DISEASES OF ROSE CAUSED BY SPECIES OF CONIOTHYRIUM IN THE UNITED STATES

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INTRODUCTION

In the examination and study of many types of cankers on diseased rose stems the attention of the writer has been attracted to the great prevalence of species of Coniothyrium as the cause of such cankers. Because of the many points of similarity among the three diseases popularly known as stem canker, graft canker, and brand canker, attributed respectively to C. fuckelii Sacc., C. rosarum Cke. and Hark., and C. wernsdorffiae Laub., the question frequently arises as to the identity of the particular organism causing a certain type of canker. The present study was undertaken to determine by a comparison of the symptoms of disease and of the morphological and physiological characters of the causal fungi what distinguishing features might be of value in diagnosing the diseases. The immediate occasion for the study was the recent discovery, in the United States, of brand canker, caused by C. wernsdorffiae, hitherto reported in Europe and Canada.

SURVEY OF LITERATURE

Brand canker was originally described in 1905 by Laubert (10) as a disease attacking branches of Rosa in many localities in Germany. From a morphological study of the organism causing the canker, he concluded that the fungus was a species of Coniothyrium. The species, however, did not correspond with C. fuckelii, which had been reported by Saccardo (19) as occurring on dead or wilted branches of Rosa and other hosts. Therefore Laubert established the fungus as a new species, C. wernsdorffiae. Kock (9) in the same year reported a disease on tea roses in Austria which he ascribed to C. fuckelii. He believed that this was the same disease as that found by Laubert and that the slight difference between the spore measurements given for Saccardo's fungus and those of his own Austrian specimens was an insufficient basis for establishing a new species. He would make the fungus causing the disease merely a variety of C. fuckelii. His spore measurements and description of the disease symptoms corresponded rather closely with those given for C. wernsdorffiae by Laubert (10), and it would seem that both writers were describing the same disease.

In a later publication Laubert (11) compared the fungus causing the brand canker with Coniothyrium fuckelii. He considered the latter fungus a harmless saprophyte with pycnidia and spores much smaller than those of C. wernsdorffiae.

Güssow (4, p. 226) described the symptoms and causal organism of a similar disease found upon hybrid tea roses and Wichuraianas in

1 Received for publication Dec. 30, 1929; issued May, 1930. These studies were conducted in cooperation with the Department of Botany, Brown University, Providence, R. I.
2 Reference is made by number (italic) to "Literature cited," p. 826.
Ireland. He considered the causal organism to be *Coniothyrium fuckelii*, since his spore measurements "practically agreed with the size of the spores of Saccardo's fungus." Moreover, he believed that the disease on the Irish specimens was identical with that observed by Laubert and agreed with Kock that Laubert was not justified in establishing a new species. From Güssew's description and the accompanying illustrations it would seem very probable that his specimens were infected by *C. fuckelii*, as he stated, and that he was not dealing with the disease described by Laubert.

In Rostrup's Danish Fungi (13, p. 436) appeared the following note concerning *Coniothyrium wernsdorffiae*: "A true parasite, attacking the bark of the branches of cultivated *Rosa* spp. for the first time found 11/6/03 [June 11, 1903], quite common."

O'Gara (16) found a species of Coniothyrium causing cankers on rose in the United States which proved by cultural methods and cross inoculations to be identical with a species occurring on cankers on apple twigs. The fungus appeared to correspond in every respect with the description of *C. fuckelii* given by Saccardo. O'Gara stated that the organism fruited readily in cultures, "producing typical pycnidia and spores varying somewhat in size, depending upon the medium, but all within the limits of the species."

From these investigations it seemed apparent that two different species of Coniothyrium caused diseases of rose. Since the time of O'Gara's investigations, however, several writers have stated that *C. wernsdorffiae* was probably identical with *C. fuckelii*.

Saccardo (19) designated *Coniothyrium fuckelii* as the "spermagonial" form of *Leptosphaeria coniothyrium* (Fckl.) Sacc. The same statement was made by Massée (15). Experimental inoculations and cultures to determine the exact relation of the perithecial and pycnidial forms were successfully made by Stewart (20) on various species of Rubus. Martin (14), in a discussion of the polymorphism of *L. coniothyrium* on rose, reported Coniothyrium as one of the phases of the fungus. So far as known at the present time no perfect stage for *C. wernsdorffiae* has been reported.

In 1925 the first reports of the occurrence of brand canker in North America were published by Howitt (6) and by Martin, following the identification of *Coniothyrium wernsdorffiae* as the cause of cankers on material collected in Guelph, Ontario. Later the occurrence of the disease in the United States was reported by Jenkins and Martin (8) and was tentatively described by Jenkins (7), Westcott, and the writer (25). Its occurrence in the Netherlands (18) was also reported.

The third disease of rose attributed to a species of Coniothyrium was described by Vogel (22) as graft disease. From his study of the causal fungus and from the results of his inoculation experiments he concluded that the disease was caused by *C. rosarum*. This fungus was originally reported by Cooke and Harkness (2) as occurring on stems of *Rosa* in California. No report of the perfect stage of this fungus has been made.
DISTRIBUTION OF THE DISEASES

In the summer of 1926 a serious epiphytotic of a disease similar to that originally described in Germany by Laubert as brand canker caused by *Coniothyrium wernsdorffiae* was discovered by the writer on climbing roses in the floriculture gardens at Cornell University, Ithaca, N. Y. (Fig. 1.) No report of the occurrence of this disease in the United States had been published at that time. Later the disease was reported by Jenkins and Martin (8) as occurring also in Minnesota and Pennsylvania. So far as known at the present time, this disease occurs principally on out-of-door roses. Jenkins and Martin (8) reported it on rose plants grown in the horticulture greenhouses of the Minnesota Agricultural College. With this exception all reports and collections have been from garden plants.

The disease commonly known as stem canker (fig. 2) and caused by *Coniothyrium fuckelii* is reported as widespread throughout the United States. It is found at some time in nearly every rose garden and in many greenhouses. It is more prevalent, however, on hybrid tea roses than on other types of roses.

The so-called graft disease attributed by Vogel to *Coniothyrium rosarum* was considered to be limited to rose grafts in forcing frames and greenhouses and to plants in the rose garden which had contracted the disease while in the greenhouse but which proved to be partially resistant. During 1927 a similar disease (fig. 3) was particularly prevalent in greenhouses throughout the middle and eastern parts of the United States.

The accompanying map (fig. 4) shows the distribution of these three diseases in the United States as known at the present time. This map was prepared from data published by Martin and Jenkins and from the results of the writer's studies. Undoubtedly the diseases are more widely distributed than this map signifies, but possibly they are not sufficiently serious to attract particular attention in those States from which a report of their occurrence has not been received.

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MATERIAL USED IN THE PRESENT STUDY

The material used in the present study was collected from various localities in the United States and from plants grown under widely differing conditions of environment. The specimens of brand canker were collected by the writer at Ithaca, N. Y., and were a portion of the material reported by Jenkins and Martin (8), who attributed the disease to Coniothyrium wernsdorffiae. The term "stem canker" is
applied throughout this study to the disease which is commonly considered to be caused by *C. fuckelii*, the imperfect form of *Leptosphaeria coniothyrium* Sacc. A few specimens of *L. coniothyrium* were studied by the writer and were compared with exsiccati specimens among the "Fungi Saxonici" collected by K. W. Krieger. In the study of this material, cultures from the ascospores produced a species of Coniothyrium which corresponded with that isolated from the stem cankers. Therefore the fungus causing such cankers has been called *C. fuckelii*. The name "graft canker" has been applied only to the disease characterized by the formation of cankers at the union of stock and scion. The fungus isolated from such cankers was compared with that occurring on specimens from the herbarium of the Iowa State College of Agriculture and Mechanic Arts, which were collected by I. H. Vogel, who attributed the disease to *C. rosarum*.

**BRAND CANKER**

**SYMPTOMS**

The symptoms of the disease known as brand canker have been described in detail by various investigators. Therefore it is necessary in this article only to call attention to those characters which are distinguishing features.

The cankers can be readily detected, even by a casual observer. This is due to the fact that the light or warm buff of the central portion and the taupe-brown or dull purplish black margin stand out in sharp contrast with the green of the adjacent healthy stem.

Small longitudinal slits in the bark of the diseased area, caused by the protruding of the pycnidial ostioles, are characteristic of these cankers. Only in rare cases were the spores of the fungus extruded.

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*The color terms mentioned in the text are according to the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.*
upon the bark in sooty masses. In Laubert’s description of the
disease (10), however, he stated that the spores are frequently collected
on the surface of the bark “in Form sehr kleiner russschwarzer
Fleckchen.” Since the majority of the cankers of this disease which
were examined by the writer were of comparatively recent infection,
it may be that the accumulation of the spores occurs only on older
cankers. Laubert also mentions the occurrence of wound callus
at the margin of 2-year-old cankers (10). No such callus formation
has been found in connection with the cankers examined by the
writer. This again may be due to the fact that young cankers only
have been observed in this country.

MORPHOLOGY OF THE CAUSAL FUNGUS

Mycelium

In the host tissue the mycelium of the causal fungus showed no
distinguishing features. In culture, however, although the mycelium
varied with the kind of agar used, certain distinguishing features of
color and amount of growth were apparent. The aerial mycelium
of the fungus grew exceedingly slowly on all the media used but eventu-
ally became luxuriant. On corn-meal and potato agars it was light
gray; on malt, dextrose, and synthetic agar, a greenish gray turning
to brownish gray. On sterilized sliced potato, the actively growing
mycelium was a very light gray, becoming darker with age until, in an
old culture, it was almost black.

Formation of Pycnidia and Spores

The formation of a pycnidium is first indicated by close inter-
weaving and anastomosing of numerous rapidly growing hyphae.
This usually occurs in some intercellular space at a point within the
zone of the three outermost cell layers below the epidermis of the
diseased area. As this tangled mass or primordium grows larger, the
epidermis together with the two or three underlying cell layers becomes
widely separated from the inner layers of the bark tissue. In the
center of the primordium a cavity is formed by the absorption of some
of the hyphal cells. By this lysogenous activity the cavity is gradually
increased in area until it attains its characteristic size. In a mature
pycnidium the cells lining the cavity are hyaline, thin walled, and
finely granular. These cells are sporogenous in function. There is
no pigmentation in the cell walls of this tissue. The next two or
three layers are similar to these in appearance. These few layers of
sporogenous cells are closely packed and more or less regular in shape.
This arrangement, together with the granular contents of the cells,
gives the appearance, in sections, of a subhyaline ring of tissue
surrounding the cavity. This ring is especially characteristic of the
pycnidia formed by the brand-canker fungus. (Fig. 5.) Adjacent to
these layers are several rows of larger cells, devoid of granular cell
contents and containing a brown pigmentation in the cell walls.
These layers of cells form the enveloping wall of the pycnidium.
The pycnidial cavity may vary considerably in shape, and the pyc-
nidia are usually so closely grouped that they appear stromatic.

9 The synthetic agar was made according to the formula given by Leonian (18) as follows: Dihydrogen
potassium phosphate, 1.25 gm.; magnesium sulphate, 0.625 gm.; peptone, 0.625 gm.; maltose, 6.25 gm.;
malt extract, 6.25 gm.; distilled water, 1,000 c. c.; agar, 1.5 to 2 per cent.
The fungus is characterized by complex pluriloculate pycnidia rising from several confluent primordia. Mature pycnidia are frequently found in which the intervening walls between the aggregate pycnidia have broken down, leaving one large irregular cavity.

In Laubert's description of *Coniothyrium wernsdorffiae* (10, p. 460) he described the pycnidia as "mit ektostromaartiger Papille," penetrated by a broad ostiolate canal. From a study of sections from a number of pycnidia on the host tissue it was found that, when the formation of the pycnidial cavity is completed, there develops at the top of the pycnidium, just below the epidermis, a small cone-shaped mass of thin-walled cells forming a "buffer tissue" (3). (Fig. 5.) The walls of these cells are at first hyaline, but as the tissue grows and finally ruptures the epidermis, the walls become subhyaline, then brown, and finally almost black. The cells along the central line of this tissue dissolve or disintegrate, and a canal ending in a papillate ostiole results. From the cells lining this canal arc produced short filamentous cells resembling the periphyses commonly found in papillate or beaked ostioles of perithecia. These periphyses may disappear as soon as the pycnidium becomes mature, but the buffer tissue often remains for some time. It is this buffer tissue which caused Laubert to describe the pipilla as "ektostromaartige."

In culture the pycnidia show much distinctly than in nature the beaklike papilla through which the ostiolar canal develops. The buffer tissue, however, was present only preceding the formation of the papilla and ostiole. It was frequently found that a secondary cavity was formed in the beaklike papilla. (Fig. 7.) The two cavities are at first separated from each other by a wall several cell layers in thickness. Eventually, however, the separating wall is broken down and an ostiole is formed through the outer wall of the upper cavity.
Laubert (10) further stated that the spores were abstricted from the innermost layer of cells in the pycnidial cavity and that sporophores were lacking. The manner in which the spores are produced in certain species of Coniothyrium has been considered by Von Höhnel (5) as the basis for separating these species of the genus into a new genus, Sclerothyrium. The distinguishing characters of this new genus are the absence of conidiophores and the endogenous formation of the spores.
In the present study of the brand-canker fungus it has been found that the spores are produced by budding from the innermost layer of cells lining the pycnidial cavity. (Fig. 8, A.) These cells appeared to be coated with a gelatinous substance, probably resulting from the lysigenous activity in the process of cavity formation. The first indication of spore formation is the protrusion of a very slender filament of cytoplasm from a cell of the sporogenous layer into the gelatinous coating. The tip of this filament gradually swells and finally emerges from the coating. It then has the ovoid or spherical form of a mature spore and has a thin gelatinous film surrounding it. This ovoid tip remains connected with its parent cell by a thin strand of cytoplasm until the spore reaches maturity. A wall is then formed between the spore and its parent cell and the cytoplasmic strand
FIGURE 8.—A, Section of the sporogenous layer of cells in a pycnidium of the brand-canker fungus, showing near the center a developing spore. × 2,600. B, Germinating spores of the brand-canker fungus. × 1,300. C, Spores of the brand-canker fungus which became septate before germination. × 1,300
disappears. This type of spore formation has been described for certain species of both Phoma (1, 24) and Coniothyrium (17), which were placed respectively in the genera Sclerophoma and Sclerothyrium by Von Höhnel (5). It is apparent that the spores are not formed endogenously, nor are they produced on typical conidiophores. It seems advisable, however, to designate the species as of the genus Coniothyrium until a revision of Von Höhnel's Endogenosporae has been made.

The ovoid spore remains hyaline until it reaches its mature size, at which time pigmentation begins to appear. The pigmentation is confined to the exospore of the spore wall. The spore contents remain colorless.

The spores are abstricted singly from the parent cells. Because of the gelatinous film surrounding each spore, they frequently adhere end to end and give the appearance of chains of spores produced from one cell.

SIZE AND SHAPE OF SPORES

The question of spore size has been considered of importance in determining whether the three rose diseases are caused by the same or different species of Coniothyrium. Laubert's measurements for C. vernsdorffiae (10) are given as 4.5μ to 6μ by 5μ to 8μ. Kock (9) found that the spores of his specimens gave an average length of 5.6μ and width of 4μ. The length of the spores was very variable, some as long as 5.7μ, others 6.6μ, and still others 4.7μ.

In the present study spores of the brand-canker fungus on the host were obtained by crushing on a slide several mature pycnidia. The spores were mixed thoroughly, and a few loopfuls were mounted on a slide in a solution of potassium acetate, 10 gm.; pure glycerine, 200 c. c.; 95 per cent alcohol, 300 c. c.; distilled water, 500 c. c. The spores were allowed to stand in a drop of the solution on a slide for 24 hours, and 50 spores from each of two slides were then measured with a filar micrometer. The measurements of the two sets were then combined, and the means and standard range for the 100 spores were calculated. One hundred spores from several pycnidia produced in polysporic cultures on potato agar were similarly measured. Extruded spores only were used for this series of measurements. The origin of the material used and the results obtained are shown in Table 1. The mean ratio of length to width is given in Table 2.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Source of spores</th>
<th>Length</th>
<th>Width</th>
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</thead>
<tbody>
<tr>
<td>Brand canker</td>
<td>Host; garden; New York</td>
<td>Mean (μ)</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>5.29</td>
<td>0.533</td>
</tr>
<tr>
<td>Do</td>
<td>Host; garden; District of Columbia</td>
<td>5.61</td>
<td>0.667</td>
</tr>
<tr>
<td>Do</td>
<td>Host; Rhode Island</td>
<td>3.09</td>
<td>0.358</td>
</tr>
<tr>
<td>Do</td>
<td>Host; grafted plants; greenhouse; Georgia</td>
<td>2.98</td>
<td>0.427</td>
</tr>
<tr>
<td>Do</td>
<td>Host; cuttings; greenhouse; Maryland</td>
<td>3.27</td>
<td>0.405</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>3.37</td>
<td>0.436</td>
</tr>
<tr>
<td>Do</td>
<td>Host; greenhouse; Pennsylvania</td>
<td>2.75</td>
<td>0.368</td>
</tr>
<tr>
<td>Do</td>
<td>Host; greenhouse; Iowa</td>
<td>3.04</td>
<td>0.426</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
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</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>3.2</td>
<td>0.376</td>
</tr>
</tbody>
</table>
TABLE 2.—Ratio of length to width for spores of fungi from brand canker, stem canker, and graft canker

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Source of spores</th>
<th>Mean ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand canker</td>
<td>Host</td>
<td>1.35</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>1.32</td>
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<tr>
<td>Stem canker</td>
<td>Host</td>
<td>1.47</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>1.35</td>
</tr>
<tr>
<td>Graft canker</td>
<td>Host</td>
<td>1.39</td>
</tr>
<tr>
<td>Do</td>
<td>Culture</td>
<td>1.49</td>
</tr>
</tbody>
</table>

The actual range of the spores measured was 4.0μ to 7.2μ by 3.0μ to 5.4μ. There is a slight difference between these measurements and those given by Laubert (10) for Coniothyrium wernsdorffiae. This is probably due to the fact that spores larger than 7.2μ are exceedingly rare. Many pycnidia from cultures were examined, the pycnidia being crushed on a slide in an attempt to force out all the spores. In one case where two large pycnidia were crushed on a slide, only 12 spores on the entire slide could be found whose length exceeded 7.2μ; these ranged from 7.4μ to 9.4μ. Another slide with two crushed pycnidia showed a larger number of spores exceeding 7.2μ in length. The largest spore measured was 10.6μ by 10.4μ. The width also varied in these larger spores. Their actual measurements may be given as 7.4μ to 10.6μ by 5.2μ to 10.4μ. Among the spores on the slide, however, were many that had begun to germinate. It is therefore possible that the large size of these spores is merely a phenomenon of germination and that these measurements do not indicate the normal size of a mature spore. Some of these large spores were uniseptate. As will be mentioned later, this septation sometimes occurs preceding germination.

PHYSIOLOGY OF THE CAUSAL FUNGUS

DESCRIPTION OF CULTURES ON VARIOUS MEDIA

MALT AGAR.—The fungus produced a moderately abundant, fluffy, aerial mycelium which in mass was greenish gray. Pycnidia were rare and were first noticeable about four weeks after inoculation. Another three or four weeks of growth was necessary to bring them to maturity.

CORN-MEAL AGAR.—A slight amount of grayish mycelium was produced. Only a few pycnidia were formed. The rate of growth was relatively the same as on malt agar.

POTATO AGAR.—The mycelium was moderately abundant, white to mouse gray, and mature pycnidia were present about six weeks after inoculation. This medium proved very satisfactory for the production of pycnidia and spores.

STERILIZED POTATO SLICES.—A dense, fluffy, mouse gray to dark gray aerial mycelium was formed, producing abundant mature pycnidia in six to eight weeks after inoculation. The pycnidia were large with a pronounced tendency to aggregate or become complex. The spores were exuded in the form of spore horns.

DEXTROSE AGAR.—The dextrose agar was made with 2 per cent dextrose and 2 per cent agar. A moderate amount of greenish gray aerial mycelium was produced, but no fruiting bodies were developed to maturity. A few appeared to form in the mycelium along the edge of the agar slant, but they failed to develop spores.

SYNTHETIC AGAR.—The synthetic medium developed by Leonian (12) for the production of pycnidia in cultures of Sphaeropsidales was used. The medium was more satisfactory than the others with the exception of the sliced potato. A fluffy, greenish gray mycelium was formed, and pycnidia appeared after about eight weeks.

ROSE-STEM AGAR.—A medium made from a decoction of rose stems with agar was tried, but the results did not prove of value. No mature pycnidia were produced.
STERILIZED ROSE STEMS.—Stems of climbing and hybrid tea roses were cut into convenient lengths, placed in flasks with 10 to 20 c.c. of distilled water, and sterilized. When cool the stems were inoculated with mycelium, with spores, or with entire pycnidia. Growth was very satisfactory, and pycnidia were produced abundantly.

**Spore Germination**

Germination tests were made on several different media. In hanging-drop cultures with tap water the spores showed evidence of germination in 24 to 36 hours. (Fig. 8, B.) In distilled water the number of germinating spores was very much smaller and the rate of germination was very much slower. Tests were also made by sowing spores on a thin film of agar on a microscope slide. The agar mounts were kept moist in a Petri dish and were examined at 24-hour intervals. The agars used were malt, potato, dextrose, and Leonian’s synthetic. The spores germinated on all these agars with about the same readiness as in tap water.

Germination was preceded by a swelling of the spores. Very frequently this increase in size was followed by septation. (Fig. 8, C.) The first septum usually originated as a slight separation of the cytoplasm near the center of the spore. The separation gradually extended outward to the spore wall, and a definite septum then became apparent. In some cases the spores became constricted at the septum. A second septum sometimes appeared, either parallel with or at right angles to the first septum. In these septate spores germination may take place from each of the cells. The tendency of spores of Coniothyrium species to become septate has been previously reported by Archer (1), but no extensive investigations have been conducted to determine the possible relation of septation to changes in environment. It is apparent, however, that spores of certain species of Coniothyrium may show the same tendency toward septation as do those of species of Sphaeropsis.

**Pathogenicity of the Organism**

**Inoculation Experiments**

Since facilities for growing roses for inoculation under greenhouse conditions were not available to the writer, it was necessary to devise other means for growing rose cuttings. Several pots of sterilized sand were prepared, and in these pots were placed cut stems of a climbing rose. The leaves were removed and the cuttings were allowed to grow about one week before they were inoculated. One series of inoculations was made by placing the inoculum, composed of a portion of a mature pycnidium with exuding spores, directly in the axil of a bud without injuring the bud or bark in any way. In a second series the inoculum was introduced into the stem tissue through a wound in the bark made by a sterile scalpel. The inoculum used was obtained from polysporic cultures grown on sterilized potato slices. In each series of experiments one pot with a cutting was set aside as a control. In all cases the cuttings were kept in a moist atmosphere under bell jars.

All the cuttings produced new healthy leaves from the two uppermost buds. With the exception of those buds in the axils of which the inoculum was placed, the buds remained green and healthy, although they developed no leaves. The inoculated buds soon turned brown and in about six weeks were found to be covered with pycnidia. The spread of the fungus in the bark tissue, however, was very slow. The infected bark at first became a reddish brown, then a dark brown. As
the pycnidia appeared and the tissue of the affected areas became dried and dead, the color of the central portion changed to a light brown with a margin of reddish brown. Before the cankers had reached any considerable size, however, the cuttings died. The fungus was re-isolated from the infected areas. On the canes that were inoculated through wounds made with a sterile scalpel, the infected areas increased in size more rapidly and pycnidia were produced more abundantly.

At the same time a series of similar inoculations was made on red-raspberry canes. In all cases small cankers appeared on the bark of the canes before the death of the cuttings. As the bark became dried following infection, it peeled off in shreds. This characteristic was noted by Laubert (10) in cankers on rose canes caused by *Coniothyrium wernsdorffiae*. The small prickles on the raspberry canes became infected and in some cases a pycnidium was formed within the prickle, the fungous hyphae thus taking the place of the disintegrated host tissue. Cankers were formed from infected buds as well as from inoculated wounds.

No reference in literature could be found to the occurrence of *Coniothyrium wernsdorffiae* on parts of rose plants other than stems or branches. Therefore, tests were made with hybrid tea and climbing rose leaves, as well as with the hips of *Rosa rugosa*, to determine whether the fungus would infect them and possibly overwinter in this way. In no case, however, did the fungus infect the leaves or hips in a moist chamber.

**Penetration of the Host**

In Laubert's (10) discussion of the symptoms of brand canker caused by *Coniothyrium wernsdorffiae*, he stated that the majority of cankers were found around the dormant buds. This is an especially noticeable symptom of the disease in the United States. Of the many cankers examined, the majority appeared to have had their origin in the vicinity of a dormant bud. A few, however, originated without a wound or a bud as the locus of infection.

The results of the inoculation experiments in the present study indicate that the fungus is capable of infecting dormant buds. Growth was more rapid, however, when the germ tubes were able to gain entrance through wounds.

**STEM CANKER AND GRAFT CANKER**

In Vogel's discussion of the rose-graft disease (22) his conclusions as to the identity of the causal fungus were based upon his inoculation experiments, the size of the spores and pycnidia, and the comparison of cultures of the organism found on rose with those of *Coniothyrium fuckelii* isolated from black-raspberry canes. He stated that "a characteristic symptom of this disease is the occurrence of lesions on the scion at the union, and just above the union." In the present study, therefore, only those specimens which showed definite cankers at the union of stock and scion were considered as affected with the graft disease. The fungus causing these cankers was closely compared with that occurring on the so-called stem cankers. Therefore the symptoms, morphology, and physiology of these two fungi will be considered together.
SYMPTOMS

In the case of well-developed graft cankers, the color is darker than that of stem cankers. It varies from Dresden or cinnamon brown at the center to hazel or auburn at the margin. The main portion of the stem cankers, however, varies in color from wood brown or cinnamon buff to snuff brown or Saccardo's umber. The margin is frequently army brown.

In both cases the presence of the fungus can be detected by means of a hand lens because of the sooty covering of spores over the slightly erumpent pustules. A splitting of the bark along the margin of the canker and the succeeding callus formation are characteristic of both of these diseases.

MORPHOLOGY OF THE CAUSAL FUNGI

MYCELIUM

The mycelia in the tissue of the two types of cankers appeared to be similar. No distinguishing features could be observed. This also was true of the mycelia developed in cultures. On corn-meal agar the mycelia were comparatively scanty and were white without any conspicuous tinge of gray. The gray was more pronounced, however, on the dextrose and synthetic agars, on which also the aerial mycelia were luxuriant. The mycelia on malt agar were not so luxuriant and were a slightly darker gray. On potato agar and sterilized potato slices the mycelia were very luxuriant and the color varied from white to light gray.

FORMATION OF PYCNIDIA AND SPORES

The pycnidia and spores of both the graft-canker and stem-canker fungi were formed similarly to those of the brand-canker fungus. In the case of the graft-canker fungus, however, the majority of the pycnidia on the host were simple globose unilocular structures. (Fig. 9.) The stem-canker fungus appeared to form complex pluriloculate pycnidia arising from several confluent primordia. (Fig. 10.) In this respect it resembled the brand-canker fungus.

In Saccardo’s description of Coniothyrium fuckelii (19) he stated that the papillate ostiole is scarcely prominent. Thomé (21) described the pycnidia of the same fungus as having an inconspicuous, scarcely prominent papillate ostiole. The original description of C. rosarum by Cooke and Harkness (2) did not mention the type of ostiole or the method of spore discharge. In the diagram given by Vogel (22) the spores of his C. rosarum appear to be exuding through a pore in the upper wall of the pycnidium. This method of spore discharge would correspond with that observed by Archer (1, p. 51) in his study of C. concentricum. He stated that “no definite ostiole is formed but it seems that usually there is merely a rupture or dissolution in the upper wall, at a point just below a stoma, which allows for the discharge of the spores.”

A papillate ostiole was found in the pycnidia of both the fungi studied. The manner of its formation and its general character were similar. A buffer tissue was developed, as in the pycnidia of the brand-canker fungus, with an ostiolate canal and periphyses. The buffer tissue was not so conspicuous, however, and both buffer tissue

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and periphyses became disorganized when the pycnidia reached maturity and thus eventually disappeared. The ostiolate canal was very short, and, as Thomé (21) stated, the papilla scarcely breaks through the epidermis.

In culture the pycnidia were developed in much the same manner as on the host. No buffer tissue, however, was found in connection with either fungus. The first pycnidia formed were usually simple uniloculate structures. Later both fungi developed complex pluriloculate pycnidia arising from confluent primordia. Vogel (22) stated that in culture the pycnidia of his Coniothyrium rosarum were larger than those of his C. fuckelii isolated from black raspberry. It is possible that he was comparing the complex pycnidia of one fungus with the simple pycnidia of the other.

Saccardo (19) described Coniothyrium fuckelii as "basidiis non visibilibus." Thomé (21), in his description of the same fungus, stated that the conidiophores are not perceptible. No mention of the conidiophores is made by Cooke and Harkness (2) in connection with C. rosarum. Vogel also (22) does not mention them, and from the diagram included in his article it would appear that he failed to find them.

The spores obtained from the two types of cankers studied by the writer were formed by budding, in exactly the same manner as those of the brand-canker fungus. No evidence of an endogenous spore origin could be found.
Size and Shape of Spores

Vogel (22), in the discussion of his Coniothyrium rosarum, distinguished this species from *C. fuckelii* as follows:

![Figure 10.—Aggregate pycnidia of the stem-canker fungus. X 160](image)

The spores *C. rosarum* are single celled, globose to ellipsoid, brown, 3μ by 3μ to 4μ in size. These measurements are the same as given by Cooke and Harkness for *Coniothyrium rosarum*.

The measurements of *C. fuckelii* given by Massec are, pycnidia 180μ to 200μ in diameter; spores 3μ to 4μ by 2μ to 3μ.
Saccardo (19) reported the spore measurements of _Coniothyrium fuckelii_ as 2.4μ to 5μ by 2μ to 3.5μ, and of the variety of _C. fuckelii_ on Rosa as 3μ to 5μ by 3μ. The description of _C. fuckelii_ given by O’Gara (16) corresponds with that by Saccardo except for the spore measurements, which he gave as 2μ to 4.5μ by 2μ to 3.5μ. The difference in the measurements given for spore length might be explained as the result of an accidental transposition of the decimal point and the figure 4.

It was impossible in the present study to formulate a plan for measuring the spores produced by the fungi from the two types of cankers which would eliminate from consideration the modifying influence of all contributory environmental factors. The fact that the so-called graft canker is produced typically on young grafts under greenhouse conditions and the stem canker on older plants in either the greenhouse or the garden creates difficulties in making a satisfactory comparison. Moreover, the smallness of the spores and the difficulty of selecting for measurement spores of equivalent maturity add to the possibility of inaccuracies in the calculation and comparison of spore size. For these reasons it is evident that the data on spore measurements here presented do not constitute a comprehensive biometric study, but show the results obtained from certain specimens grown under the conditions stated.

The spores were measured according to the procedure already described for the spores of the brand-canker fungus. In order to increase visibility, a stain consisting of 10 gm. of erythrosin was added to the mounting medium. The source of the material and the results obtained are given in Table 1, and the ratio of length to width in Table 2.

In the actual measurements the spores were found to vary considerably in both length and width. The actual range of the spores was as follows: Graft-canker fungus, 2.0μ to 4.0μ by 1.6μ to 3.6μ; stem-canker fungus, 2.0μ to 4.4μ by 1.2μ to 3.0μ. It is apparent that these measurements, respectively, show the same range as those of Cooke and Harkness for _Coniothyrium rosarum_ and of Massée for _C. fuckelii_. As in the case of the brand-canker fungus, spores larger than those of the ranges given were exceedingly rare and were usually found only in mounts bearing germinating spores.

**PHYSIOLOGY OF THE CAUSAL FUNGI**

**DESCRIPTION OF CULTURES ON VARIOUS MEDIA**

Comparative series of cultures were made of the fungi isolated from the two types of cankers. Specimens of the cankers were placed in moist chambers, and as soon as the spores exuded from the pycnidia in a gelatinous mass a few of them were transferred to the culture tubes. Transfers were always made on the same day and under conditions of environment as similar as possible. As previously stated, no distinguishable differences could be detected between the organisms from the two types of cankers in either the mycelial growth or the production of pycnidia. The characteristics of growth upon the various media were as follows:

**MALT AGAR.**—The young mycelium was almost white, that is, just barely tinged with gray. As development proceeded the aerial mycelium became
moderately abundant and fluffy with a more decided grayish tinge. The young advancing mycelium, however, appeared white. In about two weeks after inoculation the pycnidia began to develop. They were produced in moderate abundance and were usually single. In an old culture, however, the tendency toward aggregate pycnidia was noticeable.

**Corn-meal agar.**—A small amount of white mycelium was produced, which was more closely matted than on malt agar and showed no conspicuous tinge of gray. Pycnidia were developed in abundance and were at first a yellowish brown, deepening to dark brown at maturity. The black sooty mass of spores at the ostiole was particularly noticeable. The rate of growth was relatively the same as on malt agar.

**Potato agar.**—The mycelia in all the cultures on potato agar were white or very light gray and moderately abundant. Numerous dark-brown fruiting bodies were formed. The rate of growth was slightly more rapid than on the other media.

**Sterilized potato slices.**—This medium proved very satisfactory for the production of both mycelium and fruiting bodies. Dense white aerial mycelia and complex pycnidia were formed in all the cultures.

**Dextrose agar.**—As in the case of the brand-canker fungus, the mycelial growth was medium in amount. Comparatively few pycnidia were formed in the grayish-white mycelium.

**Synthetic agar.**—Excellent cultures resulted from the use of this medium. The mycelium was abundant, fluffy, and white slightly tinged with gray. Good-sized pycnidia were produced in moderate amount, both single and aggregate structures being found.

**Rose-stem agar.**—The organism produced only a moderate growth; a few pycnidia being developed to maturity.

**Sterilized rose stems.**—Portions of rose stems were prepared as already described in connection with the brand-canker fungus, and were inoculated with spores, mycelium, or pycnidia of the organisms from the graft cankers and the stem cankers. This method was the most successful for obtaining an abundance of mature pycnidia and spores.

**Spore germination**

Germination experiments, comparable to those for the brand-canker fungus, were made in tap water, distilled water, and the various media. The spores from both cankers germinated readily after 24 hours in tap water, but not quite so readily in distilled water. Moreover, the percentage of germination was greater in tap water than in distilled water. On the agars used the rate and the percentage of germination gave results similar to those from the hanging-drop cultures in tap water. In all cases a pronounced swelling of the spore preceded germination. (Fig. 11.) Septation of the spores, which was a characteristic of the spores of the brand-canker fungus, occurred only rarely.

**Pathogenicity of the organisms**

**Inoculation experiments**

Rose cuttings grown in pots of sterilized sand were inoculated as in the experiments conducted with the brand-canker fungus. In all cases cankers were produced on both the cuttings inoculated in the axil of a bud and those inoculated through wounds. The fungus was reisolated from the diseased areas. The pycnidia produced on the cankers showed both simple and complex structures.

Cuttings of red-raspberry canes were similarly inoculated, and cankers resulted. The pycnidia differed from those produced on the rose in that they showed a tendency to be single and unilocular rather than complex plurilocular.
Inoculations of leaves in moist chambers proved unsuccessful. Since a collection of specimens of hips from hybrid plants of *Rosa rugosa* showed an infection by a fungus resembling *Coniothyrium fuckelii*, a number of healthy hips were inoculated in moist chambers with the spores of the fungus isolated from the graft cankers and the stem cankers. Pycnidia and spores were readily produced on the inoculated hips in all cases. (Fig. 12.)

**Penetration of the Host**

From the results of the inoculations on the rose cuttings it is evident that infection may take place through either dormant buds or wounds. An examination of many specimens of stem canker in nature leads to the conclusion that the germ tubes are capable of entering the tissue even if no wounds or buds are present. Such infection, however, seems to be extremely rare. The diseased areas usually occur around wounds such as those caused by the breaking or pruning of stems, by the rubbing of the prickles of one stem against the bark of another, by the accidental removal of the prickles, or by insects. Rarely does one find a pruned stem which does not have a few pycnidia on the cut end at the inner margin of the bark. The same condition is very frequently found on rose cuttings, particularly those made from a plant affected with stem canker. Graft cankers also appear to result from infection through wounds or through the callus at the point of union of stock and scion. In the present study it was found that the fungus may also infect through dormant buds.
CONCLUSIONS

It is evident from this study of the morphology and physiology of the fungi isolated from the three types of cankers that the brand-canker fungus is a different species of Coniothyrium from that causing the so-called stem cankers and graft cankers. This corroborates the statements made by Laubert (10) and by Jenkins and Martin (8), who attributed the disease to Coniothyrium wernsdorffiae. In the case of the fungi isolated from stem canker and graft canker there was no evidence of any morphological or physiological differences between them. Since the fungus obtained in cultures from the stem cankers was similar to that produced in cultures from spores of Leptosphaeria coniothyrium, the writer felt justified in considering it to be C. fuckelii. The results of the present study would seem to indicate, therefore, that the graft cankers were produced by the same fungus that caused the stem cankers. The graft-canker fungus, moreover, resembled that collected and identified by Vogel (22) as C. rosarum in so far as its morphology under natural conditions is concerned. Unfortunately, type specimens of C. rosarum were not available for examination. Regardless of this fact, however, the conclusion may be drawn that the two types of cankers are caused by one and the same fungus, which resembles the imperfect stage of L. coniothyrium described by Saccardo as C. fuckelii.

SUMMARY

Three diseases attributed to species of Coniothyrium have been described as occurring upon the genus Rosa in the United States. Because of the similarity of the symptoms of the diseases, there has been considerable doubt as to the identity of the causal fungi. The present study was undertaken to determine what morphological and physiological characters might be of value in diagnosing the diseases.

The fungus isolated from the so-called brand-canker disease may be distinguished from other species of Coniothyrium on rose by the pronounced buff-like tissue and papilla as well as the long ostiolar canal of its pycnidia, the size of its spores, and the grayish color of its mycelium in culture. Spore germination and the rate of growth in culture were relatively slow. The spores of the fungus were produced by budding from the layer of cells lining the pyenidal cavity. Inoculation experiments showed that the fungus was capable of infecting rose and red-raspberry cuttings through dormant buds and...
through wounds. Inoculations of rose leaves and hips were not successful. The fungus is designated as *Coniothyrium wernsdorffiae* Laub.

No morphological or physiological differences could be detected between the fungi isolated from the so-called stem cankers and graft cankers. The pycnidia varied from simple uniloculate to complex pluriloculate structures and developed an inconspicuous buffer tissue and papillate ostiole. The mycelium in culture was white or light gray. The spores were produced by budding, as in *Coniothyrium wernsdorffiae*. Rose and red-raspberry cuttings were successfully inoculated through dormant buds and through wounds, with the production of cankers. Rose hips became readily infected following inoculation, but no growth occurred on rose leaves. The results of this study seem to indicate that the stem cankers and graft cankers were caused by the same species of Coniothyrium, which is designated as *C. fuckelii* Sacc.

**LITERATURE CITED**

(1) **Archer, W. A.**


(2) **Cooke, M. C., and Harkness, W. H.**


(3) **Dodge, B. O.**


(4) **Güssow, H. T.**


(5) **Höhnel, F. von.**


(6) **Howitt, J. E.**


(7) **Jenkins, A. E.**


(8) —— and **Martin, G. H.**


(9) **Kock, G.**


(10) **Laubert, R.**


(11) ——


(12) **Leonian, L. H.**


(13) **Lind, J.**

1913. Danish fungi as represented in the herbarium of E. Rostrup. 648 p., illus. Copenhagen.