THE COMPOSITE LIFE HISTORY OF PUCCINIA PODOPHYLLI SCHW.1

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The common rust, Puccinia podophylli Schw., presents certain features which make it of special interest to the student of the biology of this great group of plant parasites. The interest in this species lies primarily in the peculiar sequence in the seasonal appearance of the spore forms. This species is an opsis-form, possessing aecia and telia only, and occurs on the common mandrake or May-apple, Podophyllum peltatum L., practically throughout its range.

Puccinia podophylli produces teliospores at two different periods during the season; the first crop appears early in the spring on the sheath leaves or bud scales, on the stem usually at the base and sometimes on the sepals (PL 1, A and B); the second crop appears later in the summer on the under side of the fully expanded and matured leaves (PL 4, B). Between these two crops of teliospores the aecia, usually preceded by pycnia, are developed on the expanding leaves (PL 2). It is also of interest that telia often develop in association with the aecial clusters on the leaves and to all appearances on the same mycelium before the appearance of the second crop of teliospores, which obviously develop from aecial infection (Pis. 2, 3, and 4, A). The explanation of the origin of these early telial sori and the determination of the life history of this species are the objects of the study reported in this paper.

HISTORY

The only previous attempt to explain the life history of this species and the sequence of the spore forms was made by Olive (6). Based primarily on cytological evidence, he reached the conclusion that the first crop of telia and the pycnia and aecia arise from an intermingled growth of the mycelium of the gametophytic and sporophytic generations which are independently and simultaneously perennial in the host plant. He conducted no culture work nor did he attempt to verify this assumption by an histological study of the root stalk or overwintering buds.

The following summary of Olive’s work is presented in some detail, largely by quotation, in order to bring out clearly his cytological observations and the conclusions he drew from them since it will be necessary to refer to these details later in this paper.

Olive found that the mycelium in the lesions on the sheath leaves that bore the early telia was prevailingly binucleate, as one would expect. The preparations also show, occasionally, a few aecidium cups on these same sheaths * * *. I have not yet found spermogonia on the sections of these young sheaths; but their occasional occurrence in such situations may be expected from the fact that a small amount of uninucleate mycelium occurs, especially in the region surrounding the aecial sori, there forming the meager pseudoparenchyma. But the rust mycelium of the sheath, in contradistinction to that in the young leaves, is undoubtedly prevalently binucleate. Further, I

1 Received for publication May 24, 1924—issued April, 1925. Joint contribution from the Department of Botany, Purdue University Agricultural Experiment Station and the Department of Plant Pathology, Cornell University.

A report on the experiment conducted at Ithaca, N. Y., together with an interpretation of the life history essentially the same as that given in this paper, was presented before the Botanical Society of America at the Pittsburgh meeting of the American Association for the Advancement of Science in 1917 by Prof. H. H. Whetzel (not published). The problem had been freely discussed with me during the two previous years and my interest aroused. It was mutually agreed that a more detailed cultural study would be desirable and this work was begun at LaFayette, Ind., in 1917, having been planned in collaboration with my associate, Dr. E. B. Mains, who carried out the details of the cultures. It was finally decided to present the combined results in a joint paper, the preparation of which has been my responsibility.—H. S. Jackson.

Reference is made by number (italic) to “Literature cited.”

Journal of Agricultural Research,
Washington, D. C.
13949—25†—5 (65)

Vol. XXX No. 1
Jan. 1, 1925
Key N. Y. (Cornell)-5
A.—Telia of *Puccinia podophylli* on the sepals. Note theaecial-like character ofthese telia. Natural infection slightly enlarged
B.—Telia on bud scales and on the stem. Natural infection. Compare with Plate 4. B. Natural size.
am convinced that the aecidia which are borne on the sheaths arise, not from gametophytic cell fusions, but only from preexisting binucleate hyphae; therefore being secondary and sporophytic in character, and thus similar in origin to the teleutospores.

He found in the lesions on the leaves that a much more abundant uninucleate mycelium is present in the younger tissues and that the pycnia arise from this mycelium.

In those older leaves, however, in which the aecia have begun to form their chains of spores, binucleate mycelium has become quite prevalent in all the sections examined, especially at the bases of the aecial cups. These sporophytic hyphae intermingle with the uninucleate mycelium, often entering the broad, caecoma like base of the young aecia, there functioning directly as basal cells of the rows of the binucleate aecidiospores. In still older stages on leaves, binucleate mycelium apparently prevails by time the aecidium cups have for the most part broken open to discharge their spores, * * *.

His examination of the latter telia which form from aeciospore infection shows that they arise from a localized binucleate mycelium.

In interpreting the results of his observations he assumes that the uninucleate, or gametophytic mycelium, and the binucleate or sporophytic mycelium, are independently but simultaneously perennial in the plants from which his material was taken and that the pycinia are the only structure borne on the uninucleate mycelium, while both the early teliospores and the aecia develop from the binucleate mycelium. He assumes that basidiospore infection would result in a localized mycelium on which pycinia and aecia would be developed, the expectation being that this would be prevalently uninucleate.

In connection with a discussion of the principle that in perennial rusts the gametophytic mycelium in young shoots grows more vigorously early in the season than the sporophytic, the following quotation is of interest:

The fact that the teleutosori break out very early from the tissues of the leaf sheaths, some distance below the tip of the stem, and that the binucleate mycelium prevails in these sheaths almost to the exclusion of the uninucleate, to my mind, vitiates the above statement, which applies only to the younger tissues of the shoot. I interpret these facts somewhat as follows: the uninucleate mycelium grows with especial vigor into the rapidly expanding tip and young leaves of the new shoot, growing somewhat ahead of the lagging sporophyte. The latter apparently chooses ordinarily the more mature tissues for its most vigorous growth and thus early comes to predominate in the riper tissues of the poorly nourished leaf sheaths of Podophyllum, as well as later in the older leaf tissues.

As a part of Olive's discussion of the probable reason for the development of the early telia on the sheaths and later the secondary aecia on the leaves from the same binucleate mycelium, the following significant statements are found:

It is obviously quite impossible to suppose that this early teleutosporic stage in *Puccinia podophylli* has arisen in any other way than from hibernating sporophytic mycelium, which has grown up with the infected buds in the spring. It could not come from an early infection since the aecidiospores, which we assume to be the only spores from the inoculation of which the teleutospores could arise, have not yet begun to form. I will have to confess, however, that I have not been able to apply de Bary's test to this species and to look for hibernating mycelium in the underground parts.

**CULTURE EXPERIMENTS**

**EXPERIMENTS AT ITHACA, N. Y., 1916-1917**

One of the authors of this paper (Whetzel) has had Podophyllum rust under observation since about 1906. During 1912 he collected and fixed much of the material on which Olive's cytological work was based and furnished him without reservation all the information gained from the field observations which had then been made. This assistance is freely acknowledged by Olive in the paper referred to above.4

The elaborate, though entirely orthodox, explanation of the situation finally made by Olive did not seem to Whetzel adequately to explain the phenomena which had been observed in the field. On this account it was decided to test the validity of Olive's hypotheses by culture experiments. The first of these was conducted at Ithaca, N. Y., during 1916 and 1917.

The plants used for the experiment had been brought several years before from a patch of Podophyllum in which no rust was to be found and were planted on a wooded bank in Whetzel's yard far away from any other patches of Podophyllum. Since the planting was along the path by which he reached the road on his way to the office each day, he was able to determine by careful inspection each year that no rust ever appeared either on the bud scales, stems or leaves of these plants. In the summer of 1916 he collected a large quantity

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4 A detailed manuscript record of these experiments, together with the correspondence which passed between Prof. Whetzel and Dr. Olive on the Podophyllum rust, is deposited in the library of Cornell University under the title, "Notes on the Life History of Puccinia podophylli," by H. H. Whetzel.
A.—Upper surface of leaf of Podophyllum peltatum inoculated with teliospores from stem sori. Note abundance of telia in aecial lesions and telia at base of stem

B.—Upper surface of aecial lesion showing telia along the veins. Natural infection

C.—Lower surface of same lesion as B, showing aecia. Natural infection
of Podophyllum leaves which bore the second, or summer crop, of teliospores in abundance, put them between two sheets of window screen and placed this over one end of the established rust-free planting in his yard. This plot had developed so that it was about 8 feet long and rather narrow. The screen packet was about 12 by 18 inches. During the fall and winter the Podophyllum leaves in the screen rotted and the spores fell with the débris through the meshes upon the surface of the soil. Early in the spring of 1917 the empty screen was removed and careful observations were made on all the plants in the plot at frequent intervals as they developed. Telia soon appeared on the sheath leaves and stems of nearly all the plants which came up in the area covered by the screen packet. No rust appeared on plants elsewhere in the bed during the entire season. A seedling in the infected area also developed aecial lesions on the leaf blade in the spring of 1917. Two nearby seedlings showed telia only on the stems. One of these seedlings stood directly under that bearing aecia and when all three were removed on June 4, 1917, it showed the whitish lesions of the developing but unopened summer telia. However, no summer crop of telia appeared on any of the other plants in the patch during 1917. In 1918, while leaf sheath and stem lesions bearing the early telia were common, only a single plant developed an aecial lesion. This was allowed to remain, and later nearly every leaf blade in the patch showed a more or less abundant development of the summer telia. Since that time this patch of plants has been rusted more or less each year, all stages of the rust appearing in the manner usual for this species.

This experiment, while not in itself conclusive, strongly suggests that the interpretations of the life history made by Olive are not in accord with the facts. It would appear from this experiment that the infection on the sheaths and stems, resulting in the early crop of telia, arose directly from the basidiospores developed from the late crop of teliospores which were used as the inoculum and that these teliospores were responsible also for infection on the seedling leaves, resulting in pycinia and aecia. Since the aecial-bearing seedling was removed along with the seedling showing the initial stages of the summer telia, and since no other plant in the patch showed summer telia during 1917, it would further appear that the spring crop of teliospores was responsible the next season for the development not only of another crop of early teliospores on sheath and stem but also for pycinia and aecia later.

EXPERIMENTS AT LA FAYETTE, IND., 1917-1921

Since this experiment was conducted entirely out of doors and the usual objections to such experiments would naturally arise, it was decided to repeat the work with variations, in part at least under controlled conditions, and to conduct this work in connection with the rust investigations under way at the Purdue University Agricultural Experiment Station at La Fayette, Ind.

EXPERIMENT 1.—During the summer of 1917 patches of Podophyllum were located in the woods, some of which were heavily rusted and some of which showed no rust. An equal number of plants, about 25, were dug in November from each of two plots, one of which was rusted and the other not. The rhizomes were very carefully washed and cleaned of all soil or dead parts and planted in a single row in a vegetable garden (Jackson's), with a stake dividing the two lots. A frame 18 inches by 48 inches wide was placed on the ground in the middle of the row in such a position as to include 5 plants from the rusted patch and 5 from the one which showed no rust. A peck or less of surface soil and débris taken from a patch of Podophyllum which had been heavily rusted during the previous summer was evenly spread over the soil in this frame. No protection was given the plants during the winter.

In the spring of 1918 all 10 plants in the frame showed the early telia on the bud sheaths or stems and later about half of them showed pycinia and aecia in fair abundance on the blades of the leaves. No infection of any sort occurred on the other 40 plants at either end of the frame. The experiment was not continued since the ground was needed for other purposes.

Were the rust perennial in the rhizomes some of the plants outside the frame which had been dug from the rusted patch should have been rusted. The infection on the plants in the frame presumably resulted from the teliospores present in the top soil used as a mulch.

EXPERIMENT 2.—In November, 1917, about 50 rhizomes dug from a patch of Podophyllum which showed heavy infection on nearly every plant during the spring were thoroughly washed.
Some of these were placed in flats, covered with potting soil, and placed out of doors with a heavy covering of straw. Some were potted at once and placed in the greenhouse. The material which was placed in flats was brought into the greenhouse in March, 1918, when the leaves were just emerging from the sheaths. No rust developed on either set of plants, though they were grown to maturity.

In March, 1918, before the buds had swollen to any extent, a second lot of rhizomes were dug from a patch which was known to have been heavily rusted during the previous summer. These were thoroughly washed, potted in potting soil, and allowed to develop in the greenhouse. No sign of rust infection appeared on any of them.

An inspection of the remaining plants in the patch of Podophyllum from which the last-mentioned rhizomes had been dug was made at intervals during the spring and summer of 1918 and the plants were found to be heavily infected, showing the early development of telia on the sheaths and stems, pycnia and aecia later on the leaves, and finally the late crop of telia on the under side of the leaves.

Stems showing the early telial sori and the attached rhizomes were collected in the spring of 1918 and were studied in an effort to determine the extent of the mycelium. Free-hand sections were made, beginning in the region of the sori and progressing down the stem into the rhizome. The sections were mounted in chloral hydrate and iodine, which furnishes a satisfactory medium for the study of such sections. The mycelium is easily distinguished by this method and was found only in the immediate region of the sori. No mycelium was discovered lower down in the stem or in the rhizome.

This series of experiments taken in conjunction with the one made at Ithaca would seem effectively to dispose of the hypothesis put forward by Olive that the rust is perennial in the buds or rhizomes.

A number of other experiments were considered desirable, however, in order to determine as accurately as possible just what did occur. The question naturally arose as to whether there might be some difference in the teliospores formed early in the season on the stems and sheaths and those formed later from aeciospore infection, either in their ability to cause infection or in the sequence of resulting spore forms. It was also considered desirable to conduct some of the experiments with somewhat more refined methods and under as strict control as is feasible in dealing with growing plants and with a fungus which can not be grown in pure culture.

**Experiment 3.**—The telial material which had been used for the following inoculation experiments was obtained in the spring and summer of 1917 in the same patches of Podophyllum from which the rhizomes used in the previous experiment were taken. The first material was collected May 28, 1917, and consisted entirely of sheaths and stems bearing the early crop of telia. These were placed out of doors on the surface of potting soil in flats and left till the following spring, 1918. On July 17, 1917, material of the late crop of telia which develop from aecial infection and occur abundantly scattered on the under surface of the leaves was collected and handled in the same way as the sheath and stem material mentioned above. Finally, in the fall of 1917, after the Podophyllum plants had died down and the leaves were more or less disintegrated, surface soil from a patch of heavily infected Podophyllum was collected and placed out of doors to winter. At the same time rhizomes were again collected from a patch of Podophyllum which had shown no infection during the year and were placed in flats, covered with potting soil, and left out of doors with protection during the winter. In March, 1918, these rhizomes were brought into the greenhouse, thoroughly washed, and potted. Some of the pots were given a surface coating of the surface soil obtained from the infected Podophyllum patch, some were mulched with the soil and stems from the flat prepared in May, 1917, and some with the soil and leaves from the flat prepared in July, 1917, as described above. It should be pointed out that the sheaths, stems, and leaves had largely disintegrated after overwintering and most of the teliospores were mixed in the surface soil of the flats in which they had been kept; hence the reason for using the soil as a mulch. As the buds of the potted plants developed they pushed up through this mulch and became exposed to infection.

The results of this experiment were as follows: In one of the pots mulched with soil, sheaths, and stems bearing the early teliospores, pycnia and a few aecia developed on the leaves of one plant and pycnia and aecia together with a few telia immediately associated with the aecia on another. In the pots mulched with soil and leaves bearing
the late crop of teliospores, four plants showed pycnia and aecia on the leaves in numerous spots, some of which on all four plants in later stages of development showed accompanying telia. In the pots mulched with soil from the infected patch one plant showed a few pycnia and abundant telia on sheath and stem, together with pycnia and aecia on the leaves in numerous spots in association with which telia were not uncommon, and the other plant showed numerous spots with pycnia and aecia, with some of which were associated telia. In all cases where telia accompanied the aecia on the leaves they were most commonly found on the veins which were involved in the areas of aecial infection. A control series of pots in which the rhizomes were mulched with ordinary potting soil showed no infection of any sort.

**EXPERIMENT 4. — In this experiment overwintered teliospores from the late crop on the leaves, which by previous test were known to be germinating, were sown at three different dates on Podophyllum plants taken from an uninfected patch. This work was all done in the greenhouse. The first sowing was made on April 27, 1918, on leaves and stems of four plants, three of which had the folded leaves well out of the sheaths and the fourth just emerging. The result showed development of pycnia and aecia on leaves of all plants and telia on the stem of one. Epiphyllous telia developed in a number of the aecial areas, mostly on the veins. The second sowing was made on May 6, 1918, on six plants of Podophyllum all of which were well out of the sheath with the leaves partly expanded. Pycnia and aecia were developed on the leaves of all plants. Some of the aecial lesions on each plant showed telia in immediate association. In one case telia were developed in association with pycnia without aecia being present. In the third sowing on May 11, made on the leaves of more mature plants, numerous lesions bearing pycnia and aecia developed on two of the plants. Telia were also developed later in association with the aecia on a majority of the spots. On both plants a few spots containing aecia developed in association with which no pycnia were observed.

**EXPERIMENT 5. — This experiment dealt with the infection from aeciospores. Three sowings were made under control conditions in the greenhouse. The first sowing was made on May 28, 1917, using aeciospores collected in the field from plants which showed only aecia and with which no pycnia were observed. The inoculation was made on the expanded leaves, and fully mature telia developed on June 9. The second sowing was made on May 3, 1918, using aeciospores from plants collected in the field on which the aecia were accompanied by pycnia. Sowings were made on one mature plant and three younger plants. An attempt was made to inoculate the sheaths and stems as well as the leaves. Telia appeared, however, only on the leaf blades of the three younger plants, being fully mature on May 20. A third sowing was made on May 10, 1918, using aecia obtained from the inoculations described in Experiment 3. The inoculation was made on several plants in various stages of maturity. Telia developed only on the leaves of the younger plants.

In all of these sowings the telia obtained were of the type occurring progressively in spots on the under side of the leaves, commonly found in the field in midsummer, and referred to in this paper as the late crop of telia.

These experiments confirm those of Arthur (1) and effectively dispose of the idea that repeatingaecia might be present in the life history of the Podophyllum rust.

**LATER EXPERIMENTS. — During 1919, 1920, and 1921, other experiments were conducted, which confirmed the results of those outlined above in all details. In a few cases telial material which was observed to be germinating in hanging-drop tests was transferred to the plant. The results were the same as where less refined methods were used.

It may be noted that only a few records of infection on the sheath leaves are made in these experiments. This, it should be explained, is due to the fact that some difficulty was encountered, under rather dry greenhouse conditions, in preventing the sheaths from drying up before the telia developed. In many such cases, however, evidence of infection was observed, and in the later experiments of 1919 to 1921, this phase of the problem was given special attention.

**SUMMARY OF RESULTS OF EXPERIMENTS**

A careful analysis of the results of these experiments shows that the writers have obtained the development of telia on sheaths and stems, and pycnia and aecia with associated telia on the leaves, by inoculating with germinating teliospores of both the early and the late crops.
No basis was found for the idea that the rust is in any sense perennial or that any condition approaching an "unlimited infection" is evident. It would appear that all of the structures develop from a localized mycelium and that this mycelium in all cases (except the late crop of telia) arises from basidiospore infection. It is also evident that the first spore structure borne on this mycelium may be either telia, or pycnia accompanied by aecia, and in rare cases aecia without pycnia.

These experiments when taken together with field observations made at Ithaca, N. Y., and La Fayette, Ind., would seem to make necessary an interpretation entirely different from that made by Olive.

LIFE HISTORY AND SEQUENCE OF SPORE FORMS

It seems desirable, therefore, as a basis for further discussion, that the writers here set forth in some detail what they conceive to be the regular sequence of events in the life history of this rust.

In the spring, at the time when the buds of the Podophyllum plant are emerging from the ground, the teliospores of *Puccinia podophylli* are present in the surface layer of soil around the developing buds. These teliospores are derived both from the spring crop of telia on leaf sheaths, stems, and sepals and those associated with aecial lesions, and from the summer crop produced on the leaf blades. They have either fallen to the ground during the previous summer, as the telia are of the pulverulent type, the pedicles breaking close to the spore, or they have been liberated by the rather complete disintegration of the host tissues. The spore wall is beset with sparsely scattered rather long spines which would serve to prevent the spores from being carried too deeply into the soil or washed away during heavy rains.

At the time when the sheathed leaf buds begin to push their pointed tips above the surface of the soil, these teliospores (of both crops) begin to germinate in the normal way for teliospores, that is, by the development of a typical promycelium and sporidia. The scale leaves form a sheath about the base of the stem, through which the young plant emerges. These scale leaves usually extend slightly above the ground level. The lobes of the two peltate leaves of the flowering stalk are folded about the stem in a convolute manner and the single blossom bud is partly exposed.

The basidiospores fall upon the portions of the plant which are exposed in the early stage of development and infection results. The sheath leaves or bud scales are the first organs exposed. As the aerial stalk pushes forth from within the enfolded bud scales, both the base of the stem and the leaf blade are also exposed to infection. The upper part of the stem is rarely infected as it long remains protected by the enfolded leaf blades. The two sepals of the flower are, however, not uncommonly infected, for they are usually partly exposed as the young plant emerges (Pl. I, A and B).

The period of basidiospore production (that is, teliospore germination) apparently is relatively early and short. Very numerous infections occur on the scale leaves and on the base of the stems. This is perhaps due to the fact that they are nearer the soil surface and in the more effective zone of basidiospore dissemination. At Ithaca it has been noted in some seasons that only certain scale leaves, such, for example, as the second from the top, show infection. This observation (Whetzel's) was referred to by Olive as a "curious fact." The explanation is, however, comparatively simple. These "second" scale leaves were the only ones exposed in the particular patch under observation at the time of inoculation. There are three or more of these buds scales at the base of the stem. The top scale had not emerged from within the protecting outer second scale and the shorter third scale was still covered by leaf mold. It has since been observed, in the same patch, that all the scales and the base of the stem may be infected. It would appear that these differences are the result of seasonal variation in the coincidence of basidiospore production and development of the host and of variations in the extent of exposure of the bud scales above the surface of the leaf mold.

From the basidiospore infections on the scale leaves, the base of the stem, and the sepals, telial sori are developed almost exclusively. Pycnia and aecia are occasionally found intermixed with the telia, the latter usually occurring singly. Both are, however, rather rare, and by far the greater number of lesions include telia only. In one case noted at LaFayette an isolated lesion on the stem bore a group of pycnia surrounded by a ring of telial sorii, exactly similar in appearance to many true microforms of *Puccinia*.

The occasional development of pycnia and aecia among the telia on sheath
leaves is readily understandable when one has the fresh specimens in hand. These always occur on the thicker, flesher parts of the sheaths. These parts mature more slowly than the margins, thus simulating the conditions obtaining in the longer-growing tissues of the leaf blade where aecia normally develop from this primary infection, as noted below.

The infection on the sepal is not commonly observed because they soon drop off. If a search is made, however, at the right time, they can usually be found in considerable abundance on the ground among flowering stalks, and if infection is abundant in the patch on stems and sheath leaves these fallen sepal will almost invariably be covered with telia. Infected sepal still attached to the plant have, however, been found both at La Fayette and Ithaca (Pl. 1, A).

When basidiospore infection occurs on the blade of the leaf, aecial lesions almost invariably result. These show at first pycnia on the upper surface, followed shortly by the aecia below. It should be emphasized that the basidiospores which cause this infection may result from the germination of either the early or late crop of teliospores of the previous season.

The aecial lesions are rarely evident until after the teliospores on the sheath leaves, base of stem, and sepal have begun to mature. Fully developed aecia are seldom found earlier than a week after an abundance of mature teliospores appears on sheaths and stalks. It is obvious that these early telia could not have been produced from aecial infection. The delayed appearance of the aecia is perhaps due to the slower development of the fungus in the less rapidly maturing tissues of the leaf blade.

As the aecial lesions begin to mature, that is, when the cups are open and the spores are being discharged, one often finds, usually on the upper surface, about the margins of these lesions or more commonly on any larger veins which may be included in the lesion and even in the center of the spot among the pycnia, a rather abundant production of telial sor among with more or less development of epiphyllous aecia (Pls. 2 and 3). These telia are like those developed on sheath and stem and are certainly developed on the same mycelium as the aecia. A careful study made by one of the writers (Jackson) of the sor in such lesions has revealed the fact that occasionally a greater or less production of chains of aeciospores may be found in these telial sor in similar situations aecia may occasionally be found with a few teliospores in the margin. In one specimen studied a sorus was found, one-half of which consisted of aeciospore chains and one-half of teliospores.

Occasionally aecial lesions may be found in which pycnia do not develop and quite commonly only a very few pycnia are found. When infection takes place on leaves which are relatively quite mature the number of telial sori formed may be greater than the number of aecia (Pl. 3, A and B). In one instance noted in the culture results, telia accompanied by pycnia, but with no aecia present, resulted from basidiospore infection on the leaves. Such a condition exactly parallels the normal result when infection occurs on the sheaths and stems, and is comparable to the usual condition of a micro-Puccinia.

It has been observed at Ithaca, N. Y. (Whetzel), that a very much smaller number of leaf blade infections resulting in aecia occur than of infections on scale leaves and stems resulting in telia. Patches of Podophyllum have often been noted in which the early crop of telia on sheaths and stems was almost universal, but in which no aecial lesions on the leaf blades developed during the season. In that locality no cases have been observed where more than a small percentage of plants showed aecial infections. This, however, is not the case at La Fayette, Ind. Aecial lesions on the leaves are usually found in considerable abundance in infected patches. It would be interesting to have data on this point from other sections of North America, particularly from regions at the northern limit of the range of the host species.

The aeciospores are scattered by the wind and cause infection of the surrounding expanded leaves of the Podophyllum plants. This infection is usually very profuse and general, and after a period of ten days or two weeks angular yellowish spots appear, on the underside of which are developed the gregarious, pulverulent telia bearing the second or summer crop of teliospores (Pl. 4, B). These mature and fall to the ground to mingle with those of the early crop, which have already been largely disseminated.

Reference has repeatedly been made to the two crops of teliospores. Those which appear early in the season on the sheaths, stems, sepal, and in association with the aecia on the leaves form the first crop, and those which arise from the infection of aeciospores form the second crop. There is, however,
A.—Upper surface of leaflet inoculated when quite mature with overwintered teliospores of late or summer crop. Note abundant production of telia and scanty infection.

B.—Lower surface of same leaflet shown in A. Note development of telia almost to the exclusion of aecia.

C.—Upper surface of leaflet inoculated when young with overwintered teliospores from the early crop on the stems. Note abundant production of telia among the pycnia and the vigorous infection.

D.—Lower surface of leaflet shown in C. Note abundant development of aecia with an occasional telial sorus. Contrast with B.
in addition to the difference in seasonal appearance, a noticeable difference in the character of these two sorts of telia, difficult to describe but easily recognizable when one has the specimens before him. In the early crop, the telia are usually somewhat larger, less definitely circular in outline and somewhat longer covered by the cinereous epidermis. They also cause a slight but distinct hypertrophy of the host tissue similar to that produced by the aecia. When on succulent tissue they also appear to have their origin much deeper in the tissues. This difference is perhaps best brought out by comparing Plate 1, A and B with Plate 4, A and B.

It seems probable that this difference in type of sori is correlated with the fact that the early crop of telia on sheaths, stems, and sepals, as well as those which occur in association with the aecia, have a gametophytic origin, while the late crop which develops from aeciospore infection is sporophytic in origin.

The writers have been unable to obtain any evidence either from observation or cultures to show that this species possesses secondary or repeating aecia. Cultures conducted both in the field and in the greenhouse under control conditions have shown that the aeciospores give rise to the summer crop of telia.

SUMMARY OF LIFE HISTORY

The teliospores of both the early and late crops are functionally and morphologically indistinguishable and the basidiospores developed on the promycelium of either sort may, after overwintering, cause infection on any exposed portion of the Podophyllum plant. When infection takes place on the sheath leaves, stems, or sepals, telia are at once produced. They may or may not be accompanied by a few pycnia or aecia. When, on the other hand, infection takes place on the blade of the leaf, pycnia followed by aecia are developed. Aecia may develop without accompanying pycnia. Telia similar to those on sheaths and stems may or may not accompany the aecia on the leaves, and when present develop from the same mycelium as the aecia.

The late or summer crop of telia develops from aeciospore infection. There is no evidence of repeating aecia.

GENERAL DISCUSSION

It is evident from the above that the writers' interpretation of the life history differs radically from that of Olive. It now becomes necessary to attempt to correlate this new interpretation with Olive's cytological observations. The writers have at present no basis for criticizing these observations nor do they see any reason, for purposes of discussion, for not accepting them as matters of fact.

It must be admitted, however, that in view of the experimental results recorded above, it would be highly desirable that the cytological situation in Puccinia podophylli be reinvestigated. It is apparent that we are dealing with a rust showing considerable plasticity in its development since from basidiospore infection teliospores are the first spore form produced under some conditions, while under others, pycnia followed by aecia occur.

It will be recalled that Olive found a small amount of uninucleate mycelium with the developing telial sori on the sheaths and an abundance of such mycelium in lesions on the leaves, but that the aeciospores as well as the teliospores developed on a binucleate mycelium. No so-called sexual fusions were observed to take place at the base of the aecial cups. It was assumed that all the telia and aecia were formed on a perennial binucleate mycelium and the pycnia were formed on a perennial uninucleate mycelium. The aecia were then assumed to be secondary in nature and sexual fusions would not be expected. They were to be expected, according to this view, in the primary aecia which would originate from basidiospore infection. These Olive assumes he had not seen, and evidently considered it doubtful whether they would be found to be present in the life history.

In view of the evidence already presented it would hardly seem necessary to consider seriously the possibility of a systemic perennial mycelium in the case of this parasite. However, the paper by Olive has given this theory such general acceptance that it seems necessary to emphasize the fact that the writers have not been able to obtain the slightest evidence that the rust is in any sense perennial, or even systemic, in the host plant.

According to the view of the writers, accepting the cytological findings of
A.—Upper surface of leaf showing aecial lesion which involves the disk and the veins. Note the pycnia and the abundant development of telia. Natural infection

B.—The late or summer crop of telia on upper and lower surface of leaves. Compare with A and with Plate 1, A and B. Natural infection
Olive, the conjugate condition may be assumed to arise on the mycelium before the primordium of the sorus develops, or at least in a very early stage of its development. In connection with the sori on the sheaths, stems, and sepals, the change from a uninecule to a binucleate condition would be expected to take place soon after infection, which would account for Olive having found uninecule mycelium only in small amount. On the leaves, however, the change would be expected to take place later, after the pycnia are formed, and hence, as Olive found to be the case, a considerable mass of uninecule mycelium would be expected.

Once the binucleate condition has been established in the mycelium there are no difficulties from a cytological point of view as to the spore form produced, since both aeciospores and teliospores arise from binucleate cells. It is very likely that the type of spore produced is determined by the nutritional conditions prevailing in the host. However, it should be borne in mind that the tendency to an unstable condition is quite certainly inherent in the rust itself and the nutritional factors are best interpreted as secondary, serving primarily to explain the situation as we find it and in view of our present, perhaps incomplete, cytological knowledge.

That telia are the first structures developed from infections on the sheaths, stems, and sepals is perhaps correlated with the influence of the rapid maturity of the tissues of these organs and the nature of the available nutrient. This influence was recognized by Olive. He used it, however, to explain why telia were formed instead of "secondary" aecia on the "perennial" sporophytic mycelium.

The occurrence of telia in association with the aecia on the leaf blades, which has been repeatedly mentioned, is readily correlated with Olive's cytological observations and can best be explained also by the factor of food relations. Olive finds that the aecia are borne on a binucleate mycelium. It would appear quite logical to expect that this same mycelium might produce telia when the factors which determine teliospore production become operative.

The development of telia in association with aecia usually occurs sometime after the majority of the aecia are mature. It would seem reasonable to suggest that the development of telia under these conditions may be due to the depletion of the local food supply by the mycelium during the development of the aecia and aeciospores, or directly to the more mature condition of the tissues. Perhaps, however, their formation is best explained by a combination of these two influences. In some cases the telia that develop in association with the aecia appear practically simultaneously with them. This is especially true when the lesion involves the midrib or one of the larger veins. In such cases the explanation is similar to that given above to account for the development of telia only on stems, sheaths, and sepals. The tissues of the veins and midrib are quite comparable in the character of the food supply to that of the stem. It has already been noted in the discussion of the life history that when mature leaves are infected with basiospores, telia may predominate over the aecia in the resulting lesions and in rare cases may be formed exclusively.

It has been repeatedly observed in connection with many long-cycle rusts, that as the hosts approach maturity there is a gradual reduction in the development of repeating spores and a corresponding increase in the development of the teliospores either in the same or separate sori. It would seem that this situation exactly parallels that in the Podophyllum rust except that since there are no repeating spores the teliospores developed in association with the aecia normally occur in separate sori. As noted in the discussion of the life history, however, teliospores may rarely develop in the aecial cups.

It is important to note, however, that only a few other cases have hitherto been observed where aecia have been replaced by telia. In most rusts the maturity of the tissues affects aeciospore production largely by reducing the number of aecia produced or the quantity of aeciospores. It has been repeatedly observed in the laboratory at La Fayette in connection with heteroecious culture work that when over-mature leaves or those in a poor growing condition are inoculated, while pycnia may be formed to some extent, few if any aecia develop. The fact that aeciospores are replaced by teliospores in the Podophyllum rust is therefore another indication of the plastic and unstable condition of this species.

The assumption that the conjugate condition arises at an early stage in the development of the mycelium can also be used to explain the fact that aecia may occasionally be developed without the pycnia. In such cases the change may have taken place...
very early, before a sufficient mass of mycelium has been formed to enable the pycnia to develop. Either aecia or telia might then develop on the mycelium as the first spore form following infection, dependent on the available food supply or the maturity of the tissues.

It is well to recall at this point that the telial sorus on stems, sheaths, and sepals strongly suggest the normal situation in microforms, and it is enlightening to review briefly the cytological situation which has been found in the species of short-cycle Puccinia and Uromyces which have thus far been studied.

It is convenient to consider these in two groups. In the first the primordium of the telium is made up of uninucleate hyphae, and the conjugate condition arises as a definite process, by cell fusion or nuclear migration, in a manner similar to that found in aecia. This is the general situation in the following species: Puccinia (Nephytites) transformans Ellis and Ev. investigated by Olive (5), P. (Polythelis) tinea (Pers.) Wint. by Favolini (7), P. malvacearum Bertero by Worth and Ludwig (8), P. buxi DC. by Moreau (4) and Uromyces scutellatus (Schr.) Lev. and P. rossiana (Sacc.) Lagh. by Kursanov (3). Uromyces laevis Körn., studied by Kursanov, probably also belongs in this group, but the actual process of the formation of the conjugate condition has not been observed.

In the second group the primordium of the telium is made up of binucleate hyphae, and the vegetative mycelium is also primarily binucleate. The transition from a uninucleate to a binucleate condition in these forms has unfortunately not been observed, but it is assumed by the authors that it occurs very early in the vegetative growth of the mycelium. This group includes Puccinia fergussoni Berk. & Br., P. asarina Kze., P. aegopodii (Schum.) Mart., P. conferta Diet. & Holw.? on Artemesia, and Uromyces gagae Berk. as investigated by Kursanov (3) and P. adoxae Fück. and Uromyces scillarum (Grev.) Wint. by Blackman and Fraser (2).

It should be pointed out that, except for Puccinia malvacearum, P. buxi, and P. rossiana, the species of the first group normally develop pycnia; while in those of the second group, without exception, pycnia are normally absent. Uromyces ficeariae Wint. is especially interesting in this connection. Blackman and Fraser (2) record that—

the general mycelium appears to exhibit single nuclei, but the mass of mycelial hyphae round about the teliospore sorus as well as those directly connected with teliospore formation appear to have conjugate nuclei.

They did not find how or where the change took place. Moreau (4), however, found all the mycelium uninucleate and the binucleate phase beginning in lower cells of the telial primordium. Kursanov (3), on the other hand, more nearly confirms the findings of Blackman and Fraser (2). He finds, however, only a small amount of uninucleate hyphae, the vegetative mycelium being prevalingly binucleate.

A more detailed review of the literature of this subject would show that in the first group there was considerable variation in the amount of sporophytic tissue developed between the origin of the binucleate condition and the formation of teliospores.

We apparently have, therefore, in some forms of short-cycle rusts a very short sporophyte, while in others the origin of the conjugate condition has arisen at varying distances back from the mother cell of the teliospores, sometimes only a little way, sometimes far, possibly even near to the point of infection by the gametophytic basidiospore.

It seems to the writers that the cytological situation, especially as found in the second group of species mentioned above, parallels very closely that described for P. podophyllumi by Olive. It is unfortunate that in these short-cycle forms the fusions have been observed only in the cases where the change takes place at the base of the telium. This circumstance, however, serves to point out the wealth of problems awaiting solution in this field, and it is hoped that students of cytology will soon give us the necessary link to make the chain of evidence complete.

PHYLOGENETIC CONSIDERATIONS

It is pertinent to ask what is the significance of the peculiar life history as shown by the Podophyllum rust. Without attempting at this time to go into the evidence which has determined their position, it may be stated that the writers of this paper are among those who believe that, regardless of what the most primitive rust may have been, the long-cycle species in the group Pucciniaceae at least are the older and that the short-cycle forms as exemplified by the microforms of Puccinia and Uromyces are derived or reduced forms and are relatively more recent.

It follows, then, that during the process of development from a long to
a shorter type of life history some intergrading conditions are to be expected. It is not at all surprising also to find evidences of a profound readjustment and an apparently unstable condition in the nuclear phenomena in some forms.

The writers believe that *Puccinia podophylli* is a form which still exhibits evidences of the sort of changes which may have taken place in the development of the reduced forms from the more complex.

**SUMMARY**

1. *Puccinia podophylli* shows no evidence of being perennial or systemic in the host.

2. The early crop of teliospores which occur on the bud scales, stems, and sepals preceding the aecia arise directly from mycelium produced by basidiospores from overwintered teliospores and are usually not accompanied by pycnia.

3. The aecia which normally develop on the blade of the leaf also arise in a similar manner from the same source. Pycnia are usually found associated.

4. The late or summer crop of telia are produced on mycelium developed from infection by aeciospores.

5. There is no evidence of repeating aecia in this species.

6. Basidiospores from either the early or late crop of teliospores may result in the production of either the early telia or the aecia.

7. Telia may develop in association with the aecial lesions and arise directly from the same mycelium.

8. When mature leaves are infected telia may predominate over the aecia, with or without the development of pycnia.

9. *Puccinia podophylli* exhibits evidences of being in an unstable or plastic condition as to life history.

10. It is suggested that the food conditions of the various tissues invaded have an important influence on the spore form developed.

11. This species is believed to be a form which still exhibits evidences of the sort of changes which may take place in the evolutionary development from the complex to the simpler forms of life history.

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