Synchytrium vaccinii*).

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With 10 textfigures.

Synchytrium vaccinii is a parasite of *Vaccinium macrocarpon* and other species of the *Ericaceae* and causes the so-called red rust or red gall disease of the cranberry. According to Halstead (1889), it was collected first by Smith, entomologist of the New Jersey Experiment Station, in 1886 in the Marian Cranberry Bog near Browns Mills, Burlington County, N. J. In July, 1887, Brakely sent some of the diseased plants to the Division of Entomology, U.S.D.A., where the galls were identified as those caused by a gall mite of the genus *Phytoptus*. However, in March, 1889, Thomas identified the etiologic agent as a species of *Synchytrium* which he named *S. vaccinii* without giving a diagnosis of it. Halstead (1889) visited the Marian Bog on several occasions and determined the abundance and distribution of the disease. He gave a lengthy discussion of the disease, reported its occurrence on other ericaceous species such a *Azalea viscosa, Chamaedaphne* (*Cassandra*) *calyculata, Clethra alnifolia, Gaultheria procumbens, Gaylusscia* sp., and *Kalmia angustifolia,* and described and figured the variations in the structure of the galls on different hosts. In the following years (1890, '91) he devoted considerable time to a study of the disease and means of controlling it. Subsequently, *Synchytrium vaccinii* was reported on *V. macrocarpon* in Michigan by Kaufman (1928) and in Nova Scotia by Conners (1933, '36, '37, '38, '39 and '48). Conners reported it on *Andomeda glaucophylla, Chamaedaphne baccata, Ledum groenlandicum,* and *Rhodora canadense* in addition to the hosts noted above.

However, according to hebarium specimens in the National Collections at Beltsville, Md., the herbarium of the University of Wisconsin, and elsewhere, *S. vaccinii* has been collected in several other localities: on *V. macrocarpon,* May's Landing, N. J. and on *Ledum*

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sp. and *V. macrocarpon* at Kingman, Me. (C. L. Shear and N. E. Stevens, 8-23-1917 and 9-26-1917, resp.); on *V. macrocarpon* at an unidentified locality in Massachusetts (H. J. Franklin, 9-3-1912); on *V. macrocarpon* at Bournedate, Mass. (A. C. Goheen, 6-16-1954) and on *V. macrocarpon* at Medford, N. J. (C. Varney, 6-4-1956). In this connection it may be noted that the specimen labeled *S. vaccinii* on *Vaccinum canadense* collected by an unknown person near Bozeman, Mont. is a rust. Also, the specimens on *Gaultheria procumbens* collected by R. Thaxter, Apr. 1921 at Kittery, Me. is not *S. vaccinii,* but may prove to be *S. aureum.*

Thomas described the thallus of the fungus as a resting spore which germinates in the spring and produces infective zoospores, altough he did not observe the manner of spore germination. Halstead also implied that the thallus is a resting spore but he referred to it frequently as a sporangium. Probably, as a consequence other students of the disease, including Shear (1907), Schwarze (1917), Shear, Stevens and Bain (1931) continued to describe the thallus as a sporangium. Tobler (1912), on the other hand, followed Thomas' description and interpreted it as a resting sorus or spore. Accordingly, from these conflicting descriptions it is impossible to tell whether *S. vaccinii* is long-cycled and develops sori, sporangia, zoospores and resting spores, or short-cycled and forms only resting spores and zoospores. Obviously, if it forms only resting spores which germinate as sporangia it is not a species of *Synchytrium.* Therefore, it is essential that *S. vaccinii* be studied carefully and fully before its relationships and proper classification can be determined.

In an attempt to solve these problems I made a search for *S. vaccinii* in June, 1956, at Browns Mills, N. J. where it was collected originally. No evidence of it was found there, but Dr. Charles A. Doehlert, Research Specialist of the Cranberry and Blueberry Research Laboratory at Pemberton, N. J. informed me that it had been found recently in another area. Under his direction a search in the Gardner Brother's bog of the Woolman Tract two miles south of Indian Mills, N. J. on U. S. Route 206 revealed an abundance of the fungus. Almost all cranberry plants in an area of approximately half an acre were heavily infected, and among these were *Chamaedaphne (Cassandra) calyculata* and numerous seedlings of *Acer rubrum* which were infected also. Infected leaves and petioles of these hosts were fixed in killing solutions in the field, and an abundance of herbarium material was collected. At the same time pieces of sod, 12" × 18", with heavily infected cranberry plants were dug up and transferred to the greenhouse at Purdue University for the purpose of observing the progress of the disease and development of the parasite. This sod has been maintained successfully under bog con-

ditions for more than a year, and the cranberry vines have spread considerably.

The same site in New Jersey was carefully examined again in June, 1957, and only a very few cranberry vines were found to be infected. Apparently, the degree of infection varies from year to year and may even disappear entirely in a specific locality. At least, this is suggested by G o h e e n's (1950) report that no evidence of the red gall disease could be found in the type location of *S. vaccinii,* at Brown Mills Junction, N. J. I searched this location also in 1957 without finding any diseased plants.

A morphological, cytological and developmental study of the parasite on the three hosts listed above has shown that *S. vaccinii* is a valid short-cycled species of *Synchytrium* which develops only resting spores in the so-called summer phase. The present contribution gives the results of this study and concerns the reaction of the host to infection, development and characteristics of the galls produced, and the developmental cycle of the parasitic so far as it is known.

As previous workers have described, *Synchytrium vaccinii* infects the epidermal cells of the leaves, young stems, petioles, flowers and fruits of the cranberry, and if these organs are heavily parasitized when young they become stunted and abort. The galls which result from the infection are brilliantly raspberry- or lavender-red, large in size, and have a characteristic shape so that their presence can be detected readily with the unaided eye. They are largely superficial on the host and somewhat chalice-like in shape, as Thomas pointed out. However, the basal portion is usually inflated instead of stalked as in a typical chalice, but occasionally it is constricted beneath the inflated region so that the galls appear to be stalked. The apical portion may be as tall as or taller than the base and consists of an outwardly-flared rosette of greatly elongate thin-walled cells (fig. 1). Accordingly, when viewed from above the rosette resembles a cup with a brillantly yellow spore in its bottom. Longitudinal sections of galls, however, show that the spore and host cell usually lie in the upper part of the gall base so that the infected cell is partially exposed, as shown in fig. 1. The gall shown in this figure is almost symmetrical with a slight basal constriction, but other galls may vary markedly in this respect. The basal portion may be mound-like or almost hemispherical without a constriction and sits directly on the surface of the host. In such cases the entire gall may be almost cylindrical in shape if the rosette does not flare-out markedly. Also, the height and degree of flaring of the apical rosette may vary markedly on *V. macrocarpon*, and as a result the galls may be quite variable in shape and size. H a l s t e a d (1889), also, noted that although the galls on the other ericaceous hosts may vary considerably,

they, nevertheless, have the same basic structure. On *Clethra alnifolia,* however, he found them to be much larger than those on other hosts and covered with numerous hyaline hairs.

As noted previously, the galls are comparatively large and may be 494 to 720 μ broad at the base by 520—830 μ high on leaves of the cranberry vines. The sheath at the sides of the infected cell varies from 2—5 cells in thickness, and the walls of its cells are not markedly thickened. Most of these cells are filled with a densely-stained material which may be continuous, reticulate or globular, so that the galls in stained sections have a dark and chromatic appearance. This material occurs abundantly in cells of healthy leaves also, and its occurrence is not due to the presence of the parasite. Accordingly, the material has been omitted from the cells shown in figs. 1—5. The surface of the cranberry leaf immediately opposite the gall may bulge out fairly often (fig. 1), and this appears to be due to division and outward elongation of the palisade or mesophyll cells or cells derived from them.

These galls are the result of the reaction of the host to the presence of the parasite, and their development involves cell enlargement and cell division. After the parasite enters an epidermal cell the latter begins to enlarge and protrudes slightly above the surface of the leaf. Within a short time the adjacent healthy epidermal, mesophyll or palisade cells are stimulated to divide, and thus form the rudiments of the sheath. As a result the enlarging and protruding host cell becomes enveloped partially by meristematic healthy cells with its outer surface exposed, and the incipient gall emerges as a low mound-shaped protuberance wih the large infected cell at its center. The cells derived from epidermis, palisade or mesophyll continue to divide and enlarge, so that the incipient sheath keeps pace with the enlargement of the host cell. The healthy cells immediately beneath the latter, however, appear to proliferate more rapidly so that the host cell is carried outward and eventually occupies the upper portion of the developing gall. Usually part of its surface is naked and exposed at the apex (fig. 2). At the stage shown in this figure development of the basal portion of the gall may be described as being complete, and this portion now protrudes conspicuously and may be almost hemispherical or highly mound-shaped. Subsequent growth results in the development of the cup-shaped apical rosette, which occurs fairly rapidly. The rosette originates from the outer or epidermal and inner sheath cells which begin to elongate upward and divide around the upper portion of the infected cell. The outer or epidermal sheath cells also may be involved in this process, but most of the rosette appears to originate from the inner apical sheath cells. These cells elongate and inflate fairly rapidly and seem to burst out of the apex of the gall, and in some cases the outer epidermal

layer may be pushed outward sligthly (fig. 4). An early stage of rosette development is shown in fig. 3, and here the outer cells have divided and been lifted up by the proliferating cells beneath. Figure 4 shows a later stage in which the inner sheath cells have elongated further, divided and become inflated at their apices. Also, they have begun to flare outward to produce the characteristic appearance of the rosette. These cells may divide after this stage, but the further development of the rosette appears to be due primarily to cell elongation. In tall mature galls such as shown in Fig. 1, some of the rosette cells may be up to 416 μ long. In the living condition they are comparatively thin-walled and distended with a lavender-red sap, but in fixed and stained sections they are filled with the densely-stainable substance noted previously. As the galls attain maturity and dry out, the rosette usually collapses inward on two sides and thus partially closes up the conical opening or crater to the infected cell. The two collapsed sides of the rosette look like lips with a narrow slit between.

As noted earlier *S. vaccinii* has been reported only on species of the family Ericaceae, and for this reason mycologists have regarded it as being limited to one family of hosts. In the Gardner Bog, however, it was found on numerous one- and two- and a few three-year old seedlings of *Acer rubrum,* a host far-removed from the *Ericaceae* in most systems of classification. Numerous galls in various stages of development and abortion were present on the stems, petioles and lower leaves, and these had the same raspberry or lavender-red color characteristic of galls on *V. macrocarpon.* Examination of the stems of 3-year old seedlings showed areas where they had been infected in previous years. Scars and remnants of aborted galls were present in such areas and looked like those illustrated by Halstead (1889) on the older portions of stems of *V. macrocarpon.* Several of the young maple leaves were heavily infected, wrinkled, curled and stunted in growth. A cytological study of such leaves showed that *A. rubrum* is susceptible to infection but resistant to the development and maturation of the parasite. As a result very few, if any, mature and viable resting spores are formed on this host. Galls of various sizes, shapes and degrees of complexity develop from infections, but the thallus of the parasite aborts, dies, and may be eliminated from the host before it reaches maturity.

Although infection has not been observed in *A. rubrum* the zoospores apparently infect the epidermal cells of young leaves and petioles as they emerge in the spring. Such cells as well as adjacent healthy ones react in the same manner as described for *V. macrocarpon* and form the rudiments of the gall. This is illustrated in fig. 5 which shows the enlargement of the infected cell and recent division of the adjacent epidermal, mesophyll and palisade cells. Even at this

early stage the cytoplasm and nucleus of the host cell appear to be clumped, necrotic and degenerating around the parasite, and probably as a result the latter is deprived of nourishment and may not develop much beyond this stage. In such instances abortion seems to be necrotic and acute, and further host reactions may be limited to the discharge of the infected cell and fungus from the host. So far as is known at present, acute necrotic abortion seems to be comparatively rare, and in most cases studied abortion was chronic with fairly extensive host reaction and gall development. Such galls may develop to a height and breadth of 200 and 250 μ, respectively, and in some instances become almost as large and complex as those on *V. macrocarpon*. A young gall illustrating early stages of chronic abortion is shown in fig. 6. The infected cell lies in the apex, but is not fully enveloped by the sheath. The outer sheath of epidermal cells is fairly well developed but its cells are not much larger than normal ones. Apparently, this layer keeps pace with the outward enlargement of the gall by frequent division of its cell, as is suggested in fig. 8. The inner sheath cells, on the other hand, begin to enlarge and elongate outward, and this is the first indication of the mechanism by which the infected cell and parasite are eventually eliminated or discharged from the host. This elongation apparently carries the infected cell outward so that it occupies an apical position in the gall. In this figure also the host cell protoplasm appears clumped and necrotic, but the parasite seems to be normal in appearance except for the irregular nucleus.

A larger gall on *A. rubrum* is shown in fig. 7 in which the cells under the infected one have divided several times and enlarged to form a meristematic mass of cells. Here also the cells immediately beneath the host cell have elongated outward and invaginated its lower surface. The parasite fills the host cell completely and its nucleus and cytoplasm appear abnormal and necrotic. In other galls studied the elongation of the inner sheath cells was very pronounced as shown in fig. 8. In this gall the outward growth of these cells has caused the infected cell to collapse, and reduced it to a lunate cavity in the apex of the gall. The parasite also has collapsed and appears to be dead. Eventually, the host cell and parasite may degenerate intact, or the cell may rupture and release the content of the fungus as shown in fig. 9. Whichever happens, a brown scar or necrotic area remains at the tip of the gall which heals spontaneously. As noted earlier, gall development may proceed even further to the formation of the apical rosette, and in such instances the galls may look somewhat like those of *V. macrocarpon*. This occurs fairly frequently, but few of the galls are symmetrical. The rosette may develop partly or completely on one side and be lacking on another so that the galls are irregular in shape. Fig. 10 shows the upper portion of a gall whose

upper part has proliferated to form an abortive rosette. The shriveled infected cell and its degenerated parasite have been carried upward and lie at the apex of the elongate cells. In other galls they may lie at one side of the rosette. Some large galls may contain spores, but these are small, usually hyaline and aborted. In all galls studied so far no mature and normal spores were found, but it is not improbable that such spores may be formed occasionally. It should be noted here that the infected cell, parasite and gall may abort occasionally on *V. macrocarpon* also.

Conners (1939) reported that specimens of *Synchytrium* on *Rubus hispidus, Amelanchier,* sp., *Ilex* sp., and *Spirea* sp. were collected in the same bog with *S. vaccinii,* and in a note accompanying the specimens Dr. Groves stated that they might be *S. aureum* instead of *S. vaccinii.* My discovery that the latter species will infect *Acer rubrum* suggested that these specimens might be *S. vaccinii* also, and through the generous loan of material by Drs. I. L. Conners and D. B. O. Savile I had the opportunity of studying them. This study showed that they are all *S. vaccinii.* Most of the galls were aborted in varying degrees, but in general they were more extensively developed than those on *Acer rubrum.* A few of them had a well-developed and flared rosette at the apex, and resembled very closely those on *Vaccinium macrocarpon.* Also, most of them contained small hyaline to light-yellow resting spores which were aborted in varying degrees, and I doubt that any of them were viable. At least, all attempts to germinate them failed.

This study has swohn thus that *S. vaccinii* is not limited in host range to species of the *Ericales* and that it may infect members of the *Aceraceae, Rosaceae, Aquifoliaceae,* and *Primulaceae* as well. Quite likely, many other hosts will be found to be susceptible to infection when extensive host range studies are made. So far as is known, however, all hosts besides species of the *Ericaceae* appear to be resistant to the development of the parasite, although they are readily susceptible to infection.

The reactions of *A. rubrum* and the other non-ericaceous hosts noted above are similar in many respects to those described by Kusano (1929), Köhler (1927, 1931), Karling (1954), Sinski (1956), and Hartmann (1958) for other resistant or immune hosts which are parasitized by *Synchytrium* species. However, acute or almost immediate necrotic abortion of the parasite and host cell does not occur very often on *A. rubrum* so far as is known at present. Instead, abortion is commonly chronic. The parasite usually develops to considerable size before degeneration begins and this is accompanie by slight to extensive gall development. Eventually, the host cell and parasite are eliminated or discharged primarily by elongation of the inner sheath cells. So far no necrosis of the sheath cells such

as occurs in resistant potato varieties has been observed. In the potato variety, Prussian, infected by *S. endobioticum,* for instance, Köhler noted that the adjacent cells become necrotic and filled with gummy degeneration products. These eventually die and thus cut off the food supply of the infected cell and parasite. In *A. rubrum* also many of the sheath cells are filled with a dense, deeply stainable substance (fig. 5—9), but its presence apparently is not due to infection by *S. vaccinii.* Such material occurs commonly in fixed and stained sections of healthy uninfected leaves of *A. rubrum.*

As noted earlier, *S. vaccinii* is a short-cycled species and develops only resting spores in the so-called summer phase. These spores develop in the same manner as those of other species of the subgenus *Pycnochytrium* so that the process need not be described in detail. After the parasite enters the epidermal cell it increases in size as the host cell enlarges and finally becomes a typical resting spore. At maturity it almost fills the host cell completely (fig. 1—6) and is enveloped by a sparse or fairly abundant amount of reddish-brown residue. Its wall is smooth, light-amber, comparatively thin, and its content is brilliantly chrome- to lemon-yellow.

In cranberry bogs the infected leaves and their galls fall to the ground in the late summer, fall and winter, and the spores apparently germinate within the disintegrating galls during the spring months. The custom of flooding the bogs in the winter to prevent desiccation of the cranberry vines and the subsequent freezing over appear to be conducive to maturation of the spores and their germination in the spring. Certainly, flooding the bogs creates ideal conditions for zoospore dissemination and quite probably almost countless zoospores are present in the water as the new leaves of the host emerge in the spring. In Nova Scotia Conners (1939) reported that infection of the unopened terminal buds of *V. macrocarpon* takes place prior to or on May 23. However, when a bog was reflooded for 48 hours on July 5 and 6, infection was evident on July 15, ten days later. This suggests that the resting spores may germinate at any time in nature when temperature and moisture conditions are favorable.

However, it is not certain what are favorable conditions for germination. During the past three years all attempts to germinate the spores outdoors and in the laboratory at Purdue have failed. Mature spores were dissected out of the galls onto wet filter paper, flooded with charcoal-treated water in petri dishes, and kept in a refrigerator for several weeks to simulate winter temperature conditions in cranberry bogs. These were thawed out in the laboratory and observed for several months, after which the same treatment was repeated twice. Also, spores in petri dishes were kept outdoors over the winter during which time they were subject to freezing and thawing.

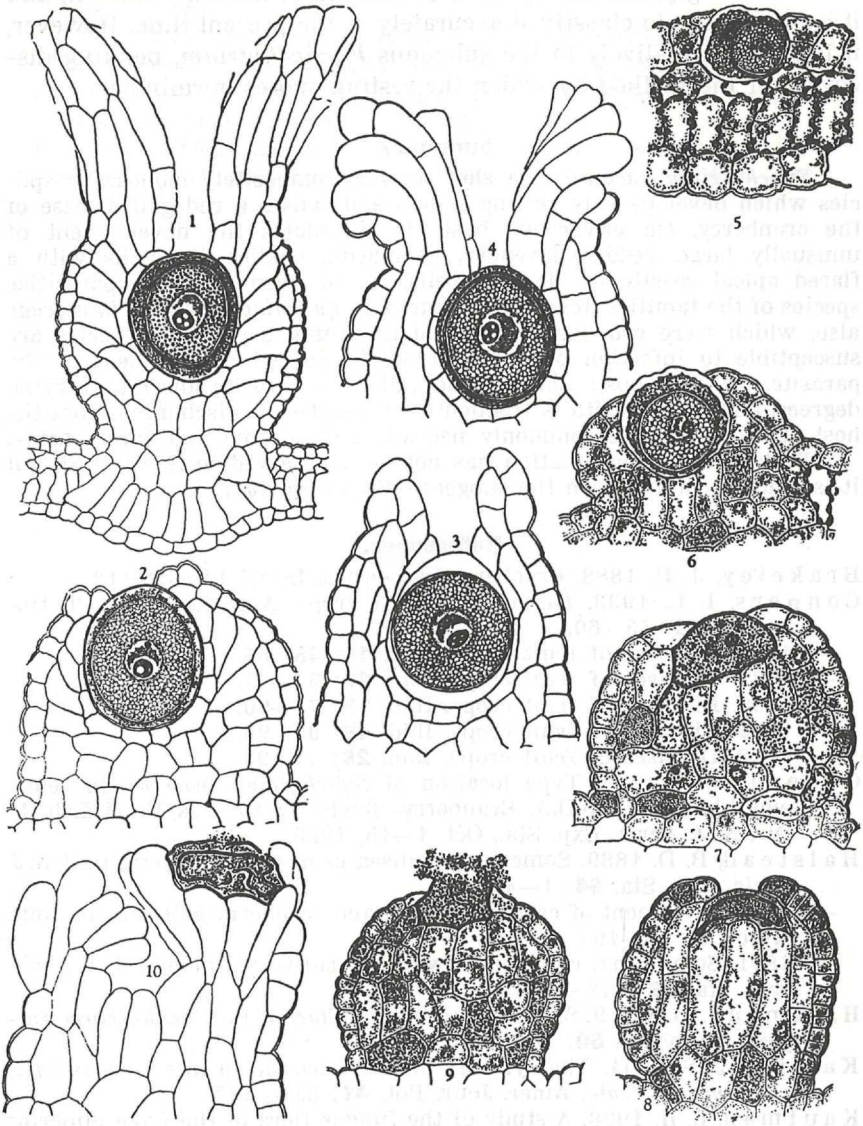

Fig. 1—10. *Synchytrium vaccinii.* Fig. 1. Longitudinal section of mature gall on *V. macrocarpon.* X124. Fig. 2. Young gall before development of apical rosette. X124. Fig. 3, 4. Stages in development of apical portion of gall. X124. Figs. 5, 6. Stages in abortive gall development and abortion of infected cell and parasite on *Acer rubrum* X140. Fig. 7, 8. Expulsion of infected cell and parasite by elongation of central sheath cells of *Acer rubrum.* X140. Fig. 9. Rupture of infected cell and parasite at apex of abortive gall on *Acer rubrum.* X120. Fig. 10. Remnants of infected cell and parasite at tip of apical rosette on *Acer rubrum.* X140.

Accordingly, the life cycle of *S. vaccinii* is not fully known, and it is impossible to classify it accurately at the present time. However, it is placed tentatively in the subgenus *Pycnochytrium,* pending discovery of the method by which the resting spores germinate.

Summary.

Synchytrium vaccinii is a short-cycled, compositely monogallic species which develops only resting spores and causes a red gall disease of the cranberry. On ericaceous hosts it stimulates the development of unusually large, reddish-lavender, composite, chalice-like galls with a flared apical rosette of elongate cells. It has been found to parasitize species of the families *Aceraceae, Rosaceae, Aquifoliaceae* and *Primulaceae* also, which were growing in infected cranberry bogs. Such species are susceptible to infection but unfavorable to normal development of the parasite. On such hosts most of the galls and parasite abort to varying degrees, and the parasite is frequently eliminated or discharged from the host. The abortion is commonly necrotic and chronic and rarely acute.

Resting spore germination has not been observed in *S. vaccinii,* but it is placed tentatively in the subgenus *Pycnochytrium.*

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