PATHOGENICITY AND IDENTITY OF SCLEROTINIA LIBERTIANA AND SCLEROTINIA SMILACINA ON GINSENG

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INTRODUCTION

For a number of years two species of Sclerotinia have been recognized as probable causes of the rotting of ginseng roots (*Panax quinquefolia*), but the pathogenicity and identity of these fungi have not been proved by by inoculation experiments.

The purpose of this paper is (1) to report inoculation experiments establishing the pathogenicity of these organisms, and (2) to detail the experimental data and considerations on which the conclusions as to the identity of the two pathogens are based.

WHITE-ROT OF GINSENG

The white-rot of ginseng was first reported by Whetzel (1907, p. 89).[2] Sclerotia were found, but the identity of the fungus was not determined. Subsequent workers, Rankin (1910), Osner (1911), and Whetzel and Rosenbaum (1912, p. 34–45) have attributed the disease to *Sclerotinia libertiana* Fuckel. These writers based their observations on the association of the sclerotia of the fungus with the host and the general resemblance of the organism on the host and in culture to the widespread *Sclerotinia libertiana*. No inoculation experiments have been reported.

PATHOGENICITY

During the spring of 1913 the fungus was isolated from diseased ginseng roots grown at Newtown, Pa., Mentor, Ohio, and Edenville, Mich. The isolations were made by washing the roots, immersing them for 10 minutes in a solution of mercuric chlorid (1 to 1,000), peeling back a portion of the external tissues, and transferring small bits of tissue from the inside of the root to poured plates of hard potato agar. Pure cultures were obtained in the majority of cases from the first planting. In addition to the cultures isolated from ginseng, inoculations on healthy ginseng

[1] The writer is indebted for many suggestions to Dr. Donald Reddick, of Cornell University, under whose direction this work was done.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 297.

roots were also made with a culture of *Sclerotinia libertiana* obtained from lettuce from South Carolina. The procedure followed in the inoculations was as follows: Healthy ginseng plants with the tops still attached were selected and the soil carefully removed from one side of the root. By means of a flamed scalpel longitudinal cuts were made in the side of the root. These cuts were approximately one-fourth of an inch in length and about one-eighth in depth. A piece of agar containing mycelium from young cultures was inserted within these cuts and covered with soil. Check roots were treated in a similar manner.

During the summer inoculations were made as shown in Table I. The checks in every case remained healthy.

TABLE I.—*Results of the inoculation of ginseng with Sclerotinia libertiana from various sources*

Date.	Source of culture.	Number of roots inoculated.	Number of checks.	Percentage of infection.
July 14	*Sclerotinia libertiana* from South Carolina from lettuce	6	2	100
15	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	6	2	100
15	*Sclerotinia* sp. from Newtown, Pa., from ginseng.	8	4	100
15	*Sclerotinia* sp. from Edenville, Mich., from ginseng	6	2	83+
Aug. 1	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	4	1	100
1	*Sclerotinia libertiana* from South Carolina from lettuce	4	1	75

Plate XXVIII, figures 1 and 2, is reproduced from photographs of ginseng roots from two of the above series. Figure 1 shows a root inoculated with *Sclerotinia libertiana* isolated from lettuce. Figure 2 shows three roots (on the left) inoculated with a species of Sclerotinia isolated from ginseng.

Reisolations were made from the inoculations of July 15 and the fungus was again grown in pure culture. Inoculations made with the reisolated culture gave positive results.

Infection was evident in from three to seven days after inoculation. The root at the point of inoculation becomes soft and the rot spreads gradually in all directions, causing the entire root to beame soft and doughy. After the mycelium has penetrated throughout the tissues of the root, it forms tufts of cottony-white felt, in which large black sclerotia rapidly develop. Sclerotia on the outside of the root have in some cases developed within 10 days after the inoculations were made. When the inoculations are made near the crown of the root, the mycelium spreads to the stem, where it develops similar sclerotia on both the inside and the outside of the stem. The rapidity with which the disease progresses in the inoculted roots depends upon moisture conditions.

During a rainy period infection is evident within a much shorter time. All attempts to produce the disease without previously injuring the root gave negative results.

IDENTITY OF THE SPECIES

In order to further prove that the species of Sclerotinia from ginseng is identical with *Sclerotinia libertiana* Fuckel, a comparison was made with cultures from different sources. In addition to the four strains mentioned above, there was also used a pure culture isolated by Dr. Donald Reddick, of Cornell University, from celery. The comparison of the strains consisted in (1) growing the cultures on different media, both acid and alkaline; (2) production of apothecia, measurements of asci, ascospores, and a study of the manner of germination; (3) cross-inoculations on lettuce. These topics are briefly discussed in the following paragraphs.

GROWTH ON DIFFERENT MEDIA.—Cultures were made on potato agar, nutrient agar, bean plugs, ginseng stems, and Raulin's synthetic fluid. In the case of potato and nutrient agar both acid and alkaline media were used (±10.5 Fuller's scale). On all the media the various strains made a good growth, but no differences were visible.

PRODUCTION OF APOTHECIA, ETC.—In order to obtain apothecia from the various strains, the sclerotia produced in pure culture were placed on sterile moist sand in dome-shaped preparation dishes. The sclerotia were covered with a very thin layer of the sand, and the dishes were placed on a shelf in front of a window. The time required for these apothecia to develop varied greatly, the limits being from three weeks to three months. The size of the apothecia likewise varied even in the case of sclerotia from the same strain and produced in the same test tube. However, the apothecia were alike in general appearance in all the strains. Plate XXVIII, figure 3, shows apothecia from the celery strain, and Plate XXVIII, figure 4, shows the same from the ginseng strain. A large number of measurements made of asci, paraphyses, and ascospores showed no marked variations, and agreed with the description of *Sclerotinia libertiana* Fuckel as given in Saccardo. In figure 1, *A*, is shown a camera-lucida drawing of asci, ascospores, and paraphyses from a fresh preparation of the Mentor strain.

Crushed pieces of apothecia were placed in drops of water in order to observe the ascospore germination. Within four hours after being placed in water the first signs of germination became visible. Figure 1, *B*, shows the ascospores within the asci, germinated by sending germ tubes directly through the walls of the ascus. No differences were noted in the germination of the spores from the different strains.

INOCULATIONS ON LETTUCE.—Mature lettuce plants were selected and inoculated with the various strains of the fungus. Inoculations were

made on injured and uninjured plants, which were then covered with bell jars for 4 days. At the end of 12 days most of the plants showed signs of rotting. Unlike the ginseng roots (Pl. XXVIII, figs. 1 and 2) previously discussed, infection occurred not only on the injured, but also on the uninjured plants.

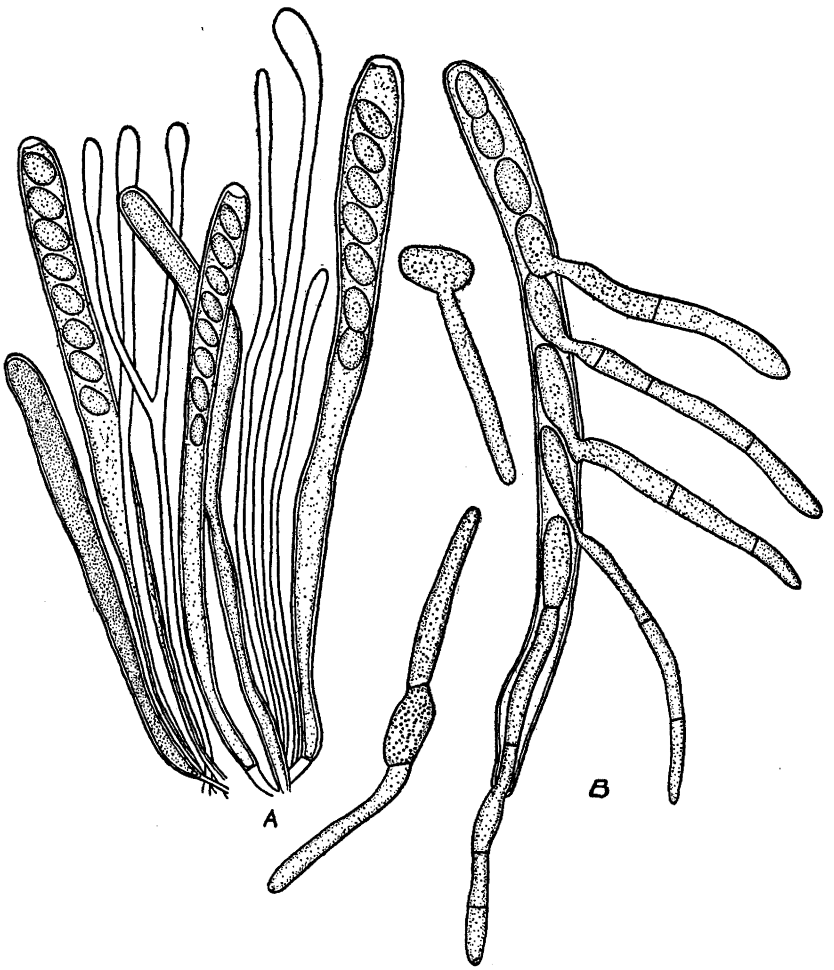

FIG. 1.—*Sclerotinia libertiana: A*, Camera-lucida drawing showing branched and unbranched paraphyses, asci, and ascospores; *B*, camera-lucida drawing showing methods of ascospore germination. Those within the asci germinate by sending germ tubes directly through the walls of the ascus.

BLACK-ROT OF GINSENG

Van Hook (1904, p. 181–182) first mentions a species of Sclerotinia as the cause of a black-rot of ginseng. Rankin (1912) reports the discovery of the apothecia and established a new specific name for the fungus. No inoculations were attempted, either on the ginseng roots or on other hosts known to be attacked by species of Sclerotinia closely allied to this one.

PATHOGENICITY

In the spring of 1912 the writer received a number of black-rotted roots from Wisconsin showing various stages of development of the disease. Isolations were made from these roots by making plantings from the inner tissues of the roots on poured plates of hard potato agar. The fungus was obtained in pure culture, where it produces a characteristic black growth.

Inoculations on healthy roots made at various times during the summer gave negative results, as would be expected from the nature of the fungus, since the disease always develops in beds during the winter. In October of the same year (1912) six roots were washed clean and inoculated by placing a piece of the agar pure culture in a small cut made in the tissues of the root. Three similar roots were injured and used as checks. All the roots were planted in soil which had never grown a crop of ginseng. The following March an examination of the roots showed the characteristic symptoms of the disease. Some were entirely black, while others were only partly blackened. The fungus was easily reisolated from these roots. Plate XXIX, figure 1, shows two inoculated roots, together with a check root. One of the inoculated roots is entirely black, while the second shows this black color only in part.

In October, 1913, inoculations were again made on ginseng roots. These roots were not injured, but the fungus was placed on the old stem scar. The next March the roots were black, as in the previous year. Reisolations were again made, and the fungus which was obtained produced the characteristic black growth.

IDENTITY OF THE SPECIES

The growth of the fungus in culture and the general behavior of this organism differed so greatly from the known species of Sclerotinia that it has always been an interesting question as to the source of the fungus which appeared in isolated gardens throughout the country. One plausible explanation is that the fungus, being associated with wild ginseng roots or with one of the common weeds, was brought in from the woods, as many growers make a practice of using leaf mold in preparing their beds. Since the fungus from the description resembled *Sclerotinia smilacina* Durand, it seemed advisable to determine whether the species of Sclerotinia on ginseng could produce a black-rot of the rhizome of *Smilacina* spp. and whether the two were also identical in other respects.

INOCULATIONS ON SPECIES OF SMILACINA.—In October, 1913, six rhizomes of *Smilacina racemosa* were inoculated with a pure culture of the black-rot fungus obtained from ginseng. The inoculations were made by slightly injuring the rhizome and inserting the mycelium of the fungus in the cut. Check plants were also injured. When examined the following March, the rhizomes showed the characteristic symptoms of black-rot

as exhibited by ginseng roots. The check plants remained healthy. Plate XXIX, figure 2, is a reproduction of a photograph of two of the inoculated and one check rhizome. Reisolations were made, and the fungus which was obtained resembled the original culture isolated from ginseng.

COMPARISON WITH TYPE SPECIMEN.—To determine further the relationship of the *Sclerotinia* sp. from ginseng to that on *Smilacina* spp., an examination was made of the type specimen of *Sclerotinia smilacina* Durand, deposited by Dr. Durand in the herbarium of the botany department of Cornell University. The specimens showed the black coloration as exhibited by the inoculated rhizomes of *Smilacina racemosa* as well as the ginseng roots.

Apothecia on ginseng are rare, and though attempts to produce them were made no success can be reported up to the present time. It is of interest, however, to compare the measurements as given in the original descriptions by Durand (1902, p. 462–463) and Rankin (1912) as shown in the following table:

Species.	Sclerotia.	Apothecia.	Asci.	Ascospores.
	Gm.	*Gm.*	*μ*	*μ*
Sclerotinia smilacina . .	0.1 by 0.2 to 2.	0.75 to 3 . .	120 to 140 by 6 to 8.	12 to 15 by 4 to 5.
Sclerotinia panacis	0.3 to 1	1.5 to 2.5 . .	125 to 137.5 by 6.4 to 6.5.	11.7 to 16 by 4.8 to 7.5.

Measurements made by the writer from the type material of these species have shown that the asci and ascospores are not to be distinguished either in form or size and agree with the measurements given above.

CONCLUSIONS

1. (A) The pathogenicity of *Sclerotinia* sp. causing the white-rot of ginseng has been established. (B) This species of Sclerotinia is identical with the *Sclerotinia libertiana* Fuckel occurring on lettuce, celery, and a number af other hosts.

2. (A) The pathogenicity of *Sclerotinia* sp. causing the black-rot of ginseng has been established. (B) A consideration of the following facts indicates that *Sclerotinia panacis* Rankin is identical with *Sclerotinia smilacina* Durand: (a) Inoculations with a species of Sclerotinia from ginseng on *Smilacina racemosa* gave positive results. (b) Measurements of asci and spores made by the writer from the type material of both species agree. There is a close agreement in all distinguishing characters, as given in the original description of the two species. (c) The lesions produced by the inoculations are similar on the two hosts and identical with those on diseased plants as they occur naturally.

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PLATE XXVIII

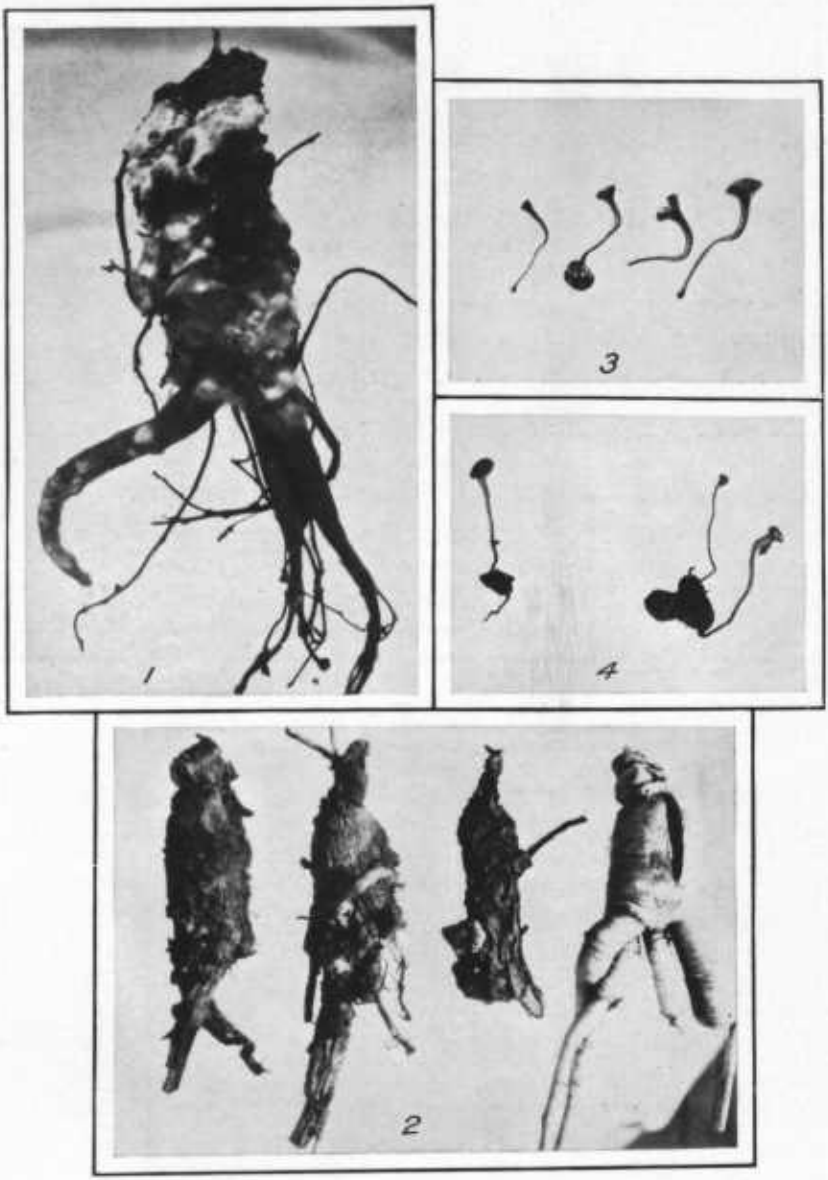
Sclerotinia libertiana:

Fig. 1.—Root inoculated with *Sclerotinia libertiana* from lettuce. Note the white mycelial felt and the production of sclerotia.

Fig. 2.—Three roots (on left) inoculated with *Sclerotinia* sp. from ginseng. Healthy check root (on right).

Fig. 3.—Apothecia from sclerotia from celery strain.

Fig. 4.—Apothecia from sclerotia from ginseng strain.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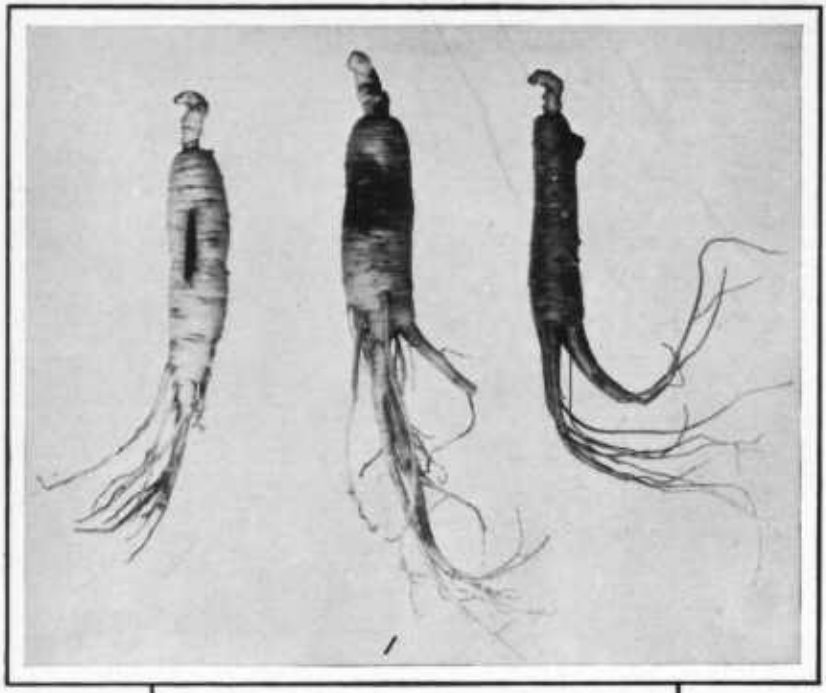

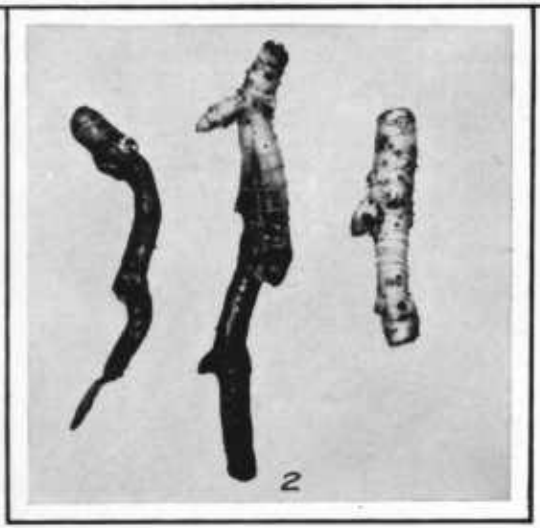

PLATE XXIX

Sclerotinia smilacina:

Fig. 1.—Ginseng roots showing the characteristic black color from artificial inoculation. The root on the left is the check.

Fig. 2.—Rhizomes of *Smilacina racemosa* inoculated with a species of Sclerotinia isolated from ginseng. The rhizome on the right is the check.