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A Consideration Of The Physiology And Life History Of A Parasitic Botrytis On Pepper And Lettuce

George L. Peltier
University of Nebraska-Lincoln

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A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC BOTRYTIS ON PEPPER AND LETTUCE.[1]

BY GEORGE L. PELTIER.

INTRODUCTION.

During the autumn of 1911, while gathering the peppers *(Capsicum)* in the vegetable plat of the Missouri Botanical Garden, the writer observed a fungus which was appearing as a parasite on the fruits. The peduncles were covered with a dark brown mass of conidia and mycelium, which so weakened the tissues that the fruit soon fell to the ground. On opening the peppers a number of large, flat, crust-like sclerotia, 1 cm. long and .5 cm. wide, were found to fill the interior. In most cases the seeds were also covered with the crust-like masses. The conidial stage was identified as *Botrytis cinerea* Pers. (Botrytis vulgaris Fr.). Although *Botrytis cinerea* has been observed parasitic on a large number of hosts, this is the first time it has been reported as a parasite on the peppers.

Later, while carrying on infection experiments, it was introduced into one of the vegetable greenhouses, where it became quite a serious disease of the lettuce, causing the characteristic "drop" and a "damping-off" of the seedlings. Thus a very good opportunity was afforded for the study of the development of the parasite and its action on several hosts.

HISTORICAL.

By using a part of the artificial classification suggested by Duggar (17), the genus *Sclerotinia* may be divided into four distinct groups:—

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[1] Abstracted from a thesis presented to the Faculty of Washington University, in candidacy for the degree of Master of Arts, June, 1912.

1. Species comprising in their life cycle not only apothecia, but also a monilia stage, *i. e.,* with conidia produced in chains, the latter frequently separated one from another by special structural devices known as disjunctors.[2]

This group may be further divided into two subheads:—*(a)* Species in which both spore forms may be produced upon the same host; *(b)* species whose life cycle is not complete on a single host.

2. Species which may embrace a form of *Botrytis* as a conidial stage. Under this head we may include forms which produce sclerotia and which germinate to produce conidia directly, the apothecial stage having been entirely lost.

3. Species in which no conidial stage has been convincingly demonstrated.

4. Species which produce neither conidia nor apothecia.

Under *(a)* of the first group may be included forms like *S. vaccinii* (Wor.), Rehm. on *Vaccinium vitis idaea, S. oxycocci,* Wor. on *V. oxycoccus* and *S. fructigena,* Schröeter on *Prunus,* whose life history and development have been so well worked out by Woronin (69). Under *(b)* may be included *S. heteroica,* Wor. and Nawasch. on *V. uliginosum* and *Ledum palustre* and *S. rhododendri,* Fischer on *Rhododendron ferruginosum* and *Rhododendron hirsutum.* It is interesting to note that these fungi are truly parasitic, but that in all forms attacking species of *Vaccinium,* infection takes place through the flower where the spores, while germinating can live saprophytically on the stigmatic exudation.

The second group includes only one species, *S. Fuckeliana* DeBary, which in its conidial stage is the well-known facultative parasite, *Botrytis cinerea* Pers. De Bary (15) first

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[2] Thom, in a recent paper, throws a new light on the origin of these devices. He states that the newly-formed conidium is cylindrical, and quickly swells to assume a spherical or elliptical form. During the process the connective appears when the primary wall of the original tube fails to follow the change in form and leaves an open space between itself and the new walls of the adjacent conidia in the chain. (The connective between conidia of *Penicillium.* Science, **35** : 149. 1912.)

connected the conidial stage of *Botrytis* with the apothecial form produced from the sclerotia of *Botrytis* on grape. However, up to the present time this connection has been disputed, as many observers have failed repeatedly to secure, under favorable conditions, the perfect stage from sclerotia produced by *Botrytis*. The utmost confusion exists since many authors have tried to connect the botrytis stage with *Sclerotinia Libertiana* Fuckel, whose conidial stage has probably been completely lost.

In Europe, *Botrytis cinerea* is important as the cause of a disease on a number of hosts, but it is especially destructive, at times, on the leaves and fruit of the grape. Pirotta (39), in 1881, again connected *Sclerotinia Fuckeliana* with the imperfect stage of *B. cinerea,* and was also able to prove that *S. Libertiana* was distinct from the above forms. The material was found on the fallen leaves and fruit of the grape. The work of Pirotta can be summed up by adding the following table, which he gives in his summary:—

ASCOSPORE

1. Sclerotia	1. Sclerotia	1. Botrytis (mycelium)
2. Botrytis (mycelium)	2. Ascospores (apothecia)	2. Sclerotia
3. Botrytis (conidia)		3. Botrytis (mycelium)
4. Sclerotia		4. Botrytis (conidia)
5. Ascospores (apothecia)		5. Sclerotia
		6. Ascospores (apothecia)

This table of Pirotta, starting with the ascospore, represents the three lines of development which he was able to trace.

Brefeld (9) at first accepted the results of De Bary, but later he discredited them, being unable to obtain apothecia from the sclerotia after many attempts, germination resulting always in the production of conidia. Viala (61) and Ravaz (43), in France, and Brizi (10), in Germany, described a rot of the grape shoots and grafts due to *Botrytis*. Numerous sclerotia were produced on the stems. Later, in 1897, Prillieux (41) was able to produce ascospores from the sclerotia formed on the shoots of grape. Tubeuf (61), after a number of attempts, obtained only conidia, while Zopf (71) found that he was able to produce apothecia after the sclerotia remained dormant at least one year.

During the next few years a number of writers in France, Germany and Italy described maladies affecting different parts of the grape plant. Finally, in 1905, Istvanffi (28), in an excellent paper, established the connection of *Botrytis cinerea* with *Sclerotinia Fuckeliana*. The author finds that the botrytis form of the fungus lives saprophytically on fallen leaves, twigs and fruits, during the early season. The conidia develop with great rapidity on the arrival of warm, moist weather and, when transferred to the growing foliage, germinate to produce a parasitic mycelium. The *Botrytis* attacks the ripening fruit in autumn, often causing great loss. Sclerotia are formed abundantly on the fruits and also on the cuttings made during the summer. The fungus may be carried over the winter in stratified cuttings and young shoots.

Istvanffi finds that the sclerotia develop on every part of the plant, appearing as small yellow papilla, consisting of a gelatinous mass which soon becomes hard and darker in color. The mycelium passes through the wood by way of the medullary rays, and sclerotia may be formed under the bark of the stems and shoots.

It is sufficient to say that the sclerotia are of the cartilaginous type, composed of two parts, a cortical layer, formed by the swelling of the filaments at the surface, and the pseudoparenchyma inside, formed by the coalescing of the mycelium. During the formation of the sclerotia large drops of a colorless fluid are exuded. In addition to the larger sclerotia, small cone-shaped, striated pseudo-sclerotia are formed which, after a brief period of rest, germinate in the fall to produce large tufts of conidiophores.

The sclerotia germinate in the fall or late spring in one of two ways; either producing conidiophores from the swollen branches of the hyphae or from the stromatic tissue, which is formed as a sort of pseudo-parenchyma on the surface of the sclerotia.

The production of apothecia from sclerotia Istvanffi found to take place anywhere on the surface, from two to six developing from a single one. The apothecia arise by the breaking down of the cortical layer and the pushing out of the swollen hyphae in parallel tubes. At this point the hyphae grow out and branch, forming the cup of the apothecium. The process takes place, in some cultures, in less than nine days after the sclerotia are placed in moist sand. Repeated infection experiments by the above author with ascospores resulted in failure, although they germinated in water. However, by using bits of the apothecia he was successful even on the leaves and fruit of the grape.

The origin and functions of the organs of attachment are discussed at length. The author finds that they serve several purposes, such as attaching the fungus to the host, breaking the cuticle of the fruit and stem and forming, together with the mycelium, tufts which are able to live over the winter.

A disease of hemp, similar to that of lettuce, has been reported from Europe on several occasions. The infected stems are permeated with mycelium, which soon causes the entire collapse of the plant. Behrens (5) found both *Botrytis cinerea* and *S. Libertiana* present on the above host, and at first believed them to be stages of the same fungus. However, he subsequently concluded that the two forms were probably distinct. Tichomiroff (58) observed a disease of hemp having the same symptoms which he attributed to a new *Sclerotinia,* called by him *S. Kaufmaniana.* De Bary was able to infect the hemp plants with the ascospores of *S. Libertiana,* and decided that the disease described by Tichomiroff was nothing more than *S. Libertiana.* Later Hazlinsky (25), in Hungary, and Hiltner (26), in Germany, noticed a disease of the hemp identical with the above. In both cases the disease was attributed to *Botrytis,* no sclerotia appearing on the plants. It would seem that we have a case where both forms, *Botrytis* and *S. Libertiana,* are associated, but entirely distinct.

In a like manner Frank (18) noticed a disease of rape, and found both *Botrytis cinerea* and *Sclerotinia Libertiana* present, consequently concluding that the two stages were of the same fungus. De Bary, however, discredited Frank's conclusion as to the connection of the two stages. It appears that, like the disease of hemp, we have the two fungi associated, though distinct.

Again, in a rotting of the stems of the potato in various parts of Europe, the same confusion exists. After reviewing the literature on this subject the identical conclusions were reached; that is, the two forms, *Botrytis* and *Sclerotinia Libertiana,* although causing the same symptons and generally associated, are always distinct forms.

In Holland the confusion of the different forms is still greater, as a separate species is made for every new host. However, here again the two forms stand out distinct. They are associated on the same host, causing a widespread disease of the tulip and other bulbous plants. The *Botrytis,* which is not typical *B. cinerea* on account of the short branching conidiophores which it produces, is known as *B. parasitica* Cav., while the sclerotia, ascribed to *S. bulborum* Wak., resemble *S. Libertiana.*

Other epidemics, in Europe, of some magnitude have been ascribed to attacks by *Botrytis* on a number of fruits, vegetables and greenhouse plants resulting, in most cases, in a complete rotting and collapse of the host.

A number of diseases caused by *Botrytis* alone have been reported from Europe at various times, the lily disease in England, observed by Ward (66), is one of the best known examples. The *Botrytis* attacking the lily, while not typical *B. cinerea,* is at least of the *B. cinerea* type. Kissling (31), in the same year, reported at length on *Botrytis* as a parasite on *Gentiana lutea,* the horse chestnut and a number of greenhouse plants.

Under the name *B. Douglasii,* Tubeuf (59) describes a disease of the Douglas fir *(Pseudotsuga Douglasii)*. It is characterized by the withering, curling up and death of the shoots toward the tips of young seedlings and the lower twigs of older trees. In the fall, sclerotia about the size of pinheads break through the epidermis under the old bud scales and on the needles. When placed in a moist chamber tufts of conidiophores immediately arise from the sclerotia. The conidia germinate directly in water and infect young plants, which are killed in a few days. Tubeuf also reports this same fungus on *Prunus triloba* and *Juniperus communis.*[3] Ritzema Bos (45) found that *B. Douglasii* was parasitic on a number of conifers. Smith (48), while working in Europe, observed a similar infection on the linden, attributing it to *B. cinerea.*

Brooks and Bartlett (12), in England, described a disease of the gooseberry due to *Botrytis,* its chief characteristic being the ability to penetrate the woody tissues of the plant. The first indication of the trouble is the wilting of the foliage, followed by the death of the branches attacked. Sclerotia were found beneath the bark, and in some cases conidia.

Botrytis cinerea seems to be the less frequent cause of the lettuce "drop" in the greenhouse, although it is a more serious parasite than is generally supposed. Since Humphrey (27) reported it in Massachusetts in 1892, it has been observed in at least ten states, causing more or less loss. The disease is especially destructive in the lettuce-growing centers along the Atlantic coast. Up to 1900, *Botrytis cinerea* was supposed to be the sole cause of the "drop." However, Smith (48), who had been working on the problem for a number of years, found that most of the "drop" in Massachusetts was not due to *Botrytis,* as generally believed, but to *S. Libertiana.* Up to the present time there is still considerable doubt as to which form is the primary cause, although it seems that both fungi are capable of infecting the lettuce under favorable conditions.

Humphrey (27) described a typical attack of *Botrytis* on

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[3] From his figures, *B. Douglasii* appears to be identical with *B. cinerea.*

greenhouse lettuce, and from his description there is no evidence whatever that *S. Libertiana* was present. In the same year, Jones (29) in Vermont mentions a lettuce rot due to a *Botrytis*. Under the term "mildew," Taft (57), in 1894, gives an account of a lettuce disease, attributing it to a species of the same genus. Galloway (19) states that "wet rot," produced by *Botrytis*, is the result of too much moisture. Bailey (2) believed that the lettuce "rot" was caused by *Botrytis*, which is at first saprophytic on decaying matter in the soil, but under conditions unfavorable to the plant, attacks it and acts as a parasite. He figures a plant infected with the fungus which is characteristic of the "drop." Selby (47) mentions a disease as lettuce "rot," attributing it to *Botrytis*.

Stone and Smith (54), in 1897, describe a "drop" due to *Botrytis*. Their description of the disease agrees very well with that of Humphrey. Again, in 1898 and 1899, they refer to the lettuce "drop", still attributing it to the same fungus, which produced a number of small flat sclerotia on the infected plants and in the cultures. Garman (20) at this time refers to a "rot" of lettuce due to *Botrytis* and other organisms. It is interesting to note that the disease causing the so-called "drop" was in all cases attributed to *Botrytis cinerea*.

Smith (48), in 1900, in a paper in which he brings together a large number of observations, concludes that there are two distinct forms which cause the lettuce "drop." The first he designates as the Botrytis type which, besides causing the "drop," produces a damping-off of the young seedlings. The fungus growing in the cultures and on the plant produced an abundance of conidia with but little mycelium. Small sclerotia were formed in large numbers, both in the cultures and on the infected plant. The second type he refers to as the "no-Botrytis" type, belonging to the fungus *S. Libertiana*. This form produces a cottony mass of mycelium with sclerotia, but no conidia. Infection takes place directly by the mycelium. Smith, unable after numerous attempts to germinate the sclerotia, was of the opinion that the bulk of the disease is due to a degenerate form of this no-Botrytis type, while *Botrytis* causes the "drop" only in rare instances, on account of its inability to attack vigorous plants under normal conditions.

Ramsey (42), several years later, described a lettuce "drop" caused by *Botrytis* during the fall in one greenhouse and by *S. Libertiana* in late winter, in a second. The former was characterized by a sort of watery decay, followed by the production of conidia, while the latter produced an abundance of sclerotia and a loose cottony mycelium.

In the past year Stevens and Hall (53) attribute the lettuce "drop" in North Carolina to *S. Libertiana.* They were able to germinate the sclerotia readily and found that infection was brought about by the ascospores as well as by the mycelium. Both ascospores and mycelium are comparatively short-lived, even under favorable conditions, so that they do not, to any great extent, serve as a means of carrying this fungus over considerable periods of time. The sclerotia, on the other hand, are long-lived and are capable of carrying the fungus over unfavorable seasons, so that the ascospores are able to produce infection on the next crop of lettuce. Here, also, the ascospores are for a short time, at least, saprophytically nourished before they infect the plants. Contrary to Smith's observations, the mycelium did not appear abundantly on the soil and was unable to migrate any distance through it. No mention whatever is made of the fungus *Botrytis cinerea.*

Botrytis cinerea has been reported several times in this country on greenhouse plants. Atkinson describes and figures a *Botrytis* parasitic on carnations. Halsted (22) remarks that *Botrytis,* previously nourished on the blossoms, is the cause of the decayed patches upon many greenhouse plants. Spaulding (52) described carefully the disease on chrysanthemums, *Euphorbia pulcherrima* and *Primula obconica grandiflora.*

The two best known fungi which may be included under the third group are *S. Libertiana* Fuckel and *S. trifoliorum* Eriks. The latter has always been regarded as an entirely distinct form with no conidial stage. It is occasionally very destructive to species of clover *(Trifolium)*. It is regarded by some to be identical with *S. Libertiana.*

The utmost confusion exists with regard to *S. Libertiana,* and it is generally associated with *Botrytis.* However, we may accept De Bary's work as conclusive that the former is always distinct and has no conidial stage whatever. In Europe it has been reported on a large number of hosts. In the United States it is certainly one of the causes of the lettuce "drop."

S. rhizoides may be taken as an example of a *Sclerotinia* which produces neither conidia nor apothecia. Stout (56), in a recent publication, states that in no way has he been able to induce germination of the sclerotia.

MORPHOLOGY.

As has already been mentioned, the first indication of the disease on the pepper is the wilting and subsequent browning of the leaves and stems. Later the stems show numerous tufts of conidiophores of *Botrytis cinerea* bursting through the bark. Careful examination shows that the conidiophores arise from the mycelium which permeates the stem. The peduncles of the fruits are also covered with a dark mass of conidia. About this time the tissues of the peduncle are so weakened that the fruits fall to the ground. Mycelium and conidia are present at the point of attachment, but otherwise the fruits seem to be quite healthy. However, on opening the fruits, a number of flat sclerotia are found filling the interior. Little mycelium is present. The sclerotia are very large; some of them, in fact, being over 1 cm. in length and half as wide. These were preserved during the winter and attempts made to germinate them.

When the stems, from which the conidiophores of *Botrytis* arise, are cut transversely, a characteristic discoloration of the wood is evident. The whole stem, with the exception of a narrow peripheral zone of light color, is stained a dark brown. Upon cutting longitudinal sections of the diseased stem, the well-marked hyphae of *Botrytis* are seen ramifying abundantly. Very few sclerotia are produced in the stems.

Since the infection occurred late in the year, it was impossible to make further observations as to the parasitic nature of the fungus. There can be no question but that the warm, wet weather of October had some influence on the virulence of the disease. The *Botrytis* was obtained in pure culture and it was while trying infection experiments on young seedlings of the pepper that it was introduced into the greenhouse, causing a destructive disease of the lettuce.

The vegetable greenhouse was given over almost entirely to the growing of lettuce during the winter months. The epidemic was first observed in February, the most obvious symptoms being the drooping of the outer leaves, followed in a day or so by the complete collapse of the plants into a slimy mass. The first evidence of the disease was a number of small brownish spots on the stem next to the ground and on the lower leaves. This was soon followed by the wilting and drooping of the outer leaves. A day or so later the whole plant was affected, the outer leaves falling to the ground, only the central head remaining erect. The leaves at this stage had the appearance of being scalded. A few days later the whole plant collapsed into a fetid, slimy mass. The above description is characteristic for most of the infected plants.

At times other plants, to all external appearance healthy, fell to the ground. In this case the fungus worked its way up through the stem into the center of the head, developing there and finally working its way out to the exterior. This method of infection was rather common. Again another type was occasionally noticed, one side of the plant rotting away, while the opposite side remained unaffected.

No conidia are produced until marked decomposition has been brought about within the leaves by the mycelium. The conidiophores are visible to the eye as small brownish masses scattered over the decayed parts. The most profuse development of the conidia takes place on the under side of the leaves and in other protected places where there is an abundance of moisture.

Little mycelium is to be found on the decayed plants, although mycelium with conidia was abundant on the soil throughout the beds, growing on dead organic material. On examining the tissues of the lettuce, the mycelium appears comparatively large and coarse, varying in diameter somewhat, with many septa and no regular method of branching, except when forming organs of attachment. The latter are formed abundantly in cultures and on the plants, appearing as numerous short branches from near the tip of an ordinary hypha. Each primary offshoot produces branches that again branch, thus forming a compact tuft. At first they are continuous with the main axis, but later become separated by a wall. On touching any object they flatten at the tips, forming disk-like appressoria which adhere to the surface.

The sclerotia do not develop until the lettuce plant has practically decayed. They vary in shape, the smaller ones being spherical, while the larger are, as a rule, irregularly cylindrical or flat. In size there is also much variation, the smaller ones being no larger than the head of a pin, the larger ones the size of a pea. The number of sclerotia produced on one plant varies considerably; fifty-two, unequal in size but all well-developed, were found on one plant. The sclerotia are of the cartilaginous type with an outer black cortical layer and in an inner white, closely packed pseudo-parenchyma, formed by the pressing together of the mycelium.

Sclerotia obtained from plants produced only tufts of conidiophores, no apothecia being obtained. Sclerotia left lying on the soil in the houses gave the same results. The conidiophores are produced by the growing and pushing out of the ends of hyphae in the pseudo-parenchyma, and may be formed on any part of the sclerotium. The conidiophores and conidia which arise in this way are in no respect different from those arising directly from the mycelium.

On moist sand the sclerotium germinates in about two days, the development of the conidia being very rapid. This method, then, is efficient in the production of rapid infection, while the sclerotia serve to tide the fungus over adverse conditions.

Young lettuce seedlings, planted in flats, were on several occasions destroyed by *Botrytis*. Here the symptoms were practically the same as in the "drop," except that the stems were encircled near the ground by the mycelium and cut off, producing a typical "damping-off." Conidia and a few sclerotia were developed from the mycelium, which lives saprophytically on the dead tissues of the fallen seedlings.

In the same manner, tomato seedlings in a number of flats were destroyed by the fungus, causing the characteristic "damping-off."

In November the poinsettias *(Euphorbia pulcherrima)* were infected by a *Botrytis*. In general, the symptoms of the disease were similar to those described by Spaulding (52), the flowers, green leaves, and in some cases even the colored leaves of the involucre being attacked. The fungus seemed to flourish on the stigmas, the spores, after germination, apparently living for some time saprophytically on the stigmatic exudation before becoming parasitic. The mycelium soon permeated the flower clusters, which fall off after a short time. In falling, the flowers strike the leaves and in some cases adhere to them, bringing about conditions favorable for new infections. On the leaves the disease first appears as light brownish spots. The fungus seems to attack the leaves in such a manner that the latex of the plant is excreted through the diseased spots and hardens on exposure to the air. Here again the mycelium first lives as a saprophyte and later becomes parasitic. The excretion of latex is the most characteristic symptom of the disease, as it is found on all plants attacked.

Another peculiar characteristic of the disease is that the infected areas are localized, considerable portions of the leaf remaining free from the parasite. Finally the petiole becomes weakened and the leaf drops off. In this way the whole stem is defoliated, leaving only the red-colored leaves at the top, which are less easily affected. In the later stages, conidia arise from the diseased areas. This *Botrytis* did not appear to be typical *B. cinerea,* because of its low manner of growth and the short branching conidiophores. However, when the fungus was obtained in pure culture and grown on suitable media, sclerotia were produced which showed that it was *Botrytis cinerea.* It also resembles in many ways the *Botrytis* described by Ward, which is in all probability a form of *B. cinerea.*

During the month of May the *Botrytis* in the greenhouse, where the infection experiments were tried, was in some manner carried over to a neighboring house given over to the growing of garden beans, where, under conditions favorable to its development, it attacked the flowers and pods. The spore germinating on the flowers developed into a parasite, and the diseased flowers falling on the pods rapidly spread the disease. Several times during the spring *Botrytis* has been observed on plants killed by a light frost; here conidia were formed abundantly, but no sclerotia.

PHYSIOLOGY.

The fungus was obtained in pure culture by plating on potato agar. From a rather abundant growth of mycelium conidia were transferred to suitable media in test tubes. All subsequent cultures from lettuce and other sources were secured in the same manner. The fungus has been kept in pure culture since October, 1911, and its behavior on various media studied to ascertain the one most suitable to employ in experimental work. All cultures, unless otherwise stated, were grown at room temperature.

Media: Upon lettuce leaves in Petri dishes, a rather vigorous mycelium, with many conidia and few sclerotia, was produced.

In lettuce broth, mycelium developed abundantly, but no conidia or sclerotia were produced until the culture was rather old and the mycelium had formed a thick mat on the surface. In no case were conidia developed in the liquid itself.

Tomato, pepper and apple broths gave about the same results, the mycelium growing equally well in all the media. Potato broth furnished a better medium, for, after the mat of mycelium was formed on the surface, conidia and sclerotia were produced in abundance.

On a two per cent lettuce agar, growth was rapid for about two days, afterwards decreasing slowly. In seven days the medium was covered with a thin layer of mycelium, which then produced conidia. Only a few sclerotia, about the size of a pinhead, arose from the hyphae. A similar growth was obtained on apple, pepper and tomato agar. Growth on potato agar was more vigorous, conidia and numerous sclerotia being formed. The spherical sclerotia, as a rule, were larger than the others mentioned above, being the size of a small pea.

Very little mycelium was obtained on nutrient gelatin and bouillon, no sclerotia and only a few conidia developing. When the study of the parasitism of the fungus was undertaken, a large number of cultures were made on sugars, fats and acids. The results of this work will be discussed under parasitism.

Sterilized corn-meal, saturated with various juices, gave good results, inducing rapid development of sclerotia. Apple, lettuce, tomato, pepper, potato and prune juices were used. Corn-meal moistened with potato and prune juices furnished the best medium for growth. Later, sterilized bread-crumbs were substituted for the corn-meal, as it was found to produce mycelium much more abundantly. In all of the above cultures growth was not rapid at first, but in five days the mycelium covered the entire surface as a loose, white, cottony mass composed of large, branching, septate hyphae. Organs of attachment were produced in large numbers wherever the mycelium came in contact with the sides of the dish.

The crowding of the mycelium led to the formation of a rather regular zone of sclerotia. As the cultures became older the latter united with one another, so that the zonate appearance was lost to some extent. The sclerotia matured in from nine to twelve days and in fifteen days formed a mass, or crust.

Five flasks, containing bread-crumbs saturated with potato broth, were inoculated February 1, 1912. On February 19, the first sclerotia were gathered. Then every three days the sclerotia were collected for a period of two months, when the medium became exhausted. An average of 512 sclerotia was obtained during this time for each culture, a surprisingly large number.

When the first cultures on bread were started, conidia developed at once, either before or while the sclerotia were forming. However, on making transfers repeatedly, the number of conidia became less, while the sclerotia increased in number proportionately. Thus cultures were obtained with very few conidia, which did not develop until all of the sclerotia had matured and the cultures had begun to dry up. This condition was noticed in cultures of the fungus obtained from several sources. When inoculations were made with such conidia on a medium containing little nutriment, conidia were immediately produced on the mycelium, but no sclerotia. It appears, then, that the conidia and sclerotia bear a definite relation to one another; that is, the formation of sclerotia requires a considerable amount of mycelium, whereas the conidia are produced when the mycelium is poorly developed and conditions for growth are unfavorable.

Acidity and alkalinity: The best development of the fungus took place on a slightly acid medium. A strongly alkaline medium retarded growth considerably, causing the mycelium to remain sterile. On the other hand, a strongly acid medium reduced the number of sclerotia, but favored the production of conidia.

Temperature: Cultures of the fungus on bread-crumbs saturated with potato broth were subjected to the following temperatures: 37°C., 30°C., 20°C. and 15°C.

From the results obtained it was clearly evident that a temperature of 37°C. was unfavorable for the growth of the fungus. Growth stopped entirely after progressing a few millimeters, no conidia or sclerotia being produced. At

30°C. the mycelium grew moderately well, covering the surface in about three weeks. At the end of five weeks the experiment was discontinued, the mycelium remaining sterile. At ordinary room temperature the best growth took place; numerous sclerotia were formed, with the conidia developing, as a rule, after the sclerotia had, for the most part, matured. Growth was somewhat retarded, but after two weeks the mycelium completely covered the surface of the plate and sclerotia developed. Only in one of the cultures were conidia produced, the abundant mycelium giving rise only to sclerotia.

From these experiments we may conclude that the fungus is unable to live long at a temperature of 37°C.; that at a temperature of 15°C. and 30°C. growth of the mycelium takes place normally, but not rapidly; a few sclerotia and, in some cultures, conidia are produced. The optimum, then, for rapid normal growth of the mycelium lies in the neighborhood of 20°C.

What the significance of the non-production of conidia at certain temperatures is we are unable to explain. Beauverie (3), working on the polymorphism of *Botrytis*, obtained a similar result. He succeeded in getting a strain of the fungus which, when kept under certain conditions (mainly of temperature and moisture), never formed conidia. He found that after controlling these factors for a long time the fungus was unable to return to its former state. When the above conditions were varied it always remained sterile.

Light and darkness: No appreciable difference was noted between the cultures grown in light and in darkness, except that the latter favored the non-production of conidia and the formation of a large number of the striated pseudo-sclerotia, while light favored the greater development of conidia. The sclerotia appeared to grow equally well in light and in darkness.

Longevity: The length of life of the mycelium varies but little, and in no case does it extend beyond two months. The conidia, on the other hand, are much longer-lived, since even after six months they are capable of germinating and producing infection. The sclerotia are not as long-lived as generally supposed, for, under suitable conditions, they germinate soon after maturity, which power decreases directly with the age of the sclerotia. When kept in moist soil for three months a large number disintegrate, leaving the hard, black exterior. But few sclerotia live more than a year. The pseudo-sclerotia produce conidia after a short period of rest; those that do not germinate rot in a few weeks, none surviving to the next season.

Germination of the sclerotia: From sclerotia developed on the lettuce and pepper plants a large number of cultures were started early in December, so that by January almost two thousand sclerotia were available. Those obtained from the peppers in the fall were kept in a dry place for three months. At this time two hundred cultures which had been gradually accommodated to the lower temperature were placed out of doors. The "drop" in the greenhouse being at its height, a large number of sclerotia were obtained from this source also. Some of them were left on the soil for further observations.

The first series of experiments was started January 15. Sclerotia from the above sources were placed in flasks containing sterilized sand moistened with sterile water. A similar experiment was set up every two weeks until May 15, a period of four months. Beginning March 1, there were added to the series sclerotia placed outside during the winter months.

In no case were apothecia formed, germination always resulting in the production of tufts of conidiophores. The results of these experiments may be summed up by saying that in from two to six days, depending on the age of the sclerotia, germination took place by the production of conidiophores bearing large masses of conidia, in no way different from those arising from the vegetative hyphæ. Cultures inoculated with these conidia gave a typical development, mainly the production of a loose, cottony mycelium which formed both conidia and sclerotia anew.

The sclerotia, after producing a number of these tufts, disintegrated and disappeared, none remaining intact. The large majority of them germinated, the others rotting within three months.

Thinking that possibly the sclerotia could be induced to germinate otherwise than by conidia, various chemical agents were used to stimulate them. Sclerotia obtained from the above sources were used in these experiments. One series was placed in a saturated atmosphere of chloroform for twenty-four and forty-eight hours. This was repeated for ether. Others were placed in desiccators, where they remained from one to seven days. The sclerotia were then placed in flasks as before.

Eighty-five per cent of the sclerotia placed in chloroform and ether vapors and fifty per cent of those exposed for forty-eight hours formed tufts of conidiophores. Germination, as before, was rapid. The cultures were allowed to run for three months, but no further development was observed. Conidia obtained from all the flasks and placed on media showed no differences in growth and development. Sclerotia, when mature, placed on moist sand, again formed the characteristic tufts of conidia. Careful observations in the field and greenhouse showed no further development of the sclerotia other than the production of conidia. About April 15, the sclerotia placed in the field formed conidiophores.

From the numerous experiments outlined above it follows that the fungus, *Sclerotinia Fuckeliana,* with *Botrytis cinerea* on lettuce as its imperfect stage, has lost its apothecial stage entirely, the life cycle being complete when the conidia, produced by the sclerotia, germinate to form a vegetative mycelium.

Germination of the conidia: The conidia germinate readily in water and nutrient solutions. Germination by one, sometimes two, germ tubes is the rule. In two days the mycelium is quite profuse and if the germinating spores are in hanging drops, organs of attachment are formed almost immediately. The conidia from the sclerotia germinate in like manner. Three generations of conidia have been observed, no difference whatever in their germination and development being noted. Sclerotia when sectioned and placed on culture media are able to produce mycelium which produces normal conidia.

Infection experiments: In no case was infection observed on healthy plants unless drops of water were present on the leaves; therefore, the plants were moistened with water just previous to inoculation. Plants sprayed with conidia in a nutrient solution like potato and prune broth produced infection in the majority of cases. Inoculated plants covered with bell jars gave almost a hundred per cent infection, the disease appearing in about three days. The progress of the disease was more rapid on cloudy days and when ventilation of the greenhouse was poor. Inoculations into wounds with spores produced the disease in all cases.

Old lettuce plants, when dusted with dry conidia, were very slightly affected; when water was allowed to run into the lettuce head the percentage of infection was much greater. Lettuce plants sprayed with conidia in water or in a nutrient solution produced, as a rule, a heart rot. During the winter months lettuce was planted from time to time in the infected beds which, in a short time, became diseased. In addition to the distribution of the disease by conidia, the mycelium, which was found to some extent in the soil, was also capable of producing infection.

With pepper and tomato seedlings no infection took place unless the spores were applied suspended in a nutrient solution and the plants covered with bell jars. In most cases the inoculated plants showed the presence of the fungus in three days, and within a week the plants collapsed. During the month of May, when the house was open and the days clear, all the above experiments were repeated, but negative results were obtained in most cases, showing that under conditions unfavorable to the fungus very little infection occurs.

Experiments with the fruit of the tomato showed that in no case could the germ tube, produced by the conidia, penetrate the cuticle, even when they were submerged in a nutrient solution. When the cuticle was broken, the conidia germinated and entered the tissues of the tomato. Here it either remained in the tissues, where it produced a rot, or the mycelium appeared on the surface and developed numerous conidiophores. The rotting of the fruit in the latter case was much slower. Carrots, potatoes and turnips, inoculated with conidia, rot slowly. The surface of these vegetables, if broken during the progress of the disease, became covered with a dark mass of conidia in less than twelve hours.

The fungus, then, was able to produce direct infection in the majority of cases if water was present on the plant. It was able to propagate itself, and cause the "drop," by its conidia, as well as by the mycelium. Infection by the mycelium always occurred on the leaves nearest to or on the ground. Infection experiments in the field were a failure in the majority of cases.

Parasitism and Saprophytism: Because the fungus exhibited so many degrees of parasitism in the infection experiments, it seemed desirable to make a rather detailed study of this subject. This same question has troubled a number of authors and conflicting results have been obtained.

De Bary (14), working with *Sclerotinia Libertiana,* found that the ascospores were not capable of producing direct infection, but must be saprophytically nourished before they possess the power of penetrating the living plant tissues. He believed that some substance, or substances, was secreted by the hyphæ, which dissolved the tissues of the host. Two phenomena could be distinguished in the breaking down of the tissues; the death of the cell contents and the destruction of the cell walls accomplished by a cellulose enzyme which dissolves, swells and breaks down the middle lamella of the cell walls.

Later, Kissling (31), investigating the development of *Botrytis cinerea,* like De Bary, found that the conidia were unable to bring about direct infection of the leaves of *Gentiana lutea,* although the young mycelium attacked the flowers. This peculiarity has been pointed out before, as one of the characteristics of the *Sclerotinia,* causing mummified fruits, and agrees with the observations of the disease on poinsettias. As was stated, this infection is due to the saprophytic nourishment afforded to the germinating spores by the stigmatic secretions. Kissling assumes that a cellulose dissolving enzyme is the sole cause of the destruction of the cell walls and the transformation of the cell contents into available food.

On the other hand, Ward (66) found that direct infection was possible in the form of *Botrytis* investigated by him. However, he observed that the infection was more virulent if the fungus was first saprophytically nourished. Like the others, he attributes the disorganization of the tissue to a cellulose enzyme secreted by the fungus. He further observed that this was accomplished by a marked swelling of the middle lamella, which preceded the breaking down of the cell walls.

Nordhausen (37), investigating the biology of *Botrytis cinerea,* was unable to make out this swelling of the middle lamella. He found that the non-cuticularized, etiolated and injured plants were much more susceptible to infection.

Smith (49) believes that too much importance has been given to this cellulose dissolving enzyme. He finds that we have two stages in the process of the breaking down of the living tissues; first, a poisoning and killing of the cells, due probably to oxalic acid; second, their disintegration and utilization by the fungus. The work of Brooks (11) can be summed up in his statement that, "Whatever may be the causes at work in the living cells which confer immunity or predisposition on the species of the host plants, or which confer virulence or impotence on the spore, they lie deeper than nutrition." We may safely say that *Botrytis* varies greatly in its ability to be a true parasite, and that in most cases it is at first saprophytic.

Tissues infected by *Botrytis* become soft and decay is very rapid. Sections of the diseased tissues show a darkening, loss of turgidity, breaking down of the cell contents, separation of the cells from one another and final disintegration of the whole tissue into a slimy mass. As was observed by Smith

(49), large areas surrounding the mycelium appear to be dead, showing that the fungus secretes some substance which kills the tissues in advance of the hyphæ. Strictly speaking, then, the fungus is always a saprophyte, because it invariably lives on dead tissue, and its ability to penetrate living tissue bougie, was quite concentrated, free from any foreign substance secreted by the hyphæ.

To carry this point a step further the writer obtained an extract from the mycelium and spores by collecting them from the cultures and grinding the material with clean quartz sand in a mortar. To obtain as concentrated an extract as possible, only a few cc. of water were added to the mass and the whole placed in a hydraulic press. The extract, passed through a bougie, was quite concentrated, free from any foreign substances and sterile.

A petiole of the lettuce submerged in the extract darkened and disintegrated very rapidly; acting for a longer time, weaker concentrations gave the same results. The point that attracted attention was that the tissues one to two centimeters above the liquid, were discolored and fell to pieces rapidly. On boiling the extract for varying lengths of time and placing the petioles of lettuce leaves in them the same characteristic changes in the tissues occurred. Sections of the tissues showed that the microscopical changes were in all cases similar to those observed in the diseased lettuce leaves.

Working along the line suggested by Smith, the next step was to ascertain the nature of the substance that caused the disorganization of the plant cells. Using several delicate tests for oxalic acid, negative results were obtained in each case. These were repeated a number of times with the same result.

Solutions of oxalic acid of different concentrations when applied to the petioles showed an action on the tissues somewhat like that of the extract, except that the tissues were bleached instead of becoming darker in color. Solutions of acetic, gallic, malic and tartaric acids of the same strength as the oxalic acid used, affected the tissues of the petioles in much the same manner. It may be, then, that the substance secreted by the fungus is an organic acid whose nature remains to be determined. That it is not oxalic acid, as is generally supposed, seems to be shown by the numerous experiments performed. Smith (49) states that he found as high as two per cent of oxalic acid in old culture media, but does not state how he demonstrated its presence. The great difficulty in this work is to distinguish sharply between the organic acids likely to be found in the plant.

The second part of the work was to determine what enzymes digested the food after the tissue was killed. Attempts were also made to ascertain whether the same enzymes were secreted on different media. Accordingly, a mineral nutrient solution (Pfeffer's) was used as a stock solution.

Two strains of the *Botrytis* were used in the experiments, one that had been obtained in pure culture from the lettuce in the greenhouse, the other secured in March, from the old infected stems of the pepper. Thus one was growing as a saprophyte, the other as a parasite.

A two per cent starch solution with the stock solution was used, which formed a paste. Growth was slow for the first two days but at the end of two weeks surpassed all of the other cultures in the production of sclerotia. In the cultures inoculated with the second strain, more conidia and fewer sclerotia were formed, which was the rule in all of the other cultures; no other differences were noted. At the end of two weeks the medium gave no reaction with iodine. Fehling's solution showed a strong reduction. With Barfoed's reägent a positive reaction was obtained, and with phenylhydranzine a brownish osazone was precipitated. The crystals, when microscopically examined, appeared to be glucosazone, which was further substantiated by their insolubility. The results show that a large amount of diastase is secreted by the fungus. Smith (49) reports that he was unable to obtain any growth whatever on starch, possibly due to some toxic substance in the commercial corn-starch used by him.

As a check on the above work, the action of the extract on a starch solution was observed. Using equal volumes of starch solution and extract, no iodine reaction could be obtained after an hour, although Fehling's solution gave negative results. However, at the end of twelve hours, hydrolysis had taken place and the final product appeared to be dextrose. The slow growth on the starch medium at first, also shows that hydrolysis must take place before it can be digested by the hyphæ. A three per cent solution of dextrose gave a vigorous growth from the beginning. The mycelial development was luxuriant, many sclerotia and some conidia being formed.

Growth on cane sugar like that of starch was rather slow at first. By the end of the first week the development equaled that in the dextrose culture. Tested in the usual manner, a strong reduction was observed, showing that inversion had taken place. As before, the extract produced similar changes in the medium.

Sugar solutions of various concentrations were inoculated with *Botrytis* conidia. Growth resulted in all cultures, the twenty and thirty per cent solutions giving the best development. Delicate tests failed to show the presence of any oxalic acid whatever.

With maltose an excellent growth developed, producing sclerotia and conidia. Lactose gave a fairly good growth after the first week. On levulose an abundant mycelium, similar to that on dextrose, developed. On dextrin, growth was slow at first, but soon a vigorous mycelium was produced. A strong reduction was noted in the cultural solution and in the medium after treatment with the extract.

In nearly all cases, Fehling's solution shows the presence of reducing sugars in originally non-reducing carbohydrate media upon which *Botrytis* has been grown, showing that a number of enzymes are secreted by the mycelium. No oxalic acid is formed in the cultures, contrary to Smith's (49) results. Careful observations were made from time to time, but in no case was alcoholic fermentation detected.

On glycerin, normal conidiophores were not produced. On testing for oxalic acid negative results were again obtained.

Tests for cellulose digestion were made with filter paper, ground to a pulp, and absorbent cotton, to both of which a stock solution was added. On the pulp a fair development of the mycelium resulted, with the production of spherical sclerotia, while on cotton only a slight growth was observed. These experiments were repeated several times, as so many authors have attributed the breaking down of the cell to a cellulose enzyme. Fehling's solution showed only a slight reduction. The mycelial extract was allowed to act on pulp for a week, and when tested gave only a slight reduction. It may be assumed, therefore, that very little cytase is formed and that too much importance has been attributed to the rôle of this enzyme.

A two per cent solution of castor oil produced a development not altogether normal. The oil lost its characteristic appearance and broke down into glycerin and fatty acids.

Malic and tartaric acids gave a fair growth of mycelium, with few sclerotia and conidia. With oxalic acid no development whatever took place, even in low concentrations.

With a two per cent solution of tannin, growth resulted in only one of six cultures and the effect of *Botrytis* on this substance was not followed out, except that the culture was finally observed to turn black. Smith found that the tannin was broken down into glucose, gallic acid and a dark coloring matter which he was unable to identify.

Amygdalin gave an excellent development of mycelium and conidia, but no sclerotia. Again no attempt was made to study the effects of the fungus on the medium.

A solution of peptone produced an excellent growth and no changes in the medium could be detected.

All of these experiments show that most of the carbohydrates, fats and protein derivatives used can serve as the sole carbon source for the fungus and that probably substances in the plant cells belonging to the above classes may be digested by the enzymes secreted by the hyphal elements. The poisoning of the plant cells may be due to one or a number of organic acids, or it may be that the fungus secretes some other toxic substance which causes the death of the cells.

DISCUSSION.

There can be no question but that the disease on the peppers, lettuce and other plants in the greenhouses of the Missouri Botanical Garden was caused by *Botrytis cinerea,* which possessed the power of penetrating the plant tissues and destroying them.

The lettuce "drop" due to *Botrytis cinerea* has about the same characteristic symptoms as when caused by *Sclerotinia Libertiana.* It is, indeed, interesting to note the similarity between the symptoms of the disease described by Stevens and those noted by the writer. Even the physiology of the two fungi agree to a large extent. The difference, of course, is that *Botrytis* produces only conidia, whereas *S. Libertiana* only ascospores. In this connection I might say that I have been able to develop from *Botrytis* a mycelium that does not produce conidia. From my observations it appears that the more parasitic the *Botrytis* becomes, the greater is the number of sclerotia and the fewer the conidia produced.

That *Botrytis* can become a serious disease was very well shown in the greenhouse the past winter. To become parasitic, however, it must have suitable conditions. The life cycle of the fungus is complete with the germination of the sclerotia, resulting in the production of conidiophores. This appears to be the prevailing type of sclerotium germination. Istvanffi (28) is the only one who in recent years has been able to obtain ascospores from sclerotia of *Botrytis.* Since he was unable to produce infection by means of the ascospores, he failed to complete the life cycle of the fungus. Apothecia were rare; the sclerotia formed conidiophores more commonly. It may be safely stated, then, that the *Botrytis* causing lettuce "drop" and similar diseases is a degenerate form which has lost the apothecial stage.

Work on the parasitism of *Botrytis* is still in its infancy and many further experiments will be necessary before the interrelations with its host are fully understood. My results show that we have two stages in the disintegration of the plant tissues. There can be no question that some substance is secreted by the fungus which kills the plant cells in advance of the mycelium. The author disagrees with Smith (49) that the poisoning effect is due to oxalic acid, since delicate tests for this acid gave only negative results. Even in old sugar cultures on which the fungus has been growing for several weeks, no oxalic acid was present. Smith reports that he found as high as two per cent of the acid under like conditions.

The harmful substance may be some organic acid other than oxalic, or it may be a toxin of some kind, which, however, is not destroyed by heating to 100°C. The writer has found that weak concentrations of malic, tartaric, oxalic, gallic, and acetic acids have an action on the lettuce tissues similar to that of the mycelial extract.

From the cultural work I have found that a number of enzymes are secreted by the fungus. These enzymes are diastase, invertase, cytase and lipase. It was also shown that glucosides were broken down and that the fungus was able to live on protein derivatives. The *Botrytis* investigated secretes very little cytase and it seems that too much importance has been attributed to this enzyme. It may be that diastase assists the cytase in breaking down the cellulose and in digesting the amylodextrin formed. In agreement with the other authors, the experiments show a varying ability of the conidia to cause direct infection of the host. This ability is always directly proportional to the conditions favoring the fungus.

SUMMARY.

1. The *Botrytis cinerea* causing the diseases of lettuce, peppers and greenhouse plants mentioned, at the Missouri Botanical Garden, is the imperfect stage of *Sclerotinia*

Fuckeliana De Bary. It is a degenerate form, having lost the apothecial stage entirely, the life cycle being completed when the sclerotia germinate by the production of tufts of conidiophores.

2. In all cases *Sclerotinia Fuckeliana* has no connection whatsoever with *Sclerotinia Libertiana* Fuckel.

3. Two stages in the parasitism of the fungus have been noted: first, the killing of the cells in advance of the fungus, due to some organic acid (not oxalic) or to some toxin (not destroyed by boiling) secreted by the fungus; second, the digestion of the dead tissues by a number of enzymes which are produced in varying quantities by the fungus.

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EXPLANATION OF PLATES.

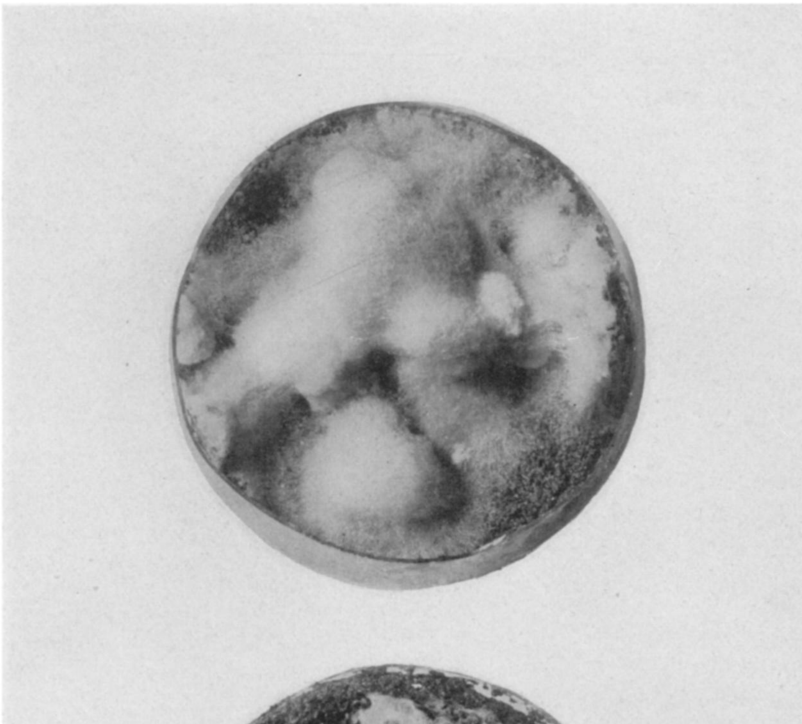
1. Two typical cultures of *Botrytis cinerea* on bread-crumbs saturated with potato broth, showing abundant growth of mycelium and developing sclerotia. No conidia are present in the cultures, although a few were formed later. Cultures are eight days old.

2. A pure culture of the fungus on bread-potato broth mixture kept in the refrigerator at a temperature of 15°C. for five weeks. A number of sclerotia are scattered through the culture but conidia are lacking.

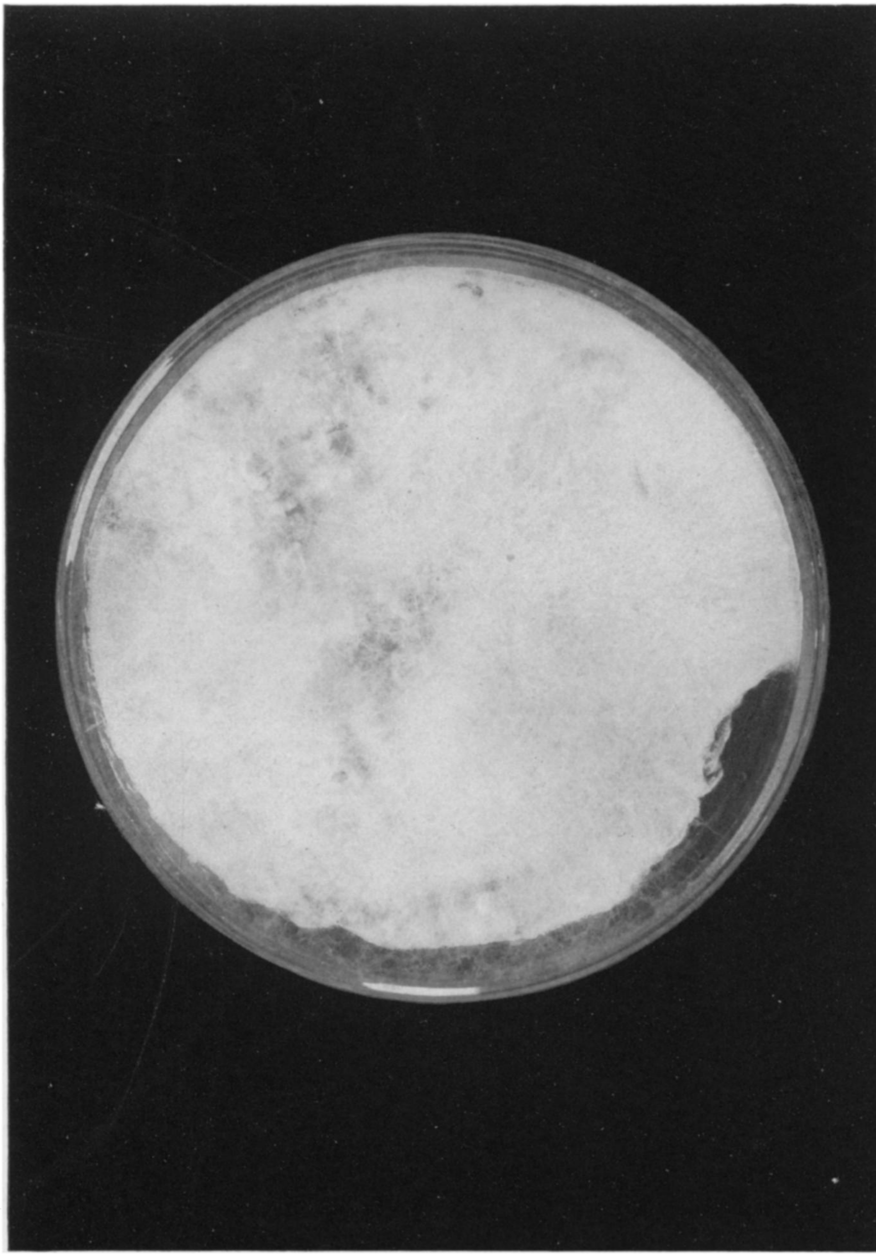
3. Tomatoes inoculated with conidia, showing conidiophores arising at the point of inoculation.

4. *Botrytis cinerea* infecting beans.

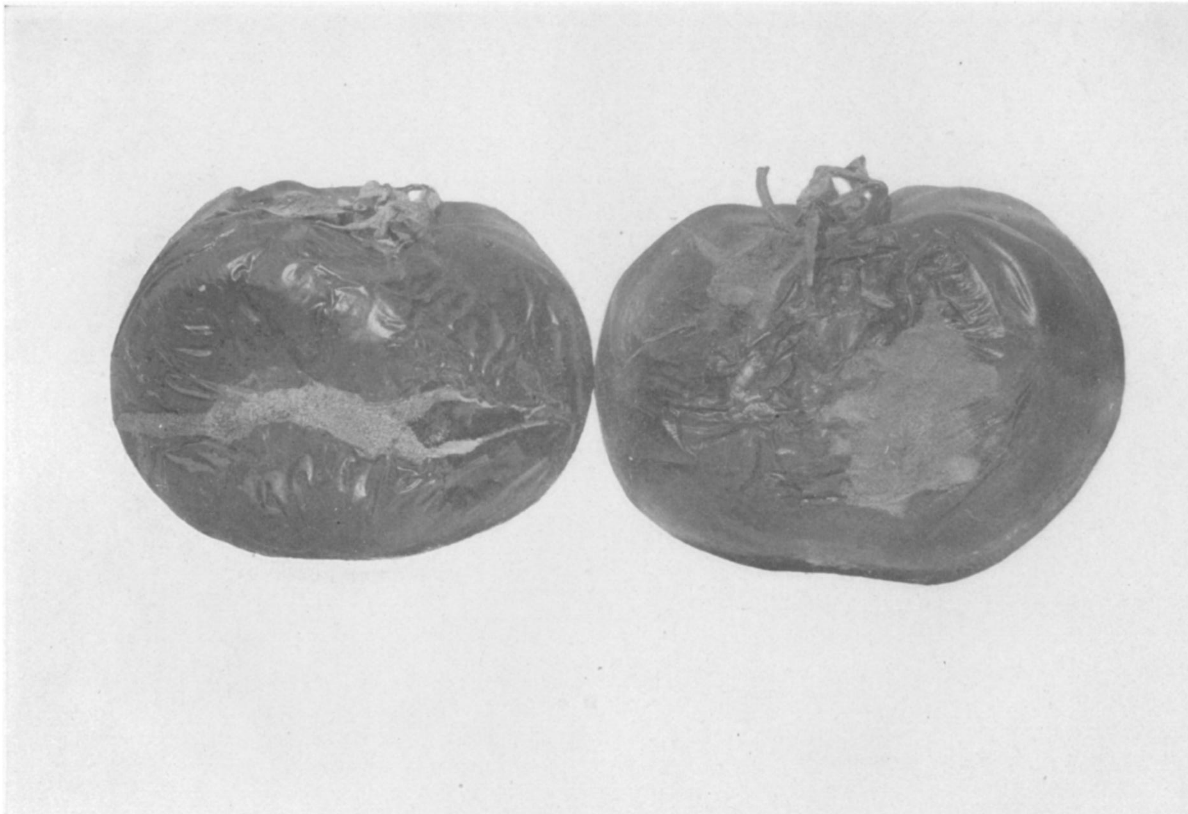
5. Germinating sclerotia, showing the formation of large tufts of conidiophores.



BOTRYTIS CINEREA.



BOTRYTIS CINEREA.



BOTRYTIS CINEREA.

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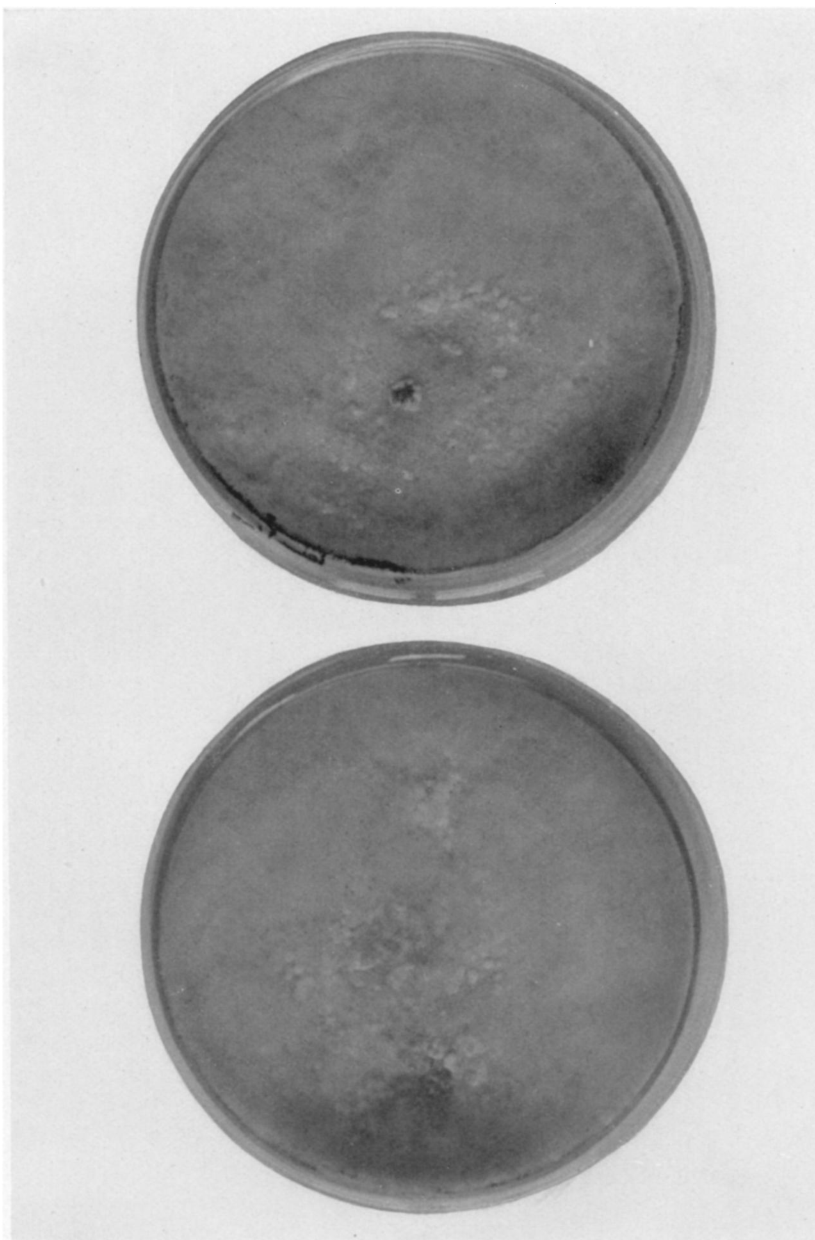

BOTRYTIS CINEREA.