A Study of the Rust Fungus, Puccinia Antirrhini, on Antirrhinum Majus

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A STUDY OF THE RUST FUNGUS, PUCCINIA ANTIRRHINI, ON ANTIRRHINUM MAJUS

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Charles Christian Rees

ENTITLED A STUDY OF THE RUST FUNGUS, PUCCINIA ANTIRRHINI, ON ANTIIRRHINUM MAJUS

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS

In Charge of Thesis

Head of Department

Recommendation concurred in:*
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction and history</td>
<td>1-2</td>
</tr>
<tr>
<td>II. Acknowledgement</td>
<td>3</td>
</tr>
<tr>
<td>III. Experiments and methods</td>
<td>3-15</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>15-17</td>
</tr>
<tr>
<td>V. Control</td>
<td>17-18</td>
</tr>
<tr>
<td>VI. Summary</td>
<td>18</td>
</tr>
<tr>
<td>VII. Bibliography</td>
<td>19</td>
</tr>
<tr>
<td>VIII. Explanation of Plates</td>
<td>20-22</td>
</tr>
</tbody>
</table>
Introduction and history

The rust fungus, *Puccinia antirrhini*, (Diet. & Holw.) which has been known since 1879 to be parasitic on the cultivated snapdragon, *Antirrhinum majus*, has, of recent years, come to be a pest of considerable economic importance. The increase in damage caused by the rust is not believed to be due to an increasing virulence of the fungus but rather to the fact that the snapdragon, as a cultivated greenhouse and outdoor plant, has lately undergone a steady increase in popularity, until now it is grown generally throughout the United States.

Concerning the early appearance of the rust, W. C. Blasdale (3) has said in part:

"During the summer of 1895 the writer found near San Leandro, California, the uredo stage of a rust growing on cultivated forms of *Antirrhinum majus*. Somewhat later the same rust appeared on some plants of the same host growing in my own garden at Berkeley. Both uredo and teleuto stages were produced and the fungus ultimately destroyed the entire group of plants. Specimens were submitted to Mr. E. D. W. Holway and to Dr. Dietel, and the species was published by them under the name, *Puccinia antirrhini* (6). Since that year the fungus has appeared every season in which an attempt was made to raise this annual, and in every case destroyed the plants shortly after they had reached the flowering stage. Further observations have shown that the disease is a common one in the region about San Francisco bay, though I have no knowledge of its occurring elsewhere in the state."

That Blasdale was not entirely familiar with the location of the rust in his own state when he wrote this article is evidenced by an extract from a letter sent by Dr. J. C. Arthur to the writer in November, 1914, which reads: "In my herbarium I have specimens of the rust fungus, *P. antirrhini*,}
from the following localities: Santa Cruz, Cal., 1879; Berkeley, Cal., 1897; Ukiah, Cal., 1902; Whittier, Cal., 1909; Portland, Ore., 1909."

During the summer of 1913, the first serious damage to be caused in this part of the United States was reported to the Floricultural department of the University of Illinois. The report came from residents of the region north of Chicago who stated that the loss to their snapdragons, as a result of attacks of this rust, had been almost total. Specimens of this infection were received and identified as Puccinia antirrhini. Material was sent to Dr. J. C. Arthur who verified this identification.

Between that time and the fall of 1914, the presence of the rust was reported by no less than a dozen growers from different parts of Illinois, Indiana, and Ohio and by January 1915, Wisconsin, Iowa, and Massachusetts had been added to the list of states in which P. antirrhini had been found. F. D. Bailey (2) who reported the presence of the rust in Oregon late in January, 1915, has furnished the most recent addition to our knowledge of the disease in this state. However, herbarium specimens of the rust from Oregon have been in the possession of Dr. J. C. Arthur since 1909.

The seriousness of this sudden outbreak of a disease that had been, of recent years, so little in evidence, especially in this part of the country, was largely responsible for the starting of this investigation. It has been the aim of those in charge of the work to promote the general knowledge concerning this fungus and its host relationships and to establish if possible, suitable means of controlling the pest. All experimental work has been done in the greenhouse under conditions as nearly like those necessary for the successful culture of snapdragons as possible. Especial emphasis was given to the economic aspects of the disease.
Acknowledgement

Grateful acknowledgement is hereby made to Professor F. L. Stevens, under whose direction the experimental work of this investigation has been conducted and whose helpful criticism has made this thesis possible; to Dr. J. C. Arthur for his many helpful suggestions and to Mr. F. W. Muncie for his assistance and counsel in matters pertaining to the chemistry of the subject.

Experiments and methods

In order to determine whether or not the disease was being carried about from place to place on seeds, a quantity of them, representing 36 commercial varieties in all, were procured from several different sources. Most of them had been produced at different seed farms in the eastern part of the United States and the remainder came from European growers. From these seeds some 1800 plants were grown to maturity without developing a single case of rust infection. In the light of these results, it can be said, if seeds are at all responsible for the transportation of the disease, that the market supply has not as yet become generally infected.

According to Schroeter, P. antirrhini is one of the Hemi forms, only the uredinial and telial stages being known. The urediniospores are produced in sori (10) or cushions which are generally in the form of small circular dots although they have been found, especially when produced on the stem of the host, to occupy linear areas. The spores are hyaline or yellow to dark brown in color, generally spheroidal (19 to 24 microns in diameter) and are born upon pedicels which are deciduous. The spore walls are echinulate or warty. The spores germinate readily in five percent aqueous solution of gelatine.

The method of germinating urediniospores is quite simple. A drop of
a five percent aqueous solution of gelatine is placed on a glass slide. Then, by means of a sharp knife, the spores are removed from the host and dusted upon the drop of medium. The spores should not be completely submerged in the medium as air is essential to germination. The gelatine furnishes no qualities of nutriment to the germinating spore but simply checks the evaporation of water from the medium. After placing the spores upon the medium the slide is placed in a petri dish, the bottom of which, in order to insure an abundance of moisture, has been covered with water-soaked filter paper. Germination usually takes place inside of 24 hours.

The telial sori (10) are inclined to be disposed in a linear manner along the stems of the host. The teliospores are thick-walled, two-celled resting spores, somewhat club-shaped (20 x 48 microns), deep brown in color and bearing a persistent pedicel. Repeated efforts were made to cause them to germinate but without success. They were subjected to an alternate wet and dry condition for days at a time: kept in a running stream of tap water for periods of 48 hours: alternated between room temperature (24°C), and the temperature of the ice box (11°C), and subjected to outdoor conditions through the months of December, January, February, and March. None of these treatments induced germination.

On account of the close relationship that exists between the genera Antirrhinum and Linaria, both of which are members of the Scrophulariaceae (Figwort family), and taking into account the fact that L. vulgaris, commonly known as toad-flax, grows wild in abundance it was believed that perhaps this wild form might be susceptible and might prove to be a transporting medium for infection. With this idea in mind, 50 plants of the wild form, L. vulgaris, were transplanted from outdoors into pots in the greenhouse. Seeds of six commercial varieties of Linaria were also obtained and from
them 169 plants were grown. From the time the Linarias were mere seedlings until they had fully matured, repeated but unsuccessful attempts were made to infect them with P. antirrhini. Failing to germinate the teliospores, only the urediniospores were used and hundreds of inoculations were made. Similar results were obtained when, in a like manner, an attempt was made to infect L. vulgaris. Under the same conditions, using similar material and applying like methods, scores of inoculations were made on snapdragons. The majority of these inoculations caused infection. The failure to infect the closely related Linarias leads to the conclusion that under normal greenhouse conditions they are not susceptible to attacks of this fungus. Knowing this, little or no credence can be placed in the idea that this closely related genus is in any way responsible for the spread of this disease.

In order to gain knowledge of the life history of this fungus 100 inoculations were made on as many plants. Being unable to germinate the teliospores, urediniospores were used, they having been previously tested and found capable of germination. Each plant before the inoculation was made thereon was thoroughly syringed with tap water with an ordinary hand atomizer. Spores were then removed from a sick plant on the tip of a scalpel blade and carefully placed on the upper side of the leaf, care being taken not to rupture the epidermis. The inoculated leaf was marked by tying a white thread loosely around its stalk. The plant was then placed in a Wardian case and shaded. Here it was left for 48 hours. This precaution, which prevents excessive transpiration, keeps a high percentage of surface moisture on the leaves and practically insures infection.

Usually within 9 to 14 days after making the inoculation, minute yellow spots began to appear on the under side of the leaf. These spots increased in size and shortly presented a swollen appearance. In most cases, within 48 hours after the yellow spots had appeared, small rusty brown specks were
formed in the center of each yellow swollen area. These brown specks or sori are made up of masses of urediniospores which are capable of germinating at once and reinfecting the host plant. Occasionally, later in the season or under conditions less favorable for propagative reproduction, teliospores are developed. Neither pycnia nor aeciospores have been seen. These forms may either not exist or they may, according to Duggar (8), be present upon some other host.

In order to determine the exact extent of the migration of mycelium within the plant, 12 healthy snapdragons of a yellow variety, each about eight inches tall, were selected for inoculation. The subjects were divided into three groups of four plants each and, for convenience in taking records and tabulating results, were numbered from 101 to 112, inclusive. The plants of the first group were inoculated with virulent urediniospores on leaves at their extreme growing tips. Inoculations in the case of the second group were made near the middle of each plant and to each of the four remaining subjects the spores were introduced to the basal leaves. The plants were inoculated in this way in order that the experiment might result in the establishing of a relationship between the extent of the internal migration of mycelium in parts of the host of different ages. Inoculations were made according to the method that has already been described.

The plants were kept inside of a case built over the bench in order that air currents might not interfere with the results by carrying spores from one plant to another. Each plant was fitted with a covering of oiled paper which left no part of the host, below the point of inoculation, exposed. The oiled paper protection kept spores from dropping from the infected leaves upon the healthy leaves below. As an additional means to the same end the plants were watered only from below.

In the course of 14 days after the inoculations were made, the eight
plants inoculated at the growing tips and middle leaves had developed brown pustules. The inoculations made on the basal leaves failed to produce a single infection. After the pustules appeared the plants were watched closely for two months and in no case did an infection spread beyond the leaf originally inoculated. The infected areas were measured and the extent of each is given in Table 1. From these results it can be seen that the spread of the mycelium is purely local, as is generally the case with rust fungi. The failure to cause infection on any of the basal leaf inoculations also leads to the conjecture that possibly the advanced age of the plant tissue is responsible for the condition of immunity in this part of the host.

Table 1

<table>
<thead>
<tr>
<th>Position on plant</th>
<th>Tip leaf</th>
<th>Middle leaf</th>
<th>Basal leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mm.</td>
<td>20 mm.</td>
<td>no infection</td>
</tr>
<tr>
<td></td>
<td>5 mm.</td>
<td>23 mm.</td>
<td>no infection</td>
</tr>
<tr>
<td></td>
<td>4 mm.</td>
<td>15 mm.</td>
<td>no infection</td>
</tr>
<tr>
<td></td>
<td>9 mm.</td>
<td>12 mm.</td>
<td>no infection</td>
</tr>
</tbody>
</table>

The force lent by the spray in syringing rust-infected snapdragons is a very important factor in the spread of the disease. The practice keeps the plant's surface plentifully supplied with moisture which is most conducive to the germination of spores, once they reach the host. An experiment was planned to ascertain the advantage of underneath watering over syringing of snapdragons as a means of preventing the spread of rust infection.

In order to carry out this test, two sets of healthy snapdragon plants, growing in four inch pots, were selected. There were 23 plants in each set.
and both were arranged on the same bench but eight feet apart. The arrangement of the plants in each set was after the manner shown in Figure 1 of Plate I, the plants being placed 10 inches apart. By conducting the two tests in such proximity to each other similar physical conditions were insured without danger of interfering with the results of either in caring for the other. The XX indicates the location of badly rusted plants which were placed in those positions at the start of the experiment.

When the plants had been arranged, the experiment was placed in charge of a greenhouse employee who attended to all watering. He was instructed to water one lot only from below, that is to introduce water to the soil around the roots, taking care not to wet or disturb the foliage. The other lot was to be watered from above which would insure a complete wetting and considerable disturbance of the foliage. The two sets of plants were cared for after this fashion for a period of sixty days, at the end of which time each plant was closely examined and a record taken of its condition. The results produced by the two methods of watering are clearly indicated in Figures 2 and 3 of Plate I. The XX indicates the location of each sick plant at the start of the experiment. Each subsequent infection of a new host which can safely be attributed to the method of watering employed, has been marked by a single X. Figure 2 represents the plants that were watered only from below. Not a single plant became infected. Figure 3 represents the plants that were syringed. Sixteen out of a possible 23 became infected.

The results of this experiment are striking enough to make conclusions quite obvious. The practice of watering Antirrhinums only from below, in addition to showing a striking advantage over the syringing method, was accompanied by no apparent disadvantages. The time that was required to water each lot of plants was practically the same and the plants that were
watered only from below were in as good condition, physically, as were those that were syringed. For these reasons it would seem advisable to practice underneath watering of snapdragons in the greenhouse to prevent the spread of rust infection.

The characteristic growth made by certain varieties of plants, the variance in thickness of the epidermal cell walls, the presence of a cutinized epidermis or of protective appendages, such as plant hairs etc., are oftentimes found to be influencing factors in the matter of immunizing these varieties against attacks of plant diseases. This relation was especially noticeable among certain of the 30 different varieties of snapdragons used in this investigation. Accordingly an experiment was conducted in order to ascertain whether or not any one of these varieties was more or less susceptible to attacks of this rust fungus than were the others.

Three plants of each of 30 varieties were used as subjects for inoculation in this experiment. Each plant was inoculated in three places, urediniospores being introduced on the growing tip, middle and basal leaves. The usual method of inoculation was used and the inoculations were made April 3, 1915. Nine days later and every 48 hours thereafter the plants were examined and any advances made in the progress of infection were recorded. The records are pictured in a condensed manner in the graphs included in Plates II, III, IV, V, and VI.

It is seen that in order to secure an accurate average of the time required for the complete infection of every variety such an experiment would necessarily have to be duplicated many times. However, the results obtained serve well to indicate that these varieties, generally speaking, are equally susceptible since all became infected between nine and 16 days after inoculation.

The following percentages, figured from records taken in connection
with the preceding experiment are quite striking. Nine days after inoculating, 54% of the tip leaves showed infection as against 21% of the middle leaf inoculations while none of the basal leaves were infected. Fifteen days after inoculating, 62% of the inoculations made on middle leaves and 5% of the basal leaf inoculations showed infection. Again, 18 days after making the inoculations, 97% of the tip inoculations, 69% of those made on middle leaves, and 15% of the basal leaf inoculations had contracted the disease. The last records were taken 23 days after the inoculations had been made, when 97% of the tip inoculations, 81% of the middle leaf inoculations, and 20% of the inoculations made on basal leaves were found to be infected. The relationship between the three infection curves is clearly pictured in the graph, represented in Plate VII, in which the ordinates represent the number of inoculations made and the abscissas the length of time, in days, over which the experiment was allowed to run.

As can be seen from the graph, the middle leaves, although a little more time is required for their infection, are but slightly less susceptible than those at the tip. It is the basal leaf in nearly every case that shows considerably less of a tendency to become infected than the others. This is probably best explained by the fact that in a large majority of cases the basal leaves, after being inoculated, die before the minimum time (nine days), for the appearance of infection has elapsed. Death is probably caused by old age and the lack of sufficient sunlight and nourishment. Those few basal leaves that did live and failed to become infected were less turgid than the middle and tip leaves on the same host. The fact that in older tissue the cell walls are thicker may be introduced as having significance in this connection. Another explanation of this fact is that rusts are fungi of the most highly parasitized type and therefore
grow best on those parts of the host that are at their maximum as regards vitality. A looseness of cell structure and an open condition of the stomata, concurring to admit air freely to the intercellular spaces, presents a condition highly conducive to the entrance of rust infection. This state of affairs rarely if ever occurs in the basal leaves of the snapdragon.

The effect of the application of aqueous solutions of commercial fertilizers and of liquid manure upon the total acidity of the cell sap of the plant and the relation of this condition to immunity was made the last problem for consideration in this investigation.

Three hundred vigorous snapdragon seedlings, 50 each of six different commercial varieties, were selected as subjects for the feeding experiment. The plants were set on strips of glass on a bench in the experimental house in order to prevent the entrance of any foreign commercial feeding substances through the bottom of the pots. Aqueous solutions of sodium nitrate (2.5 grams per litre), di-sodium phosphate (2.5 grams per litre), and ammonium sulphate (1.25 grams per litre) were prepared. For the fourth feeding substance, liquid manure, taken from the greenhouse supply, was used.

Beginning January 25, 1915, ten plants of each of the different varieties were fed with sodium nitrate, ten with di-sodium phosphate, ten with ammonium sulphate, ten with liquid manure, and the last ten of each variety were taken as checks and not fertilized. Each variety, exclusive of the checks, was given these four different treatments. Applications

a. The liquid manure was prepared by allowing one half bushel of cow manure to leach in a 50 gallon barrel of water for four days. At the end of this time fermentation had taken place and the manure water was drawn off and used.
of 75 cc. portions of these aqueous solutions of commercial fertilizers were made weekly until six had been given.

Determinations of relative total acidity were then made on plants from the different series. The method was essentially that used by Astruc (1) and described by Detmer (5) with the exception that the stems were frozen before the sap was expressed. To freeze the stems (9), they were placed in clean, dry test tubes (25 x 130 mm.) and tightly stoppered. These tubes were then immersed in an ice-salt bath (-15° C.) for a period of from 12 to 24 hours. The procedure was as follows. The sap, upon expression, was filtered and tested at once although it can, upon the addition of a drop or two of xylol, remain at ice box temperature for days without a noticeable change in acidity taking place. With a standardized, dry pipette one cc. of the sap was transferred to a test tube (25 x 130 mm.) and, to insure the complete removal of the sap from the pipette, it was washed out three times with one cc. portions of previously cooled carbon dioxide-free water. The sap solution was then further diluted to a volume of from six to eight cc. and three drops of a one percent solution of phenolphthalein in 50% alcohol were added. Standard carbonate-free KOH, approximately \( \frac{N}{50} \) was then added from a Mohr burette, drop by drop, until the solution was of a faint rose coloration. The results were calculated as cc. of normal acid per cc. of cell sap. The results obtained by the use of this method give a measure of the reserve acidity of slightly ionized acids or their acid salts as well as the hydrogen ion concentration in the sap.

a. Dixon and Atkins have shown that the osmotic pressure of successive portions of sap, expressed from plants after freezing to destroy the permeability of the cell membranes, is the same. The sap so expressed is probably an accurate representative of that within the plant as regards acidity also.
The data appearing in Table 2 shows the amounts of normal acid found in a cc. of a composite sample of cell sap taken from each lot of plants fed differently.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Yellow</th>
<th>Fairy Queen</th>
<th>Giant Purple</th>
<th>Striped</th>
<th>Atroccineum Dark</th>
<th>Scarlet</th>
<th>Tom Thumb Scarlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>.0064</td>
<td>.0073</td>
<td>.0054</td>
<td>.0073</td>
<td>.0087</td>
<td>.0095</td>
<td></td>
</tr>
<tr>
<td>Liquid Manure</td>
<td>.0076</td>
<td>.0062</td>
<td>.0051</td>
<td>.0075</td>
<td>.0102</td>
<td>.0090</td>
<td></td>
</tr>
<tr>
<td>Sodium Nitrate</td>
<td>.0073</td>
<td>.0067</td>
<td>.0056</td>
<td>.0078</td>
<td>.0082</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Di-sodium Phosphate</td>
<td>.0095</td>
<td>.0084</td>
<td>.0048</td>
<td>.0078</td>
<td>.0099</td>
<td>.0106</td>
<td></td>
</tr>
<tr>
<td>Ammonium Sulphate</td>
<td>.0074</td>
<td>.0070</td>
<td>.0075</td>
<td>.0075</td>
<td>.0085</td>
<td>.0098</td>
<td></td>
</tr>
</tbody>
</table>

To prove that the acid determined after this method was not due to carbon dioxide present in the sap, the following experiment was conducted. One cc. of distilled water, saturated with carbon dioxide, was placed in a test tube to which was added five drops of kerosene (tested and found to be neutral) and a small piece of porous plate. These were added to prevent excessive bumping and frothing. The contents were then boiled under reduced pressure for one or two minutes by warming in the hand. After the addition of three drops of phenolphthalein, one drop of KOH(\(\frac{1}{36}\)) turned the contents of the tube a deep red color, showing that the removal of the carbon dioxide from this solution by this method had been complete. Two samples of sap of one cc. each were then taken from a row of check plants. One sample was subjected to this treatment and the other was not. When titrated against KOH, using three drops of phenolphthalein as an indicator, the
sample to which suction had been applied required .74 cc. of alkali for neutralization while the other sample required .76 cc. This fact is significant in that it shows there was no carbon dioxide to be liberated and therefore that the acid reactions obtained in the tests run in connection with this experiment were not in any way due to its presence.

Ammonium sulphate caused a somewhat perceptible increase in the acid content of the cell sap, only one sample running lower than the check. The increase in sections where di-sodium phosphate was used was even more noticeable although in this case, as in that of the ammonium sulphate sections, one sample ran below the check. In no instance was the increase in the acid content sufficiently striking to lead to the supposition that the degree of susceptibility of these hosts to rust attacks would be materially changed thereby.

Inoculations were made, ten plants of each variety including two plants of each treatment being taken as subjects. Urediniospores were used and the usual method of inoculation was followed. The plants were inoculated 3-8-15 and by 3-25-15, 17 days later, every plant showed infection.

O. Comes (4) has published an article on the "Connection between the acidity of the cell sap and rust resistance in wheat". His work, which deals with results obtained previously by himself and others, is believed to justify the general conclusion that the biochemical factor which constitutes the means of resistance of an organ to disease may be estimated from the acidity of the cell sap; that this acidity, rather than density and compactness of tissues, enables plants to resist parasitic fungi; and that the normal production of sap more or less rich in sugars or in acid is hereditary, but capable of modification by cultivation, manuring, and elevation. He also claims that in order to preserve the highest degree of acidity in the cell sap and at the same time maintain fertility of soil the
phosphatic fertilizers, especially superphosphates, should be used instead of nitrogenous manures.

It is possible that, as a host, Antirrhinum majus is not compatible with Come's theory of the relation that exists between the acid content of cell sap and immunity from attacks of rust fungi. Perhaps, taking into consideration the medium in which the subjects were grown, the fertilizers applied to increase the acid content were not applied for a long enough time prior to testing the cell sap. Had the subjects been rooted and grown in sand or some other sterile medium during the time the feeding experiments were being carried on the results would very probably have been different. The soil, in the fall when the subjects were potted, was neutral or slightly alkaline to litmus, which may be taken as a significant fact. Even after six weeks feeding with a substance that ionizes to react acid, the acid content of the soil might possibly not have reached concentration high enough to allow a very perceptible amount of it to be taken up by the plant. The presence of basic substances in the soil may have had much to do with the neutralization of acid, formed upon the ionization of the ammonium sulphate. However this may be, the addition of commercial fertilizers mentioned, under conditions as outlined in this experiment, has in no way created a condition of immunity among snapdragons, as regards attacks of this fungus.

Discussion

In the main, these experiments have been instrumental in establishing a number of facts which ought to serve, as stepping stones to the ultimate solution of the control of this pest.

The experiment which disclosed the local nature of the mycelium of this fungus is a striking example of the meaning of this statement. The necessity of establishing this point was early realized, since without this knowledge the practice of picking off diseased leaves to prevent the spread of infec-
tion could not have been recommended.

The outcome of the experiment in which the relative merits of overhead and underneath watering were tested was conclusive enough to justify the recommendation that snapdragons be watered only from below. Any grower of this floral crop in the greenhouse can well afford and by all means should adopt this method of watering. By so doing, he will not only succeed in checking the spread of the disease, should it already be present, but by keeping the foliage dry, the chances of introducing the infection will be materially lessened.

Care in handling and watering and the exclusion of strong air currents are the factors that have been largely responsible for the successful culture of some 25,000 snapdragon (subjects for this investigation) from seedlings to mature plants without contracting a single accidental infection. Quite a number of these plants were grown on the same bench with plants in the last stages of rust infection and the others were propagated under the same glass although not in the same house with them. The ease with which the infection, introduced for experimental purposes, was kept in check during the winter months, convinced the writer that with the exercise of a moderate amount of care the spread of rust infection in the greenhouse can be largely prevented during this season.

In addition to the plants used in this investigation the superintendent had growing, for purposes of demonstration, in a distant corner of the range, a number of snapdragons of the yellow and white varieties. These plants remained free from rust during the entire fall and winter but with the coming of spring and the arrival of bumble-bees, aided by the fact that these plants, not being a part of the investigation, were watered regularly from above, they soon contracted the disease. Bumble-bees are known to be carriers of spores of this rust fungus and as transporting agents of in-
fection from one locality to another, within a reasonable limit, they in all probability play an important part.

The shipping of diseased cuttings from place to place is largely responsible for the introduction of this rust infection into many new regions. Cuttings are extremely susceptible to infection and many instances of the disease entering a locality on them have been brought to the attention of the writer. For the sake of the grower of snapdragons in the future, the practice of sending out cuttings without first submitting them to a rigid inspection and destroying those diseased, should by all means be discontinued.

Considerable experimental work remains yet to be done in connection with this fungus. Owing to an oversight in placing the order for seeds, the varieties, Buxton's Pink, Phelps' White, and Nelrose were omitted from the list of commercial varieties used. These varieties have been almost universally recommended by florists as having a tendency to be less susceptible than others to attacks of rust but until tested by experimental practice these recommendations must remain unconfirmed.

The question of whether or not the seeds borne on rusted plants will give rise to plants similarly affected remains yet to be settled. An experiment to prove this point has already been undertaken and although the outcome can not be reported on at this time, results will soon be forthcoming.

A few preliminary experiments on the effect of different fungicides on the fungus have been conducted in the greenhouse. However, in view of the fact that extensive plans for outdoor experimental work along this line have been made, to be carried out in the near future, no report will be made on work done in this connection at this time.

Control

With our present knowledge of this rust fungus, P. antirrhini, it would
seem advisable to observe the following precautions:

(1) Cover with straw and burn all plants left out of doors in the fall.

(2) Do not propagate from a plant showing the slightest signs of rust or from a plant that has in anyway been exposed to the disease.

(3) Examine carefully all cuttings and refuse to accept them if there are any signs of rust present.

(4) Water the plants only from below. Do not syringe. Should the soil dry out too rapidly, apply a mulch to the bench.

(5) Examine plants carefully and remove and burn all signs of infection.

(6) If rust becomes too general, discontinue the growth of snapdragons for a period of two years.

Summary

(1) Thirty different commercial varieties of snapdragon were employed in this investigation.

(2) The market supply of seeds is free from rust.

(3) Different varieties of the related genus, Linaria, under greenhouse conditions, are not susceptible to attacks of this rust fungus.

(4) Only the uredinia and telia spore forms are known.

(5) The mycelium is purely local.

(6) Overhead watering is conducive to the introduction and spread of infection.

(7) Each one of the thirty varieties employed is equally susceptible.

(8) The leaves at the growing tip of the host are more susceptible than the leaves at the middle while the basal leaves are little or not at all apt to contract the disease.

(9) The application of different commercial fertilizers for a period of six weeks did not increase or decrease the susceptibility of the host.
Bibliography


(5) Detmer and Moor, Practical Plant Physiology (1898), p. 324.

(6) Dietel and Holway, Hedwigia, 36(1899), p. 298.


(10) Rees, Chas. C., The rust on antirrhinum. Am. Flor., 43(1914), No. 1386, p. 1198; Flor. Rev., 35(1914), No. 886, p. 18; Flor. Ex., 28(1914), No. 21, p. 1156.
Explanation of Plate I

Figure 1 shows the arrangement of the potted snapdragons and pictures the location of the sick plants (XX) at the start of the experiment. The pots were placed 10 inches apart.

Figure 2 shows the lot of plants that was watered only from below for a period of 60 days. With X representing the plants that were infected as a result of the method of watering used, it can be seen at once that this method was effective in preventing the spread of the rust. Not a single plant became infected.

Figure 3 shows the lot of plants that was syringed for a period of 60 days. With X representing the plants that were infected as a result of the method of watering used, the undesireability of this method is evident. Sixteen out of a possible 23 plants became infected.
Explanation of Plates II to VI (inclusive)

The graphs included in these plates were prepared for the more ready interpretation of the time required to complete the infection of each of 30 different commercial varieties of snapdragon with the rust fungus, *P. antirrhini*. Three plants of each variety were inoculated and, accordingly three graphs under the head of each variety appear in the plates. Each graph has been constructed with the numbers 1, 2, and 3 along the axis of ordinates, representing the three points of inoculation on the plant, the basal, middle, and tip leaves, respectively. Twenty-five divisions, each representing a 24 hour period, have been made on the axis of abscissas. Each dotted line connects the point established by the first appearance of infection (yellow swellings) with the point showing the time when brown pustules first appeared. The solid line joins the points located by the appearance of brown postules at each place of inoculation namely, at the tip, middle, and basal leaves.
Explanation of Plate VII

The graph represented in Plate VII was prepared in order that the relation existing between the basal, middle, and tip leaves of the snapdragon, as regards their tendency to become infected, might be more clearly shown. The ordinates represent the number of inoculations made and the abscissas the length of time, in days, over which the test was allowed to run.
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Graph showing rate of infection after inoculation

- Tip leaf
- Middle leaf
- Basal leaf

Time: 5, 10, 15, 20