STUDIES ON SUNFLOWER RUST,

PUCCINIA HELIANTHI SCHW.

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Introduction

Sunflower rust caused by the fungus Puccinia helianthi has attracted the attention of numerous investigators. It is a rust of considerable economic importance, since it frequently develops to an injurious extent upon sunflowers cultivated for forage, seed, or ornament. Aside from the economic features, sunflower rust is of interest because it is one of the few known rusts in which the life-cycle is not constant; the cluster cup stage is sometimes omitted entirely. The conditions resulting in this shortening of the life-cycle are as yet very inadequately known, and studies of this problem are included in this paper. The situation regarding the physiologic specialization of P. helianthi is also in need of further study, as is indicated by the results of hybridization experiments reported herein.

The recent discovery that P. helianthi is hetero-thallic has added much interest to the consideration of the problems of sex and hybridization in this rust.

Sunflower rust is euautoecious, i.e., all the spore stages are, or may be, produced upon the sunflower plant. This fact, and the readiness with which the teliospores will germinate at any season of the year, makes the rust a favourable one for studies relating to life history and pathology.

The investigations reported here were begun in the autumn of 1930, and have been continued up to the present. Wherever Helianthus annuus is mentioned, the cultivated plant is to be understood.

Historical

Sunflower rust caused by Puccinia helianthi was first described by Schweinitz (13) in 1822, from material collected in North Carolina. His original description of the telial stage may be translated as follows: "Pustules rather small, orbicular, aggregated, black; spores globoid-oval, bilocular, very long pedicelled. Common on many Helianthi. Spores fuscous yellow, pedicel white, pellucid". In a later work (14) published in 1832 he noted that this rust occurred also in Pennsylvania, and "on cultivated H. tuberosus it occupies the lower surface of almost all leaves".

In his 1822 work Schweinitz published his Aecidium helianthi mollis, with a brief description, and reported it as "frequent on the under side of the leaves of Helianthus mollis". Schweinitz of course had no idea that the two stages described belonged to the same rust. He did not observe the uredinial stage of the rust, or at least did not recognize it as distinct from the telial stage; had he noted it, he would doubtless have described it as a species of Uredo, for it was not then even known that one rust may have both uredinial and telial stages. Since Schweinitz described the Aecidium on an earlier page than the Puccinia, the combination Puccinia helianthi-mollis (Schw.) H.S. Jackson, has been made; but usage favours the retention of the name P. helianthi Schw., and this also was the first name applied to the telial stage of the rust.

Since its first description, P. helianthi has been reported from most of Europe and from Australia, and is recorded in North America from Canada to Mexico and the West Indies. Many species of Helianthus are considered to bear this rust; Arthur (3) records it on 36 species of Helianthus and one species of Heliopsis in North America. Cultivated Helianthus annuus is often so seriously affected that much defoliation occurs, and the yield is thereby greatly lessened.

Jacky (9) made a number of cross-inoculations with sunflower rust. He inoculated the following hosts with

telia collected on H. annuus, and reports as follows:- H. annuus, H. cucumerifolius, H. californicus, became infected, H. tuberosus, H. maximiliani, H. multiflorus, H. scaberimus, and H. rigidus, did not show any infection. He concludes that the rusts on H. annuus, H. petiolaris, and H. mollis, are identical, with the probability of a separate or distinct form existing on H. tuberosus. Woronin (18) using telia collected on H. annuus, in two successive seasons was unable to produce infection of H. tuberosus. Kellerman (10) from inoculations with uredinia decided that there is but one species of rust inhabiting the several species of Helianthus. Carleton (5) apparently early observed the occurrence of short-cycling tendencies. He summarizes his observations as follows:- "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 sperm-agonia, so often with the uredo, may be accounted for by the fact that uredo is often produced by direct teleuto-sporic infection."

Bailey (4) noted the tendency of P. helianthi to form uredinia following telial inoculation. He says, "There is a distinct tendency for the rust to omit the aecia, and develop the uredinia after the production of Pycnia." He was unable to induce short-cycling by adverse conditions, but concludes that, "Although short-cycling was not frequent, it occurred often enough and

was so clear that no doubt remains that it actually does take place". In the same paper he gives a full account of the viability of aeciospores, urediniospores, and teliospores, and states that a small percentage of teliospores will germinate without a rest period. In 1922, by the use of eight varieties of sunflowers he was able to differentiate at least three Biologic Forms of P. helianthi, and concludes that, "As the wild species of Helianthus are not pure lines, and the resistance of each species is extremely viable, the results obtained by testing the reactions of particular samples of several species to given forms of rust can not be applied generally".

Craigie (6) in his studies of sex in rusts, has shown that P. helianthi is heterothallic and is normally dependent upon diploidization for the production of aecia. He says, "Compound pustules formed by coalescence of two simple pustules, each pustule originating from monosporidial infection, form aecia after uniting, or no aecia appear. When not more than 2 mm. apart about 50 percent of compound pustules form aecia. When two sporidia of opposite sex (+) and (-) are sown close together on a sunflower leaf fusion takes place, to give rise to normal binucleate aeciospores, but when two sporidia of the same sex, i.e., two (+) or two (-) are sown close together, the two monosporous mycelia come into contact but do not fuse, and consequently aecia do not occur".

Craigie (7) has also shown that simple pycnial pustules of P. helianthi may form aecia spontaneously. From 228 pustules he notes that aecia appeared in 11, but he concludes "there was no regularity in their time of appearance".

Hanna (8) made a study of the method of origin of the binucleate condition. He worked with Puccinia graminis, and presented evidence indicating that conjugate nuclei result from possible cell fusion, and consequent nuclear migration, in mycelial wefts at the base of the aecium. He states, that "Sporidia are uninucleate, and about 48 hours after mixing pycnial nectar, nuclei at the base of each weft of hyphae, situated at the base of the pustule, become enlarged. Neighbouring hyphae then fuse in pairs, and two nuclei become associated in each fusion." Allen (1) however is rather inclined to the view that conjugate nuclei appear in the mycelium connecting the pycnium and the aecium. She says in part, "Between a pycnium and a young fertile aecium are many haploid and a few diploid hyphae." Andrus (2) found "receptive hyphae" associated with the pycnia, which appeared to serve to initiate the diploid condition after a pycniospore of opposite sex came in contact with them.

Materials and Methods

The telial material used throughout the course of this study was collected on cultivated Helianthus annuus, and the following native species; H. tuberosus, H. petiolaris, H. subtuberosus, and H. maximiliani. The telial material was kept stored dry in the laboratory at ordinary room temperature until used for inoculation.

Telial material used for inoculation was first soaked in tap water for a period varying from twenty-four to forty-eight hours, it was then dried for a week and again soaked for twenty-four to forty-eight hours, followed once more by a drying period of a week. At the end of the second drying period, soaking for four hours was found sufficient to allow the material to become thoroughly wet. The treated telial material was then transferred to Petri dish covers lined with moist filter paper, and these were inverted over seedling plants of H. annuus, which had been encircled with glass chimneys in a manner similar to that outlined by Newton, Johnson and Brown (12). The plants were then kept in a moist chamber for two days. At intervals during the incubation period, the petri dish covers were revolved, to ensure a more even distribution of sporidia. This method gave excellent infections, and is illustrated in Figure (1). Inoculations with aeciospores and uredinio-spores were made with a small flattened needle, similar

to the procedure followed in the culture of Cereal rusts.

The host plants were grown from seed of cultivated H. annuus, and were usually inoculated after the first pair of leaves had developed above the cotyledons. The native species of Helianthus, were grown from roots collected in the neighbourhood of the Agricultural College, Winnipeg.

Normal Life History of Puccinia helianthi

Puccinia helianthi is typically an eauutoecious rust; pycnia, aecia, uredinia and telia, follow in regular sequence on the same host.

Under ordinary greenhouse conditions, pycnia appear in from seven to eight days following inoculation with sporidia. Young aecia may become visible two to four days after the pycnia appear. Uredinia usually appear in eight or ten days after inoculation with aeciospores, or urediniospores, but telial formation is somewhat delayed - usually not occurring until a month or six weeks after the appearance of uredinia. High average temperatures however hasten the appearance of the telial stage. This was particularly noticeable during the prevailing high temperatures of July 1931. At that time plants inoculated with uredinia on July 9th. had passed completely into the telial stage by the 25th. of the month. The rust employed during this trial was collected

on Helianthus tuberosus, and was cultured on Helianthus annuus. This response to temperature confirms the finding of Bailey (4) that prevailing high temperatures of from 75°F. to 85°F. together with low soil moisture hastened the production of telia. He also found that correspondingly low temperatures, about 55°F. influenced the slow formation of uredinia and their replacement by telia in two weeks. This ready production of telia is apparently a response to unfavourable conditions. The fact that telia may germinate and form sporidia without a rest or dormancy period, is the possible explanation for the occasional appearance of pycnia and aecia throughout the growing season.

Inoculations with Basidiospores

Inoculation with basidiospores obtained from teliospores was made at varying temperatures from 10° to 20°C. Teliospores floated on distilled water in syracuse watch glasses germinate readily up to 25°C. Relatively high temperatures were found to have a profound influence in inhibiting teliospore germination; above 25°C. infections resulting from teliospores suspended over the plants fell off sharply, and at 30°C. very few infections have been observed. At 15°C. excellent infections have always been secured from telia suspended over seedling plants. Teliospores floated on distilled water in syracuse watch glasses germinated by abnormal promycelia, in many respects similar to the germ tubes of urediniospores. Occasionally this promycelium became divided at the tip into four

segments, but did not evince any tendency to form sporidia, or basidiospores. Teliospores germinating in moist air, however abstract sporidia. Sporidia allowed to fall upon a glass slide germinated in moist air in a comparatively short period of time. After 24 hours most of the sporidia were found to have produced germ tubes.

Freshly gathered telia will result in a slight infection if suspended over plants in a moist chamber for 48 hours, after having been soaked for 24 hours without any drying period. Toward spring, from the beginning of March to the end of April, teliospores germinate well with only one thorough soaking previous to suspension over seedling plants, and a generous infection results. From the time of collection in the months of September and October, until the beginning of March it was found that best infection results were obtained by successively soaking and drying the telia. Towards the end of April germination apparently diminished. The method of repeated soakings followed by drying periods, seemed sufficient to induce the germination of the teliospores, and to give infection results comparable with those obtained during what might be termed the optimum period for telial germination, namely March and April, for telia collected during the previous September and October.

That teliospores remain viable for a considerable period of time when stored dry at room temperature, was demonstrated with teliospores collected on September 8, 1930. On August 10, 1931, the teliospores were subjected to the methods already described, and were suspended over H. annuus seedlings on September 1, 1931. On September 10th., the resulting pycnial infection was as abundant as at any time during the year. Unfortunately, this material was destroyed before further tests could be made. It was evident however, that the teliospores had remained viable for almost one year.

Variations in the Life History of Puccinia helianthi

As nothing definite has been described with regard to the nuclear condition of the uredinia formed without the prior appearance of aecia, a phenomenon that has been called "short cycling", it was reasoned that the prevention of the intermixing of the pycnial nectar, might serve to prevent the incidence of short-cycling. Observations during the course of experiments conducted during the winter of 1930-1931, failed to establish a direct relationship between the intermixing of pycnial nectar from separate pycnial pustules, and the omission of the aecial stage. (Fig. (2) and Fig. (3).) Short cycling uredinia formed telia normally, and required a period of time to change completely to telia similar to that of cultures established from infections following

the regular life cycle. Attempts to germinate teliospores from cultures that had apparently short cycled gave inconclusive results due to the somewhat indifferent specimens obtained. Early in the month of January 1931, further evidence of short cycling did not occur when telia collected the previous September was used as a source of inoculation. Owing to the rather sporadic occurrence of short cycling, very little opportunity was given for an intensive study relative to the conditions essential to its appearance.

At the close of the summer of 1931, telial material was collected at Beaver Creek, Saskatchewan, on Helianthus petiolaris, and on Helianthus subtuberosus. This material was subjected to soaking and drying periods beginning on September 4th. On September 19th. seedling plants of H. annuus were inoculated by suspending over them telial material that had been examined and found to be germinating vigourously. A week later copious infection could be detected, and on the 29th. of September, ten days from the date of inoculation, uredinia appeared from every infection without any evidence of pycnial formation. There is a possibility that these uredinial infections came from urediniospores remaining viable amongst the teliospores used for inoculation. The repeated soakings and subsequent dryings of the inoculum were however, considered sufficient to germinate any urediniospores present, but germination tests were not made to determine if the uredinia still remained viable. Microscopical examination of the

telial material demonstrated that urediniospores were present to the extent of only about one percent of the number of teliospores.

In view of the unexpected results obtained from the inoculation of September 19th., it was reasoned that inoculum of a host species different from that upon which the inoculum developed might have been a factor in inducing short cycling in P. helianthi, if short cycling had really occurred in the experiment just mentioned. With this view in mind the experiment given in Table 1 was conducted during the month of November. The results obtained from this experiment, however, did not support this assumption, as uredinial infections appeared in all the cultures; and this time the uredinia when developing on H. annuus from inoculation with rust from H. annuus and H. petiolaris, were associated with pycnia, as one expects in cases of short cycling. The two uredinia developing from rust collected on H. tuberosus were however without pycnia as in the preceding experiment. It scarcely seems possible that the uredinia could this time have developed from urediniospores falling from the inoculum. Some of the infections did not show the phenomenon of short cycling, since several of the pycnial pustules apparently not connected with uredinia formed aecia in a few days after the production of nectar. It is however assumed that the formation of uredinia was not dependent upon the mixing of nectar, as both uredinia and pycnia appeared at the same time.

Table 1. The results of inoculation with sporidia, when using H. annuus seedlings as a host, and telia collected on Native and Cultivated Helianthi.

Source of telia	Host inoculated	Number of Uredinia associated with pycnia	Number of Uredinia not associated with pycnia	Number of pycnia only
H. annuus	H. annuus	5		10
H. subtuberosus	"		2	
H. petiolaris	"	40		20

This experiment was repeated during the month of December, with very similar results, uredinia again being formed in many cases simultaneously with pycnia. The isolated pycnial pustules in this experiment were kept under observation for almost six weeks, but they did not show any tendency to form either aecia or uredinia. At the end of the period of observation, the nectar of these isolated pycnia was mixed in such a manner that it came from composite pustules, to guard against the possible mixing of like pustules only. Aecia later appeared in a majority of the pustules mixed, with a small percentage remaining in the pycnial stage. No evidence of uredinia forming in the place of aecia could be detected. In this experiment as in the former the uredinia when present appeared associated with very closely grouped

infections, and only very occasionally did single pustules of uredinia appear not in association with very heavy infections. In table 2 the number of uredinia appearing in the cultures studied is given together with the number of pycnial pustules that formed aecia after the nectar was mixed.

Table 2. The results of inoculation with sporidia, in which the pycnial nectar was not mixed for nearly six weeks following the appearance of the pycnia.

Source of telia	Host inoculated	Number of Uredinia associated with pycnia	Number of Uredinia not associated with pycnia	Number of isolated pycnial infections forming aecia after nectar mixing
H. tuberosus	H. annuus	5		7
H. petiolaris	"		50	
H. maximiliani	"	7		3
H. annuus	"	20		30

The manner in which uredinia form directly from basidiospore infection, apparently without nuclear fusion, is very imperfectly understood. Infections that were thought to have formed uredinia by the suppression of aecia, were examined after six days had elapsed from the date of inoculation, the uncertainty with which short-cycling occurs making earlier examination almost

impossible. Microtome sections 5 to 7 microns thick were stained by Heidenhien's Iron Alum Method. The uredinia were clearly diploid, and conjugate nuclei were also detected in the mycelial wefts beneath the uredinia.

Experiments in Hybridization

Recent investigations have shown that hybridization between physiologic forms of wheat stem rust can be successfully accomplished. Stakman, Levine, and Cotter, (15) have published results of experiments wherein they were able to hybridize and produce an entirely new form between the rusts Puccinia graminis tritici, and P. graminis agrostidis. Stakman, et al, (16) also have recently given rather convincing proof that physiologic forms of P. graminis tritici, hybridize naturally on the alternate host, Berberis vulgaris, in the United States. Waterhouse (17) working in Australia where only a limited number of physiologic forms of P. graminis tritici are known to exist, successfully produced two new forms, one of which had not been identified previously. The telial material he used in his study was collected in widely separated districts, and in the uredinial stage had been identified specifically as physiologic forms 34 and 43, both of these forms differing distinctly in pathogenicity from the resulting hybrid forms. Newton, Johnson, and Brown, (12) have shown that some physiologic forms of

P. graminis tritici, will give rise to numerous new forms, when completing their life cycle on Berberis vulgaris, under controlled conditions - the pycnial nectar of each culture being selfed - and the possible foreign mixing of the nectar being eliminated. The same authors (11) have further demonstrated that two physiologic forms of *P. graminis tritici*, pathogenically different, will hybridize to give rise to a new form in the F_1 generation. This new form when carried through its life cycle, with pycnial nectar selfed will segregate into numerous new physiologic forms.

In view of these results, it was naturally thought that physiologic forms I and II of *P. helianthi* as described by Bailey (4) both of which will infect H. annuus, Fig. (4), would hybridize in a manner similar to that found in *P. graminis*. This seemed particularly feasible, because former investigations had shown rather definitely that *P. helianthi* is composed of but one species. The following experiments were therefore conducted, using telial material collected during the month of October 1930, on H. annuus and H. tuberosus.

The telial material was treated before inoculation in a manner similar to that described earlier in this study. H. annuus seedlings were used as a host for both cultures of rust, and were inoculated by suspending the telia of forms I and II over the plants. Six or seven days after inoculation, when infection had taken place

and whitish flecks appeared on the leaves, the plants were enclosed in fine mesh wire screen cages. The resulting nectar, appearing in about ten days after inoculation, was mixed at the end of a twelve day interval from the date of inoculation. The period of mixing varied however, as it was found that the amount or length of sunshine had a profound effect in stimulating the secretion of pycnial nectar. A copious production usually accompanies bright sunshine, and an average temperature of 70° to 75°F.

Pycnial nectar from the culture resulting from inoculation with form I collected on H. annuus, was transferred to the pycnial pustules of the culture resulting from inoculation with sporidia from teliospores of form II collected on H. tuberosus, and vice versa. Simultaneously with the mixing of pycnial nectar between the forms I and II, several pustules of each form were "selfed", by the artificial mixing of the nectar between pustules arising from the same physiologic form. For example, the nectar from two pycnial pustules arising from an inoculation with form II collected on H. tuberosus, was intermixed. All the pustules in which the nectar had been intermixed were then mapped out on labels attached to the plants, together with a record of the date of mixing. In this manner a complete record of the subsequent behaviour of the pustules under observation was always available. The wire cages were at all times kept over the plants,

Fig. (5), and accidental mixing of the nectar was considered negligible. In from eight to ten days from the time of mixing the nectar, aecia arose in all pustules that had been "selfed", with both forms I and II, but the pustules from which pycnial nectar had been transferred from one culture to the other, remained in the pycnial stage only and continued to exude nectar. Pustules which will form aecia usually do not exude nectar after mixing has taken place. In view of the failure of form I and II to hybridize, the experiments were repeated at intervals during the months of November, December, April and May, with consistently similar results. The results of these experiments are given in Table 3.

To check these results further, crosses in which the pycnial nectar had been previously intermixed, and aecia had not formed, were used, and mixing of the nectar was again carried out, after a twelve day period had elapsed from the date of the first mixing. In this second mixing care was taken to mix the nectar exactly as had been done in the first mixing. At the end of an eight day period no further evidence of aecia appeared. A third time the pustules were intermixed, with still negative results. The pycnia continued to increase and to produce nectar, and some of these were "selfed" after ten days had elapsed from the time of the third mixing, and all such pustules formed aecia in less than eight

Table 3. The results from intermixing pycnial nectar between form I collected on H. annuus, and form II from H. tuberosus.

Source of pycnial nectar	Pustules intermixed	Pustules forming aecia
Form II x Form I	8	0
Form II x Form I	8	0
Form II x Form I	9	0
Form II x Form I	6	0
Form II x Form I	8	0
Reciprocal		
Form I x Form II	4	0
Form I x Form II	12	0
Form I x Form II	10	0
Pustules "selfed"		
Form I	45	39
Form II	23	21

The host for both cultures in this experiment was H. annuus. (Cult.)

Date of Experiments, November and December 1930. April May and June 1931.

days from the date of "selfing". Identical methods of mixing were followed when the pustules were "selfed", as when they were crossed, and this is considered to eliminate the possibility that the seeming inability to hybridize between the two forms of P. helianthi, is due to faulty technique in the transfer of nectar from one culture to the other. Since both forms were cultured on H. annuus it was thought that the host might be a factor influencing this unexpected behaviour between physiologic forms of P. helianthi. The ease and regularity with which each form produced aecia when "selfed" however eliminates this possibility. To obviate any doubt that host played a part in the inability of the two forms to hybridize and form aecia, the following experiment was conducted. Identical methods of inoculating, covering of plants, and transferring pycnial nectar were followed, as in the trials already described.

Young plants of H. tuberosus, were inoculated with sporidia from telia collected on H. tuberosus, coincident with the inoculation of seedling plants of H. annuus, with sporidia from telia collected on H. annuus. Pycnia appearing in both cultures resulting from this inoculation, were after ten days intermixed, by the transfer of nectar from the culture on H. annuus, to the culture on H. tuberosus, and also reciprocal transfers were made. (Table 4). Several pustules of each culture were selfed to serve as

checks. All pustules were carefully recorded on labels, and the plants covered as in the former experiment. Particular care was exercised to ensure that the pycnial nectar was not accidentally mixed by excessive moisture adhering to the leaves, due to water being allowed to come into contact with the plants at the time of watering. In six days from the date of mixing the pycnial nectar almost all pustules that had been "selfed" in each culture formed aecia normally, but the pustules that had been crossed by the transfer of nectar from one culture to the other, remained in the pycnial condition, continuing to exude nectar. A further intermixing of these pustules exactly as they had previously been mixed, gave still negative results.

Table 4. Results of intermixing pycnial nectar between cultures of Forms I and II of P. helianthi. In this experiment Form II was cultured on H. tuberosus, and Form I on H. annuus.

Source of pycnial nectar intermixed	No. of pustules intermixed	No. of pustules forming aecia
Form II x Form I	7	0
Form II x Form I	6	0
Form II x Form I	9	0
Reciprocal Mixing		
Form I x Form II	11	0
Form I x Form II	8	0
Results of "selfing"		
Form I	15	12
Form II	12	9

Experiment conducted during April and May 1931.

A further series of experiments was conducted during the winter of 1931-32, using telia collected on H. subtuberosus, H. petiolaris, and H. maximiliani in addition to H. tuberosus and H. annuus. In the preceding experiment, telia from the two latter hosts only were used. Whatever the physiologic forms involved may have been, the cross inoculation results were strikingly similar to those just reported, except that in the case of a mixture of nectar from the rust on H. petiolaris and H. annuus, aecia developed in seven out of nine pustules intermixed. It appears therefore, that the forms of rust on various wild Helianthi do not hybridize with each other. The results of these experiments are given in Tables 5 to 8 . Aecia did not develop in any of the cases in which pycnial pustules were left unmixed as checks, but aecia did form in all pustules that were "selfed".

This ability of the rust from H. petiolaris and H. annuus to hybridize might be taken as an indication that the two forms in question are identical; this conclusion would, however, be premature until their infection characteristics are known on a wider range of hosts. The failure to get aecial formation as a result of intermixing pycnial nectar between cultures originating from sporidial inoculation using telia collected on H. annuus, H. tuberosus, H. maximiliani, and H. subtuberosus, suggests a distinct genetic variance. Should adequate hosts be available it is not unreasonable to suppose that

distinct cultural differences could be detected, in both the uredinial and the telial inoculation results.

Discussion

The evidence submitted in the experiments that have been under consideration, would appear to agree with the finding of former investigators, with regard to the unstable condition in the life history of *P. helianthi*. The sporadic occurrence of uredinia associated with the pycnia, is however difficult to explain. The uredinia appear simultaneously with the pycnia, and are apparently not dependent upon pycnial functions to become diploid in nuclear condition, as is necessary when diploid aecia are formed. It is very important in consideration of this phenomenon to exclude the possible infection of the host by viable urediniospores remaining amongst the telia, although if that had occurred, we must assume that the pycnia present arose from simultaneous infection by sporidia. If the pycnia and uredinia arise as a result of sporidial infection, then it appears that *P. helianthi*, which is normally a heterothallic rust dependent upon the mixing of the pycniospores to change from the haplophase to the diplophase, with diploid aecia normally resulting, must change spontaneously in a manner similar to that of homothallic rusts.

Considerable investigation will be necessary to clarify this interesting phase in the life cycle of *P. helianthi*. Investigations of the early changes in

the mycelium are rendered very uncertain owing to the fact that one cannot be sure that uredinia will appear associated with pycnia.

The experiments with crossing physiologic forms of P. helianthi indicate that these forms will not hybridize, as do the forms, or even in some cases the races, of Puccinia graminis. Repeated attempts to produce hybrids between the forms collected on H. tuberosus, H. subtuberosus, H. annuus, and H. maximiliani, failed to produce results. This failure to secure aecial formation when intermixing pycnial nectar between forms, can scarcely be attributed to faulty technique, nor to inability of the rust to develop upon the host, since in all cases aecia developed when the pycnial nectar of each form was "selfed". The rust on H. petiolaris is, however, sufficiently compatible with form I on H. annuus, to produce aecia in most cases when the pycnial nectar is intermixed, Fig. (6). It is unwise, however, to consider the two forms in question as separate or distinct physiologic forms, without further study to determine their respective host ranges. In the experiments conducted with the forms that repeatedly failed to form aecia, when pycnial nectar was intermixed from one to the other, and vice versa, the possibility that pustules of similar sex only were intermixed, was avoided by the transfer of nectar from composite pustules to simple pustules. The failure of the forms of Puccinia helianthi to hybridize is indicative

of a surprising difference existing between them. Further investigations using a wider host range would undoubtedly yield much interesting information with respect to the physiologic forms of P. helianthi.

Summary

1. Puccinia helianthi is typically an eueutoecious rust in which all spore stages develop upon the same host.
2. Teliospores of P. helianthi may germinate to a limited extent without a rest period.
3. Repeated soakings in tap water, with successive drying periods, seems sufficient to overcome dormancy of the telia, without an accompanying freezing period.
4. Telia will remain viable for a considerable period of time when kept stored dry at ordinary room temperature, or stored in a refrigerator at a constant temperature of 40° F.
5. Uredinia are often associated with pycnia even after precautions are taken to insure that all remaining uredinospores in the telial material are given adequate opportunity to germinate.
6. The uredinia associated with pycnia do not appear to be dependent on intermixing of the pycnial nectar to become diploid.

7. If the uredinia associated with the pycnia are not the result of infection from viable uredinospores, but are the direct outcome of sporidial infection, then P. helianthi can "short cycle" by the suppression of aecia.

8. Aecia follow normally after the pycnial nectar has been intermixed although an occasional pustule has been observed to form aecia without any apparent mixing of the nectar.

9. Intermixing of the pycnial nectar between forms of rust on different species of Helianthus, has failed to produce aecia, except in the case of those collected on H. annuus and H. petiolaris.

10. The cultivated sunflower, Helianthus annuus, appears to be a common host for all forms of Puccinia helianthi in both the pycnial and the uredinial stages.

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Table 5. Experimental results from the intermixing of the pycnial nectar between cultures resulting from sporidial infection from telia collected on H. subtuberosus, and other wild species of Helianthi, together with H. annuus.

Source of pycnial nectar intermixed	No. of pustules intermixed	No. of pustules forming aecia
H. subtuberosus x H. annuus	5	0
H. subtuberosus x H. tuberosus	2	0
H. subtuberosus x H. annuus	7	0 x
H. subtuberosus x H. petiolaris	6	0
H. subtuberosus x H. annuus	8	0 xx
Source of pycnial nectar when "selfed" as a check		
H. annuus	10	10
H. subtuberosus	6	6 xxx
H. tuberosus	3	2

x, Pycnia were intermixed from composite pustules.

xx, Pustules were intermixed at three different times exactly as they had been at first.

xxx, Pustules forming aecia after "selfing", were pustules that had been previously intermixed with nectar from the H. subtuberosus culture.

Date of inoculation 14-10-31. Date of intermixing 2-11-31.

Table 6. Experimental results obtained when intermixing pycnial nectar from cultures of P. helianthi, established from sporidial infections from telia collected on H. petiolaris with H. annuus and various wild species of Helianthi.

Source of pycnial nectar intermixed	No. of pustules mixed	No. of pustules forming aecia
H. petiolaris x H. tuberosus	10	0
H. petiolaris x H. subtuberosus	7	0
H. petiolaris x H. maximiliani	5	0
H. petiolaris x H. annuus	9	7
H. petiolaris x H. petiolaris	8	8
Source of pycnial nectar when "selfed" as a check		
H. tuberosus	11	9
H. maximiliani	5	5
H. subtuberosus	6	6

The date of inoculation in the above experiment was 29-9-31. Pycnial nectar was intermixed and selfed on 10-10-31. Aecia appearing in selfed pustules on 16-10-31.

Table 7. Experimental results obtained from the intermixing of the pycnial nectar of a culture established with sporidia from telia collected on H. tuberosus, with pycnial nectar from rust cultures arising from sporidial inoculation from telia collected on other Helianthi species.

Source of pycnial nectar intermixed	No. of pustules intermixed	No. of pustules forming aecia
H. tuberosus x H. annuus	5	0 xx
H. tuberosus x H. subtuberosus	7	0
H. tuberosus x H. petiolaris	8	0
H. tuberosus x H. tuberosus	10	8

The xx denotes that on H. annuus the nectar from composite pustules was transferred.

In this experiment all the pustules that had been intermixed were latterly "selfed" and aecia formed in a majority of the pustules. Sufficient time, however, had elapsed to make certain that the aecia did not result from the intermixing or transfer of nectar from one culture to the other.

Table 8. Experimental results obtained when intermixing pycnial nectar from cultures of P. helianthi, established from sporidial infections from telia collected on H. annuus and various wild species of Helianthi.

Source of pycnial nectar intermixed	No. of pustules mixed	No. of pustules forming aecia
H. annuus x H. tuberosus	10	0
H. annuus x H. subtuberosus	4	0
H. annuus x H. subtuberosus	7	0
H. annuus x H. annuus	6	6
Source of pycnial nectar when "selfed" as a check		
H. tuberosus	2	2
H. subtuberosus	4	3

The telia was collected on September 1, 1931, and the experiment was conducted on the following dates:- Inoculation with telia, 10-10-31. Mixing of the pycnial nectar, 2-11-31. Aecia appearing in the selfed cultures 8-11-31.

Fig. (1). Method of inoculating H. annuus seedlings, by means of glass chimneys and inverted Petri dish covers containing the telial material, suspended over the plants.

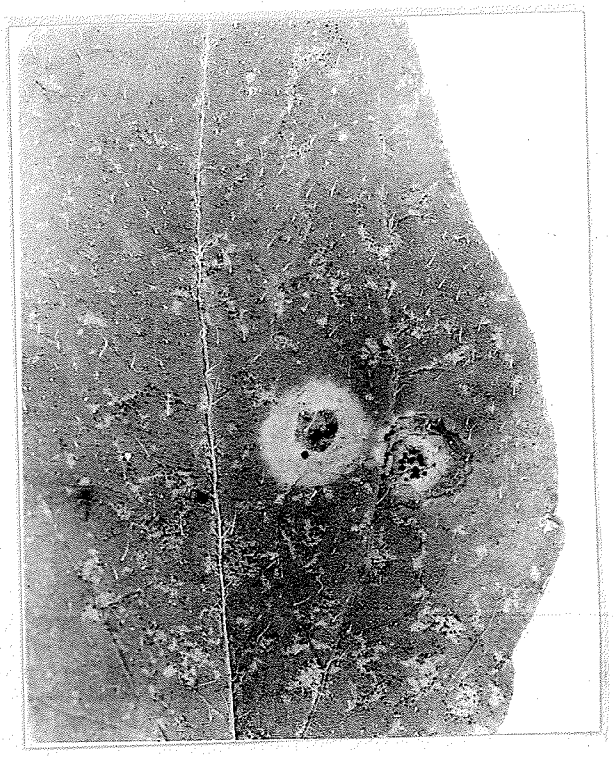

Fig. (2). The upper surface of a leaf from H. annuus, after inoculation with sporidia. Uredinia are associated with pycnia.

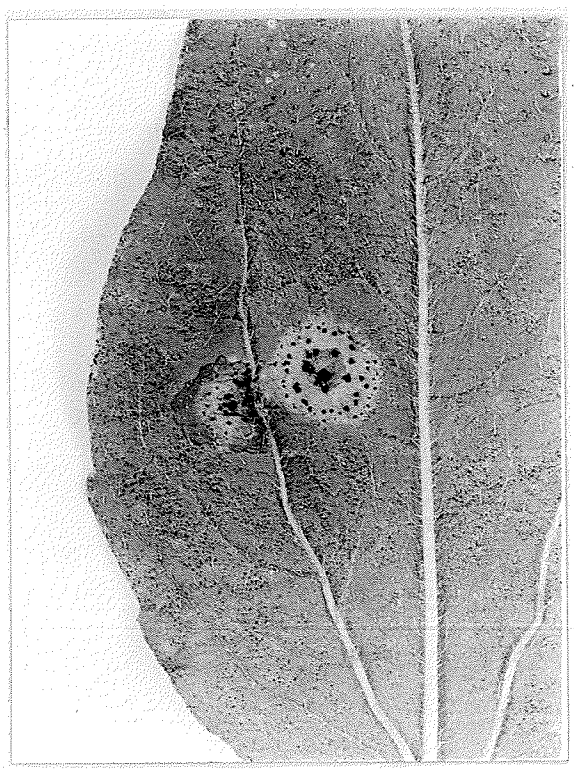

Fig. (3). The lower surface of a leaf from H. annuus, following inoculation, presumably with sporidia. The aecia are suppressed and only uredinia have formed.

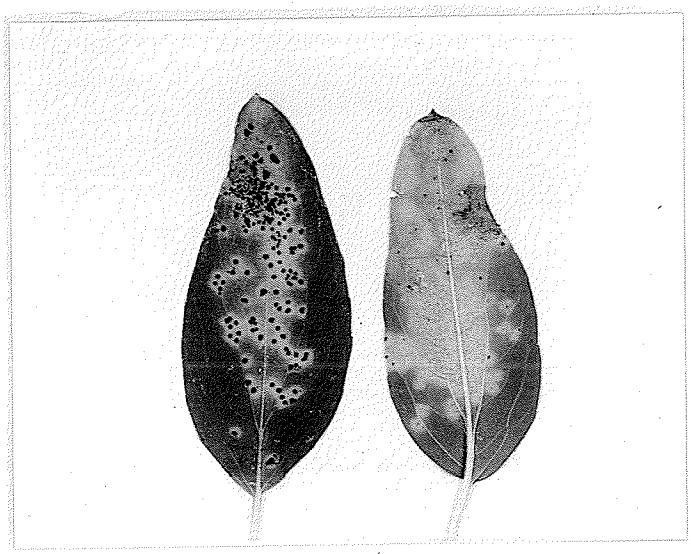

Fig. (4). At left, underside of H. annuus leaf with uredinial infection resulting from inoculation with urediniа collected on H. annuus. (Form I). At right, underside of H. annuus leaf with uredinial infection from inoculation with urediniа collected on H. tuberosus. (Form II).

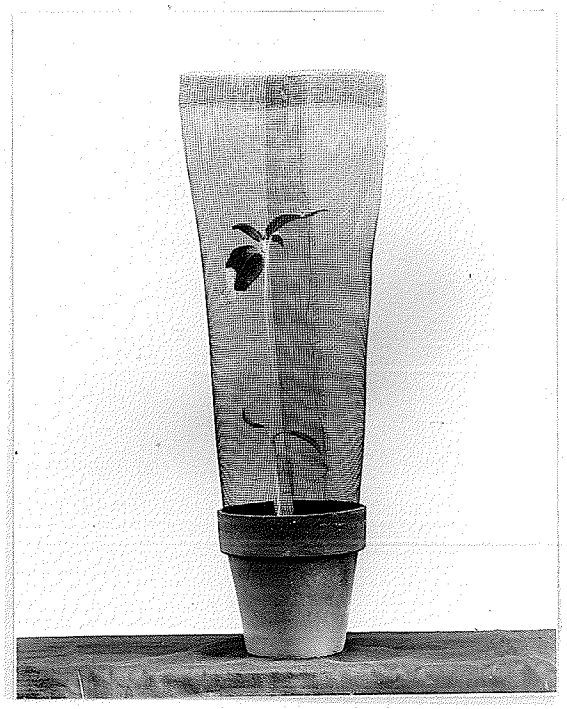

Fig.(5). Plants that have been inoculated and have formed pycnia are protected by means of wire screen cages.

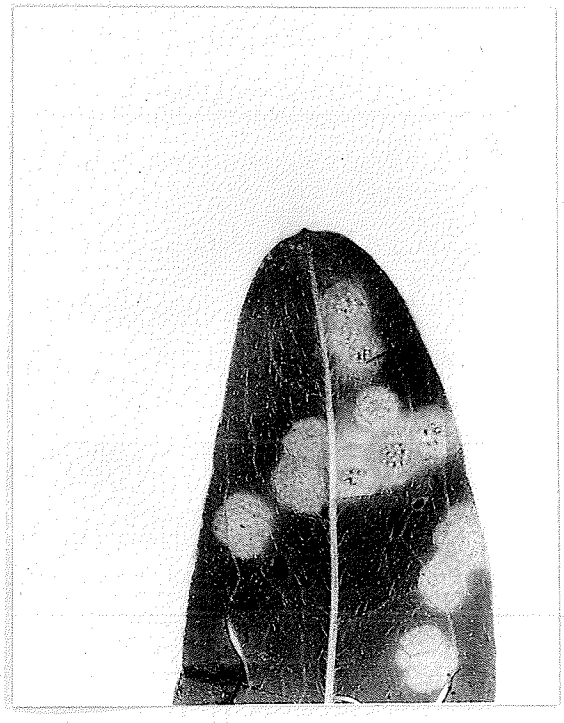

Fig. (6). Pycnia from inoculation with sporidia from telia collected on H. petiolaris. All pustules "selfed" have formed aecia. Pustules that were intermixed with pycnial nectar from an H. tuberosus rust culture are still in the pycnial condition.

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