately for Magnolia Gardens and for four other gardens in which collections were made (table 8). The first infection was obtained March 1 at the Tea Farm, and infections occurred in collections from other gardens a little later, but none was obtained at Magnolia Gardens until March 15. The disease was much less serious at Magnolia during 1938 than at the other gardens and the proportion of infective insects was also much lower, being 7.2 percent of those collected at Magnolia Gardens and 21.8 percent of the same species collected at other gardens.

Relationship of Insects to Primary Infection

In the preceding experiments the insects collected on azalea flowers during the early part of the seasons of 1937 and 1938 did not transmit azalea flower spot until after primary infections had developed to the limp-blight stage. These experiments showed that the insects observed to be visiting azalea flowers were not introducing the disease from some other wild or cultivated host. Other experiments were being conducted at the same time to determine whether insects might still be involved in the initiation of primary infections.

Honeybees or Their Hives as a Source of Infection

Because honeybees were the most common insect visitors on azaleas early in the season, the possibility that they were responsible for the introduction of the disease, either from other flowers or from their hive, was investigated.

In 1937 and 1938 honeybees were collected at the entrance of the colony in a trap made by fitting the bottom from a coffee can with a detachable cover. A screen cone with a small hole in the apex was soldered in the top of the can so that bees entering the can could not escape but could be released by removing the detachable bottom. In 1937 no hives of bees were available, but captures were made at the entrance to a colony occupying the hollow limb of a tree in Magnolia Gardens. In making the captures the trap described above was applied to an adapter made from a piece of screen on a metal frame nailed over the opening in the tree. In 1938 a similar adapter for capturing bees was placed on two beehives which contained colonies that were brought into the gardens in 1937.

In 1937, 36 tests were made between February 9 and March 23 with bees captured as they entered or departed from the tree. Only 1 infection was obtained by inoculations of 78 flowers with 2,027 bees, and that occurred in the last test. In 1938, a total of 76 tests with 3,536 bees from 2 colonies were made between February 27 and March 22, but no infections developed on 324 exposed flowers. In addition, 77 flowers were placed individually for 2 to 7 hours in the entrances to 4 hives during the period March 1 to 10 so that hundreds of bees walked over them each hour as they entered or left the hives. Four infections developed on 3 flowers exposed on March 7 in the entrances of 2 colonies whereas all other exposed flowers remained healthy.

This information indicates that the honeybees are unlikely to harbor the flower spot organizm in their colony or to initiate early-season primary infections.

SOIL AS A SOURCE OF PRIMARY INFECTION

As a result of a survey of the occurrence of primary infections and their location on early flowering azaleas at Magnolia Gardens and Drayton Hall conducted February 1 to 13, 1937, the summary shown in table 9 is made from observations on 26 plants that ranged from 3 to 10 feet in height.

TABLE 9.—*Locations of primary infections on 26 plants of azalea flower spot with reference to height above the ground*

Foci of infection (number)	Infections	Infected flowers	Height from ground
	Number	*Number*	
7	67	23	Touching ground.
12	54	25	2–6 inches.
6	10	10	7–12 inches.
4	4	4	1–2 feet.
3	3	3	2–3 feet.
2	5	5	4 feet.
1	3	1	5 feet
1	1	1	7 feet.
Total 36	147	72	

In these observations 25 of the 36 foci and 131 of the 147 primary infections occurred on 58 of the 72 infected flowers that were touching the ground or within 12 inches of it. The remaining infections occurred in decreasing numbers to a height of 7 feet. These observations, made before the source of primary infection from apothecia had been determined, led to a further investigation in 1938 of the soil and the animals inhabiting it as an early-season source of the disease.

The following observations in 1937 are presented as an illustration of further circumstantial evidence that the soil is a source of primary infection. On February 11 primary flower spot infections were general on flowers of the variety Early Lavender where the plants were growing in the same beds as in 1936 and where they had been generally attacked by flower spot. In contrast to the general infection on the established plants was the scarcity of infection on flowers of plants moved the preceding December from this bed to a new bed a few rods distant. Only 1 infected flower close to the base of 1 plant was found on 20 plants examined, and none were found on many transplanted plants in other beds. Because the transplanted plants were located so near the undisturbed plants it appeared that infection in the latter took place from the soil or from some other very local source. If the disease had come from a distance or was transported by insects it should have been present on the transplanted plants as quickly as on the nearby undisturbed ones, unless infective insects emerged from the soil about the base of the latter plants and immediately caused infection.

Further experiments were made in 1938 to determine whether the soil or its inhabitants were the source of primary flower infection by

the splashing of water and soil on the flowers in the absence of air currents. Three series of tests were made February 24 to 28 at 10 places in the Wood's Nursery and 1 series on February 28 at Drayton Hall, all located in areas where soil or mulch had not been recently disturbed and beneath branches of azalea plants that were infected in 1937.

In performing the tests, clean flowers were removed from an incubation can beneath a celluloid box with arm holes in opposite sides and laid on the soil. A small jet of water from a spray pump directed on the soil splashed drops of mud and water on the flowers, which were then either returned to the incubation cans and incubated in the laboratory or covered with celluloid cages and incubated in place. The results are summarized in table 10.

TABLE 10.—*Summary of flower infections in soil-splashing tests, Magnolia Gardens and Drayton Hall, Charleston, S. C., 1938*

Location	Date of splashing	Flowers		Total infections
		Exposed	Infected	
		Number	*Number*	*Number*
Wood's Nursery	Feb. 24	46	5	7
Incubated in laboratory	Feb. 25	75	1	1
	Feb. 28	64	3	3
Incubated in place	Feb. 24	23	2	4
Drayton Hall	Feb. 28	23	2	4
Unexposed checks		42	0	0

In these tests 19 infections developed on 13 of 231 flowers exposed to the splashing of soil and water. Infections were obtained beneath plants of several varieties of azaleas some of which were in flower but not infected, while buds of other plants were not yet showing color. After this demonstration that infection can be obtained from the soil near previously infected plants, further tests were made to determine the possibility that insects or other soil-inhabiting animals might be responsible for transmitting the causal organism to the low-hanging azalea flowers.

SOIL-INHABITING ANIMALS AS VECTORS

Insects and animals in soil from areas in which infections resulted in the splashing tests were extracted in a modified Berlese funnel (fig. 8, *B*). At each of the 10 places where infections had been obtained samples of soil, 8 by 9 inches in surface area and 2 inches deep, were taken, placed in the screen compartments of the funnel, and left for the soil animals to emerge. During the period from February 26 to March 18 a total of 48 samples, or 24 square feet of soil area, were thus handled and the following varied fauna of insects, mites, spiders, centipedes, and diplopods was obtained:

SOIL FAUNA COLLECTED UNDER AZALEA BUSHES

	Number of individuals
Centipedes:	
Lithobiidae:	
Neolithobius latzeli (M.)	1
Benthobius sp	1
Geophilidae:	
Arenophilus watsingus Ch	1
Not further identified	2
Diplopods, not further identified	2
Spiders:	
Erigone sp	1
Habrocestum sp	1
Herpyllus sp	1
Pardosa sp	2
Pellenes sp	1
[illegible]s	(in all) 18
Galumnidae.	
Nothridae.	
Pediculoididae.	
Trombidiidae.	
Collembola	56
Coleoptera:	
Larvae (Carabidae):	
Platynus sp	1
Pasimachus sp	2
Adults (Carabidae):	
Harpalus nitidulus Chd	1
Tachys sp	10
Stenolophus humidus Ham	1
S. conjunctus (Say)	1
Clivina americana Dej	1
Adults (other families):	
Aleocharinae (Staphylinidae) (gen. and sp. uncertain)	2
Atheta sp. (Staphylinidae)	4
Euplectus sp. (Pselaphidae)	2
Acrotrichis sp. (Ptiliidae)	1
Paria sp. (Chrysomelidae)	1
Diptera:	
Diplosis group	1
Hymenoptera:	
Eulophid	1
Pheidole dentigula M. R. Smith	6
Ponera trigona var. *opacior* Forel	20

The collections are believed to be fairly complete except that many mites and Collembola were not recovered. These soil animals were tested for infectivity, and 2 infections developed in one test on flowers exposed on March 5, all the other 113 exposed flowers remaining disease-free. The species responsible for disease infection in the one test could not be determined since several of the foregoing groups were represented.

INSECTS EMERGING FROM SOIL AS VECTORS

An experiment was planned to determine whether insects and animals wintering or breeding in the soil or leafmold about azalea plants would, upon emergence during the flowering period, carry some stage of the flower spot organism to the azalea plants and cause infection. On March 2, 1938, 6 cheesecloth-covered cages, each covering an area of 4 square feet, were set among azalea plants in the areas

where infections were obtained by splashing dirt over the flowers, and from which the soil samples were taken. These cages were examined at least once daily until March 26, and during this period a total of 39 insects and 3 spiders were collected and used in 14 inoculation tests with azalea flowers.

No infection was obtained with the spiders, *Clubonia* sp., or with *Tetragnatha laboriosa* Hentz, with the ceratopogonid *Culicoides canithorax* Hoff., with the cecidomyiid flies identified as belonging near the genera *Diplosis*, *Lestremia*, or *Neolasioptera*, respectively, or with *Oscinella* sp. or another dipterous form identified as belonging to the Actiinae. Twenty-one individuals of the ant *Crematogaster ashmeadi* Mayr were taken in the cages and a single infection was obtained in one test with a single adult on March 22, at which time the flowering season was nearing the end. It is probable that this infection resulted from the conidial stage of the disease rather than from ascospores that are produced earlier in the season.

Other Relationships of Insects to the Disease

Infection of More Than One Azalea Flower by an Insect Visitor

Information was obtained in the following described experiment to answer in part the question whether a conidia-bearing insect rapidly releases all spores in its visit to the next flower or gradually releases them and thereby may infect several flowers in sequence.

Thirty-five insects of 6 species (table 11, series 1) were captured March 21, 1938, while they were visiting limp-blighted flowers of azalea at the Tea Farm, Summerville, S. C., and were used individually in the inoculation of 3 sets of 2 flowers each. Each set of flowers was incubated individually. A second set of tests with 24 insects collected at Drayton Hall (table 11, series 2) was made on March 25. When it was revealed that in some instances nearly as many infections occurred in the third set of flowers exposed to each insect as occurred in the first set, a third series of tests extended to 5 sets of flowers was made on March 26 with 30 individual insects collected at the Tea Farm. The major disease epidemic had passed at the time all these collections were made. Most of the flowers had been destroyed since March 17 by having passed through the limp-blight stage and were drying on March 21 to 26. As the few remaining flowers had been infected during various stages of the disease, the insects were collected in a period in which they had greatest opportunity to become contaminated. The results of the first 3 sets of flowers exposed in series 3 are combined with those of series 1 and 2 in the summary, while the results of series 3 are also summarized separately (table 11).

It is evident that the 61 insects belonging to the genus *Bombus* caused more infections on the first set of flowers than on flowers in succeeding sets. In the tests with 24 adults of *Emphoropsis floridana* more infections occurred in the third set than in either of the first 2 sets in series 1 whereas the reverse was true in the other series. No infections occurred in the first set of flowers exposed in 3 tests to *Tetralonia rosae* and *Xylocopa micans*, but infections did occur in later sets.

TABLE 11.—*Infectivity of insects carrying spores of Ovulinia azaleae in repeated contacts with azalea flowers, Charleston and Summerville, S. C., 1938*

Species of insect in test	Series No.	Total tests	Infective insects and infections caused in each series in sets— 1		2		3		4		5	
			Insects	Infections	Insects	Infections	Insects	Infections	Insects	Infections	Insects	Infections
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Bombus americanorum	1	2	2	20	1	13	1	18				
	2	1	0	0	1	8	0	0				
	3	7	5	34	5	25	2	21	2	16	2	12
B. bimaculatus	1	4	4	9	3	8	2	6				
	3	5	5	23	4	15	5	14	4	12	3	12
B. griseocollis	2	1	1	29	1	9	1	19				
	3	3	3	5	1	1	2	3	0	0	1	1
B. impatiens	1	14	14	143	13	109	13	87				
	2	10	9	218	9	81	10	65				
	3	14	12	86	13	70	10	42	12	46	12	39
Emphoropsis floridana	1	13	7	27	6	18	10	39				
	2	11	9	51	9	34	7	23				
Tetralonia rosae	1	1	0	0	1	1	1	2				
Xylocopa micans	1	1	0	0	1	2	0	0				
	3	1	0	0	0	0	0	0	0	0	1	1
X. virginica	2	1	1	6	1	5	1	2				
Summary of all tests		89	72	651	69	399	65	341				
Summary of series 3		30	25	148	23	111	19	80	18	74	19	65
Average infestations per individual tested: All series				7.3		4.5		3.8				
Series 3 alone				4.9		3.7		2.7		2.5		2.2

Tests that have been combined in the data of table 11 may be discussed individually as follows: Among 10 tests with *Bombus americanorum* 2 bees caused infection in the second set of flowers but did not in the first or later sets. Three others caused infection in the first set, 2 infections in the second, and none in the succeeding ones. Among 9 tests with *B. bimaculatus* all the bees caused infection in the first set of exposed flowers and fairly uniform infection in the succeeding ones. With *B. impatiens*, 3 out of 38 did not cause infection in the first set of flowers but did in the second or third. All individuals of *B. griseocollis* caused infection in the first set of exposed flowers and irregular infections in the succeeding 4 sets. Considerable irregularity in infection occurred in the tests with *Emphoropsis floridana*. Five of 24 individuals did not cause infection in the first set of flowers but 2 of these 5 caused infection in the second and third sets, while the other 3 did not cause infection until in the third set. Three others did not cause infection in any of the sets of exposed flowers. In 1 test *Tetralonia rosae* did not cause infection in the first set but did in the second and third sets. In 2 tests with *Xylocopa micans* no infection occurred in the first set of flowers, whereas infection occurred in the second set in one case and not until the fifth in the other.

In the 3 series of tests with 89 insects of 8 species, 4 individuals caused no infection in any test, while 14 others caused no infection in the first set of exposed flowers but an average of 1.1 infections in the second and 1.6 infections in the third. Considering the third series separately, 4 of 30 insects caused no infection in the first set and 0.75, 0, 0.5, and 0.5 infections, respectively, in the second to fifth

sets of exposed flowers. Comparative infections by all insects in each series are shown in the last 2 lines of table 11.

It may be seen that the average infection for all insects is higher on each set of exposed flowers than occurred in tests with the 14 insects not causing infection in the first set, as discussed in the preceding paragraph. These 14 insects that caused no infection in the first sets caused only small numbers of infections in any succeeding set, which indicated that they bore small numbers of spores. Those insects causing large numbers of infections regularly caused infections in all exposed flowers.

A total of 1,530 infections occurred on all exposed flowers. On flowers exposed in the first 3 sets of all tests, 46.8, 28.7, and 24.5 percent, respectively, of the 1,391 infections occurred on the first, second, and third sets. In the third series 31.0, 23.2, 16.7, 15.5, and 13.6 percent, respectively, of the 478 infections occurred on the first to fifth sets.

The foregoing experiment shows that an infective insect loses its load of spores gradually and can infect at least 10 flowers. The maximum number of flowers that might be infected was not determined.

Insects that were apparently bearing only small numbers of spores were irregular in causing infections and did not in some instances infect the first set of exposed flowers although they did infect later ones. This reflects somewhat upon the reliability of procedure in determining infective and noninfective individuals in the seasonal testing of insects from azalea, particularly early in the season when no or only an occasional infection was obtained. When it is considered, however, that the insects were enclosed with the flowers in the cans and had opportunity to walk over the flowers after the initial exposure in the inoculation chamber, it was possible that they did so and thereby added to the reliability of the method. Because large numbers of insects were tested over a period of several years it is believed that a fairly reliable index of the seasonal infectivity of the various species was obtained.

TRANSMISSION OF AZALEA FLOWER SPOT TO ISOLATED PLANTINGS

A nursery planting of azaleas was separated 0.4 mile from the main display at Magnolia Gardens. No flower spot infection had appeared during 1935 or 1936 in this planting of almost completely unshaded azaleas located at the edge of a wind-swept field. Because bees had been captured in this planting that were carrying mixtures of pollen and the fungicidal dust that was being applied to plants in the main garden, they were evidently flying between the 2 plantings. Among 100 insects of 6 species captured in this nursery, 16 individuals belonging to 5 species caused infection in transmission tests (table 12). It is probable that these insects picked up the organism in the infected plantings and transported it over the intervening distance. It is also probable that, although the insects must have repeatedly dropped viable spores in flowers at this nursery, prevailing conditions were unfavorable for development of infection. This point was further borne out by 2 other tests in which approximately 50 bees of *Bombus*, *Emphoropsis*, and *Xylocopa* were caged on each of 2 azalea plants in this nursery. In both cages they mutilated the flowers (fig. 2) in their efforts to obtain food, yet no infection developed on the plants located

in the sun, although infection did develop in flowers taken from each of the caged plants and incubated in the laboratory.

TABLE 12.—*Summary of tests in transmission of azalea flower spot by insects captured in a disease-free nursery, Magnolia Gardens, Charleston, S. C., 1936*

Species tested	Tests	Infective insects	Species tested	Tests	Infective insects
	Number	*Number*		*Number*	*Number*
Bombus americanorum	17	3	*Emphoropsis floridana*	19	1
B. bimaculatus	10	1	*Xylocopa virginica*	4	0
B. griseocollis	1	1			
B. impatiens	49	10	Total	100	16

Further evidence on the transmission of flower spot to uninfected plantings was obtained in 1937. On April 9 a collection of insects was made in a nursery about 1 mile from Middleton Place Gardens, a nursery in which no flower spot had been observed during the current or the 2 previous seasons. Infections in transmission tests were caused by 1 of 16 bees of *Emphoropsis floridana*, 2 of 21 bees of *Bombus bimaculatus*, and in a single test with *B. impatiens*. Incidentally, the disease occurred in this nursery in 1938 even though no azalea plants had been moved into it.

Although the disease was not found in Hampton Park in Charleston in 1936 or previously, it was observed there late in the flowering season of 1937. Since all available information indicated that no plants had recently been brought into this park it is possible that insects may have carried the organism from infected plantings located approximately 3 miles away.

INSECT TRANSMISSION OF FLOWER SPOT TO NATIVE HOSTS

Since flowers of several native plants had been found to be naturally infected with azalea flower spot, tests were made in 1937 with insects on two of these hosts, *Rhododendron nudiflorum* and *Vaccinium* spp., growing in the nearby woods. Both hosts had been shown to be susceptible by artificial inoculation and the former to be naturally infected.[6]

In these tests 4 species of insects captured from the same diseased azalea plants were individually used to inoculate each of 3 groups of flowers of *Rhododendron nudiflorum* and each of 10 groups of flowers of *Vaccinium* spp., and then each insect was used to inoculate 2 azalea flowers, after which all the flowers were incubated. Typical infection with spores developed on the 3 groups of flowers of *R. nudiflorum*, but none was found on the flowers of *Vaccinium* spp. A summary (table 13) of infection by these bees on the azalea flowers indicates that a relatively high percentage of infective individuals were used in these tests and they should have left spores on *Vaccinium* spp. as they did on *R. nudiflorum*.

[6] See footnote No. 3, Circular 550.

TABLE 13.—*Summary of infections on cultivated azaleas after they had been inoculated by insects previously used in inoculating other hosts, Magnolia Gardens, Charleston, S. C., 1937*

Species of insect	Insects tested	Insects found infective	Total infections
Insects which had been used in tests on *Rhododendron nudiflorum*:	*Number*	*Percent*	*Number*
Bombus bimaculatus	5	60.0	6
B. impatiens	3	33.3	3
Xylocopa virginica	5	100.0	12
Insects which had been used in tests on *Vaccinium* spp.:			
Bombus bimaculatus	7	42.8	8
B. impatiens	3	33.3	5
Emphoropsis floridana	11	45.4	8
Xylocopa virginica	19	52.6	22

Bumblebees, including *Bombus bimaculatus* and *B. impatiens*, the carpenter bee *Xylocopa virginica*, and also *Emphoropsis floridana* have been observed to visit flowers of *Rhododendron nudiflorum* and *Vaccinium* spp. in the woods near infected azalea gardens. In one case, at Cypress Gardens, flowers on a plant of *R. nudiflorum* growing one-half mile from the nearest cultivated azaleas and showing typical feeding punctures of *Xylocopa virginica* were found upon incubation to be infected with flower spot. No natural infections have been found on flowers of *Vaccinium* spp. While this apparently natural transmission of the disease by insects to a wild host is of interest, its importance depends on whether viable sclerotia are produced. If the infection were a continuing one, this host or others could serve as reservoirs from which the disease would spread or be distributed to cultivated azalea the following season.

PROTECTION BY A FUNGICIDAL DUST AGAINST INFECTION BY INSECTS

After a series of tests with fungicidal dusts and sprays had been made at the laboratory, frequent applications of a dust containing 20 percent of a monohydrated copper sulfate (guaranteed to contain 26 percent of copper) and 80 percent of kaolin for disease control were made to azaleas during the flowering seasons of 1936 and 1937 at Magnolia Gardens.

To test the effect of this fungicide on the transmission of flower spot by insects, 3 series of inoculations with insects were made, March 23 to 25, 1937, in which insects were captured from the same diseased azalea plant and nearly equal numbers were used in inoculating pairs of undusted flowers and others dusted with the above-mentioned fungicide. The results of the tests, shown at the end of 3 days, are given in table 14 and are summarized as follows: In these tests with 202 bees, 63 caused 220 infections on undusted flowers and 33 caused only 69 infections on dusted flowers. There were 47.6 percent fewer insects that caused infections on dusted flowers and a reduction of 68.6 percent in numbers of infections, and this may be attributed to the protective fungicidal effects of the dust.

TABLE 14.—*Comparison of flower spot infection on undusted azalea flowers and on those dusted with copper-sulfate kaolin previous to inoculation by insects, Magnolia Gardens, 1937*

Species of insect	Treatment of flowers	Tests	Insects causing infections	Infections	
				Maximum	Average for all individuals
		Number	*Percent*	*Number*	*Number*
Bombus bimaculatus	Undusted	11	72.7	6	3.00
	Dusted	11	63.6	8	1.45
B. fraternus	Undusted	1	0	0	0
	Dusted	1	0	0	0
B. griseocollis	Undusted	1	100.0	1	1.00
	Dusted	1	0	0	0
B. impatiens	Undusted	8	37.5	3	.87
	Dusted	10	20.0	2	.50
Emphoropsis floridana	Undusted	17	41.2	4	.7
	Dusted	16	25.0	2	.62
Xylocopa micans	Undusted	6	33.3	8	1.83
	Dusted	6	0	0	0
X. virginica	Undusted	57	73.7	33	2.74
	Dusted	56	35.7	11	.67
Summary	Undusted	101	62.4	33	2.07
	Dusted	101	32.7	11	.68

REPELLENT EFFECT OF ACETIC ACID SPRAYS ON INSECTS

During the 1938 flower season frequent applications of a spray of dilute acetic acid were made to azalea flowers at Magnolia Gardens. All species of bees were apparently repelled by the spray, and when one alighted on a sprayed flower it promptly left in considerable excitement, apparently repelled by the odor or the flavor of the liquid.

Groups of plants were sprayed with acetic acid 1–600 and records were kept of the insects visiting and feeding on the sprayed flowers and on unsprayed flowers of nearby plants.

Table 15 shows the records of the visits to these flowers of three species of bees.

TABLE 15.—*Record of insects visiting unsprayed azalea flowers and those sprayed with a dilution of acetic acid 1–600. Magnolia Gardens, Charleston, S. C., 1938*

Series and species of insect	Treatment	Insects visiting flowers after the spraying		
		The first 50 minutes	The first 70 minutes	From 60 to 90 minutes
Series 1:		*Number*	*Number*	*Number*
Bombus impatiens	Sprayed		0	
	Unsprayed		27	
Emphoropsis floridana	Sprayed		0	
	Unsprayed		14	
Series 2:				
Bombus impatiens	Sprayed	2		2
	Unsprayed	1		0
Emphoropsis floridana	Sprayed	7		18
	Unsprayed	6		8
Xylocopa virginica	Sprayed	0		7
	Unsprayed	7		8

In series 1, a total of 41 *Bombus impatiens* and *Emphoropsis floridana* were observed on unsprayed plants and none fed on sprayed plants during the first 70 minutes after application of the spray. The liquid had not dried from the flowers during the period of observation.

In series 2, 14 adults of 3 species were observed feeding normally on unsprayed flowers, while no *Xylocopa* spp. and a total of 9 adults of the other 2 species alighted on sprayed flowers but did not feed during the first 50-minute period after the application of the spray. After this time the spray had dried, and the insects fed normally.

It is evident from these observations that insects are repelled by this spray of dilute acetic acid only during the period that the flowers are wet with the material. Several applications daily would be required on most days to give a fairly complete repellent effect.

CONTROL EXPERIMENTS WITH INSECTS ON AZALEAS

During the seasons of 1935 and 1936 preliminary experiments were conducted to determine whether the various species of flower-visiting insects could be controlled by dusts, poisoned sprays, or baits.

Because extensive poisoning of honeybees has occurred in California by dusts containing arsenic applied to fruit-tree blossoms, tests were made to determine the effect of adding arsenicals or cryolite to the fungicidal dust (containing 20 percent of monohydrated copper sulfate and 80 percent of kaolin) that was being applied for disease control. Tests were also made with poisoned sugar sirup sprayed on the foliage and flowers.

The tests were conducted in screen cages 4 to 5 feet square, each covering a flowering azalea. After the bees were put in the cages they flew against the screen for some time, but if only 10 or 15 bees were introduced they usually settled down in a few minutes. In tests with dusts the material was applied after the insects were introduced, but the sprays were applied before the plants were caged.

Effect of Insecticidal Dusts on Bees

Apparently the application of combined insecticidal-fungicidal dusts has no repelling effect on bees in general. The entire gardens were dusted nearly every morning during the flowering season with monohydrated copper sulfate-kaolin (1–4), and the insects frequented the flowers in large numbers. In one observation the dust cloud had not yet settled after cryolite-monohydrated copper sulfate-kaolin had been applied to azalea plants on March 29 when individuals of *Emphoropsis floridana* and *Bombus bimaculatus* alighted on the flowers and fed over them normally. The fly *Bombylius azaleae* and bumblebees *Bombus bimaculatus* and *B. impatiens* visited flowers dusted with kaolin and lead arsenate, and when the bumblebees were captured some of the insecticide was visible on their legs or in their pollen baskets. In the tests on control some of the dusts were applied previous to the introduction of the insects into the cages, but in all cases a thorough application of dust was blown onto the caged plants immediately after the introduction of the insects. None of the dusts had an immediate effect on the insects. Particular attention was directed to the three contact dusts pyrethrum, derris, and nicotine, but no paralytic or irritating effect was observed immediately on bees that were enveloped in a dense cloud of dust while on the wing or resting on the cage or plant.

Compared with undusted checks the stomach-poison dusts apparently had no effect on the bees. Those containing derris powder,

pyrethrum powder, or nicotine sulfate killed adults of most species in 1 to 4 days.

Effect of Poisoned Sprays on Bees

The caged bees were observed in 1935 to feed on droplets of poisoned spray on the flowers or foliage. They were sluggish in 24 hours after having fed on a spray containing lead arsenate or copper sulfate but generally died no more quickly than if they had fed on sprays containing derris or nicotine sulfate.

In 1936 further cage tests were made with the following insecticides incorporated in a solution containing 5 gallons of molasses and 95 gallons of water:

(1) Derris extract to give a rotenone content of 0.031 percent.
(2) Derris powder to give a rotenone content of 0.0107 percent.
(3) Lead arsenate (4 pounds to 100 gallons).
(4) 40-percent nicotine sulfate (2 quarts to 100 gallons).
(5) Tartar emetic (2 pounds to 100 gallons).

The cages were examined for mortality at the end of 4 days.

With *Bombus impatiens*, 73.5 percent were killed by derris powder, 68.2 percent by nicotine sulfate, and 81.4 percent by tartar emetic, while 11.5 percent died in checks. Variable results were obtained with *B. americanorum*, *B. bimaculatus*, and *Emphoropsis floridana* and with the two species of *Xylocopa*.

None of the bees were observed to be attracted to the sugar sprays applied to azaleas in the garden, and none were observed to feed on the droplets on flower petals even though they fed in the corolla according to their usual habits.

DISCUSSION OF RESULTS

Early in the investigation on azalea flower spot (*Ovulinia azaleae*) circumstantial evidence pointed strongly to the importance of insects as vectors. At first it appeared impossible for a disease to become established and to spread so rapidly without aid of some other agency than the natural drift of spores, and since insects were so abundant on azalea flowers they were naturally to be suspected. As the potentialities of the organism were revealed by subsequent studies, however, the implication of insects as vectors was correspondingly reduced.

Until the overwintering phase and the early-season development of the disease had been determined, it appeared that insects were introducing the organism either from some flower outside the azalea gardens or from their overwintering quarters. Tests of all possibilities to prove this point were negative, as were tests with soil animals that might have transported the organism causing infection to the earliest flowers. This negative evidence with insects is in full agreement with the final results of tests with the causal organism, which has proved itself to be fully capable of initiating primary infections as they are observed in nature. The disease has also amply demonstrated its ability to spread locally in a series of secondary infections and to destroy practically all the flowers present.

At the present stage of the investigation it appears that insects may be chiefly involved in the introduction of the disease into local uninfected azalea gardens. The observed habits of some of the insects

to travel for at least 5 miles within a period of 8 days would indicate a potential spread by insects of several miles within a given season. The demonstrated gradual release of spores by insects in inoculation tests would indicate that they could carry the organism for some distance in flight. Insects might therefore be responsible for reintroduction of the disease into a garden from which it had been eradicated and make it necessary for any effective disease-control program to be conducted on an area basis. Insects are not efficient vectors of the disease until infection becomes general. In one garden where a disease-control program was conducted a retardation of disease spread occurred. Only one-third as many insects collected from this garden throughout the season were infective as compared with those collected in another garden where the disease passed through its unhampered course of flower destruction. The first infective insect was not collected in the first garden until 2 weeks after the first infective one had been taken in the other. During the period of greatest infectivity insects caused less than 5 percent of the number of flower infections in the garden where disease control was conducted than did those used in inoculation tests that were collected from the other. Because of the potentialities of the disease the present solution of the problem appears to be one of disease control rather than of insect control. While insects have been shown to be important vectors under certain conditions, their elimination will probably not materially affect the seasonal progress of the disease in a previously infected garden.

Although no promising control of the insect vectors was developed in the present investigation, no further studies on this point appear at present to be necessary.

On present information as a basis it is concluded that while insects may at times be efficient vectors of the organism causing azalea flower spot, the disease when once established has demonstrated its ability to initiate primary infection each season and to spread through a given garden without the aid of insects. Insects appear to be chiefly involved in the distribution of the disease to local gardens and might be concerned in reinfection in a garden from which the disease had been eradicated, thus necessitating disease eradication or control on an area basis. Disease control rather than insect control appears to be the logical means of attacking the problem.

SUMMARY

An investigation was conducted from 1934 to 1938, inclusive, on the insects visiting azalea flowers and their possible relationship to azalea flower spot caused by *Ovulinia azaleae* Weiss.

Five species of bumblebees (*Bombus* spp.), 2 species of carpenter bees (*Xylocopa* spp.), *Emphoropsis floridana*, and honeybee (*Apis mellifera*) are the most conspicuous visitors, although thrips, flies, and many others do come to the flowers. The numbers of insects vary considerably during the season and in different localities. Some species feed by preference on flowers other than azalea. From 10 to 13 flowers per minute are visited by *E. floridana* and *B. impatiens*, respectively.

In experimental tests insects caused abrasions on flowers that are similar to those found on flowers in the open. Flower spot infections occur independently of insect abrasions. Microscopical examination

of insects collected from azalea flowers showed that spores were present among the hairs on their legs.

In tests with insects to determine their ability to transmit flower spot, a fixed procedure was used that had been devised in an attempt to prevent accidental contamination yet permit natural contamination and to give the insect full opportunity to cause infection.

In 1936 to 1938, inclusive, tests with living insects showed positive cases of infection with honeybees, five species of bumblebees (*Bombus americanorum*, *B. bimaculatus*, *B. fraternus*, *B. griseocollis*, and *B. impatiens*), two species of carpenter bees (*Xylocopa micans* and *X. virginica*), three solitary bees (*Emphoropsis floridana*, *Osmia lignaria*, and *Tetralonia rosae*), one thrips (*Heterothrips azaleae*), three ants (*Crematogaster ashmeadi*, *Pheidole morrisi* var. *vanceae*, and *Prenolepis imparis*), and a fly (*Hylemya* sp.). Infections were obtained with heads and legs of *Apis mellifera*, *Bombus* spp., *Emphoropsis floridana*, *Osmia lignaria*, and *Xylocopa* spp., but in no case with pollen, even though taken from infective insects and though spores were present among the pollen grains.

Infectivity of insects varied with the season in studies conducted throughout the flowering periods of azalea in 1937 and 1938. Early in the season the usual vectors except honeybees were not present, and these did not cause infection nor did miscellaneous Diptera, Hymenoptera, and other insects and spiders that were also present on azaleas. Primary infections appeared on the azalea flowers and developed to the limp-blight stage before any insect caused infection experimentally. As the disease became more general on azalea flowers infectivity of insects increased and reached its maximum during the period that flowers were drying after a wave of general infection and the development of limp blight.

In studies to determine the source of primary infection honeybees and other insects present early in the season apparently did not bring the organism from their winter quarters or from other flowers. Primary infections were most abundant near the soil, and flowers splashed with water and soil became infected. Soil-inhabiting animals, including insects, mites, spiders, centipedes, and diplopods, did not cause infection until after the disease had appeared in azalea gardens.

In further studies insects dropped the spores gradually, since a single insect infected all of 10 flowers that were inoculated. Insects transmitted the disease to uninfected gardens over a span of 2 miles, and marked bees were recaptured as far as 5 miles away. Infection was also obtained on *Rhododendron nudiflorum*, a widely distributed native plant.

Infections by insects were reduced 68.6 percent by a fungicidal dust containing monohydrated copper sulfate that was being applied for disease control. A fungicidal spray containing dilute acetic acid repelled insect visitors only while flowers were wet with it.

In cage tests to control the insect vectors, dusts containing cryolite or arsenicals were ineffective, but those containing derris, pyrethrum, or nicotine sulfate apparently killed some species if dusted directly on them. Caged bees fed on 5-percent-molasses sprays containing various insecticides and appeared to be most markedly affected by those containing tartar emetic, derris powder, and nicotine sulfate. In field tests the insects did not feed on the sprays and were apparently unaffected by the dusts.

It is concluded from these investigations that, while insects are at times efficient vectors of the organism, they are not primarily responsible for the development of the disease when once it has become established in a given planting. They may, however, be concerned with its spread to nearby plantings of azalea. Controlling the disease, rather than the insects, therefore appears to be the logical means of attacking the problem.

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