THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Dixon Lloyd Bailey final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

Oct. 14 1921

E. C. Stakman
Chairman

H. K. Hayes

L. I. Knight

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Dixon Lloyd Bailey for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

E. C. Stakman
Chairman

H. K. Hayes

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Sunflower Rust

A thesis presented to the Faculty of the
Graduate School of the University of
Minnesota in partial fulfilment
of the requirements for the
Degree of Master of
Science

by

Dixon Lloyd Bailey

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Table of Contents

10-20 5M

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Table of Contents (Continued)

Table of Contents (Continued)

Sunflower Rust

Introduction

With the increased production of sunflowers for silage purposes sunflower rust has assumed increased economic importance and has proven an important factor in their cultivation. Puccinia helianthi Schw. is a euautoecious rust and is of considerable interest from a scientific standpoint because it has a tendency to short-cycle itself; this may possibly throw some light on the origin of hemi-forms of rust from eu-forms. Sunflower rust occurs on a wide range of wild hosts and thus affords also an excellent opportunity for the study of the fundamental problem of biologic specialization.

Economic Importance and Geographical Distribution

Sunflowers

The wild sunflower, Helianthus annuus L., from which the cultivated variety has been developed, is native in the Great Plains region from Nebraska to Northern Mexico. When Champlain explored the region in the vicinity of Georgian Bay in 1615 he found the Indians there cultivating an "herbe des soleil". Gray, who has made a careful study of the history of the sunflower, states that, judging from the breadth of the flower heads soon after its introduction into Europe, it must have assumed in aboriginal hands much of the abnormal development which distinguishes the cultivated sunflower from its wild original of the Western plains.(2)

In Europe the sunflower was first cultivated in the gardens at Madrid about the middle of the sixteenth century and, as this was shortly after the Spanish Expeditions to Peru, all the earlier botanists assumed it was of Peruvian origin. This would seem to be an error, however, since it is not indigenous anywhere south of the northern part of Mexico and there is no record of its ever having been cultivated by the natives of Peru. As early as 1576 De Lobel, a Flemish botanist, gives a good illustration of a sunflower with stout erect stems, big leaves and large nodding head, very much like our present cultivated variety. (1)

In Western Europe and America where the sunflower has been grown chiefly for ornamental purposes, or occasionally for poultry food, there has been apparently little improvement or development within the large types during the three and a half centuries of cultivation. Seeds 6 to 10 mm. in length, as large as those of ordinary varieties at the present time, were figured by Camerarius in 1586. (1)

In Russia, where the restricted use of meat has lead to the consumption of large amounts of vegetable oils and oily foods, sunflower seed has become a staple article of diet. It is eaten raw or roasted as peanuts are in America. Between 1830 and 1840 sunflower oil began to be manufactured on a commercial scale in the southern provinces of Russia and since that time a series of important industries based on the production of oil

4-21 6M

and oil cake have been developed there. This has led to the development of more prolific seed-producing varieties until now three principal varieties are cultivated in Russia - one with large white seeds which yields the largest amount of oil; one with smaller black seeds which are sweeter and regarded as best for eating; and an intermediate form with striped seed used for both purposes. There are numerous intergrading forms as may be found in most plants long in cultivation.

In the United States three principal varieties are grown for the production of seeds. The common sunflower, with no distinguishing varietal name, has nodding heads, which are 8 to 16 inches in diameter, and produces chiefly gray-brownish or striped seeds. The Mammoth Russian is a recently introduced variety with heads from 15 to 20 inches in diameter, producing seeds about one-half inch long, with black or brownish stripes or sometimes all white. The Black Giant, another variety, has heads 16 to 22 inches in diameter with rather thick black seeds about three-eighths of an inch in length. (1)

Although the sunflower has been grown for a long time in this country for ornamental purposes it is only recently that we have begun to learn from the experience of China and Russia the economic value of the plant itself and to recognize in it a possible source of wealth in this country. Much may be said of the value of the seed. The oil expressed from the seed is highly prized as an edible oil and one which, more nearly than any other known vegetable oil, has the same general properties of olive oil. The oil cake left after the extraction of the oil is extremely rich in nitrogenous matter and has a food value equal to the cake resulting from the expression of maize oil or linseed oil.

4-21 6M

In addition to this, it has the advantage of being more palatable than those just mentioned.

In this country, however, the sunflower has achieved its chief significance as an ensilage crop. The first report of its use for this purpose came from the Central Canadian Experimental Farm at Ottawa in 1893. There on advice of the chemist, based on chemical analyses of the various parts of the sunflower plant, only the heads were used for silage; and these were combined with corn and beans to form the so-called Robertson mixture which was highly recommended to Canadian farmers. From that time on a steadily increasing amount of attention has been given to the possibilities of sunflowers as an ensilage crop, particularly in those parts of Canada and the northern states where corn cannot be grown to advantage. In 1896 the Maine Experiment Station reported a yield of 24.4 tons per acre and stated that the silage was readily eaten by live stock when fed in mixture with corn silage. (3) In 1889 the Nebraska Experiment Station and in 1890 the Colorado Experiment Station carried on some work with sunflowers but no yields are reported. (3) Extensive experiments over a period of four years have been carried on in Montana on growing and feeding sunflowers. The yield varied from 31 to 44 tons to the acre under irrigation and about 10 tons to the acre in dry land tests in a season of unusually low precipitation. Thus sunflowers are promising dry-land forage producers and yield extremely well under more favorable conditions. After four year's experience (3) the Montana Experiment Station believes that sunflower silage may be used as a winter feed for practically all classes of farm stock. They have used it successfully in winter feeding of beef calves, dairy cows, breeding beef and

4-21 6M

dairy bulls, breeding ewes and to a limited extent with brood sows and believe it can be used also to good advantage to supplement pastures in dry seasons or when they become inadequate in the fall. (3) In general these results have been substantiated by extensive investigations in Michigan. (5 & 6) In the light of a steadily increasing amount of experimental results indicating the desirability and wide range of usefulness of sunflower silage it seems fair to conclude that sunflowers are destined to play an extremely important role economically as an ensilage crop in those parts of the United States and Canada where corn does not mature every season or where the increased yield resulting from their use is an important consideration. Several serious diseases of the crop, however, have appeared. One of the most destructive is the sunflower rust.

Sunflower Rust

Sunflower rust was first described by Schweinitz in 1822 from material collected in Pennsylvania. Subsequent to this description of it, Puccinia helianthi has been reported from Canada, Germany, Austria, Italy, Serbia, Roumania, Sweden and Russia. (12)

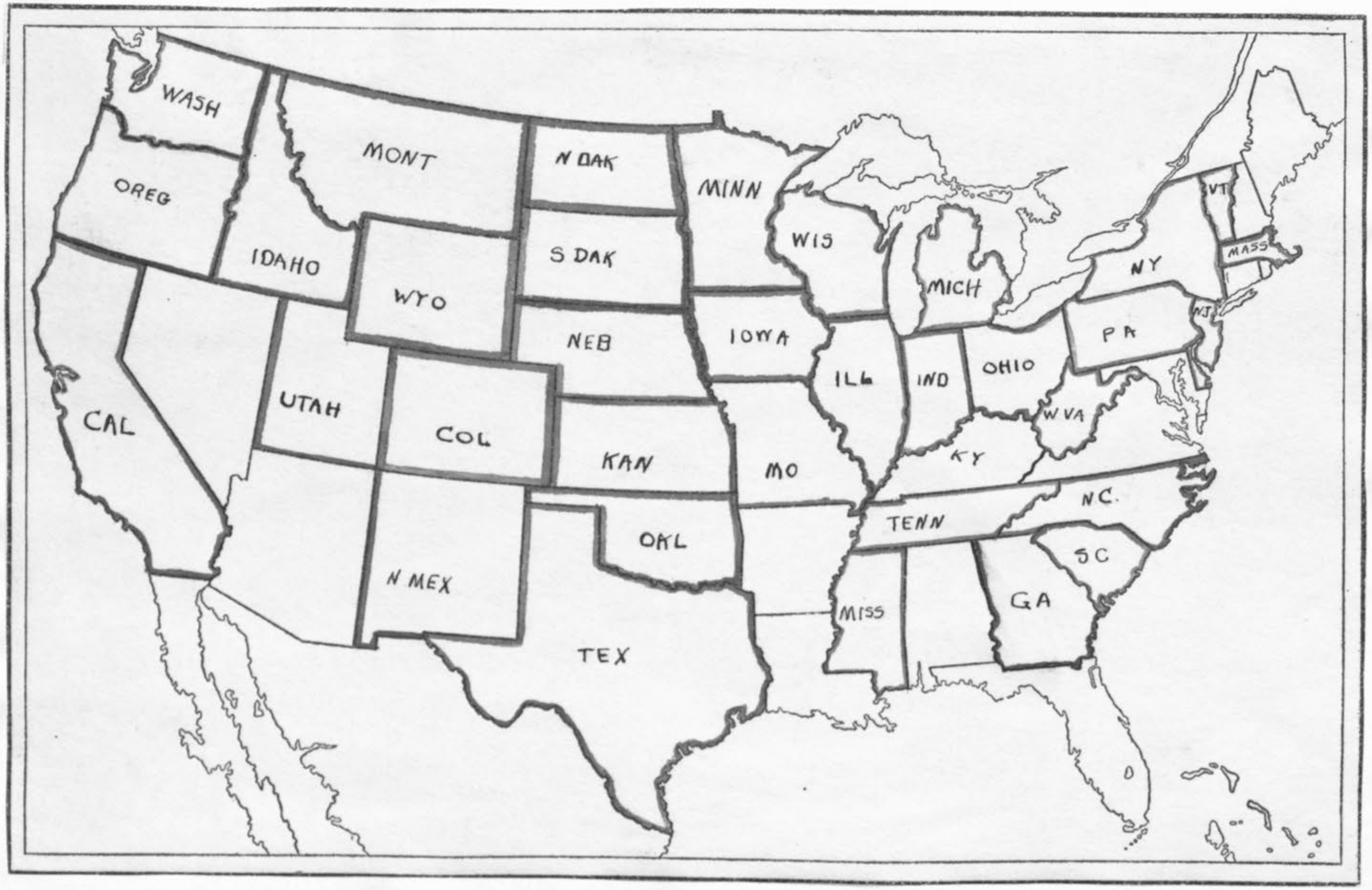
At present it seems to be common throughout practically all of the United States. In the herbarium of the Plant Pathology Department, University of Minnesota, specimens are present from the following states: Colorado, Illinois, Indiana, Kansas, Montana, Mississippi, Minnesota, Nebraska, New York, North Dakota, South Dakota, South Carolina, Texas, Vermont, Wyoming, Georgia, and Wisconsin. According to the records of the Plant Disease Survey, U.S.D.A., B.P.I. (Unpublished letter)

4-21 6M

4-21 6M

Figure I.

Distribution of Sunflower Rust in U. S.



it occurs in the following additional states: California, Idaho, Iowa, Michigan, Massachusetts, New Jersey, Oklahoma, Oregon, Pennsylvania, Washington and West Virginia. It is reported in the literature examined as occurring also in Ohio (8), North Carolina (9), New Mexico (10), Missouri, Kentucky and Utah. (11) This distribution (Fig.1.) includes not only the majority of the states but also all types of climatic conditions met with throughout the country and indicates the probability of its occurring in all the States of the Union.

According to Saccardo *Puccinia helianthi* Schw. occurs on the following hosts: *Helianthus angustifolius* L., *H. annuus* L., *H. californicus* Schw., *H. decapetalus* L., *H. divaricatus* L., *H. doronicoides* Lam., *H. grosse-serratus* Mart., *H. heterophyllus* Schw., *H. hirsutus* Rafin., *H. laetiflorus* Pers., *H. maximiliani* Schrod., *H. mollis* Lam., *H. occidentalis* Ridd., *H. rigidus* Desf., *H. strumosus* L., and *H. tuberosus* L. (7) No record could be found of *Puccinia helianthi* occurring on any genus other than *Helianthus*. Although *Puccinia heliopsidis* Schw., which is morphologically indistinguishable from it, occurs on *Heliopsis laevis* and *Heliopsis scabrae*, *Puccinia heliopsidis* is retained by Sydow as a separate species since it has not been shown to be biologically identical with *Puccinia helianthi*. The latter must be considered in the light of our present knowledge as confined to the genus *Helianthus*.

Its extensive distribution coupled with its wide host range makes *Puccinia helianthi* a serious consideration when it is desired to raise rust-free sunflowers. The fact that its wild hosts occur in greatest profusion in the Middle Western and Northern States where sunflower cultivation is assuming its chief economic importance greatly increases the seriousness of the outlook and makes the future of sunflower growing seem doubtful if the disease cannot be controlled.

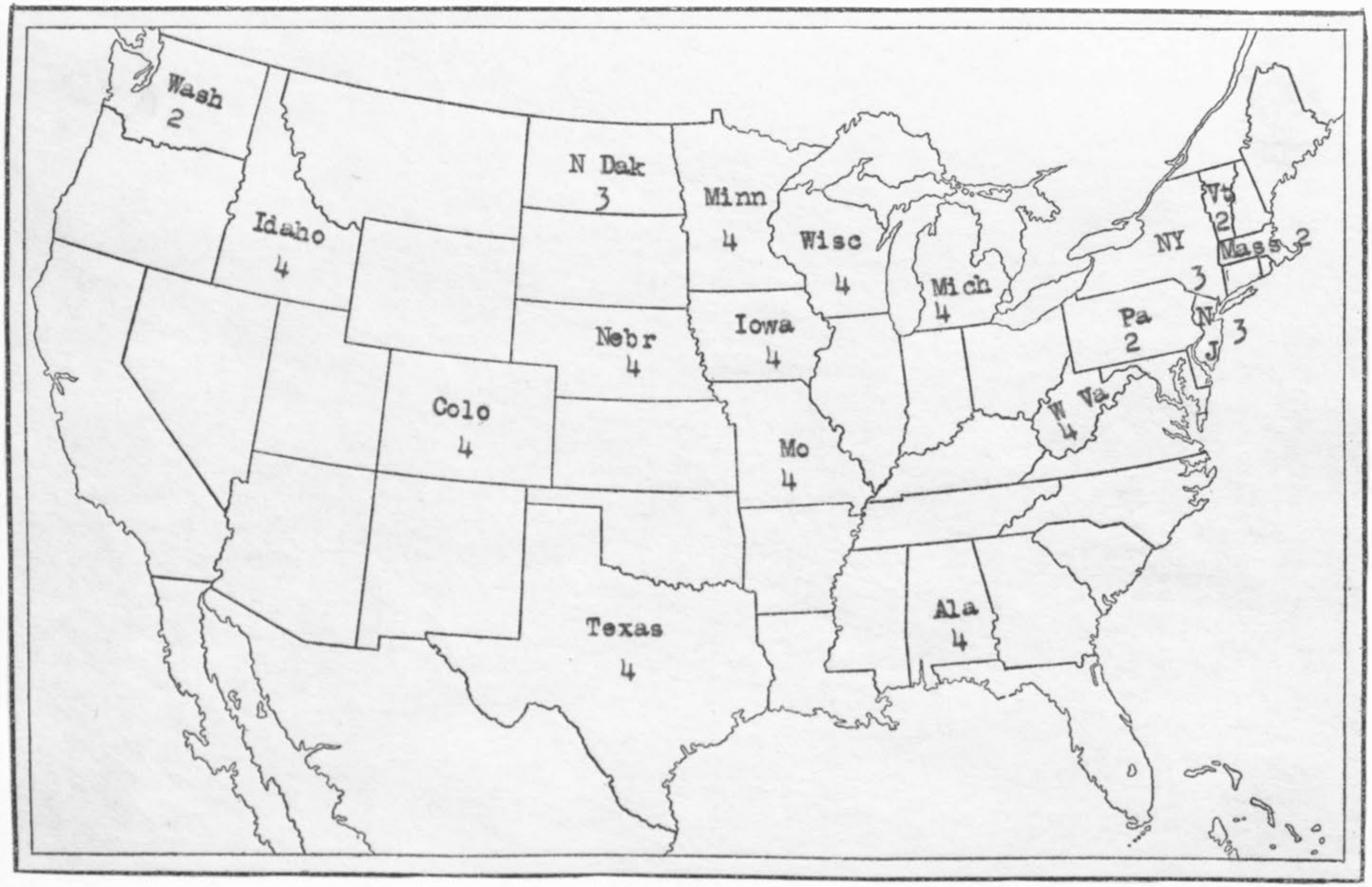
Inasmuch as sunflower rust has been of interest chiefly from a purely scientific standpoint and has assumed economic importance only recently as the sunflower itself assumed greater importance economically, estimates of the damage done by Puccinia helianthi are difficult to obtain.

In Michigan, where sunflowers have been grown rather extensively for some time, rust is considered a very important disease. At the Michigan Agricultural Experiment Station the Dubble Mixed and Burbank varieties were practically killed by rust in 1918, and again in 1919 Burbank and Russian suffered equally severely. (10) In Western Canada, where sunflowers are at present practically confined to the experiment stations, considerable damage was done throughout Manitoba and Saskatchewan in 1919. The damage was particularly severe at Morden, Manitoba which is located in the Red River Valley. Rust was also injurious in Minnesota in 1919. Fields were heavily attacked and on account of the rust the sunflowers had to be cut earlier than was considered desirable. Similar conditions prevailed in 1920 when a heavy attack of rust in Minnesota and throughout the Red River Valley caused an appreciable loss.

Two outstanding factors are involved in the loss sustained; fall of those leaves which are heavily attacked and decrease in size of the heads as a result of the reduced area of photosynthesis.

An idea of the prevalence of sunflower rust is obtained from Figure 2. which was prepared largely from information obtained through the courtesy of the Plant Disease Survey, Washington and

FIGURE 2
Prevalence of Sunflower Rust



4 Abundant
3 Common
2 Occasional
1 Slight

includes mostly only those states on which they had reports. It indicates the presence of the rust in great abundance throughout the whole region where sunflowers will be likely to become important economically.

The extensive distribution, prevalence and wide host range of sunflower rust, combined with the injury it is capable of causing, make the disease sufficiently serious to threaten the future of sunflower growing in United States unless the disease can be controlled.

Historical Summary

Although Puccinia helianthi was described in 1822, comparatively little work has been done on it up to the present. Kellerman first demonstrated that the rust as it occurs in this country is euautoecious and Kellerman, Carleton and Arthur subsequently investigated the identity of the rust on various wild hosts. Their results are conflicting and inconclusive but seem to indicate that there is some biologic specialization within the species P. helianthi Schw. and that cultivated H. annuus is a common host for all the forms of rust. Nothing further has been reported on the life history of the fungus, its relation to the host or control measures, yet these have become of great significance since the rust has become important economically.

Objects of the Investigation

The investigation was undertaken to determine (1) normal life-history of Puccinia helianthi, (2) variations from the normal life history, (3) conditions under which infection takes

4-21 6M

place, (4) the influence of external factors on the development of rust, (5) the relation between the rust on wild and cultivated sunflowers and (6) possible means of control.

Symptomology

All three stages of the rust were produced and studied in the greenhouse as well as being observed under natural conditions.

The Uredinial Stage

The disease is most striking and most commonly observed in the uredinial stage because the injury to the plant resulting from this stage is most marked.

The uredinia are chestnut-brown and are most conspicuous on the lower surface of the leaves. A marked chlorosis of that part of the leaf which is heavily infected develops soon after the uredinia are produced and is followed after a short time by the death of this part of the leaf. The remainder of the leaf lives somewhat longer but becomes an unhealthy green, dies prematurely and the leaf falls. The general effect of the uredinial stage on the plant is illustrated in Plate 1. and the injury to individual leaves in Plate 2.

The Telial Stage

This stage also affects the leaves chiefly although in cases of heavy infection the petioles and involucral bracts may be involved. On the leaves the compact, pulvinate, black or brownish-black spore masses are most conspicuous on the lower surface. (Plate 3.)

4-21 6M

The Pycnial and Aecial Stages

The pycnia appear chiefly on the upper surface of leaves and petioles. They occur in small clusters which are usually isolated but frequently coalesce and are honey-colored at first, later becomes orange. Although produced in considerable abundance they are relatively inconspicuous.

The aecial stage is comparatively rare in nature but when produced is readily observed as orange-colored, orbicular or broadly expanded, oblong lesions from a few millimeters to a centimeter and a half in diameter. They are most common on the under surface of the leaves but are also to be found on upper leaf surface, the petioles and young stems. (Plate 8.)

Etiology

The Causal Organism

Name:

Sunflower rust was first described by Schweinitz in 1822 from material collected on *Helianthus mollis* Salem in Pennsylvania and was named by him *Aecidium helianthi-mollis*. (4) Therefore the causal organism of sunflower rust is technically correctly designated as *Puccinia helianthi-mollis* (Schw.) Arthur & Bisby. (4) The cumbersomeness of the trinomial probably explains why the *mollis* has been dropped and the pathogene commonly referred to as *Puccinia helianthi* Schw.

Description:

The telia may be scattered or gregarious and are frequently confluent. They are usually oval, two to three millimeters in diameter, pulvinate, compact and brownish-black in color.

(Plate 3.) The teliospores are chestnut brown in color, oblong-ellipitical or pear-shaped, slightly constricted at the septum and smooth. The apex is thickened (6-9u) and is usually round though occasionally somewhat flattened. The upper cell is similar in color or just a shade darker than the lower and is slightly larger in size. The lower cell is usually slightly tapering toward a rounded base. The spores vary considerably in size. The range as indicated by measuring a hundred spores was 30 to 46x18 to 26u and the average was 39.I x 23.9u. The pedicels are hyaline, persistent and generally much longer than the spore, at times reaching a length of 110u.

The pycnia occur in small clusters which are usually isolated but frequently coalesce and are honey-colored at first, later becoming orange. The pycnospores are small, oval, hyaline, and appear shiny and viscous in mass.

The aecia are arranged on orbicular spots which frequently coalesce into broadly-expanded oblong lesions. (Plate 4.) The isolated clusters are three to five millimeters in diameter but the expanded spots are often a centimeter and a half in length. The aecia are orange-red in color with white laciniate margins. Ordinarily they appear from ten to fourteen days after the pycnial pustules develop and, while most common on the under surface of leaves, are also to be found on the upper leaf surface, the petioles and young stems. The aeciospores are orange-red to pale orange in color and somewhat variable in shape, typically ellipsoidal but sometimes almost polygonal. They are finely echinulate and have four median germ pores. They vary from 21 to 28 by 18 to 20 microns.

4-21 6M

The uredinia are chestnut brown in color, round, scattered or confluent and pulverulent. They present an entirely different appearance on the upper and lower surfaces. (Plate 3.) On the upper surface they are minute and located on pale green or yellowish spots, while on the lower surface the sori are much more massive, darker in color and there is no associated chlorosis. The uredinia occur on leaves, petioles, young stems and involucral bracts. The urediniospores are sub-globose, elliptic or obovate and yellowish brown in color. They are echinulate, have one median germ pore on each face. The measurements of a hundred spores varied from 23 to 27 by 17 to 22 microns and averaged 23.7 by 20.8u.

Life History

Typical Life History:

Sunflower rust is a typical euautoecious rust; that is telial, pycnial, aecial and uredinial stages are all produced on the one host. All four stages have been produced and studied under greenhouse conditions.

Telial Stage:

Under greenhouse conditions telia follow uredinia at varying lengths of time depending apparently largely on the condition of the host. Under optimum conditions for the host telia begin to form about a month and a half after the uredinial stage appears. At the end of two months both stages are present in about equal proportions and by the end of the third month the telial stage has almost entirely replaced the uredinial stage. This course is much varied, however, by external conditions; in general conditions unfavorable to the host hasten the production of telia.

4-21 6M

Thus by cutting down the light intensity telia began to form within two weeks after the uredinia were produced and had entirely replaced the latter at the end of a month. This was the normal course of events under greenhouse conditions during December and January and was induced later by allowing only weak diffuse light to reach the plants. Similarly high temperatures (75 - 85°F.) with low soil moisture induced telial formation within the same period. Under temperatures averaging aproximately 55°F. uredinia developed very slowly and were replaced by telia within two weeks.

This ready formation of telia is very important in fortifying the fungus against adverse conditions for the onset of these quickly induces the production of a resistant spore form which tides the fungus over until conditions became suitable for growth. Then the teliospores germinate, producing sporidia which cause infection and give rise to the pycnial stage.

Pycnial and Aecial Stages:

Pycnia appear from eight to ten days after inoculation with teliospores and after about the same period are followed by the aecia.

Although Woronin (15) reported in 1872 that he had been successful in obtaining the aecia of sunflower rust on *Helianthus annuus* from teliosporic infection, his observation was not confirmed until 1900, and during this period the rust was regarded by most authorities as a hemi-form. Sydow (13:93) in his first description of the rust questions the accuracy of Woronin's observations and ventures the opinion that the aecia reported on various species of *Helianthus* in North America belong either to a heteroecious form or an isolated *Aecidium*. However Kellerman in 1900 (19) and Jacky (12) and Arthur (20) in 1903 demonstrated

4-21 6M

conclusively that the rust is euautoecious. Sydow in 1903 (13:360) in correcting his earlier error concludes that on account of the rarity with which the aecial stage has been reported the fungus is able to omit the formation of aecia and propagate itself altogether through the uredinial-and telial-generations. McAlpine (14) states that the aecia never have been reported in Australia, although the rust is plentiful.

In this country the aecial stage is by no means uncommon, and it probably plays a very significant role in the life-history of the rust. Not only has it not been shown that the rust can propagate itself in this country without the aecial stage but this stage has been found in abundance as late as July 28 on the cultivated sunflowers. This would make it an important factor in the early stages of the epidemiology of the rust.

Uredinial Stage:

Uredinia are formed within five to seven days after inoculation with either aeciospores or urediniospores. This is the repeating stage and under natural conditions, infections continue to take place until unfavorable conditions of moisture or temperature prevent further infection and induce the formation of telia.

Variations from the Typical Life History:

The normal development of pycnia and aecia does not always follow teliosporic infection. There is a distinct tendency for the rust to omit the aecia and develop uredinia after the production of pycnia only. Plate 8. shows a typical infection of this type. Two uredinia developed under pycnial pustules while under the rest of the pycnia, aecia were formed in the normal

4-21 6M

way. In three instances uredinia seemed to develop, without the intervention of either pycnial or aecial stages, from infection with naturally produced teliospores which had overwintered outside. The uredinia in this case appeared quite similar to those developed from infection with urediniospores whereas those produced by short-cycling subsequent to pycnial formation had a very distinct appearance. They developed immediately below pycnia and were confined to definite, light-colored, slightly hypertrophied spots. (Fig. 8.) Therefore, although in these three instances uredinia developed apparently directly from teliospore infection the possibility of chance uredinial infection or overwintered urediniospores, though unlikely, must be entertained and the case, as an instance of short cycling, cannot be considered established until the nuclear phenomena connected with the development of such uredinia have been investigated.

Short-cycling seems to occur rather infrequently and the conditions governing it are not well understood. It was observed only on rather heavily infected leaves which suggested that a nutrient relation might be involved.

Attempts to induce short-cycling by adverse host conditions did not yield conclusive results. The phenomenon was so erratic in its occurrence that it was difficult to ascertain definitely the cause. It occurred on plants incubated at approximately 60°F. as well as on plants kept dry at 75°F. but it did not do so consistently. Moreover an equally high percentage of it was obtained at times under conditions which were apparently entirely suitable to the host.

4-21 6M

Efforts to cause the omission of the uredinial stage were not successful. Subjecting the host to low and high temperatures, diminished light and lack of moisture resulted in the early formation of telia as already mentioned but did not cause the complete omission of the uredinial stage.

Carleton (17) apparently suspected that short-cycling occurred in P. helianthi. In discussing the comparative infrequency of the aecial stage he says: "The aecidium occurs rarely in comparison with the occurrence of other stages but is to be found on a number of hosts and occasionally in considerable abundance. This rarity of its occurrence together with the occurrence of spermagonia so often with the uredo may be accounted for by the fact that the uredo is often produced by direct teleutosporic infection". He gives nothing further as to the evidence on which his statement is based and no other reference in the literature consulted has referred to the phenomenon at all.

In our experience, although short-cycling was not abundant, it occurred sufficiently frequently and was so unmistakable in its occurrence that there remains no doubt that the phenomenon actually does take place.

Phenomena of Infection:

Methods of Inoculation:

Artificial Inoculation

The method of application of spores does not seem to be an important factor in the development of the rust. Uredinial infection is equally successful whether the spores are spread on moistened leaves or applied in suspension. Heavy infection also resulted from spreading dry spores on dry leaves and incubating

4-21 6M

in a moist chamber for forty eight hours. In this case, however, there is considerable condensation on the leaves before the end of the incubation period.

With teliospores the usual method of inoculation was direct smearing of the spores on moistened leaves followed by forty eight hours incubation in a moist chamber. After spraying spore-suspensions on the plants with an atomizer and then incubating as above, satisfactory though usually lighter infection developed. The suspension of well soaked telial material above the plants in moist chambers for three days also proved a good method of inoculation.

Heavy infection resulted when aeciospores were applied either in suspension or directly on moistened leaves and the plants incubated forty eight hours.

Natural.

Dissemination of the various spore forms in nature is probably largely by wind for sunflower plants in isolated locations are frequently found rusted. The results of artificial inoculations indicate that the manner of inoculation is relatively unimportant and that the transfer of spores by any means is followed by infection when moisture conditions became suitable.

Spore Germination:

Teliospores

Length of the rest period:

Teliospores germinate over a long period of time. In October cultivated *Helianthus annuus* was inoculated with mixed uredinial and telial material from both wild and cultivated *H. annuus*. In all about forty five plants were inoculated and, along with the uredinial infection that developed, five pycnial

4-21 6M

pustules were produced. This indicated that a small percentage of teliospores germinate without a rest period and as soon as freshly-produced telia were obtained in the greenhouse further investigations were made. With the exception of one instance, where a heavy infection was obtained, such spores produced consistently a light infection of one to six pycnial pustules on each leaf. (Plate 4.) The percentage of teliospores that germinate without any resting period must therefore be very small. This is further indicated by the failure to demonstrate any germination of freshly-produced spores in Van Tieghem cells.

As time went on increasingly heavy infection resulted from telial material produced and stored out-doors so that by the middle of February from fifteen to twenty percent of the spores were germinating and consistent heavy infections were being obtained. How long these spores will retain their vitality is not known, but since collections of the pycnial stage have been made by the writer in Saskatchewan as late as the latter part of July, germination obviously continues over a long period.

Telial material collected November 16, 1930, was stored in darkness and light out-doors and inside, as well as at the following temperatures in darkness: ice-box (6-9°C.): 18°C.: room temperature, 30,40,45. Inoculations made from all of these on March 5, showed the spores kept at 40° and 45° were no longer viable. Those at 18° produced about the same infection as spores stored out-doors and this infection was much heavier than that from spores kept in the ice-box. This agrees with Woronin's (15) observation that the teliospores of this rust germinate equally well whether kept dry in a room or taken from leaves which had lain under the snow all winter. At this time somewhat better infection was obtained from material stored in light than in darkness.

4-21 6M

No satisfactory explanation of the varying requirements in regard to a resting period by the teliospores was discovered. Efforts to break up the dormancy of resting spores by the citric acid treatment as used with success on teliospores of *Puccinia graminis tritici* by Thiel and Weiss (18) were altogether unsuccessful. Varying the strength of the citric acid from one to ten percent and the time of treatment from ten minutes to three hours likewise gave no positive results. Acetic and hydrochloric acid were substituted for citric in similar treatments and the spores were exposed to ether vapour for various intervals but only negative results were obtained. The recent observation pointed out above that spores stored at room temperature produced heavier infections in March than those stored under more humid conditions in the ice-box and out-doors seems to indicate that drying is an important factor in determining the length of the resting period. This phase of the problem will be investigated as soon as material becomes available.

Time, manner and effect of temperature:

In Van Tieghem cells teliospores normally germinate within twelve to twenty four hours and produce a much elongated, four-celled promycelium, each cell of which bears a hyaline, globose sporidium . (Plate 5. Fig. 1&2) The sporidia germinate soon after production remaining attached or breaking away from the promycelium. (Plate 5. Fig. 3)

Rather frequently the promycelium, instead of forming sporidia, branches and grows indefinitely as shown in Plate 5. fig. 4. This type of germination has also been observed when examining the epidermis of artificially inoculated leaves.

4-21 6M

The teliospores are not sharply limited in their temperature requirements as they have been found to germinate well from 6 to 21° C. The optimum is approximately 18° C.

Germination of Aeciospores

Method:

Practically one hundred percent of freshly produced aeciospores germinate within a few hours. Each spore sends out one germ tube from a median germ pore.

Vitality:

Aeciospores do not retain their vitality over long periods. Greenhouse material kept indoors in darkness gave only a trace of weak germination at the end of three weeks and none at all at the end of four.

Germination of Urediniospores

Time required:

Urediniospores produced in the greenhouse were found to germinate within an hour and a half. Within two hours fifteen percent of the spores had germinated and some of them had produced germ tubes 46u in length. This is of great significance in insuring the development of rust as short periods of favorable moisture conditions, even a light dew, would be sufficient for spore germination.

Manner:

Each spore sends out a single germ tube from a median germ pore.

Effect of temperature:

The optimum temperature for the germination of urediniospores is approximately 18°C. and the maximum probably slightly above

4-21 6M

28°C. Germination tests were carried out in incubators running at higher temperatures but gave negative results with the exception of one case. In this instance five percent germination had taken place and the incubator registered 36°C., when the spores were removed. As this result could not be obtained again, it seemed possible that the temperature of the incubator must have dropped and allowed germination to take place. The relative germination of urediniospores at various temperatures is shown in Table 3.

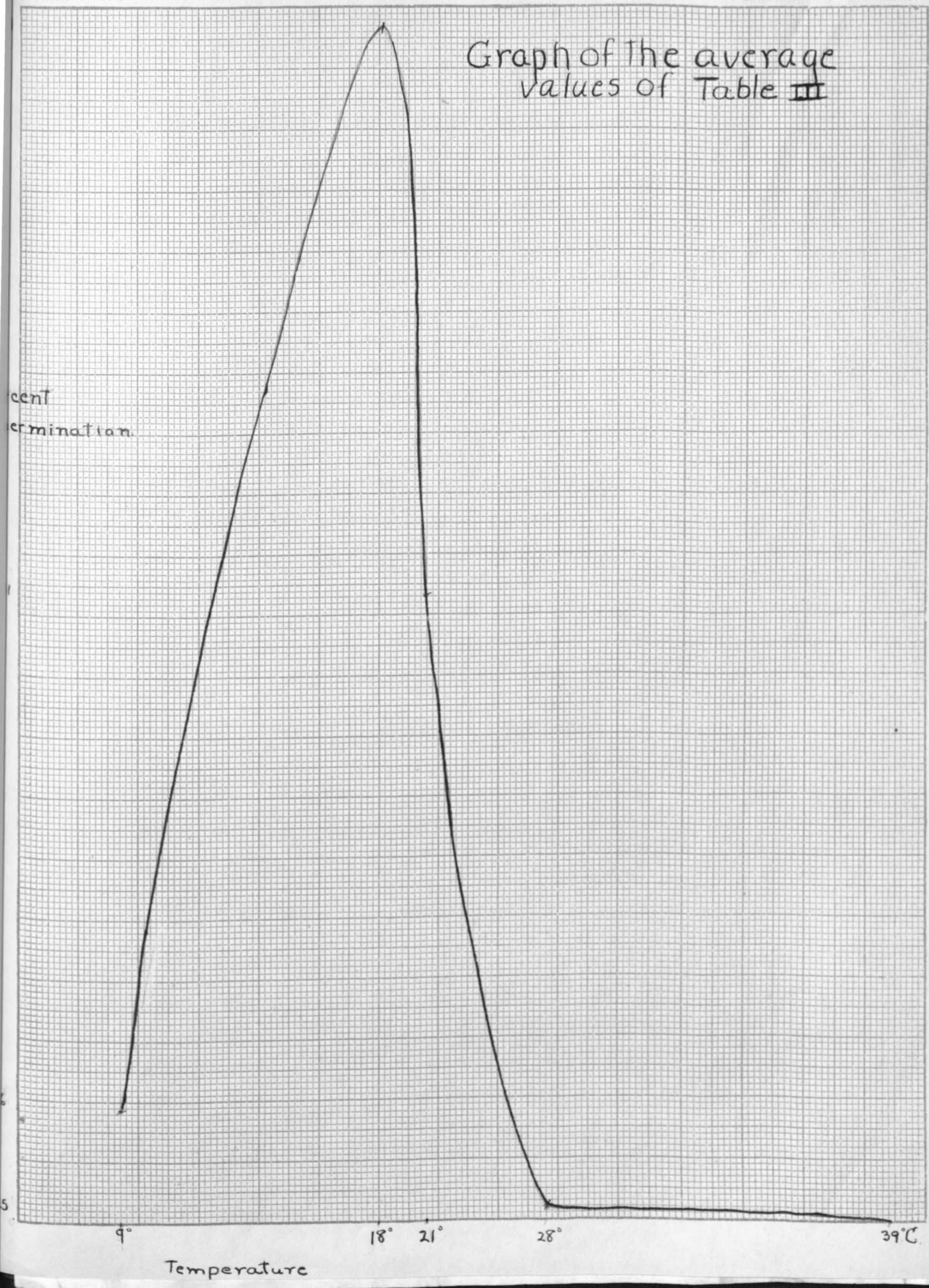
Table 3.

Percentage of Germination of Urediniospores
At Various Temperatures

	Temperatures				
Trial	6-9°C.	23-26°C.	18°C.	30°C.	40°C.
	Percentage of Germination				
1	3.5	30	40	+	0
2	+	16	17	+	0
3	+	44	41	1	0
4	0	40	68	+	0
5	+	38	48	0	0
6	0.6	36	49	0	0
7	5.5	18	51	0	0
8	5.6	4.3	9.8	1.2	0
9	+	61	56	+	0
10	3	25	36	5	0

+ = less than one percent germination
Each percentage the average of two or four hanging-drop cultures.

4-21 5M


Graph of the average values of Table III
cent
ermination.
9°
18°
21°
28°
39°C.
Temperature

Effect of oxygen supply:

A light smear of paraffin oil on the cover slip of Van Tieghem cells was found to have a marked influence in increasing the germination of urediniospores. This influence did not usually extend to the spores submerged in the center of the drop but was very pronounced around the edge. Using a spore suspension without an oil film in hanging drops it was found impossible to get more than a trace of germination even under optimum temperature condition. The results are given in Table 4.

Table 4.

Effect of an oil film on Urediniospore Germination

Percent germination in hanging drops at 18°C.

With an oil film	Without an oil film
53	0
59	0
32	+
95	4
31	+
48	+
43	0
94	+

+ = less than one percent.

The effect of the oil smear does not seem to be explicable on a direct chemical basis, because of the marked inactivity of the saturated hydrocarbons. The physical action would seem to be largely a matter of reducing surface tension thus increasing the ease of wetting and regulating the oxygen supply. That an oxygen relationship is largely involved is indicated by the fact that spores will not germinate even if an oil film is present if the lower part of the cell contains potassium pyrogallate solution; and if the spores are floated on water in open dishes they germinate almost equally well whether an oil film is present or not. (Table 5.) Moreover if spores are floated on the drops used in Van Tieghem cells, instead of using a spore suspension, the spores germinate well in the absence of an oil film.

Table 5.

Relative efficiency of various methods of Urediniospore Germination

Method	Percent Germination Trial 1.	Trial 2.
Floated on distilled water	60	65
Same plus an oil film	65	75
Van Tieghem cells without oil	T	T
" " " with oil	83	92

Vitality:

The only lot of urediniospores which have been under observation was collected November 17 and stored out-doors. The collection consisted largely of telial material however, and the small amount of uredinial material present made it impossible to obtain percentage germinations which would be at all significant in representing fairly the longevity of the spores. A few of these spores germinated as late as February 8. At this time also an infection resulting from inoculation with this material developed two uredinia and the incubation period indicated they were probably from urediniospores and not by short-cycling.

Urediniospores produced in the greenhouse were stored at 18°C. and 6° C. under relative humidities of 100,80,60,40,20, and 0 percent. At the end of three weeks the series at 18°C. would not germinate and of those at 6° C. the lot in saturated atmosphere germinated 66 percent but was the only one that was viable. This represents only one trial and all that can be inferred safely from it is that greenhouse material is not comparable in viability to material produced out-doors.

Entrance into the Host Plant:

Time Required

The time required by the fungus to enter the host was determined by the influence on infection of different periods of incubation in moist chambers. This was investigated in detail for the uredinial stage. Twenty four hours incubation was followed by very heavy infection and longer periods showed little difference in effect as long as the plants remained uninjured. The infection became lighter as the incubation period was reduced below twenty four hours although it was found possible to obtain perfectly normal infection with only six hours incubation. This is of great significance when correlated with the fact that sunflower rust flourishes in regions that are normally dry, for evidently the occurrence of even light dews enable new infections to develop sufficiently to be independent to a large extent of external conditions.

Methods of Entrance

Method of Investigation:

The most satisfactory method of investigating the method of entrance into the leaf of the germ tubes from the various spore forms was the following. The cotyldeons, which can be infected heavily with the rust in any of its stages, were inoculated with a particular spore form and incubated in a moist chamber for two or three days. Then the epidermis was peeled away from the rest of the leaf and after staining in weak aqueous eosin solution, was examined directly under the microscope. Both the inner and outer surface of the epidermis could be observed in the same mount and the position of the spores made the outer surface easily distinguishable. Prepared slides of the aecial and uredinial stages also were made by the paraffin method.

The Sporidia:

The method of penetration of the sporidia has not been definitely determined. The germination of the sporidia _in situ_ complicates matters somewhat because, after germination has proceeded to some extent, the sporidium is not distinguishable from the promycelium. In other cases the promycelium actually branches and grows out indefinitely without forming any sporidia. (Plate 4. Fig. 4.) There were several instances where such a germ tube or one from a sporidium seemed to have penetrated directly through the epidermis. On account of the extreme

4-21 6M

thinness of the epidermis however it was impossible to decide positively from the focus and the observation will have to be verified histologically. Direct penetration seems still more probable, because, of the absence of any evidence whatever of stomatal penetration.

The Aeciospores:

Figures 5 and 6 of Plate 4. show the method by which the germ tube of the aeciospore enters the leaf. Both are views of the inner surface of stomata three days after inoculation and demonstrate stomatal penetration. Substomatal vesicles beginning to send out hyphae in all directions are shown in the figures and at a slightly different focus the remnants of appressoria could be seen.

The Urediniospores:

The germ tubes of the urediniospores also enter through the stomata. Here, just as in the case of the aeciospores, the germ tube forms an appressorium above the stoma and then a substomatal vesicle develops which gives rise to hyphae in all directions. This is well brought out by figs. 1, 2 and 3 of Plate 6. That the method of penetration in the other leaves is similar to that of the cotyledons is indicated by fig. 4. Plate 6. which is drawn from a section of an older leaf. The substomatal vesicle and appressorium are both visible and are connected by a tube passing through the stomatal opening.

Length of Incubation Period:

General Statement

The length of the incubation period is not sharply fixed in any stage of the rust, but is much influenced by external conditions which exist shortly after infection has taken place. Under optimum conditions pycnia follow teliospore infection after ten to twelve days and aecia are developed within the next eight to ten days. Aeciospores produce uredinial infection normally within seven days while plants fleck following urediniospore inoculation within five days. The influence of various factors on the incubation period was most studied in the uredinial stage.

Age of Host

The age of the host prolongs the incubation period but does not alter the final degree of infection. When other conditions are favorable young leaves develop uredinia in the greenhouse five or six days after inoculation. Ten inoculations of the mature leaves of flowering plants indicated that they became infected readily. The incubation period varied from six to eight days.

Light

Light has a marked effect on the rapidity of the development of rust. In two trials the incubation period in strong diffuse light was two days longer than in direct sunlight. In weak diffuse light, such as resulted from passing ordinary diffuse light of the latter part of February through three thicknesses of cheesecloth, the incubation period was increased in three trials, ten, ten and twelve days respectively. The bench on which the plants were kept was screened off by heavy cotton into compartments in which the various cultures of rust were kept. Each compartment was about a yard square and faced the south. It was found that there was enough difference in light intensity between the front and back of these compartments to prolong the incubation period

4-21 6M

a day in some cases. The development of the rust may be inhibited altogether if the light is cut down still more, but, as the plant is rendered unhealthy before this occurs, the result cannot be attributed altogether to light.

Temperature

The pronounced influence of temperature on the development of rust was brought out by the following experiment. Six pots of plants were inoculated in a similar manner with the same spore suspension on November 28 and incubated two days in a moist chamber. Four of the pots were transferred to a cold incubator where the temperature remained fairly constant between 40° and 50° F. The other two were retained in the greenhouse as checks at approximately 75° F. and developed normally, flecks appearing after five days. At the end of a week no change had taken place in the plants in the cold incubator, but one of the plants flecked two days after it was transferred to the greenhouse and rust developed normally. After two weeks a second plant was brought into the greenhouse and after three days flecks appeared and a normal infection developed. The last was brought in at the end of four weeks and reacted similarly. Thus for at least twenty eight days the mycelium remained dormant in the infected leaves without giving any evidence of its presence or losing its vitality.

A second series on the relation of temperature to rust development was carried out under more variable temperatures ranging from 36 to 70° F. but averaging approximately 55° F. The plants did not fleck until sixteen to eighteen days after inoculation. Shortly after this the plants in three of the four

4-21 6M

pots were killed by root rots but those in the fourth pot lived a month longer. The uredinia in this case were developed weakly and continued to be covered by the epidermis. Within two weeks from the time the uredinia developed they were practically completely replaced by telia, but even these remained covered by the epidermis.

The upper limit of the temperature at which infection may take place has not been determined but as a few urediniospores were found to germinate at a temperature of 30°C., high temperatures are probably not a limiting factor if moisture conditions are favorable.

The temperature relations of the rust as brought out by the above experiments are of very great significance. The wide range of temperature at which infection can develop partially accounts for the occurrence of the rust under such varied climatic conditions as were indicated in the discussion of distribution. The fact,that after infection has taken place the rust may remain dormant in the leaves for long periods if external conditions are unfavorable and develop rapidly when conditions again become suitable, offers the most satisfactory explanation yet advanced of the sudden appearance of rust over large areas. For instance, a cold spell would prevent the development of infections previously initiated but would not inhibit spore germination or the initial stages of infection and the return of favorable conditions would be marked by the rather sudden completion of development of all of these infections. That is only the latter steps in the process of inoculation and infection are inhibited by cold weather. Therefore warm weather following such a period results in the development, within a few days, of a great

4-21 6M

number of infections which would have normally been spread over the whole period.

Pathological Histology:

In uredinial infections the hyphae are largely intercellular, are binucleate and seem to have an affinity for the nuclei of the host cells (Plate 6. Figs. 5&6). All parts of the leaf are penetrated by the hyphae. In the formation of pustules the mycelium masses beneath the epidermal cells, the cells of the host are killed locally, resulting in small light-colored areas on the green leaf which is the first evidence of infection. Plate 7 shows a mature pycnial pustule. The epidermis has been ruptured, ostiolar filaments are protruding and a mass of pycnospores are being liberated.

Host Range and Biologic Specialization

Historical Review

According to our present knowledge Puccinia helianthi Schw. must be regarded as confined to the genus Helianthus L. One report of its occurrence on Rudbeckia hirta L. was investigated by Sydow who reports (12:93) the host in this case probably not as indicated but H. angustifolius L. It occurs widely throughout the genus Helianthus as specified above under hosts.

As to whether there are several forms of the rust there has been much speculation. Woronin and Jacky (12:93) claimed that the rust from H. annuus L. would not transfer to H. tuberosus L. and on this basis, after a very limited number of infection experiments, Jacky divided the species into two. He retained P. helianthi as the designation of the form on H. annuus L. and called the form on H. tuberosus L., P. helianthorum. Sydow did not con-

10-20 5M

sider the evidence sufficient to warrant breaking up the species and does not follow Jacky's division of it in his Monographia Uredinearum (12:860).

Carleton in 1901 (22) first carried on culture experiments with the rust in this country. He showed that the species was autoecious here and was of the opinion that "there is no distinction of host forms."

Arthur (20) in 1903 reported that teliospores from H. grosse-serratus Mart. were successfully sown on H. grosse-serratus and H. maximiliani but failed to infect H. strumosus. In the following year teliospores from H. mollis gave negative results on H. strumosus, H. tuberosus, H. grosse-serratus, H. rigida, and H. Maximiliani, slight infection on H. tomentosus and heavy infection on H. mollis and H. annuus. In 1905 (21) Arthur inoculated fifteen species of Helianthus with teliospores from H. mollis, H. grosse-serratus and H. laetiflorus. He summarizes his results as follows: "Each set of spores grew upon the species of host from which derived but not upon the other two species, except that spores from H. laetiflorus sown on H. mollis gave a tardy showing of pycnia without further development. Also each set of spores grew luxuriantly on H. annuus and made a feeble growth on H. tomentosus but on all other species failed to infect or make a feeble growth." He concludes that P. helianthi Schw. is "a single species having many races, for which H. annuus acts as a bridging host." From his results it would not seem that "bridging host" implied more than a common host.

Kellerman in 1905 got infection on H. annuus with teliospores from H. tuberosus but failed to get it on H. tracheii-

4-21 6M

folius, H. mollis, H. maximiliani, H. decapetalus, H. grosse-serratus, H. orgyalis, and H. kellermani. Teleutospores from H. grosse-serratus gave infection on H. annuus, H. orgyalis, H. tracheiifolius, H. kellermani, H. giganteus, H. grosse-serratus and H. decapetalus. This work shows that Jacky's division of the species as noted above is invalid. With reference to the existence of specialization within the species Kellerman says "Recent inoculation work leads me to think there is but one valid species and that there are no recognizable 'biologic' forms." The negative results reported above he considers uncertain since the results of different years have not checked in all particulars.

Experimental

Urediniospores from H. scaberrimus, wild H. annuus and H. subrhomboideus, Rydb., three wild forms which occur very widely thru the West transferred readily to cultivated H. annuus.

Teliospores from wild H. annuus, H. maximiliani and from three collections of cultivated H. annuus also infected cultivated sunflower heavily.

Eight horticultural and cultivated varieties of sunflower were inoculated with the following six cultures of rust.

1. Collected on H. annuus at University Farm, St. Paul.
2. " " " " Main Campus, Minneapolis.
3. " " " " Mora, Minnesota.
4. " " wild H. annuus in Minnesota.
5. " " H. scaberrimus " "
6. " " H. subrhomboideus Rydb. in Saskatchewan and obtained from Professor W. P. Fraser, University of Saskatchewan.

The results obtained are given in Table 6. Although there

4-21 6M

was some variation in the severity of infection all varieties were susceptible to all cultures of rust. This variation cannot be considered an indication of specialization, since it was fluctuations within susceptible varieties and not difference in resistance.

Considering all the work that has been done on the subject the indications are strongly in favor of the view that specialization does exist within the species. The conflicting results of various workers and even of the same workers in different years, as well as the results themselves support such a view but the whole subject is in a very confused state and requires much additional investigation.

Control

Under greenhouse conditions spraying with bordeaux (4:6:50) has been found to control the rust. The plants were thoroughly sprayed with bordeaux (4:6:50) and later in the same day were sprayed with a heavy suspension of urediniospores and then incubated in moist chambers for forty eight hours in the usual way. The resulting control was absolute or partial depending on the thoroughness of the application of bordeaux.

Field experiments will have to be carried on to determine whether this method of control is practical under those conditions before its general use could be recommended. It would probably be justifiable in any case, however, where sunflowers are grown as windbreaks as they are at times in Saskatchewan, for here much more is involved than the value of the individual plant.

The development of resistant varieties justifies attention particularly since it has been found in Michigan (13) that the

4-21 6M

Table 6.

Source of Rust

Sunflower variety inoculated	H. annuus Univ. Farm	H. annuus Main Campus	H. annuus Mora Minn.	Wild H. annuus	H. scaberrimus	H. subrhomboideus
	Degree of Infection					
Double California	Heavy	Medium	Heavy	Heavy	Heavy	Heavy
Chrysanthemum flowered	Heavy	Heavy	Heavy	Heavy	Heavy	Heavy
H. cucumerifolius	Heavy	Heavy	Medium	Medium	Medium	Heavy
*Giant Russian	Heavy	Heavy	Heavy	Heavy	Medium	Heavy
Orion	Medium	Medium	Medium	Heavy	Medium	Heavy
Miniature 1	Medium	Light	Medium	Heavy	Heavy	Medium
Miniature 2	Medium	Light	Medium	Heavy	Heavy	Medium
*Gray Mammoth	Heavy	Light	Medium	Medium	Heavy	Heavy

* Extensively Cultivated.

4-21 6M

Kaeurpher, a South American variety, is outstanding in its resistance and might well be used as a basis for such work.

Discussion

Significance of Results Obtained

From the facts obtained in this investigation it is evident that *Puccinia helianthi* has many characters which favor its occurrence in epidemic form.

The urediniospores are produced in great numbers and retain their viability at least four months. These are supplemented by the teliospores some of which are capable of germinating without a rest period and originating more urediniospores and the rest of which germinate over a period of at least six months. Thus an abundant source of inoculum is assured.

Temperature relations will not be prohibitive since the urediniospores and teliospores germinate through a wide range of temperature (6-28°C.)

Light conditions will be optimum under the normal bright sunny weather of the west.

The fact that urediniospores may germinate within an hour and a half and cause infection within six hours not only assures the development of rust under normal conditions but indicates that the season would not have to be very abnormal to cause an epidemic. That is, a certain loss must be expected every year and this will be greatly increased each year that is slightly wet.

Host conditions are also particularly favorable to the development of epidemics. It has been shown that the rust found on four of the most commonly-occurring wild varieties will transfer readily to cultivated sunflowers. Thus an abundance of

4-21 6M

spores will have been produced on wild varieties before the cultivated ones develop. In any year, therefore only the dissemination of spores from wild varieties and suitable weather conditions for short intervals will be necessary to produce an epidemic.

Sunflower rust seems well fortified against unfavorable conditions and particularly well adapted to rapid development when conditions become favorable.

It has been found that the onset of unfavorable conditions is followed quickly by the formation of telia. That is, a resistant spore form capable of remaining dormant over long periods is readily developed and insures the perpetuation of the fungus until the return of suitable growth conditions. On the other hand some telia can germinate any time and thus start rust anew. The urediniospores were found also to retain their vitality for four months.

Under favorable conditions the rust develops very rapidly. Short-cycling is of significance in this respect in as much as it results in the early production of the repeating urediniospores without the necessity of developing aeciospores.

Short periods of high humidity are sufficient to insure the development of the urediniospores thus formed since they may germinate within an hour and a half and cause infection within six hours. The efficiency of the urediniospores is further increased by their ability to germinate over such a wide range of temperature (6-30° C.) and infect almost any part of the host.

The striking fact that the development of the rust may be inhibited for a month after infection by unfavorable external conditions and then proceed normally, explains in a large

4-21 6M

measure the sudden appearance of the rust over large areas and has a far-reaching significance in connection with rust epidemics in general. Spore germination takes place at much lower temperatures than will permit subsequent development of the rust. Infection may therefore occur just before or during periods of cold wet weather and subsequent development takes place very rapidly with the return of favorable conditions. The sudden development of an epidemic may be explained thus and this explanation is probably applicable to the similar phenomenon in wheat and other rusts.

Future Problems:

Sunflower rust has offered a fruitful field for preliminary investigation and promises much to more extensive research. Much is still to be learned of the life history of the causal organism, its methods of overwintering and the physiology of the spore germination and development of the various stages. The conditions influencing infection and the exact nature of the injury to the host call for further study. The working out of the histology of infection offers an especially interesting field because of the phenomenon of short-cycling which occurs. The host range and biologic specialization are very imperfectly known and should be investigated because of their practical, as well as their scientific value. Finally the economic importance of the disease warrants much more extensive investigation of methods of control.

4-21 6M

<u>Summary</u>

1. Sunflower rust became of economic importance when sunflowers began to be grown for ensilage purposes; it is a serious problem in their cultivation.

2. *Puccinia helianthi* was first described by Schweinitz in 1822. It has now attained practically world-wide distribution and occurs throughout the United States.

3. *P. helianthi* occurs on sixteen species of *Helianthus* but has not been shown to go to other genera.

4. Sunflower rust is a typical euautoecious rust. All four stages were produced and studied under greenhouse conditions.

5. Sunflower rust has a tendency to short-cycle itself and may omit the aecial or pycnial and aecial stages.

6. Although most teliospores will not germinate without a rest period, a small percentage of them germinate immediately. Teliospore germination continues over at least six months.

7. Teliospores germinate from 6 to 28° C. They usually germinate by a four-celled promycelium each cell of which bears an oval, hyaline sporidium. They may also germinate by a branched promycelium of indefinite growth.

8. Aeciospores germinate readily when fresh but lose their vitality within three weeks.

9. Urediniospores may germinate within an hour and a half. Each spore sends out a germ tube from a median germ pore.

10. The optimum temperature for germination is about 18° C, the maximum slightly above 28° C. and the minimum below 6°C.

11. Urediniospores immersed in water germinate not at all or very slightly apparently due to lack of oxygen. They germinate

4-21 6M

best floating on water. An oil film on the cover slip of hanging-drop cultures increases the percentage of germination.

12. Uredinial infection develops if inoculated plants are incubated in moist chambers six hours or more.

13. Germ-tubes from the aeciospores and urediniospores enter the host through the stomata while those from the sporidia seem to penetrate the epidermis directly.

14. Under optimum conditions pycnia follow inoculation with teliospores within ten to twelve days. Aecia follow pycnia after eight to ten days. Uredinia usually develop five to seven days after inoculation.

15. The incubation period of the uredinial stage is about two days longer with mature plants than with young plants.

16. Light is essential to the development of rust. Reduced light intensity increases the length of the incubation period six to eight days.

17. Rust will not develop at temperatures below 50° C. but if infection has taken place already the mycelium may remain dormant in the leaves for a month at this temperature and develop quickly with the return of higher temperatures. At 55° C. the rust develops very slowly and the uredinial stage is soon replaced by the telial stage.

18. The uredinial mycelium is largely intercellular and binucleate. Cells are killed only where pustules are being developed.

19. Rust from four of the most commonly occurring wild-varieties of sunflower (*H. scaberrimus*; *H. annuus*; *H. subrhomboideus*; *H. maximiliani*) was found to transfer readily to cultivated sunflowers.

4-21 6M

20. Eight common horticultural and cultivated varieties of sunflower were inoculated with six cultures of rust and none of them were found to be resistant.

21. Bordeaux (4:6:50) was found to control the rust under greenhouse conditions.

4-21 6M

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Acknowledgments

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4-21 6M

Explanation of Plates

Plate 1: Two sunflower plants infected with sunflower rust (uredinial stage). The plant at the left a later stage than the other as shown by the more-shrivelled, unhealthy, lower leaves.

Plate 2: The uredinial stage on leaves. From left to right:
(1) upper surface of leaf in early stage of infection;
(2) lower surface at same stage;
(3) later stage in which the heavily infected part of the leaf has become yellow;
(4) (5)&(6) subsequent stages in the death of the leaf.

Plate 3: The telial stage on the under surface of leaves.

Plate 4: An infection resulting from a mixture of urediniospores and freshly-produced teliospores. Five aecia have been formed along with the uredinia.

Plate 5:
Figure 1. Germinating teliospore (x430).
" 2. Tip of a promycelium bearing sporidia (x430)
" 3. Tip of a promycelium and germinating sporidia (x430).
" 4. Teliospore germinating by a branched germ tube (x430).
" 5. Aecial penetration. View of inner surface of epidermis, three days after inoculation with aeciospores. Substomatal vesicle giving rise to hyphae (x430).
" 6. Similar to five (x950).

Plate 6:
Figure 1. Inner view of epidermis 3 days after inoculation with urediniospores (x315). A substomatal vesicle giving rise to hyphae is shown.
" 2. Similar to one; inner view of a single stoma(18x1/6).
" 3. Similar to one (x560).
" 4. From a cross section of leaf; showing method of penetration of uredinial germ tube. Substomatal vesicle forming from the appressorium (x950).
" 5. Binucleate condition of uredineal mycelium (x950).
" 6. Hyphae growing toward nucleus (x950).

4-21 6M

Plate 7: Photomicrograph of a mature pycnium discharging spores.

Plate 8: The lower surface of a leaf showing several aecia and two uredinia. The uredinia developed beneath pycnia, the aecial stage being omitted in these two cases.

Plate 1.

4-21 6M

Plate 2.

4-21 6M

Plate 3.

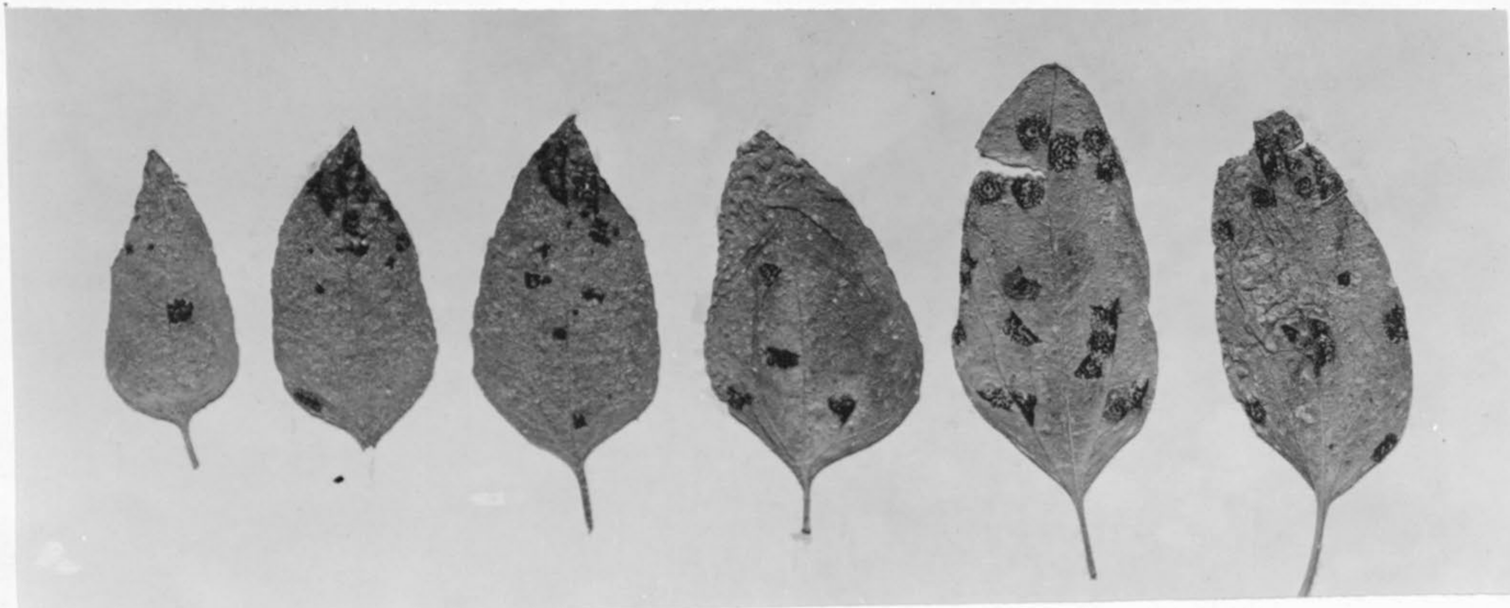
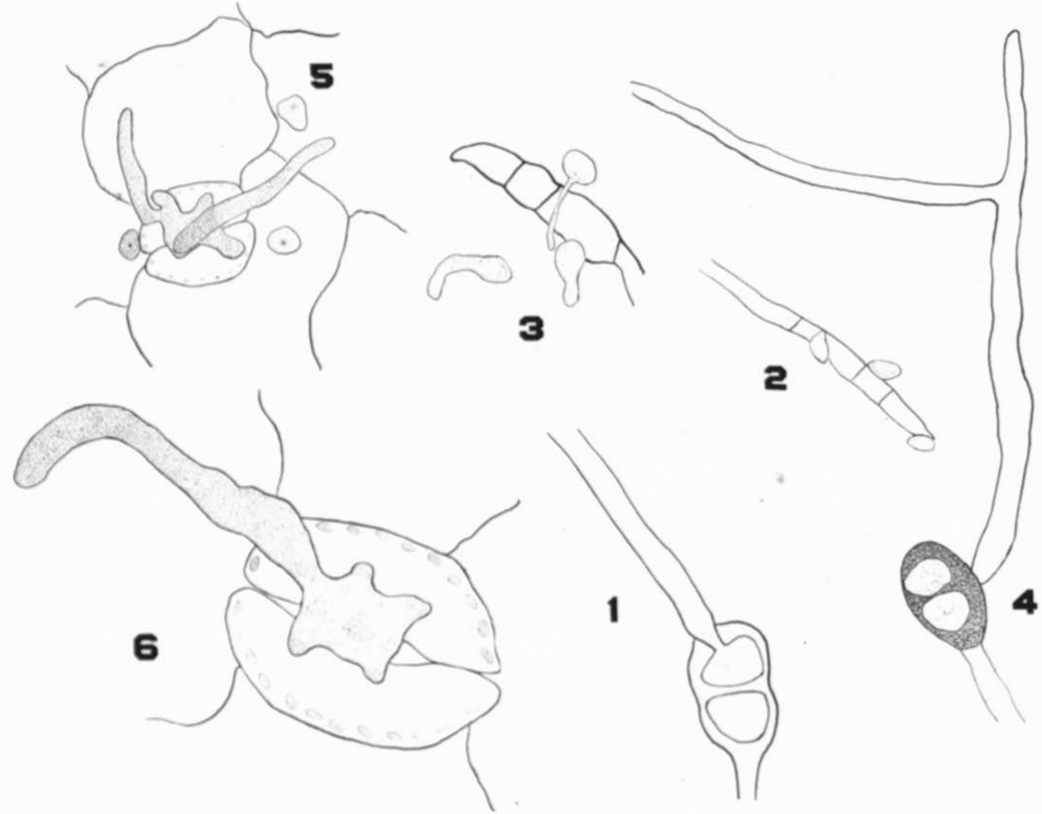


Plate 4.

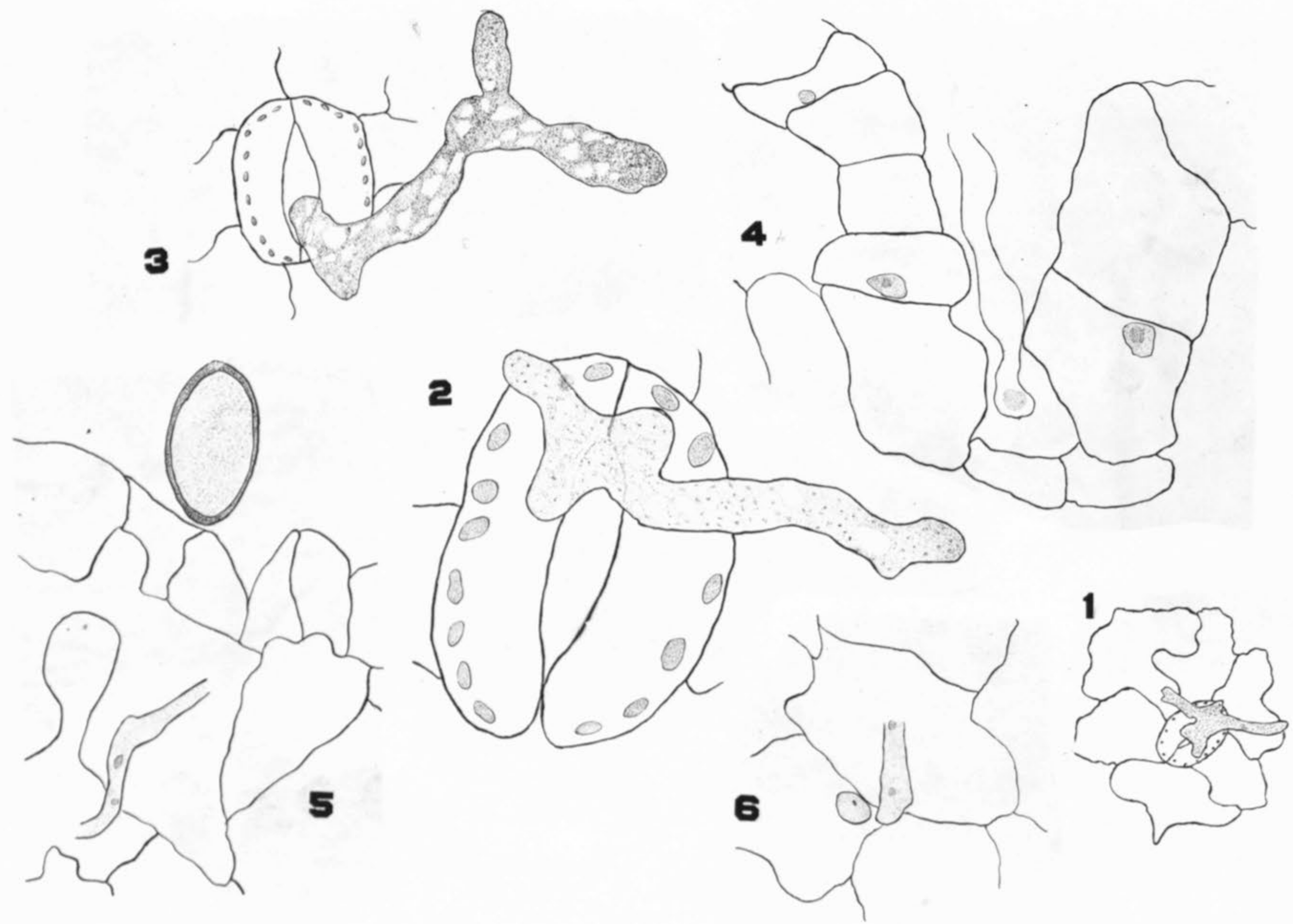
4-21 6M

Plate 5.



4-21 6M

Plate 6.



4-21 6M

Plate 7.

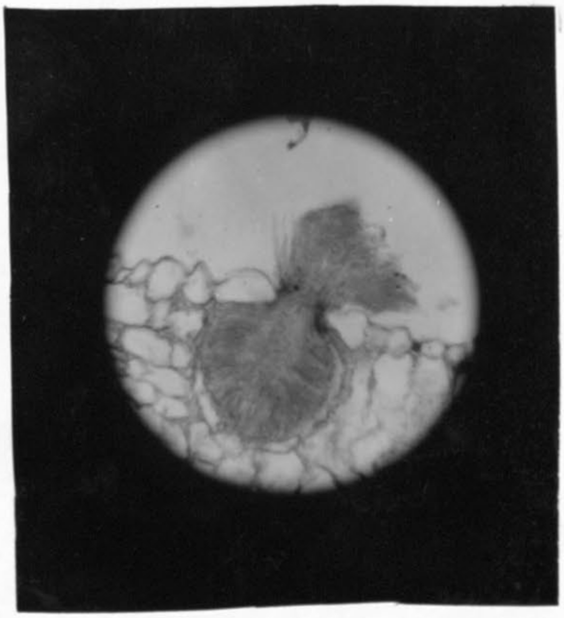


Plate 8.

4-21 6M