West Virginia Agricultural and Forestry Experiment Station Bulletins

Davis College of Agriculture, Natural Resources And Design

1-1-1934

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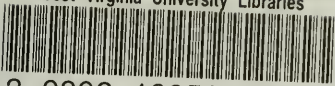
European Canker of Black Walnut and Other Trees

by
J. M. ASHCROFT

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European Canker of Black Walnut and Other Trees

by J. M. ASHCROFT *

BLACK WALNUT (*Juglans nigra* L.) is one of the most valuable of the trees indigenous to eastern United States. The durability and luster of its wood when polished has long made it a favorite in the manufacture of fine furniture, gun stocks, and for the interior woodwork of buildings, while the rich flavor of the nuts has endeared the tree to many. West Virginia is among the leading states in the production of walnut timber, and in recent years a start has been made in the growing of the fruits of this tree for the commercial market. While yet in the incipient stages of development, nut-growing holds promise of becoming in the near future a profitable business enterprise. The presence of a disease destructive to this tree, therefore, was held to be of sufficient economic importance to justify an investigation. In the following pages the results of this investigation are reported.

DESCRIPTION OF THE DISEASE

The disease appears on the axial parts (trunk and branches) of the tree as a rough sunken or flattened canker, characterized in the more advanced stages by a number of prominent ridges of callus wood arranged more or less concentrically around a central area from which very frequently the stub of a lateral branch protrudes. Each of these ridges of callus wood is produced by the host at the limits of the infected area, according to the natural wound-healing process of trees. As the disease progresses, the last-formed callus circumvallation is killed, and the following season a new callus is formed beyond the old. This new callus eventually succumbs to the same fate. Hence in a few years the typical canker develops a number of these ridges arising terrace-like about a central point. As the lateral margins of the canker continue to advance eventually the stem is completely girdled. When this occurs the parts of the tree above the canker die. Usually the growth at the margin of the canker is hyperplastic or hypertrophic. This fact lends to the cankered area, in front view, a bulged or flared appearance (Pl. I, fig. 2). Sometimes, under conditions favoring rapid growth of the tree, the diameter

of the affected region of the stem in the direction parallel with the "face" of the canker is increased to twice the diameter of the unaffected parts.

At first the bark in young cankers remains adherent to the wood beneath. But as the canker spreads, the dead bark eventually is sloughed off, first at the center of the canker and progressively as the disease advances. The wood thus exposed is seen to have the same concentric, terrace-like pattern (Pl. II, fig. 1).

Another form of the younger stages of the canker makes its first appearance as a tumor-like swelling on the trunk. This type seems not to have its beginning in infection through the stubs of broken branches. After the swelling has reached considerable size the outer cork cracks open and exposes the wood. From this point the development is the same as that described above.

On the smaller branches and twigs the concentric pattern of the canker is not so evident. This is due partly to the fact that the typical canker form is somewhat obscured by the greater hypertrophic growth of the younger parts, and partly to the circumstance that very small branches are killed in a single season. On parts large enough to support perennial cankers, however, the development, although somewhat varied in appearance, is always the same.

Under uniform conditions the successive formation of the callus ridges occurs in a fairly regular way, and the resulting canker is symmetrically formed with uniform spacings between the ridges. But many cankers are very unsymmetrical. For some reason the growth of the canker may be retarded or cease in certain parts of its circumference. At such points the callus continues to grow instead of being killed back, and much of the once exposed wood may be covered again by this callus. At other points of the circumference the canker continues to develop in the usual manner. The outer margin of such a canker, at a given time, may be any shape and be continually changing. Cankers formed at the crotch or forking of a branch are as a rule shaped somewhat like the letter Y.

The size of the canker varies greatly according to its age. Of course on the smaller limbs it never becomes very large, and develops only few ridges. But on the main trunk and larger limbs cankers have been seen to range in size from a few inches to 4 ft. longitudinally and 2.5 feet laterally, and with as many as 24 concentric ridges (Pl. II, fig. 1).

During late autumn and winter months the red perithecia of a Nectria are almost always to be found associated with the canker. They appear singly or in small clusters most frequently on the dead but still adherent bark of the later-formed ridges, but may be present on any of the dead tissue, even on the exposed heartwood. Sometimes the perithecia are produced in great abundance and again they may be found only by careful search in the deeper fissures of the dead bark. On cankers resulting from natural infection the conidial stage (Cylindrocarpon) of this fungus has never been found by the writer.

Very frequently a tree affected will have a number of cankers scattered over the trunk and limbs. In fact, a tree with but a single canker is the exception, and trees still living are commonly found on which literally dozens of cankers are scattered on the trunk and most of the branches (Pl. II, fig. 2).

Cankers for which the above description might apply with equal validity have been discovered on a number of other species of trees native to West Virginia: *Liriodendron tulipifera* L., *Acer rubrum* L., *Quercus rubra* L., *Q. velutina* Lam., *Q. alba* L., *Hicoria glabra* (Mill.) Britton, and *Juglans cinerea* L. With the exception of the last, the cankers on the trunks were all large, and typically formed: *i.e.*, they showed the same concentric arrangement of callus ridges. On *Juglans cinerea*, however, cankers were found on only one occasion, and then they were all confined to the smaller branches. In this case the small lesions were typical of the disease as it appears on black-walnut branches of equal size.

HISTORY OF THE DISEASE ON BLACK WALNUT

Although many references to a similar canker on many fruit and forest trees in Europe and America can be found in phytopathological literature, no published record of its occurrence on black walnut had appeared until 1930, when Orton (*27*) published a brief description of the canker at the beginning of this investigation. It soon became evident, however, from the replies to a request appended to that description that the canker on this host had not passed the notice of phytopathologists. Dow V. Baxter* wrote that he had seen the canker in West Virginia and other eastern states, and in Indiana and Wisconsin; that he had made collections in Wisconsin as early as 1924; and that he had sent specimens of this collection to the Office of Forest Pathology at that time. A collection of Nectria reposing in the herbarium of the Office of Forest Pathology indicates that A. H. Graves had observed the canker on walnut as early as 1910. According to the label, this specimen was taken from a badly cankered but still living black walnut tree in the neighborhood of Balsam, N. C. The label, furthermore, informs us that the disease was rather common in that section.

The writer has seen cankers bearing 24 callus ridges, each of which represents at least one year in the age of the lesion. This canker, therefore, could not be less than 24 years old. Since the tree observed by Graves was "badly cankered," we may reasonably assume that the cankers thereon were several years old. Hence it appears that the disease has been present in the Appalachian region for approximately 30 years at least.

Perennial canker of this type on apple has been known for several centuries in Europe, where it has been and still is a serious problem of apple growers. In certain sections of Germany the disease has been responsible for the complete abandonment of apple-growing on a commercial scale (14). The canker has more recently been found on the North American continent, where it is now commonly known as ''European canker,'' and in other parts of the world. At present it is probably co-extensive with the land areas of the temperate zones (34). Although this malady is an important destroyer of the apple tree, it is not confined to that host, but has been described on a large number of other deciduous trees in Europe and America. The cankers described above on *Juglans nigra, J. cinerea, Hicoria glabra, Acer rubrum, Quercus rubra, Q. alba, Q. velutina,* and *Liriodendron tulipifera* are to be considered extensions of European canker to new host species. Therefore a short review of the more important contributions to our knowledge of this disease is appropriate to a further discussion of the problem.

The early writers on the subject of canker of trees attributed it to many and varied causes, many of which to the modern reader seem to be merely expressions of the superstitions then prevalent (37). Among the explanations which possess some degree of plausability, adverse environmental factors such as poor soil conditions and frost injury were probably the most favored. The latter explanation as the primary cause of canker has persisted until very recent times (37).

In 1866 Wilkomm (47) published the results of his investigation of a disease of the young shoots of beech—a disease which he described and named the ''schwarzen Brand der Rotbuchentriebe.'' In an addendum to the same publication, however, he identifies this disease with the perennial type of canker. He found fungous mycelium growing in the tissue of the host plan. Microchemical tests showed that starch and tannin had disappeared from the invaded tissue, and that changes had occurred in the chemical composition of the walls of young wood cells and bast fibers as indicated by the change from the purple color of these cells in normal tissue to a gold-yellow in invaded tissue when both were treated with a solution of chlorozinc iodide. The cause of the disease he attributed to a fungus associated with the diseased parts. He identified this fungus as *Fusidium candidum* Link, and believed that the spores of a second associated fungus, which he identified as *Libertella faginea* Desm., represented the spermatial stage. This latter view has since proved erroneous (15) (19). Wilkomm made no inoculation studies, and his work left much to be desired in the way of proof of his claims.

Some years later (1877) Goethe (14), like many before him, concluded from his study of canker on apple that this disease was caused by frost injury; but thought that the destructive work of frost was favored by poor soil conditions, native susceptibility, and by sucking scale insects, particularly *Coccus mali*. In 1878 he discovered young

cankers on the growth produced in that same year. Since no late frosts had occurred that year, the possibility of frost injury was excluded in this instance. He (15) was therefore induced to reinvestigate the problem of causal agent. When these twigs were placed in a moist chamber for a few days, the surface of the wounds became bedecked with the conidial stromata of a fungus which corresponds closely to Wilkomm's *Fusidium candidum*. He later learned that this fungus had a second fruiting stage, which was formed on the same stroma and which identified the organism as a Nectria. This Nectria, he believed, did not differ significantly from *Nectria ditissima* Tul.

Inoculations of apple with the spores of the fungus produced the symptoms of the disease. He next inoculated wood of different ages (1, 2, and 3 years), and found that the symptoms appeared first on the 1-year-old wood, and later but less marked on the others according to age. Unwounded twigs kept in a moist chamber were infected directly through the epidermis and through the lenticels. He then extended his inoculations to other hosts. Cross inoculations of pear with the fungus isolated from apple and *vice versa* gave positive results in both cases. The same results were obtained with cross-inoculations with isolants from apple and beech on beech and apple respectively. Inoculation of *Acer pseudoplatanus* with the organism isolated from apple succeeded, but the same organism failed to infect *Æsculus hippocastanum*, and *Ulmus montana*.

Goethe also studied, microscopically, the behavior of the fungus in apple tissue. He observed the penetration of the germ tube through the epidermis and also through the lenticels. In the bark, the mycelium grew more luxuriantly in the phloem region as opposed to the cortex. The contiguity of the cells was finally broken in advanced necrosis, and the mycelium could be seen between the disrupted cells. The fungus, he states, enters the xylem by way of the medullary rays, from which it spreads into the surrounding vessels and tracheids through the pits. He noted, as did Wilkomm, that the starch had disappeared from the regions invaded by the fungus.

While Goethe was occupied with the problem of apple canker, a contemporary of his, Hartig (19), was concerned with the canker of beech and other forest trees. He collected what he considered to be *Nectria ditissima* Tul. from a number of hosts: Fagus, Quercus, Corylus, Fraxinus, Carpinus, *Alnus glutinosa, Acer campestris, Acer pseudoplatanus*, Tilia, Frangula, and Padus. Inoculations of beech with the organism from that host (beech) failed when attempted in May, but those tried later, in September and October, were successful. Hartig studied the manner in which the canker form was developed. He noted that the formation of concentric rings was due to an alternation of the growth period of the fungus with that of the host. The periodically augmented growth rate of the parasite he attributed to an increased supply of moisture. He thought the same factor was responsible for the greater destructiveness of the canker on the trunks as compared with infections on limbs and branches. He succeeded in infecting young leaves

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by sprinkling them with a spore suspension as Goethe had done in the case of apple. In his microscopical examination of the invaded region, Hartig observed within the host tissue large numbers of small, conidia-like bodies which he believed were abstricted by the fungus. He hypothesized that perhaps these bodies were instrumental in the destruction of the host cells, but that the mycelium threads only were responsible for the spread of the fungus within the host. Later workers have failed to observe any such bodies. Hartig illustrated a number of the stages in the development of the perithecium on the old conidial stroma. Although he inclined to the opinion that canker on the several hosts was caused by the same fungus, he noted the variability among the different isolants and freely admitted the possibility that more than one species is involved.

In 1892 Lapine (22) repeated the experiments of Goethe, using for inoculum pure cultures of a Nectria which he considered as *Nectria ditissima* Tul., and which he had isolated from apple. His results substantiated Goethe's conclusions in every way. Inoculations were then extended to other hosts such as Acer, Populus, Catalpa, Quercus, Cydonia, and Platanus. Of these, only the inoculations on Quercus gave positive results. Microscopic examination of the wood of the other trees showed that mycelium was growing therein, although no lesions resulted.

The workers already mentioned considered that they were justified in their claims of having demonstrated the cause of canker when they succeeded in producing infection by artificial inoculation. Aderhold recognized the possibility that such an infection might occur without a subsequent development into the characteristic concentric ringed pattern of the typical canker. Consequently he decided to settle this objection. He (1) followed the development of artificial infections on apple for a period of a year. During this time the wound margin had produced its first callus and the infection had spread into this newly-formed callus tissue. At the same time he tried inoculations on cherry and plum with the same organism which was originally isolated from apple. Although the results were positive, the resulting infections did not behave exactly as did those on apple. Furthermore, no fructification appeared on these infections. This led Aderhold to conjecture whether only sterile mycelium might be formed on these hosts, and whether new infections might depend upon spores produced on other hosts.

In this same publication Aderhold criticized the work of Brzezinski (5), who had published the results of his investigation earlier in the year (1903). Brzezinski states that his inoculation experiments with *Nectria ditissima* Tul. on apple had yielded negative results, that this species was in no way responsible for the canker, but that this disease was probably caused by a bacterium (*B. mali* Brz.) which he found constantly present in the diseased tissue. Aderhold's criticism elicited a scathing rebuke from Brzezinski and began a bitter polemic between these two workers. It should be mentioned that Brzezinski (6) reports the production of large pycnidia, visible to the naked eye, by the fungus which he alleged was *Nectria ditissima* Tul.

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Meanwhile the canker was found on apple in America. Paddock (28) reported in 1900 the discovery of cankers in Nova Scotia and Cortland county, New York. Specimens sent to Hartig for verification were identified by him as the same as the canker common in Europe.

Weese (42) concluded from taxonomic studies that *Nectria ditissima* Tul. is not the cause of canker, and that *N. galligena* Bres. is the only canker-causing Nectria. He states that he never found the former species associated with canker on any of the specimens in the various herbaria which he visited in Berlin and Vienna. On the other hand, he found almost invariably a fungus coinciding with *N. galligena*, with canker on ash, apple, and hazel. He, moreover, concluded that *N. ditissima* Tul. is synonymous with *N. coccinea* (Pers.) Fr. He distinguished *N. galligena* from *N. coccinea* (=*N. ditissima*) principally by its larger spores, by the structure of its perithecial wall, by the gross habit of its fruit bodies, and by its pathogenicity.

Voges (41) in 1914 denied Weese's statement that *Nectria ditissima* is unable to cause canker on apple, asserting that he (Voges) had worked with *Nectria ditissima*, with which he produced canker by artificial inoculation, and that he had never found *Nectria galligena*. Voges made a study of the histological differences between sound and cankered stems and showed by inoculation experiments that differences existed in the resistance and susceptibility manifested by different varieties of apples. He was unable to substantiate the claims of Lapine and Goethe that infection may occur through the lenticels. Above all he ridicules the theory of Muench, that disease resistance is dependent upon a high water content of the invaded tissue.

After an examination of material which Goethe had himself collected, and of specimens sent by Voges, Weese (43) wrote that both men had worked with *Nectria galligena* and not *N. ditissima*. Meanwhile he had found *Nectria galligena* associated with cankers on beech. This discovery strengthened his belief that *Nectria galligena* is the only Nectria which produces cankers on deciduous trees.

In 1910 Appel and Wollenweber (4) reported the results of some inoculation experiments with several canker-producing organisms which they had isolated from cankers on beech and apple trees and from apple fruits. Inoculations of several varieties of apples with these organisms gave positive results in all cases. The authors came to the conclusion that these organisms, in spite of minor variations under cultural conditions, were all the same species, *Fusarium wilkommii*, and that according to the earlier workers it must be the imperfect stage of *Nectria ditissima*.

After the appearance of Weese's first publication (1911), Wollenweber (50) concurred with the former in his belief that *Nectria galligena* is the cause of canker on apple, and that *N. ditissima* is synonymous with *Nectria coccinea*. Wollenweber created the form-genus Cylindrocarpon for the imperfect stage of the Nectrias which have subcylindrical conidia with rounded apical cells and rounded or somewhat flattened basal cells. Hence those organisms referred to in the earlier paper now were referred

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to as *Nectria galligena* and the conidial stage, *Cylindrocarpon mali* (Allesch.) Wr. Later he (*51*) reports that inoculations of beech with *Nectria galligena* isolated from both apple and beech produced infection, but did not result in typical canker, and that *Nectria coccinea* from beech on beech produced no injury at all. However, neither organism isolated from beech was associated with a canker. At the same time he reports the discovery of a Nectria which was associated with typical beech canker, and which in regard to the spore size stood intermediate between *Nectria coccinea* and *N. galligena*. He identified this fungus as *Nectria ditissima* Tul. despite his earlier agreement with Weese as to synonymy of *Nectria ditissima* with *Nectria coccinea*. Inoculations of beech with this organism gave indecisive results. Still later Wollenweber (*52*) recognized not only the three species, *Nectria galligena*, *N. ditissima*, and *N. coccinea*, but also several varieties of each.

The finding of a poplar tree affected with canker apparently caused by a small-spored Nectria corresponding to *N. coccinea*, which according to Weese is unable to cause canker, led Westerdijk and Van Luijk (*45*) to reopen the question of the pathogenicity of this species and to re-examine the evidence upon which rested the separation of *Nectria galligena* from *N. coccinea*. They concluded that such characters as growth habit, color in culture, sensitivity to H^+ concentration, measurements of curvature of conidia, structure of the perithecial wall, or pathogenicity are of negligible diagnostic value, but that by careful measurement of a large number of ascospores the two species may be separated with ease. They measured spores of a number of different specimens and found that the means of the ascospore length of the several specimens fell into one of two groups, a large-spored group which they considered to represent *Nectria galligena*, and a small-spored group which corresponded to *N. coccinea* (=*N. ditissima* Tul.).

Although positive results were obtained by inoculation with isolants from among both groups, the organisms making up either group varied considerably, both quantitatively and qualitatively, in their ability to infect the test plants, apple (several varieties), poplar, and beech. For example, among the *N. galligena* group, Malus 7 infected all three hosts; Pyrus 3 (from pear) infected apple and beech but failed on poplar; Malus 13 and 14 and Sorbus 4 infected only apple; and Sorbus 1 failed on apple and poplar, the only hosts on which it was tried. Similar behavior was shown by the *N. coccinea* group. Fagus 7 infected all three hosts; Fagus 9 infected beech and apple, but failed on poplar. Populus 1 infected poplar and apple but failed on beech; Populus 2 infected only beech; and Fagus 5 failed on beech and poplar and was questionable on apple.

In 1928 Richter (*34*) published the results of his work on the taxonomy and pathogenicity of the canker-causing Nectrias and their closely related species. His work was based upon the classification proposed for the genus Nectria by Wollenweber. Following the cultural methods outlined by Appel and Wollenweber and used by them for the genus Fusa-

rium, Richter worked out in detail the characters by means of which, he alleges, the various species and varieties may be distinguished from one another. He also studied the pathogenic behavior of each of the segregates and concluded that *Nectria galligena* is chiefly a parasite of pomaceous plants. This same conclusion had been reached by Westerdijk and Van Luijk. He attributed the cause of canker on non-pomaceous hosts to *Nectria ditissima* and regarded *Nectria coccinea* as a saprophyte. These conclusions were drawn despite the fact that some of his strains of *Nectria galligena* were able to infect beech, and *Nectria ditissima*, according to his published results, was as virulent on apple as *Nectria galligena*.

No support was found by Moritz (*25*) for the opinion expressed by Westerdijk and Van Luijk and also Richter that *Nectria galligena* and *N. ditissima* manifest any preference for pomaceous and non-pomaceous hosts respectively. But on the contrary he showed that strains which were highly virulent on apple were inclined to be so on non-pomaceous hosts. For example, with isolants from apple he secured positive results on apple, *Pyrus aria, Cratœgus oxyacantha, Fraxinus excelsior, F. americana, Acer pseudo-platanus,* Tilia, Betula, Alnus, Carpinus, Fagus, and Populus. Inoculations on Quercus failed.

In England, where the canker on apple seems to be of considerable economic importance, several contributions have been made. Wiltshire (*48*) has shown that infection of apple stems may occur through leaf scars. Cayley (*8*) studied the complete life cycle of *Nectria galligena* in pure culture, and made some important contributions to our knowledge of the development of the perithecia. She also gives the formula of a medium on which she induced the fungus to form the perfect stage.

Zeller (*57*) in America has made a detailed study of the disease on apple as it occurs under Oregon conditions. According to this writer an especially destructive form of the disease occurs in that state, where a canker may spread 20 or 22 inches in a single season. Zeller and Owens (*54*) have moreover identified the canker on the following hosts: *Quercus garyana, Acer macrophyllum, A. cercinatum, Cornus nuttallii,* and *Salix sp.* Graves (*16,17,18*) has described the canker on *Betula lenta, B. lutea, B. populifolia,* and has succeeded in infecting *Betula alba var. papyrifera* with the fungus obtained from cankers on *Betula lutea.*

Others (*7,21,32,46*) from time to time have reported the appearance of the canker in different places. Still others have shown that in addition to causing canker of the axial parts, the species of Nectria associated with canker may cause a fruit rot of apple and pear. But since the writer is not concerned with this phase of the problem, no special discussion of their results will be undertaken.

DISTRIBUTION OF THE DISEASE

The map (Pl. I, fig. 1) shows at a glance the distribution of the disease in West Virginia and the geographical relation of the counties in which the canker has been found on black walnut to one another. As

one familiar with the topography of West Virginia will readily recognize, the area in which canker is found coincides rather closely with the hilly and mountainous sections of the state. On the contrary, those sections from which the disease appears to be absent are less rugged, more open, and lower in altitude. From this it would seem that the presence of the disease in this state is correlated with environmental conditions which are most often met with in the mountainous regions, since the walnut tree is not limited to the latter sections.

In addition to West Virginia, the canker on black walnut has been found in or reported from eight other states and one province of Canada: Pennsylvania, Ohio, Virginia, Tennessee, North Carolina, Rhode Island, Wisconsin, Indiana, and Ontario. The cankers were found in Pennsylvania, Ohio, and Virginia by the writer or by C. R. Orton. The reports from the other states were accompanied by specimens or photographs from which the canker could be readily identified. Hence it is evident that this disease is widely distributed in the northeastern United States.

ORIGIN OF ORGANISMS USED

A list of fungi used in this investigation is given in Table 1. All the necessary information about these specimens will be found in the table. The same reference numbers are used throughout this discussion. A letter after any number indicates a monosporous culture obtained from the specimen. For example, the reference number 1A indicates that this culture was derived from a single ascospore from specimen No. 1 given in the table. A sub-number indicates that the specimen originated from an artificial inoculation. The reference number $1A_2$, for example, indicates that this specimen was obtained from a lesion resulting from an artificial inoculation with culture 1A.

PATHOGENICITY STUDIES

Because of the almost constant association of the perithecia of a Nectria with the canker, this fungus was from the first suspected of being the causal agent of the disease. Consequently, during the summer of 1930 sporadic attempts were made to produce, by inoculation with this fungus, infection of young black-walnut trees (2-3 yrs. old) growing in the open. In these inoculations conidia produced in pure cultures of strain No. 1, grown on oatmeal agar, were used as the inoculum. All of the inoculations yielded negative results. These attempts were not considered an adequate test of pathogenicity, however, in view of the fact that the summer of 1930 in this region was extraordinarily warm and dry.

Another attempt was made by inoculating several small trees planted in pots. A number of such trees were brought into the greenhouse in the latter part of November and subjected to treatment with vapor of ethylene chlorhydrin, as recommended by Denny (12), to force them to break dormancy. After this treatment, three of the trees were inoculated by placing conidia from pure cultures of strain No. 1 in small wounds made with the point of a sharp scalpel. The usual care was taken to prevent

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contamination. Two other trees were wounded in a similar manner but no inoculum was inserted in the wounds. These served as controls. Each of the trees was then covered with a bell jar and left standing on the floor of the greenhouse, the temperature of which fluctuated between 18° and 23° C.

After a few weeks the control plants and one of the inoculated plants began to leaf. The inoculated plant, however, soon ceased to grow, while the controls continued to develop normally. Examination of the inoculated plants revealed that all of them were dead above the point of inoculation. Small pieces cut out aseptically from the margin of the dead area and transferred to liquid media in test tubes resulted in a number of pure cultures of a fungus corresponding to the culture from which the inoculum was obtained.

With these results for encouragement a more extensive schedule of inoculatons was undertaken outdoors. Beginning January 31, 1931, a series of inoculations numbering from 6 to 22 was made at intervals of approximately a fortnight until May 10 of the same year. Thereafter the inoculations were made at more irregular intervals, one series or more being made during each month of the year except June.

All inoculations were made outdoors on the branches of large trees or on the trunks of young trees, 2-5 years old. A mass of conidia from pure cultures was transferred, with a sterile platinum needle, to a small wound made by puncturing through the bark to the cambium with the point of a flamed scalpel. The wounds were immediately covered with cotton held in place by a band of adhesive tape. A number of inoculations (1 to 10) were placed on the same limb or trunk, depending on its length, and were spaced approximately 15 cm. apart. With each series, control wounds also were made. These were treated in the same way except that the inoculum was omitted.

In the case of larger branches the control wounds were made on the same branch with the inoculations; but when the branches were small, the controls were placed on ''neighboring'' branches. When placed on the same branch, the control wounds were placed above the inoculations to prevent spores from being washed from the inoculated wounds to the control wounds by rain-water. If the inoculated branch was small, the control wounds were made on another branch in order that the results of the controls might be read even though the inoculated branch might be completely girdled, and thereby killed.

The final reading of the results was taken July 7, 1932. These results arranged in tabular form are given in Table 2. In the first column of the table is given the date the inoculations were made, in the second the isolant of the fungus used. In the last column the discrepancy between the total number of infections made on a particular date and the sum of the numbers given in the positive and negative columns is explained.

In several cases branches bearing a number of inoculations had died. Although death was undoubtedly due to the girdling of the stem by the spread of the infection, it was impossible to determine the number of

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TABLE 1—*Summary of the history of the fungi used in this investigation*

No. of Fungus	Host	Isolated from	Collected at	Collector	Date of collection
1	Canker on *Juglans nigra*	perithecia	Morgantown, W. Va.	C. R. Orton	Feb. 1930
2	Canker on *Juglans nigra*	perithecia	French Creek, W. Va.	J. M. Ashcroft	Sept. 1930
3	Canker on *Juglans nigra*	perithecia	Morgantown, W. Va.	J. M. Ashcroft	April, 1931
4	Canker on *Quercus velutina*	perithecia	State College, Pa.	Orton & Ashcroft	June, 1930
5	Canker on *Pyrus malus*	wood	Corvallis, Ore.	S. M. Zeller	Aug. 1930
6	Canker on *Juglans nigra*	—	State College, Pa.	H. W. Thurston	March, 1929
7	Canker on *Juglans nigra*	perithecia	Hundred, W. Va.	Ashcroft	July, 1931
8	Canker on *Juglans cinerea*	perithecia	Morgantown, W. Va.	Orton & Ashcroft	Feb. 1932
9	Canker on *Juglans nigra*	perithecia	Laurel Point, W. Va.	Ashcroft	Nov. 1932
10	Canker on *Quercus velutina*	perithecia	Pisgah, W. Va.	Orton	Dec. 1932
11	Canker on *Hicoria glabra*	perithecia	Pisgah, W. Va.	Orton	Dec. 1932
13	Canker on *Hicoria glabra*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
14	Canker on *Hicoria glabra*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
15	Canker on *Quercus alba*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
16	Canker on *Q. rubra*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
17	Canker on *Hicoria glabra*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
19	Canker on *Quercus velutina*	perithecia	Pisgah, W. Va.	Orton & Ashcroft	Dec. 1932
20	Canker on *J. nigra*	perithecia*	Guelph, Ontario	D. W. Welch	June, 1929
22	Canker on *Quercus velutina*	perithecia*	Hemlock, W. Va.	B. Sleeth	July, 1931
23	Canker on *Juglans nigra*	perithecia*	Greene, R. I.	W. G. Aborne	May, 1931
24	Canker on *Juglans nigra*	perithecia	Morgantown, W. Va.	Ashcroft	Feb. 1933
25	Canker on *Quercus alba*	perithecia*	Pisgah, W. Va.	Orton & Ashcroft	Feb. 1933
27	Bark disease of *Fagus grandifolia*	perithecia	Liberty, Maine	J. Ehrlich	Nov. 1932
30	Canker on *Pyrus malus*	perithecia	Corvallis, Ore.	S. M. Zeller	March, 1933

* Fungus was not cultured.

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successful inoculations on these branches. When the reading was made, the limb bearing the inoculations made December 15, 1931, was abundantly bedecked with the fruit bodies of Cytospora. This fungus had invaded the regions of the control and inoculation wounds. Hence the results of the inoculations and control wounds for that date were valueless. But exclusive of these exceptions the number of successful inoculations amounted to 83 percent (Pl. III, fig. 2), while only 1 of the 168 control wounds which could be read accurately was infected.

A number of these infections have been kept under observation for the past two years in order to determine whether they would develop into the typical form of the canker as it occurs in nature. Many of these have already formed the second ridge of callus. From others of these artificial infections the fungus has been reisolated from the wood in pure culture.

The conidial stage of the fungus has been observed on many of the young infections. The conidia are produced on minute sporodochia which usually protrude through the recently killed bark at the lenticels. In every case, conidia have been found only on lesions resulting from inoculations made during that same year. Perithecia have been observed on only a few of the inoculations, but when present they were usually produced abundantly. The isolants most fruitful in this respect are numbers 1 and 7.

In the spring of 1932 the inoculation studies were extended to other host plants. The purpose of this was to determine whether the organism found on black walnut was more or less specific for that host. The following species of trees were inoculated, as described above, between April 2 and 23: *Juglans cinerea, Hicoria ovata, Pyrus malus* (several varieties), *Acer sp., Liriodendron tulipifera, Fagus grandifolia, Sassafras variifolium, Fraxinus sp., Cornus florida, C. alternifolia, Ostrya virginiana, Quercus alba, Pyrus communis,* and *Quercus velutina.* The sources of the inoculum for all these were cultures of 2L and 2J. These were chosen because culture 2J had proved to be the most virulent in the inoculation studies reported above. Control wounds were of course made on each host with each series of inoculations. A further control was set up by making a series of inoculations on black walnut with inoculum from cultures of 2L.

During the same period black walnut, several varieties of apple, and chestnut oak were inoculated with 4B, 4D, or 4E, and black walnut and white walnut with isolant 8. The number of inoculations made on each host with each isolant ranged between 10 and 28.

The results were read December 17, 1932. Of all the inoculations only those made on black walnut and white walnut with isolant 8 were positive. The 31 inoculations on these hosts were all successful, although the fungus seemed to be much more virulent on the white walnut than on the black. In both cases the inoculations were made on small trees about 2 cm. in diameter. Whereas the black walnut still was living at

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TABLE 2—*Results of inoculations on black walnut (Juglans nigra)**

Date	Inoculum	No. of inoculations	No. positive	No. negative	No. of Control wounds	No. positive	No. negative	Remarks
1-31-31	1	22	6	8	22	0	14	Branch with 8 inoculations and 8 checks dead
2-18-31	1	20	17	3	13	0	13	
2-28-31	1	21	17	4	11	0	11	
3-17-31	1	21	18	3	8	0	8	
3-28-31	1	20	20	0	9	0	9	
4-11-31	1	11	11	0	8	0	8	
4-28-31	1	11	6	5	8	0	8	
5-10-31	1	10	2	3	5	0	5	Branch with 5 inoculations dead
7-15-31	$1A_1$	20	14	1	7	0	7	Branch with 5 inoculations dead
7-21-31	$1A_1$	17	13	0	11	0	11	Branch with 4 inoculations dead
7-24-31	$1A_1$	10	10	0	10	0	10	
8-6-31	7	10	10	0	9	0	9	
8-11-31	7	11	3	8	9	0	9	
9-4-31	7	10	3	0	10	1	9	Branch with 7 inoculations dead. Sporodochia of the Nectria fungus abundant on the dead branch
10-10-31	2J	10	8	0	10	0	10	Branch with 2 inoculations dead
10-24-31	2J	10	0	0	10	0	10	Branch with 10 inoculations dead
11-14-31	1A	10	4	0	6	0	6	Branch with 6 inoculations dead
12-5-31	1A	10	0	0	10	0	0	Branch bearing many fruiting bodies of a cytospora which had spread to control wounds and inoculations
12-19-31	1A	7	7	0	5	0	5	
1-9-32	1E	10	8	2	5	0	5	
Totals		271	177	37	186	1	167	57 inoculations and 18 checks as given above

* Final reading taken July 7, 1932.

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the time the reading was taken, the white walnut grew only poorly for a short time, and was dead by the middle of June.

The remainder of the inoculations healed over normally, as did the checks. Even the inoculation made on black walnut with isolant 2L for a control gave negative results. Therefore no conclusions as to the pathogenicity of the black walnut organism for other host species can be drawn from these experiments.

TABLE 3—*Results of cross-inoculations**

	Reference number of inoculum					
Host inoculated	1A₂	10	13	11	15	16
Juglans nigra	10/10 v.s.	10/10 v.s.	10/10 v.s.	10/10 v.s.	10/10 v.s.	10/10 v.s.
Juglans cinerea	10/10 w.					
Hicoria alba (L.) Britton	20/20 w.			10/10 s.		
Hicoria cordiformis	10/10 s.					
Hicoria glabra	10/10 w.	10/10 v.w.	11/11 m.	10/10 m.		
Hicoria ovata (Mill.) Britton	10/10 v.w.					
Quercus velutina	20/20 m.	10/10 m.	10/10 s.			
Quercus alba	10/10 m.				10/10 v.s.	

*All inoculations made between Feb. 14-18, 1933; results read May 25, 1933.
The denominator of the fraction represents the numberof inoculations tried.
The numerator of the fraction represents the number of positive inoculations.
v.w. indicates very weak virulence.
w. indicates weak virulence
m. indicates medium virulence.
s. indicates strong virulence.
v.s .indicates very strong virulence.

In December, 1932, Nectria perithecia were found associated with cankers on a number of other forest trees: black oak, red oak, white oak, and pignut hickory. So, instead of repeating in 1933 the experiments of the previous year, the plan of cross-inoculation was substituted. A freshly isolated culture from each of these trees was used to inoculate both black walnut and the host from which the culture was isolated. Conversely, a freshly isolated culture (1A₂) from black walnut was inoculated back on walnut and also on each of the trees from which cultures had been obtained. In addition white walnut and three other species of hickory were inoculated with the black-walnut organism, and one species of oak and a different species of hickory with the hickory organism. Ten inoculations were made in each case.

The results are tabulated in Table 3. In the first column the hosts which were inoculated are given. The remaining columns indicate the results obtained with the several isolants used. The denominator of the fraction represents the number of inoculations tried, and the numerator, the number thereof which were positive.

Table 3 shows that the results were 100 percent positive and that black walnut appeared to be equally susceptible to all the isolants used. On the other hand, the black-walnut organism (1A₂) was much more

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virulent on certain hosts than on others. The hickories with the exception of *H. cordiformis* (Wang.) Britton seem to be more resistant than the species of either of the other two genera. Comparisons among the results obtained with isolants $1A_2$, 10, and 13, which were isolated from three different hosts, and which were inoculated into three hosts in common, show that what differences exist among the several isolants are quantitative. None of the isolants, however, shows a specialization for the particular host from which it was obtained. The 154 control wounds all remained free of infection.

The results of these inoculations stand in striking contrast to the results of inoculations made during 1932. The difference is no doubt due to changes which occur in the behavior of the parasite under cultural conditions. The latter inoculations were made with isolants which had been in pure culture for two years, whereas the former were made with fresh isolations. Leonian (*24*) has shown that monosporous cultures of virulent strains of *Fusarium moniliforme* may sector into virulent and non-virulent dissociants. Dissociation is a property also of Nectrias in culture. The chances of segregating a non-virulent dissociant is greatly increased by the repeated transferring incident to culturing. It is necessary to use freshly isolated cultures in inoculations, therefore, to be reasonably certain that the inoculum has retained its virulence.

METHODS OF INFECTION

In the studies of pathogenicity reported above, inoculations were made in every instance by wounds which penetrated to the cambium. The question remained whether the fungus was able to infect in any other way. Goethe (*15*) found that the fungus responsible for canker on apple was able not only to penetrate through the lenticels into the living bark tissues of that species, but could also penetrate directly through the epidermis. Lapine later substantiated Goethe's work. But several other workers, including Voges, were unable to produce infection in either way. In order to throw some light on this question in the case of the black-walnut organism, the experiment described below was performed.

On April 2 a number of sound, 1-year-old black-walnut twigs about 20 cm. long and in the dormant condition were surface-sterilized by immersing for 10 minutes in a solution of mercuric chloride (1:1000), after a preliminary dip in 80 percent alcohol; and then washed twice in sterile tap water. The twigs were divided into three series of three twigs each. The first series was inoculated by a wound which penetrated to the cambium, and the second by a very shallow wounding of the outer bark. The third series was not wounded; but conidia were spread over the lenticels of the uninjured bark. The same culture of the fungus ($1A_2$) was used in all cases. About 3 cm. of the lower end of the stems was cut off and the cut end immersed in sterile tap water contained in a beaker. The whole was then set in a flat pan of water and covered with a bell jar.

At the end of a week, April 9, one twig from each series was sectioned transversely through the point of inoculation, stained, and mounted. The others were similarly treated a week later, April 16.

Examination of the sections under the microscope revealed that the twigs inoculated by wounding to the cambium had been infected in every case. Even after a week the germ tubes of conidia lying on the surface of the bark near the wound could be traced into the cortex and outer phloem, where considerable development of mycelium had occurred. The mycelium, however, had not yet penetrated to the cambium.

After two weeks the sections showed that the fungus had penetrated into the wood to a significant depth, and that the cambium and bark tissues had been killed for a considerable distance beyond the wound opening, in a tangential direction.

Sections made from the second series showed that infection had occurred in no case. However, such care had been taken to avoid wounding too deeply that in no case did the wound penetrate to the cortex. In most cases two or three rows of cork cells yet remained intact. It would seem that even this narrow barrier was sufficient to prevent infection by the canker fungus.

Sections made from the third series, like those of the second, showed no infection. Although abundant mycelium could be found ramifying in the lenticular cavity, in no case was there an indication of any ability on the part of the fungus to penetrate to the living tissue beneath. This is probably due to the fact that several layers of bridging cells remained intact at the base of the lenticel. Since the walls of the bridging cells are apparently of the same chemical composition as those of the ordinary phellem cells, which are impenetrable to the fungus, the latter is unable to reach the vulnerable tissue beneath.

PATHOLOGICAL ANATOMY

In the established parasitic relation between host and parasite three phases can be distinguished: viz., (1) the primary effect of the parasite on its host, (2) the secondary response of the host to the presence of the parasite, and (3) the changes made in the behavior of the parasite by the nature of the host response. The first of these deals with the nature of the effects of the fungus on unresisting host tissue, and might be thought of as the changes produced by the fungus in dormant host tissue. The second phase corresponds to what is called pathological histology in animal pathology. It deals with the behavior of the living, active cells of the host in the presence of the fungus. The third phase as given above is self-explanatory. All three phases are included under the heading ''pathological anatomy'' as used in this discussion.

Method

The material used in this study consisted entirely of branches and twigs on which cankers or infections had been produced by artificial inoculation. These were sectioned without embedding, as thin as the

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size of the branch would permit. Sections of the smaller twigs could be cut 10 microns, but those cut 14 microns were found to be most satisfactory for all purposes. In sections thinner than this too great a displacement of the mycelium from its normal position in the tissues was occasioned by the cutting. Larger stems could not be cut thinner than 20 microns. During the cutting, the specimen and microtome knife were kept wet with 95 percent alcohol. No doubt the fixing of the material occurred wholly or in part at this time. After cutting the sections were transferred to 95 percent alcohol and remained there until stained.

A number of staining combinations were tried. For differential staining of the host tissues the safranin—light-green combination was most satisfactory, and for differentiating the fungus mycelium the phenol-thionin-orange G combination recommended by Stoughton (38) proved excellent. For the cellulose test, sections were first treated with iodine in a solution of potassium iodide and then with 60 percent sulfuric acid. The phloroglucin-HCl test was used for lignin, and the iodine solution mentioned above for starch.

In order to separate the changes initiated and effected in the host tissues by the fungus from those attributable to the response of the living host cells, it was necessary to section infected material in various stages of development, as well as normal and wounded but uninfected branches. A number of young cankers which had resulted from inoculations made during the spring and summer of 1931 were sectioned in June, 1932. Others inoculated at the same time were sectioned in March and April, 1933. For the effects of the parasite on the dormant host tissue, the sections made in the infection studies described above were used. The host response to uninfected wounds was determined by sectioning a branch which bore healed control wounds of the previously described inoculation experiments. The wound was made in February, 1932, and the branch sectioned in July, 1932.

Primary Effects of the Parasite

In no case has the mycelium of the parasite been found in or contiguous to living cells. On the contrary, the sections show quite evidently that the host cells are killed somewhat in advance of the fungus invasion. This is most evident in the bark region. The protoplasmic cell contents are coagulated to an irregular mass which usually lies approximately at the center of the cell. Coincident with the first apparent step in this coagulating process, a change in the staining reaction of the cytoplasm occurs. In the normal cell, the cytoplasm takes the yellow stain of the orange G quite uniformly, while the cells adjacent to the infected region take the blue of the thionin stronger and stronger, depending on the stage coagulation has reached.

In the invaded region proper an apparent change takes place also in the composition of the cell wall. This also is evident from the difference manifested by normal cells and those of the invaded region in their affinity for the orange G. Whereas the normal cell walls are stained

deeply by this stain, those in the invaded region appear quite incapable of taking either the color of orange G or thionin. The walls of the phloem cells seem to lose their affinity for the orange stain most rapidly, and those of the thick-walled, outer, cortical cells least rapidly. In the latter region the yellow stain persists longest in the angles formed by the meeting of three cells. This appears to indicate that the changes taking place involve the pectic compounds of the cell wall and middle lamella. Further evidence of this is given by the fact that the bark tissues in advanced infections have about the same consistency as vegetable tissues affected with soft rot. These parts drop out from sections cut from unembedded tissue. This would indicate that the binding material between the cells has been dissolved by the action of the fungus. At the margins of the gap left by this removal, the cells of the cortex to some extent, and those of the phloem especially, appear to be almost completely disintegrated.

Further evidence of the dissolution of the middle lamella is furnished by the behavior of the fungus. At first, growth of the parasite appears to be mostly if not entirely intercellular. This point is very difficult to determine more accurately from unembedded material because of the displacement of the mycelium incident to sectioning. However, the intercellular growth habit of the fungus is very plainly evident in the thick-walled cells of the outer cortex.

These cells form a collenchyma-like girdle which abuts on the phelloderm. The middle lamella here is strongly thickened and stains a deep yellow with orange G. That this region offers more resistance to the progress of the parasite is evident from the fact that in transverse sections it, together with the cork layer, often persists as a narrow band of cells jutting out from either side after the inner cortex and phloem beneath have been completely disintegrated and have dropped away in sectioning. These cells hold their shape longer than the more delicate cells beneath, and for that reason the mycelium is much less likely to be displaced in cutting.

In this region the fungus hyphæ appear to force their way between the cell walls like a wedge splitting them asunder. This is probably more apparent than real, however. What probably occurs is that the middle lamella is gradually dissolved and its place taken by the hypha; for parallel with the growth of fungus go the changes in the ability of the cell wall described above to take a stain. Cells in this region have been observed surrounded on all sides by hyphæ, but with none in the cell cavity (Pl. VI, fig. 1). In more advanced stages the growth of the fungus could not be followed with any degree of certainty, but it appears very likely that later the fungus enters the cell cavity. However, no adequate evidence showing that the fungus had a dissolving action on cellulose could be found.

The progress of invasion of the bark tissues is very much more rapid in the phloem region than in the cortex. This was found by Voges to be the case also in apple. The apparent explanation for this lies in the weaker development of pectic compounds in that region and hence the

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more rapid disintegration of the tissue by the solvent action of enzymic secretions of the fungus.

In the xylem the destructive action of the fungus is not nearly so evident. Microchemical tests show that no dissolution of wood or cellulose occurs as a result of the fungus invasion. The only noticeable effect of the fungus is on the living cells, medullary-ray cells, and wood parenchyma. The protoplasmic contents of these cells are coagulated and at times appear to be completely dissolved. Sections treated with the iodine solution show that the starch is removed for a considerable distance around the area invaded by the fungus.

The growth of the mycelium in the xylem is entirely intracellular (Pl. VI, figs. 2, 4). The inability of the fungus to dissolve either lignin or cellulose makes this a necessary character of its growth in woody tistues. For the same reason the mycelium is forced to utilize the passageways afforded by the pits in its ramifications from cell to cell. This is the only way the mycelium has been observed to progress from a cell to an adjoining cell. Even the way in which a hypha passes through a pit bears witness to the ineffectiveness of the parasite against the chemical composition of the walls of the xylem cells. Since the diameter of the pit opening is considerably smaller than the diameter of the fungous hypha, the latter, possessing no solvent action on the wall, is of necessity constricted at this point (Pl. VI, fig. 3).

That the living cells are the sources of the nutrients used by the fungus in its growth is amply borne out by the favoritism for these cells exhibited by the parasite in its growth. Occasionally one finds a hypha in a wood fiber, but it is in the medullary rays, wood parenchyma, and vessels that the fungus grows luxuriantly (Pl. VI, fig. 2). Of course the mature vessels are not living, but they are surrounded on all sides by those parenchyma cells into which branches from the mycelium in the vessels penetrate through the pits. Parenchyma cells, medullary-ray cells, and vessels have been observed which were literally filled with fungus mycelium.

The entrance into the wood from the bark is made through the medullary rays; at least this is the only avenue through which penetration has been observed to occur (Pl. VI, fig. 4). From here the fungus spreads into the vessels and wood parenchyma chiefly at the points where these structures are contiguous to the ray cells.

Secondary Responses of the Host

Soon after the resumption of cambial activity in the spring the dead bark is separated from the living bark by the formation of a phellogen which extends from the cambium outward until it becomes continuous with that at the periphery. Meanwhile the cambium adds new cells to the wood cylinder. At the margins of the infection the cambial activity is most luxuriant, and the amount of new tissue produced in these regions far exceeds that on the opposite side of the stem. It is this excessive growth which produces the conspicuous concentric ridges of the typical

canker. The cells first produced at the edge of the wound do not develop into the normal xylem cells but remain undifferentiated and parenchymatous. Farther away from the wound the new tissue passes gradually over into the normal xylem elements.

Very frequently the parasite from the older wood begins to invade the parenchymatous tissue mentioned above. When this occurs the mycelium is excluded by the formation of another phellogen within this undifferentiated tissue. The outer cells of this second phellogen usually join the one formed earlier, and the inner extremity frequently curves back in a tangential direction away from the wound and along the line which marked the position of the cambium at the end of the previous season's growth (Pl. IV, fig. 1). This tangential extension usually reaches to where the newly formed tissue passes over into normal xylem tissue. In this manner further invasion of the parenchymatous region directly from the old wood is excluded, and further infection of this region must take place through the newly formed wood.

Very likely the undifferentiated tissue is not all produced by division of cambial cells, but considerable addition to its bulk is made by the continued division, in any plane, of the cells first cut off from the cambium. At any event, along the margins of the canker there is produced a mass of this tissue, which bulges out in the direction of the wound. Here a specialized meristematic region is differentiated which on its outer end is continuous with the normal cambium, and which curves inward toward the xylem (Pl. IV, fig. 1).

In this way the tree produces a mechanism which would eventually heal the wound if in no way prevented; for growth initiated by the curved portions of this meristem results in a gradually lengthening "lip" extending laterally over the wound. Eventually the "lip" advancing from one side of the wound meets that advancing from the other, a fusion of tissues occurs, and growth thenceforth proceeds in the normal way.

So far the discussion of the host's responses has been confined to the behavior of the extracambial tissues and those produced after infection is established. In addition, certain changes occur in the living wood which was produced before infection occurred. Apparently these changes are as much a part of the host's response as the phenomena described above, for the changes to be described were not observed in those twigs which were infected in the dormant condition and which were sectioned before dormancy was broken.

The first of these changes to be discussed is the greatly increased development of tyloses in the vessels. Tyloses appear to be a normal development in the older wood of black walnut. Normally they appear only rarely if at all in the wood of the outer layers. In the infected area, however, and extending somewhat beyond this region, development of tyloses proceeds most luxuriantly in the later-formed xylem tissue and even in the vessels of the current season's growth. Since these structures are produced only by living cells, those lying in the infected region

must have been formed at a time when the cells from which they developed lay outside the region invaded by the fungus. Frequently the wall of the tylosis becomes completely lignified and at other times remains cellulotic. The cavity of the tylosis may often be infiltrated with wound-gum.

The term wound-gum serves to introduce the second change to be discussed. Coincident with or at least soon after the development of tyloses, the other living cells (wood parenchyma and cells of the medullary ray) in the same regions in which the tyloses are formed, and frequently the tyloses themselves, become infiltrated with a yellow or golden-brown, gum-like substance which is commonly known as wound-gum, the origin of which is a disputed point.

In order to identify the substance more definitely with the substance or substances known as wound-gum, shavings of wood darkened by the infiltrations of this material were subjected to some of the treatments used by Rhoads (32,33). The shavings were first extracted repeatedly with a solution of Na OH (10%) until the alkali solution was no longer discovered by substances diffusing from the wood. From this filtrate a brown, flocculent substance was precipitated upon the addition of alcohol. After repeated washing with water the wood was then oxidized for 15 minutes by treatment with hot HCl solution to which a few crystals of $KClO_3$ had been added. At the end of this time the wood had lost practically all its dark color, yet the oxidizing liquor was not noticeably colored. Under the microscope some of the cells of the wood were observed to be filled with a bright yellowish-golden substance resembling in appearance that seen in the cells of sectioned but unstained wood.

The shavings were then divided into two equal portions. One portion was extracted for eight hours with absolute alcohol, and the other for an equal time in 10 percent Na OH solution. The sodium hyroxide solution was turned brown by the substances diffusing from the wood. But the alcoholic extract was very much lighter in color, and when evaporated to dryness yielded a residue of light yellowish-golden, resin-like substance which as it dried upon the watch glass, after the alcohol had evaporated, fractured into a reticulum of fine fissures. The fracture was distinctly vitreous. This substance is very soluble in the alkaline solution and yields a solution which in color resembles that formed by the extraction of the digested wood. Furthermore, when the wood which had been extracted with alcohol was, after washing in water, covered with the alkaline solution, no discoloration of the liquid occurred. Hence it appears that all the substance had been extracted by the alcohol, and that the substance in either case is the same.

The extract had all the appearance of the substance observed in the wood parenchyma and medullary-ray cells of unstained sections. Further, when treated with the stain employed in the preparation of the slides used in this study (0.1 gr. thionin, 100 c.c. of 5% aqueous solution of phenol), the residue was colored as it appeared in the stained sections.

In the behavior of the host there is no indication of a specific re-

sponse to the presence of the parasite. Comparisons between infected and uninfected wounds show that the behavior of the host towards the latter is qualitatively the same as towards the former. The differences observed were quantitative. In the uninfected wounds the healing callus soon covers the wound, disintegration processes in the xylem cease, and growth proceeds normally. In the infected wounds the healing process is not allowed to complete its work. The calluses are repeatedly killed back and repeatedly formed, while the disintegration in the xylem proceeds in pace.

Adjustments Made by the Parasite

The responses of the host force upon the parasite certain restrictions in its mode of living. During the dormant period the fungus, despite its invasion of the wood, appears to thrive much better in the inner phloem regions. But at the beginning of the growing season the region of the bark invaded by the fungus is separated from the living tissue by a phellogen, through which the fungus is unable to penetrate. Hence the fungus becomes a xylem parasite exclusively during the active period of the host. Its re-entry into the living bark tissues is prevented during the growing season by the repeated formation of a phellogen around any region which the parasite may succeed in entering from the wood. But at the close of the growth period the bark may again become infected, and invaded for a considerable extent before the host tissues become active again. The perennial nature of the canker therefore is seen to be dependent upon the ability of the fungus in the woody tissue to survive the period of activity of the host.

The exclusion of the parasite from the extra-cambial tissues during the summer is furthered by conditions unfavorable for the growth of the fungus. Studies to be discussed later show that the fungus is very sensitive to higher temperatures. A temperature of 30° C. is high enough to cause the almost total cessation of growth. Since the fungus kills the host cells in advance of its own invasion, the tissues in which the fungus is found are dead, and probably no great amount of water passes through them, especially as the vessels usually are occluded by tyloses. Hence in dry summers the factor of moisture may also become a limiting one. The combined effect of these two factors causes an almost total inactivity of the fungus.

Development of the Canker

After the foregoing description of the behavior of the parasite and host, the development of the typical canker can be explained very briefly by the use of a few diagrams. Figure 1, Plate V represents a transverse section of a young infected branch. The shaded area represents the tissue killed by the fungus, and (c) the point of infection. That the necrosis here represented has occurred during the dormant period of the host is obvious from the fact that the dead cambium, represented by the arc (a.b.c.), is continuous with the living cambium.

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When growth of the host is resumed in the spring, the living cambium begins to add new wood (n.w., fig. 2) to the wood cylinder. Meanwhile the cambium moves out radially. The new growth around the necrotic area imparts a sunken appearance which is accentuated by the drying of the dead bark.

Cambial activity ceases at the end of the growing period, but the fungus growth is accelerated by more favorable conditions. Re-infection of the bark follows, and more of the cambium is killed. This is indicated by the extension of the shaded area at n, fig. 2. Consequently upon the resumption of the growth of the host and the production of another layer of new wood (n.w., fig. 3), the margin of the previous year's growth (r., fig. 2) forms a circumvallation of callus tissue (r., fig. 3) around the primarily infected area (arc a.b.c., fig. 3).

The annual repetition of this process results in the formation of a number of such circumvallations concentrically arranged around the area first infected. Eventually the dead bark is sloughed off, and the wood showing this concentric pattern is exposed.
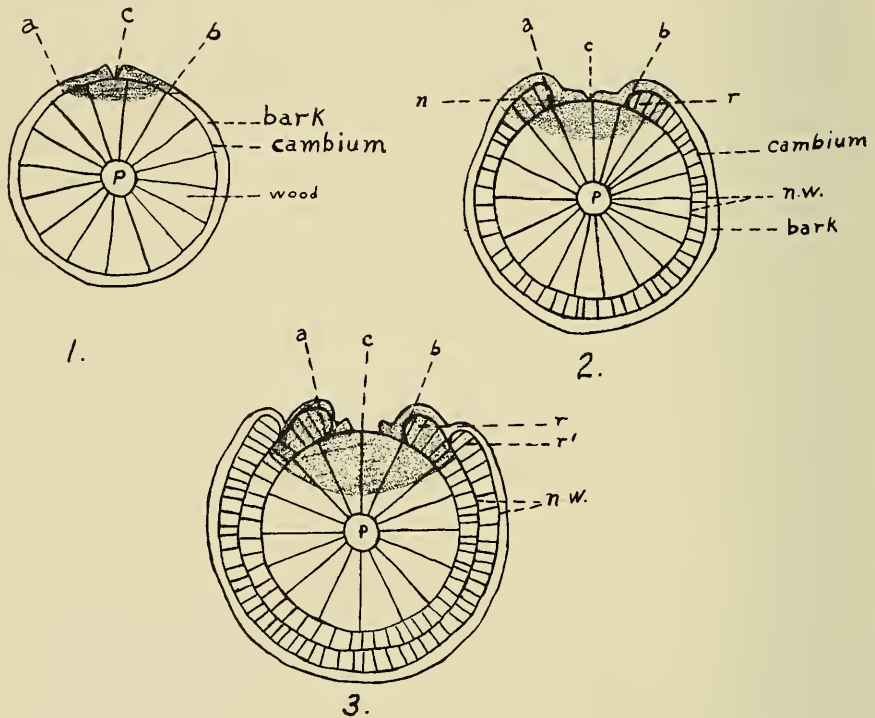
PLATE V

1—Diagrammatic representation of a young infection
2—The same for an infection which has survived one growth period of the host
3—The same for an infection which has survived two years' growth of the host.

EXPLANATION OF PLATES

PLATE I

1—Known distribution of black-walnut canker in West Virginia
2—Small canker on black walnut

PLATE II

1—Large canker with heartwood exposed
2—Black-walnut trees with numerous cankers on trunk and limbs

PLATE III

1—Perithecia of a Nectria on the dead but still adherent bark of the callus ridges (x4)
2—Young cankers produced by artificial infection

PLATE IV

1—Transverse section through the callus at the margin of a young canker resulting from artificial inoculation
2—Perithecia of the type specimen of *N. ditissima* Tul.

PLATE V (see page 26)

PLATE VI

1—Mycelium surrounding cell in the outer cortical region.
    The cells are separated by the dissolution of the middle lamella
2—Wood parenchyma cell filled with mycelium of the fungus
3—Mycelium passing through a pit
4—Mycelium in the medullary ray cell at the cambium
5—Spores of *Nectria galligena* Bres.
6—Spores of *N. ditissima* Tul.
7—Ascus and paraphysis of *N. galligena* Bres.
8—Ascus of *N. ditissima* Tul.
9—Ascus of a type distinct from those shown in figs. 7 and 8
9a—Apex of the same type of ascus shown in fig. 9 enlarged to show pore

PLATE VII

Cultures in conidial condition (cylindrocarpon) showing effect on subsequent growth after 5 days' incubation at three different temperatures. The fungus is killed during 2 hours' exposure at 36°C.
    Optimum range 15°-23°C.
    Minimum not determined, but fungus not killed by 0°C.

PLATE I

PLATE II

PLATE III

PLATE IV

PLATE VI

PLATE VII

Historical

In 1800 Persoon (*29*) described *Sphæria coccinea*. He (*30*) also included a description of the fungus in his later work (1801), to which many workers have subsequently referred as the original citation. In 1849 Fries (*13*) transferred this fungus to the genus Nectria, and it became known as *Nectria coccinea* (Pers.) Fr. Under this name‡ he includes a description of the specie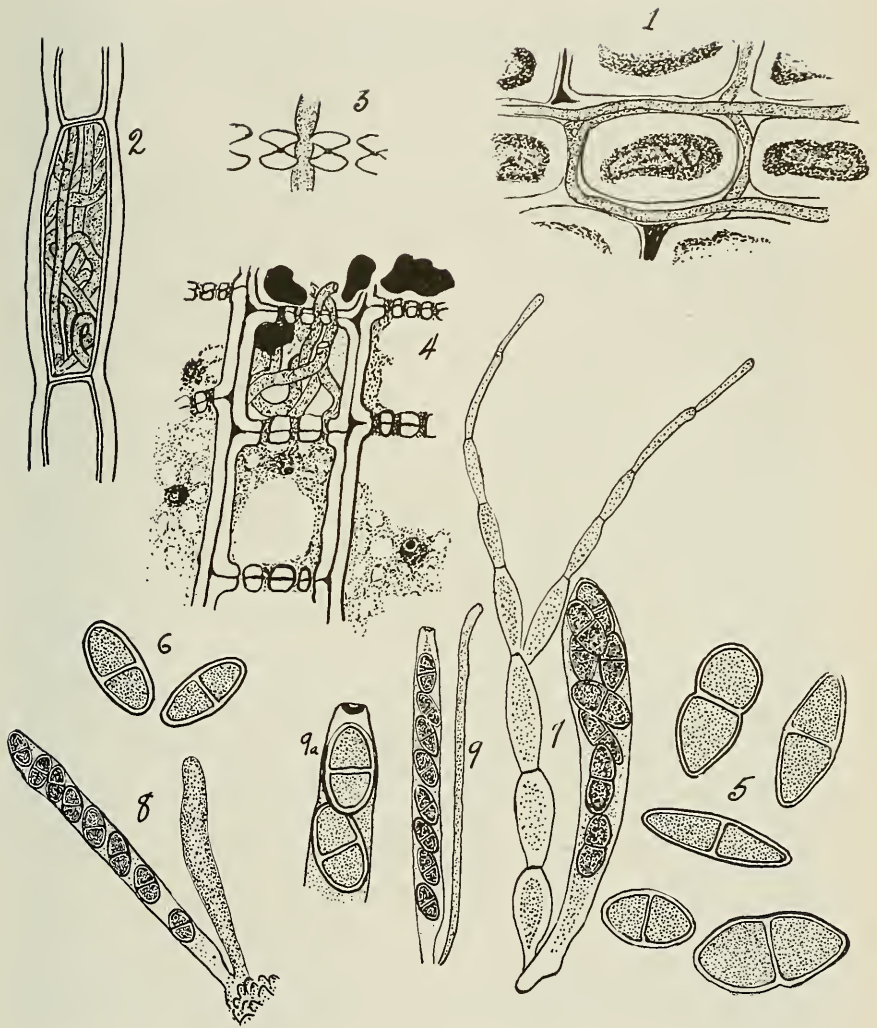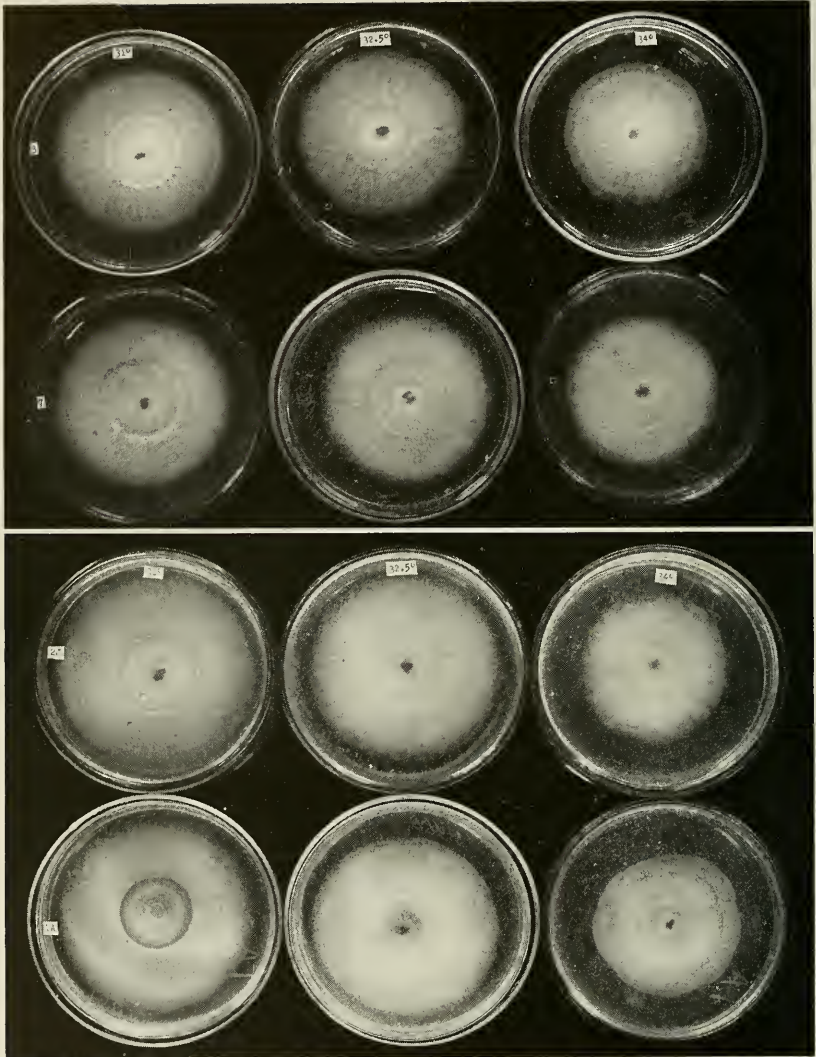s. In 1865 Tulasne (*40*) described *Nectria ditissima* Tul. At the same time he declared that the fungus described by Persoon (*30*) *under Sphæria coccinea*, as well as that appearing under the same name in Desmaziere's Plantes Crypt. France No. 380 (1829), was synonymous with *Nectria ditissima* Tul., but that Fries's description† of *Nectria coccinea* does not agree with *N. ditissima* in that he depicted that species as having elongate-filiform ascospores. Presumably neither did the description of Fries agree with *Sphæria coccinea* Pers. Hence it appears that Tulasne felt justified in renaming *Sphæria coccinea* Pers. as *Nectria ditissima* Tul., since he believed that Fries had incorrectly described Persoon's organism.

In 1909 Seaver (*35*) divided the genus Nectria on the basis of the presence or absence of a stroma. Those without a stroma were left in the genus Nectria, and a new genus, Creonectria, was created for the species which produce their perithecia on a distinct stroma. Furthermore, he lists *Nectria ditissima* Tul. as a doubtful species, declaring that, so far as he could determine, the "American specimens referred to this name" were the same as *Nectria coccinea* (Pers.) Fries.

Between 1865 and 1910 *Nectria ditissima* Tul. became the accepted name for the causal organism of canker on deciduous trees. In the latter year, Hoehnel and Weese (*21*) declared that *N. ditissima* Tul. was synonymous with *N. coccinea* (Pers.) Fr. The following year Weese (*42*) explained that his opinion of the synonymy of *Nectria ditissima* Tul. with *N. coccinea* (Pers.) Fr. was based upon a comparison of the specimen, Desmaziere's Plantes Crypt. France No. 380, with Persoon's (*30*) description of *Sphæria coccinea* Pers. However, the synonymy of *N. ditissima* Tul., Desmaziere's specimen No. 380, and the species described by Persoon as *Sphæria coccinea* Pers. had been freely admitted by Tulasne 45 years before Weese's paper appeared.

The taxonomy of canker-causing Nectrias was apparently clarified by Weese's (*42*) statement that *N. coccinea* (Pers.) Fr. (=*N. ditissima* Tul.) was not the cause of canker, but that *N. galligena* Bres., a more recent segregate, and a species quite distinct from *N. coccinea*, was responsible. The type material of *N. galligena* was found fruiting on sphæroid galls* on *Salix purpurea* in Lower Austria. Bresadola (*39*) described this species evidently under the impression that the fungus was

‡ *Loc. cit.*, p. 387.
† *Ibid.*, note 2.
* When these galls were shown to Miss N. A. Brown, of the Bureau of Plant Industry, U. S. Dept. of Agriculture, who is especially interested in "crown gall" caused by *Bacterium tumefaciens*, she said that they possessed a strong resemblance to crown gall.

the cause of the galls; hence the name *galligena*. However, *N. galligena* Bres. became accepted as the cause of canker until 1923.

In that year Westerdijk and Luijk (*45*) proved that a fungus identified for them by Weese as *N. coccinea* (Pers.) Fr. was pathogenic on apple, beech, and poplar. It should be mentioned, however, that Weese was not entirely satisfied with his own identification of this fungus.

Wollenweber (*50*) considers *Nectria coccinea* (Pers.) Fr. as a saprophyte only and as distinct from *N. ditissima* Tul. This conclusion is especially interesting in view of the fact that *Sphæria coccinea* Pers. was considered by Tulasne as synonymous with the organism he described as *N. ditissima*. Furthermore, Wollenweber revives the name *Nectria ditissima* Tul. and recognizes it as a pathogenic organism on deciduous trees. Within this species, moreover, he (*51*) recognizes two varieties: *N. ditissima* Tul. var. *arctica* Wr., and *N. ditissima* Tul. var. *major* Wr. He likewise recognizes *N. galligena* Bres. var. *major* Wr. as a variety of *N. galligena*.

Richter (*34*), working under the direction of Wollenweber, has worked out the distinguishing characters by means of which, it is alleged, the several species and their separate varieties may be identified.

Materials

One of the objectives of the present investigation was to identify the organism responsible for the canker on black walnut. No difficulty presented itself until it was necessary to decide which name, *N. ditissima* Tul., *N. coccinea* (Pers.) Fr., or *N. galligena* Bres., should be applied to the black-walnut organism. Then it became apparent that very little reliability could be placed upon the descriptive characters published by the previous workers. This necessitated a first-hand study of the various species of Nectria which have been considered as the cause of canker. The material for this study consisted of the numerous collections accumulated during the investigation and cultures obtained from these, as well as a collection of herbarium specimens obtained from various sources.

Among these collections* were small pieces of the type-specimens of. both *Nectria galligena* Bres. and *N. ditissima* Tul. A list of the specimens in this collection is given in Table 4.

The Problem

Weese (*42*) distinguished *N. galligena* from *N. coccinea* (=*N. ditissima*) by the gross habit of its perithecial stage, by the weaker plectenchymatous development of the perithecial wall, by its larger spores, and by its pathogenicity. When Westerdijk and Luijk (*45*) proved the pathogenicity of a species identified as *N. coccinea* by Weese, of course the last character became invalid. Westerdijk and Luijk came to the conclusion that the structure of the perithecia also was too varied to be of any worth as a distinguishing character. This left only the gross habit and the spore

TABLE 4—*List of herbaria and exsiccati specimens used for reference in this investigation*

Ref. No.	Name on packet	Host	Herbarium or exsiccatum	Collected at	Collector	Date
21	N. galligena (type)	Salix purpurea	Herb of Bresadola	Sonntagsberg, Niederoesterreich	P. Strasser	
26	N. ditissima (type)	Fagus	From Herb. Crypt. Mus. Paris, given by L. R. Tulasne in 1873	Pierrefonds, France	L. R. Tulasne	1857
29	N. ditissima (type)	Pyrus malus	C. Roumeguere, Fungi selecti #6655	St. Die, France	R. Ferry	1896
31	N. ditissima (type)	Pyrus malus	Herb. Station Cent. Path. Veg. Versailles, France	Ecole d' Horticulture, Versailles		1901
32	N. ditissima (type)	Fagus	Herb. Crypt. Mus. Paris	St. Cloud	Ludwig	1910
33	N. ditissima (type)	Pyrus malus	Krieger, Fungi Saxonici #2259	Kgl. Garten zu Gross-Sedlitz bei Pirna, Germany	W. Krieger	1913
34	N. ditissima (type)	Fagus sylvatica	Allescher & Schnabl. Fungi bavarici #154	Oberammergau, Germany	Schnabl	1889
35	N. ditissima (type)	(Fagus)?	Dr. F. Petrak, Mycotheca carpatica #463		Dr. F. Petrak	1925
36	N. ditissima (type)	Acer rubrum	Penn. State Coll. Herb.	Huntingdon Co., Pennsylvania	L. O. Overholls	1920
28	N. coccinea	Juglans regia	C. Roumeguere, Fungi selecti #3244	Environs de Senlis (Oise)	F. Sarrazin	1884
37	N. coccinea	Quercus	Vestergren, Micronyccetes rariores selecti #519	Suecia. Smaland Skatelof	C. J. Johanson vidit Dr. Rehm	1883
39	N. coccinea	Fagus sylvatica	Krypt. Exsic. Mus. Pal. Vindobon Fungi #1430 Ex.Herb.Bur. Pl. Ind. U.S.D.A.	Lower Austria		
38	N. coccinea	Magnolia	E, & E, Fungi Columbiani #618	Newfield, N. J.		
45	N. coccinea	Tilia americana	E, & E, Fungi Columbiani #2043	London, Canada	J. Dearness	1904
40	N. coccinea	Ulmus pubescens	E. & E. Fungi Columbiani #2238	London, Canada	J. Dearness	1904
41	N. coccinea	Magnolia fraseri	Nuttall, Flora of Fayette Co., W. Va. #(532) 1472 J. B. E. 451	Fayette County, W. Va.	L. W. Nuttall	1894
42	N. coccinea	Magnolia fraseri	Nuttall, Flora ol Fayette Co., W. Va., #J. B. E. 774	Fayette County, W. Va.	L. W. Nuttall	1895
43	N. coccinea	Robinia pseudacacia	Nuttall, Flora of Fayette Co., W. Va., #(533) 115 J. B. E. 162	Fayette County, W. Va.	L. W. Nuttall	1893
44	N. coccinea	Ilex opaca	Nuttall, Flora of Fayette Co., W. Va. J. B. E. 841	Holliday Rd., Fayette Co . West Virginia	L. W. Nuttall	1896
20	Nectria sp.	Juglans nigra	Herb, of D. S. Welch #746]	O. A. College, Guelph, Ont.	Stone, Faull, Welch	1929
27	N. coccinea?	Fagus grandifolia	Ehrlich Herb. #1071	Liberty, Maine	Ehrlich, Pierson, Nash	1932

size. Concerning the latter these workers declared that, by means of it, only in the last analysis could a separation of the two species be effected.

The distinctions between the *N. galligena* and *N. ditissima*, and their several varieties as delimited by Richter, are based chiefly upon size characters: *i.e.*, length and width of ascospores and conidia. These are supplemented by color characters in culture. *N. ditissima*, with the exception of variety *major*, is distinguished by smaller ascospores and narrower and longer conidia. Variety *major* has ascospores that are in the same range as those of *N. galligena* var. *major*, but is separated from the latter by the narrower and longer conidia, and from the other varieties of *ditissima* by its larger ascospores. Use is also made of color characters in the separation of varieties of the same species.

N. coccinea, as delimited by Richter and Wollenweber, is a saprophyte according to the former, who has tested its pathogenicity. Since, however, this species is so frequently confused with the proved canker-causing Nectrias, reference will be made to it later.

From the above discussion of the distinguishing characters it will be clear that these species have been separated by three characters: gross habit of the perithecial stage, size of spores, and color in culture. Concerning the last, little need be said. It has been shown repeatedly that this character is of no diagnostic significance. Leonian (*24*) has produced, under controlled conditions, ''dissociants'' of all shades of color, varying between a red and a purple from a single, colorless, monosporous culture of Fusarium by selection from ''sectors'' of the original culture. Although the writer has made no special study of this character, he has frequently observed the phenomenon of dissociation in his cultures of Nectrias. Duplicate stock cultures derived from a single ascospore often exhibit wide variations in color, even though their history from the time of isolation has been identical.

Although it appears at times that the gross habit of the fruiting bodies offers a ready means of identification, familiarity with this character as it is manifested under different conditions shows that it cannot be taken as a final distinction. On bark it is true that perithecia of the smaller-spored specimens tend to be grouped in larger numbers on a common, well-defined stroma, and that perithecia of larger-spored specimens, on the other hand, tend to be isolated or in small numbers on a common stroma. But on bare wood, stromata in both cases are greatly reduced or entirely lacking, and in fissures of the bark, where the perithecial stromata are crowded together, the large- and small-spored types cannot be distinguished by macroscopic appearances. Workers with this group of Nectrias have all recognized the shortcoming of distinctions based upon macroscopic appearance. Hence in the final analysis, separation of the several species has dwindled to a matter of spore-size.

Since the validity of the several species depends so greatly on this factor, it is important that we have some knowledge of its reliability. The study reported below is an attempt to evaluate spore-size as a means of distinguishing the several species mentioned above.

Method

A sample of 100 ascospores from each of 26 specimens among the collections in the writer's possession was measured carefully both for length and width. The same was done with conidia from each of 16 distinct cultures. These cultures had grown on the same medium under the same conditions and were of the same age. In a few cases in which the conidia were found to be uniform, the width of only 50 was measured. The sample from each specimen was analyzed separately by statistical methods. In all cases except length of conidia, the class interval used was 1 micron. For conidia-length a class interval of 3.3 microns was substituted. The mean spore-length and -width, the standard deviation, coefficient of variability, and the probable error of each sample were computed in the usual way.

To facilitate measurement, the spores were stained with a weak solution of methylene blue. It was found that staining differentiated the mature from the immature spores, the former taking the stain less deeply. This presumably was due to differences in permeability of the walls. At any rate mature spores could be distinguished by a method which eliminated the personal element.

In order to furnish a check on the method of measuring the spores, 212 spores from one group of perithecia were measured. These were then divided into two equal parts, and each half analyzed separately. The mean, standard deviation, and coefficient of variability computed for the one half did not differ significantly from the respective computations for the other half. In each case the difference was less than its probable error. This is shown (No. 8) together with all the other measurement-data in Tables 5 and 6.

After this test the writer assumed that the samples were random and that the mean, standard deviation, and coefficient of variability computed for them were representative of the specimen from which the sample was obtained. In the discussion which follows, therefore, the terms ''specimen'' and ''sample'' are used interchangeably.

Analysis of Data

If the means of the ascospore-lengths of all the specimens measured are arranged as in Table 7 in an ascending numerical order, it will be noted that they form a series without a gap. When Westerdijk and Luijk (45) arranged the means of their measured specimens in a similar way they found that a gap occurred between 13.34 microns and 15.92 microns. The mean of none of their specimens fell in that range. They concluded that *N. coccinea* was represented by the smaller-spored group. and *N. galligena* by the larger-spored, and that no transition forms with spores intermediate in size between the two species occurred. As can readily be seen from Table 7, 13 of the 26 specimens measured in this study fell within that range. Obviously the claim of those writers that a wide gap exists between the normal variation of the two species is without foundation.

TABLE 5—*Ascospore measurements*

Ref. No.	Host	Length				Width			
		Number measured	Mean length	Standard deviation	Coefficient of variability	Number measured	Mean width	Standard deviation	Coefficient of variability
#21	*Salix purpurea*	226	16.19 ± .06	1.4 ± .04	8.5 ± .27	100	6.67 ± .04	.66 ± .03	9.95 ± .47
#20	*Juglans nigra*	100	16.27 ± .13	1.88 ± .09	11.56 ± .55	100	6.79 ± .03	.48 ± .02	7.00 ± .33
#1	*Juglans nigra*	110	16.62 ± .12	1.90 ± .09	11.41 ± .52	100	6.43 ± .04	.53 ± .03	8.30 ± .40
#1A$_2$	*Juglans nigra*	100	14.79 ± .11	1.65 ± .08	11.20 ± .53	100	7.98 ± .04	.57 ± .03	7.19 ± .34
#23	*Juglans nigra*	100	15.43 ± .12	1.84 ± .09	11.92 ± .57	100	6.48 ± .04	.52 ± .03	8.16 ± .39
#7	*Juglans nigra*	157	15.19 ± .11	2.13 ± .08	14.03 ± .53	100	6.87 ± .05	.76 ± .04	11.12 ± .53
#8	*Juglans cinerea*	106	16.28 ± .14	2.08 ± .10	12.80 ± .60	100	6.68 ± .04	.64 ± .03	9.56 ± .46
#8	*Juglans cinerea*	106	16.35 ± .14	2.14 ± .10	13.41 ± .62				
#11	*Hicoria glabra*	100	14.77 ± .13	1.93 ± .09	13.05 ± .62	100	7.22 ± .05	.68 ± .03	9.4 ± .57
#13	*Hicoria glabra*	100	15.60 ± .11	1.62 ± .08	10.36 ± .49	100	7.45 ± .04	.55 ± .03	7.44 ± .36
#14	*Hicoria glabra*	100	15.64 ± .11	1.64 ± .08	10.47 ± .50	100	6.56 ± .03	.50 ± .02	7.69 ± .37
#16	*Quercus rubra*	100	16.46 ± .10	1.52 ± .07	9.21 ± .44	100	7.92 ± .04	.62 ± .03	7.82 ± .37
#4	*Quercus velutina*	178	14.82 ± .08	1.54 ± .06	10.41 ± .37	100	6.34 ± .03	.44 ± .02	6.95 ± .33
#22	*Quercus velutina*	100	15.35 ± .10	1.47 ± .07	9.55 ± .46	100	6.56 ± .04	.60 ± .03	8.69 ± .41
#19	*Quercus velutina*	100	16.18 ± .10	1.55 ± .07	9.57 ± .46	100	6.80 ± .04	.57 ± .03	8.45 ± .40
#1A$_1$	*Juglans nigra*	100	14.07 ± .13	1.97 ± .09	13.97 ± .67	100	7.30 ± .04	.60 ± .03	8.22 ± .39
#26	*Fagus sylvatica*	100	13.44 ± .09	1.33 ± .06	9.86 ± .47	100	6.07 ± .04	.62 ± .03	10.22 ± .49
#27	*Fagus grandifolia*	100	12.57 ± .06	.93 ± .04	7.40 ± .35	100	5.87 ± .04	.52 ± .02	8.90 ± .42
#28	*Juglans regia*	100	12.55 ± .08	1.15 ± .05	9.18 ± .44	100	5.76 ± .04	.52 ± .02	9.05 ± .43
#9	*Juglans nigra*	100	14.77 ± .10	1.46 ± .07	9.86 ± .47	100	7.33 ± .03	.43 ± .02	5.80 ± .28
#17	*Hicoria glabra*	100	15.87 ± .16	2.42 ± .12	15.27 ± .73	100	7.69 ± .95	.69 ± .03	8.95 ± .63
#17	Oatmeal agar	100	15.33 ± .13	1.97 ± .09	12.82 ± .61	100	6.77 ± .04	.56 ± .03	8.32 ± .40
#29	*Pyrus malus*	100	17.27 ± .12	1.72 ± .08	9.96 ± .47	100	6.90 ± .04	.62 ± .03	8.93 ± .43
#25	*Quercus alba*	100	19.12 ± .15	2.21 ± .11	11.57 ± .55	100	8.02 ± .05	.70 ± .03	8.73 ± .42
#30	*Pyrus malus*	100	19.45 ± .12	1.84 ± .09	9.46 ± .45	100	7.59 ± .04	.58 ± .03	7.70 ± .37
#71	*Juglans nigra*	100	16.45 ± .15	2.25 ± .11	13.66 ± .65	100	7.73 ± .05	.71 ± .03	9.12 ± .44

TABLE 6—*Conidia measurements*

Ref. No.	Host	Length				Width			
		Number measured	Mean length	Standard deviation	Coefficient of variability	Number measured	Mean width	Standard deviation	Coefficient of variability
1A	*Juglans nigra*	100	54.02 ± .46	6.79 ± .32	12.56 ± .60	50	5.62 ± .03	.33 ± .02	5.78 ± .39
1A	*Juglans nigra*	100	67.91 ± .63	9.30 ± .44	13.69 ± .65	100	5.81 ± .04	.46 ± .03	7.98 ± .48
1A₁	*Juglans nigra*	100	53.13 ± .36	5.40 ± .26	10.16 ± .48	100	6.46 ± .02	.19 ± .01	3.73 ± .18
1C	*Juglans nigra*	100	53.13 ± .31	4.60 ± .22	8.65 ± .41	100	5.61 ± .02	.34 ± .02	6.12 ± .29
2K	*Juglans nigra*	100	54.68 ± .34	5.03 ± .24	9.20 ± .44	64	6.45 ± .02	.21 ± .01	3.28 ± .20
2J	*Juglans nigra*	100	52.64 ± .27	4.02 ± .19	7.65 ± .36	100	6.24 ± .03	.44 ± .02	7.02 ± .33
4H	*Quercus velutina*	100	59.37 ± .35	5.14 ± .25	8.67 ± .41	100	6.41 ± .02	.32 ± .02	4.98 ± .24
4I	*Quercus velutina*	100	55.08 ± .36	5.29 ± .25	9.61 ± .46	100	6.08 ± .03	.49 ± .02	8.12 ± .39
4J	*Quercus velutina*	100	56.13 ± .33	4.95 ± .24	8.82 ± .42	50	6.08 ± .05	.49 ± .03	8.12 ± .55
8	*Juglans cinerea*	100	48.91 ± .27	3.99 ± .19	8.15 ± .39	50	6.38 ± .03	.33 ± .02	5.09 ± .34
9	*Juglans nigra*	100	54.29 ± .36	5.28 ± .25	9.72 ± .46	50	6.40 ± .03	.30 ± .02	4.69 ± .32
11	*Hicoria glabra*	100	61.48 ± .53	7.79 ± .37	12.67 ± .60	64	6.06 ± .04	.53 ± .03	8.69 ± .51
13	*Hicoria glabra*	100	51.41 ± .30	4.52 ± .22	8.79 ± .42	100	6.39 ± .02	.34 ± .02	5.38 ± .26
14	*Hicoria glabra*	100	48.08 ± .31	4.58 ± .22	9.52 ± .45	50	6.50 ± .00	.00 ± .00	0.00 ± .00
16	*Quercus rubra*	100	50.26 ± .34	5.00 ± .24	9.95 ± .47	100	6.50 ± .00	.00 ± .00	0.00 ± .00
19	*Quercus velutina*	100	72.44 ± .64	9.48 ± .45	13.08 ± .62	50	6.52 ± .01	.14 ± .01	2.13 ± .14

In Table 8 the means of the ascospore-length of those specimens which were associated with canker, and which incidentally are indistinguishable morphologically, are arranged according to their host relationship. Arranged so, the specimens of each host exhibit wide variations in their mean ascospore-length and variability. There is also an indication that the ascospores from certain hosts are considerably larger than those from other hosts. This is more noticeable in a comparison of the figures given for the collections from apple with those for black walnut and hickory. However, without more data no emphasis can be placed on this point.

In Tables 5, 7, and 8 the measurements indicated for $1A_1$ and $1A_2$ are of spores from perithecia produced on lesions resulting from artificial inoculations. The inoculations were made on different parts of the same limb, and the same inoculum (1A) was used for both. The culture (1A) was obtained originally from a single ascospore of specimen No. 1. Also, specimen No. 7_1 in those tables was derived by artificial inoculation from a culture of No. 7.

TABLE 7—*Specimens arranged with means of spore-length in an ascending numerical order*

Reference No.	Host	Mean length
28*	*Juglans regia*	12.55
27*	*Fagus grandifolia*	12.57
26*	*Fagus sylvatica*	13.44
$1A_1$	*Juglans nigra*	14.07
9	*Juglans nigra*	14.77
11	*Hicoria glabra*	14.77
$1A_2$	*Juglans nigra*	14.79
4	*Quercus velutina*	14.82
7	*Juglans nigra*	15.19
17	pure culture (oatmeal agar)	15.33
22	*Quercus velutina*	15.35
23	*Juglans nigra*	15.43
13	*Hicoria glabra*	15.60
14	*Hicoria glabra*	15.64
17	*Hicoria glabra*	15.87
19	*Quercus velutina*	16.18
21	*Salix purpurea*	16.19
20	*Juglans nigra*	16.27
8	*Juglans cinerea*	16.28
8	*Juglans cinerea*	16.35
7_1	*Juglans nigra*	16.45
16	*Quercus rubra*	16.46
1	*Juglans nigra*	16.62
29	*Pyrus malus*	17.27
25	*Quercus alba*	19.12
30	*Pyrus malus*	19.45

The specimens designated (*) are *N. ditissima*. The remainder are *N. galligena*. The method of identification is discussed later.

Statistical analysis shows that many of the differences between specimens from the same host-plant are highly significant. The test of significance was the ratio of the difference between corresponding statistics of any two specimens to the probable error of the difference.* Some of the ratios obtained from comparisons of the mean ascospore-lengths of different specimens are given in Table 9.

* The probable error of the difference was computed by the use of the following formula: $E = \sqrt{e_1^2 + e_2^2}.$

Table 8—*Specimens arranged according to host*

Host	Reference number	Mean ascospore length	Mean length of 5-septate conidia
Juglans nigra	1A₁	14.07 microns	53.13 microns
Juglans nigra	9	14.77	54.29
Juglans nigra	1A₂	14.79	53.13
Juglans nigra	7	15.19	. . . .
Juglans nigra	23	15.43	. . . .
Juglans nigra	20	16.27	. . . .
Juglans nigra	7₁	16.45	. . . .
Juglans nigra	1	16.62	(54.02
			(67.91
Hicoria glabra	11	14.77	61.48
Hicoria glabra	13	15.60	51.41
Hicoria glabra	14	15.64	48.08
Hicoria glabra	17	15.87	. . . .
(Oatmeal agar)	17	15.33	. . . .
			(55.08
Quercus velutina	4	14.82	(56.13
Quercus velutina	22	15.35	(59.37
Quercus velutina	19	16.18	72.44
Quercus rubra	16	16.46	50.26
Quercus alba	25	19.12	. . . .
Pyrus malus	29	17.27	. . . .
Pyrus malus	30	19.45	. . . .

RICHTER'S MEASUREMENTS

Species	Mean ascospore length & width	Mean length and width of 5-septate conidia
N. galligena	16.4 x 6.6 microns	60.0 x 5.5 microns
N. galligena var. *major*	18.0 x 6.8	58.0 x 5.8
N. ditissima	14.1 x 6.0	69.0 x 4.9
N. ditissima var. *major*	18.6 x 6.5	67.0 x 4.3
N. ditissima var. *arctica*	14.8 x 6.4	61.0 x 4.9

To what are these variations due? Since we are dealing with the sexual or perfect spore-stage, it might be supposed that perhaps this variability is due to a genetic segregation of size factors. However, the inoculum which produced the two specimens 1A₁ and 1A₂ was derived from a single ascospore, and this inoculum was presumably homozygous. But even so, a significant difference appeared between the two specimens derived from it. The difference, it is true, was not so great as the differences between certain others from the same host. But it must be remembered that the environment (the same limb) of both specimens was almost identical. Furthermore, if the variation was a matter of genetic segregation, we should expect specimens 1A₁ and 1A₂ to show less variability than the specimen (No. 1) from which it was derived. This follows if the single ascospore which was the starting-point of the specimens 1A₁ and 1A₂, was homozygous. Instead of a decreased variability, however, we find that one of the specimens has undergone a rather significant increase in variability, while the other remains about the same as the "parent" specimen. This indicates that at least some of this variability must be attributed to environmental factors.

Whatever may be the cause of the variability of size, once its possibility is accepted, the absurdity of assigning definite limits to it simply

by finding the mean and variation of a representative sample from one or a very limited number of specimens is apparent. The figures so found may or may not have any value, but the probability that they will not is by far the greater.

TABLE 9—*Significance tests of differences in mean ascospore length between specimens from the same host-plant*

Reference numbers of specimens compared	Ratio of difference to probable error of difference
11 & 17	5.8
7 & $1A_1$	6.6
1 & $1A_1$	14.4
$1A_1$ & $1A_2$	4.2
1 & 9	11.6
19 & 4	10.6
29 & 30	12.9
7 & 7_1	6.8

The writer knows no more forceful way of demonstrating the ineffectiveness of such a procedure than to outline the dilemma into which one falls if one attempts to identify the black-walnut organism by the use of Richter's measurements.* If the measurements given in Table 8 are made to fit as closely as possible the figures given by Richter, we find that, to judge from the width of the conidia, all the cultures represented in the table must belong to *N. galligena*. But if we compare the lengths, both *N. ditissima* var. *major* and *N. galligena* var. *major* are represented. Moreover, duplicate cultures No. 1A from a single ascospore apparently belong to different species! A similar situation occurs if Richter's measurements are applied to the specimens collected from *Hicoria glabra*.

If now an attempt is made to clarify this anomaly by use of the ascospore measurements applied to Richter's figures, we find that among the specimens from black walnut two varieties of *N. ditissima* as well as of *N. galligena* are represented. But in addition we have two interspecific transmutations! *N. ditissima* var. *arctica* (No. 7) produces *N. galligena* (No. 7_1), and *N. galligena* (No. 1) engenders *N. ditissima* and *N. ditissima* var. *arctica* ($1A_1$ and $1A_2$). *N. galligena* and *N. ditissima* var. *arctica* as well as a hybrid (7) between these two are apparently present on *Hicoria glabra*. On the oaks *N. galligena* and two varieties of *N. ditissima* are represented.

If we now compare the mean length of the 5-septate conidia produced in the three cultures from *Hicoria glabra*, we find that only one (No. 11) reasonably agrees with any of the figures given by Richter, and that the range for these three cultures is greater than the range given by Richter for the 2 species and their varieties. This variation in the conidia of the three specimens occurred despite the fact that they were collected within a hundred yards of one another, in a similar habitat, were isolated at practically the same time, and had received the same treatment after isolation. As a matter of fact, the range of the cultures from any host

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* See bottom of Table 8 for Richter's figures.

(provided more than one culture were measured) exceeded the range given by Richter for the 2 species and their varieties.

It is interesting to note the disposition which Richter (*35*) makes of one of the specimens he collected from *Fraxinus excelsior*. The mean ascospore-length of the specimen agreed rather closely with that of *N. galligena*. But in his own words: ''Trotzdem muszte er als die auf Esche allgemein vorkommende *Nectria galligena* var. *major* angesprochen werden, da er in der Reinkultur ploetzlich Riesensporen hervorbrachte, die sogar noch ueber den ueblichen Durchschnitt hinausgingen.''

Enough has been said to show the unreliability of making spore-size the primary criterion for the differentiation of species. So far, the study of quantitative differences furnishes no evidence that more than one species was present among the fungi listed in Tables 1 and 4. However, a study of the morphology of the different specimens was more fruitful.

Morphological Studies

It appeared to the writer that although spore-size was a worthless diagnostic character, spore form held more promise. Among the specimens there appeared a number in which the shape of the spores was very variable, and another group in which the spores were very uniform both as to shape and size. In the first group, oval, ellipsoid, spindle-shaped, or constricted spores appeared with almost equal frequency (Pl. VI, fig. 5). In the second group the spores were very uniformly oval (Pl. VI, fig. 6). The shape of the spores was, moreover, correlated with a number of other morphological characters. The asci of the uniform-spored group were predominantly cylindrical and the spores were 1-ranked (Pl. VI, figs. 5, 6), while those of the other group were predominantly clavate and 2-ranked at the upper end (Pl. VI, fig. 7). The uniform-spored group on smooth bark showed the gross-habit of perithecia used by Weese (*42*) to differentiate *N. coccinea* from *N. galligena*: *i.e.*, the perithecia were collected in large clusters on a common stroma. The other group corresponded to the habit-character given by that writer for *N. galligena*: *i.e.*, the perithecia were isolated or in small groups on a common stroma. In addition it was found that the group with uniform spores was always small-spored, although not all small-spored specimens had spores of uniform shape.

A more detailed and closer scrutiny of the specimens included in the group with uniformly shaped spores disclosed the fact that this group was not homogeneous. It was noted that some of the specimens in this group, in the structure of the ascus, had certain peculiarities which made possible a division of the group into two distinct subgroups. The ascus in these specimens was decidedly truncate at the apex, which in addition usually was extended somewhat beyond the topmost spore. These characters were said by Richter (*34*) to characterize *N. coccinea* (Pers.) Fr. as he delimits that species. A further character, which Richter does not mention, is a distinct pore present in the flattened apex of the ascus (Pl. VI, fig. 9a). This pore is very easily overlooked if the asci are not

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first colored with some stain for which the ascus-wall has an affinity. Furthermore, the pore is best seen with a good oil-immersion objective of medium power. Very high-powered objectives are not so favorable, probably because of their reduced focal depth. The objective used by the writer was one manufactured by Leitz* and was combined with an ocular with a magnifying power of 8x. The stain used was phenol-thionin (0.1 g. thionin, 100 c.c. of 5% aqueous solution of phenol).

Despite Richter's failure to mention any such pore, there can be little doubt that the subgroup described here is identical with his *N. coccinea*, for in all other respects the former agrees with the latter.

In contrast to the specimens with truncate asci, the remainder of the group with uniform spores possess asci which are distinctly rounded at the apex. The topmost spore, moreover, is usually to be found closely appressed to the apical end-wall of the ascus. (Pl. VI, fig. 8.)

Hence it appears that three apparently closely related groups can be distinguished morphologically among the specimens represented in Tables 1 and 4. The characters by means of which the distinctions are made may be summarized as follows:

Group I Spores variable in size and shape, oval-elliptical, spindle-shaped or constricted; asci broadly clavate, usually 2-ranked above; perithecia on smooth bark isolated or in small groups (2-5) on a common stroma.

Group II Spores uniform in size and shape, oval; asci cylindrical or sometimes narrowly clavate and usually 1-ranked, with apex rounded and not extended beyond the topmost spore; perithecia on smooth bark grouped in large numbers (10-50) on a common stroma.

Group III Spores uniform in size and shape, oval; asci cylindrical and usually 1-ranked; with truncate or flattened apex usually extended somewhat above the topmost spore and provided with a distinct pore; perithecia grouped as in Group II.

In Table 10 the specimens listed in Tables 1 and 4 have been separated on the basis of the above-described characters. The first point of interest to be noted is that all the specimens which were associated with cankers fall into Group I, together with the type-specimen of *N. galligena* Bres. This agrees with Weese's statement that *N. galligena* is the only cause of canker on deciduous trees.†

* Designation No. 1/7a, N. A. 0.95, magnification 54x.

† Numbers 5 and 7 could not be identified, since the writer did not have the perithecia of these cultures.

No. GROUP I	Name under which the specimen appears	Host	− Not associated with canker + Associated with canker	Remarks
21	*Nectria galligena*	Salix	−	Type specimen
1	. . . .	*Juglans nigra*	+	
2	. . . .	*Juglans nigra*	+	
3	. . . .	*Juglans nigra*	+	
4	. . . .	*Quercus velutina*	+	
7	. . . .	*Juglans nigra*	+	
8	. . . .	*Juglans cinerea*	+	
9	. . . .	*Juglans nigra*	+	
10	. . . .	*Quercus velutina*	+	
11	. . . .	*Hicoria glabra*	+	
13	. . . .	*Hicoria glabra*	+	
14	. . . .	*Hicoria glabra*	+	
15	. . . .	*Quercus alba*	+	
16	. . . .	*Quercus rubra*	+	
17	. . . .	*Hicoria glabra*	+	
19	. . . .	*Quercus velutina*	+	
20	*Nectria sp.*	*Juglans nigra*	+	
22	. . . .	*Quercus velutina*	+	
23	. . . .	*Juglans nigra*	+	
24	. . . .	*Juglans nigra*	+	
25	. . . .	*Quercus alba*	+	
29	*N. ditissima*	*Pyrus malus*	+	This specimen identified by Weese as *N. galligena.*
37	*N. coccinea*	Quercus	+	This specimen identified by Weese as *N. galligena*
33	*N. ditissima*	*Pyrus malus*	+	
30	*N. galligena*	*Pyrus malus*	+	
GROUP II				
26	*N. ditissima*	*Fagus (sylvatica?)*	−	Type specimen
28	*N. coccinea*	*Juglans regia*	−	
38	*N. coccinea*	Magnolia	−	
41	*N. coccinea*	*Magnolia fraseri*	−	
42	*N coccinea*	*Magnolia fraseri*	−	
27	*N. coccinea*	*Fagus grandifolia*	−	This is apparently the cause of bark disease present in Nova Scotia and New England
GROUP III				
32	*N. ditissima*	Fagus	−	
35	*N. ditissima*	(Fagus?)	−	
36	*N. ditissima*	*Acer rubrum*	−	
45	*N. coccinea*	*Tilia americana*	−	
40	*N. coccinea*	*Ulmus pubescens*	−	
UNDETER- MINED GROUP				
31	*N. ditissima*	*Pyrus malus*	+	Specimen immature probably belong to Group I
34	*N. ditissima*	Fagus		Spores over-ripe, germinated, no asci found
39	*N. coccinea*	*Fagus sylvatica*		Spores over-ripe, germinated, no asci found
43	*N. coccinea*	*Robinia pseudacacea*	−	Does not belong to any of the three groups
44	*N. coccinea*	*Ilex opaca*		Probably *N. applanata*

Six specimens including the type-specimen of *Nectria ditissima* fall into Group II. This disposal of the type-specimen of *Nectria ditissima* Tul. is rather at odds with the description of the species as given by Richter, for he characterizes the perithecial habit of the three varieties of that species as either isolated or in small groups but never in large groups on a common stroma. He uses the same terms to describe the habit of Wollenweber's two varieties of *N. galligena*. However, the photograph (Pl. IV, fig. 2) of the type of *Nectria ditissima* Tul. shows on the contrary that the perithecia are in large groups. Furthermore, the spores of his three varieties, to judge from his descriptions and drawings, are considerably more variable in size and shape than the spores of the type-specimen. For example, Richter gives as the ''normal variation'' the figures 12-16, 16-20, and 13-17 for *N. ditissima*, *N. ditissima* var. *major*, and *N. ditissima* var. *arctica* respectively. The type-specimen when measured by the same method had a range 11-14.

In regard to *Nectria galligena* Bres. var. *major* Wr. the same author†states that in the size of the spores this variety coincides with the type-specimen of *N. galligena* Bres. It is a little surprising to learn that the type-specimen of a species has been relegated to a varietal position under the same species of which it is the type. But if this is considered by the reader as too great a perversion of Richter's statement, it still remains to be explained how, if spore-size is made the basis of a separation of two varieties of a species, the spore-size of one happens to coincide with that of the other. But, aside from the question of theory, the statement does not agree with the facts. Reference to Table 5 will show that the mean spore-length of 226 spores of the type-specimen of *N. galligena* Bres. was 16.19 microns. This agrees much more closely with the figure given by Richter for *N. galligena* (16.4 microns) than with that for *N. galligena* var. *major* (18.0 microns). The spore-length given by Bresadola (*39*) for *N. galligena* is 18-20 microns. This appears to agree more nearly with Wollenweber's *N. ditissima* var. *major* than with his *N. galligena* var. *major*.

Five specimens fall in Group III. These agree with Richter's description of *N. coccinea* (Pers.) Fr. That this group is sufficiently distinct from the fungi in Groups II and III to justify its separation as a different species, the writer does not wish to deny. Serious objection may be raised, however, to the application of the name *Nectria coccinea* (Pers.) Fr. to this group. The characteristics of the asci of the group do not agree with the characteristics of the asci of the type specimen of *N. ditissima* Tul. which Tulasne recognized as synonymous with *Sphæria coccinea* Pers. Nor does the group agree with Fries's (*13*) description,* according to which *N. coccinea* (Pers.) Fr. is characterized by elongate-filiform spores. Hence it is difficult to understand the reason for Wollenweber and Richter's application of the name *N. coccinea* (Pers.) Fr. to a species with the characteristics of Group III.

† *Loc. cit.*, p. 59.
* *Loc. cit.*, p. 387.

No decisive evidence of the pathogenicity of the fungi of this group is at hand. It is true that one of Richter's specimens (Populus 1.) which possessed the characteristics of Group III was collected from a canker on poplar in Holland. But this fungus manifested no decisive pathogenicity when inoculated into poplar and several other hosts. Furthermore, to the writer's knowledge it has not been shown that the *Nectria coccinea* used by Westerdijk and Luijk in their inoculation experiments and that designated *N. coccinea* by Wollenweber and Richter are the same species. With these observations the final disposal of this species is left for the future.

The question of the proper name to apply to Group II is not as simple as the presence of the type-specimen of *N. ditissima* Tul. within the group might indicate. The difficulty involves the question of synonymy of *N. ditissima* Tul. with *N. coccinea* (Pers.) Fr. In 1909 Seaver (*35*) and in 1910 Hoehnel (*21*) and Weese (*42*) concluded that *N. ditissima* Tul. and *N. coccinea* (Pers.) Fr. were one and the same species. Seaver admits that his opinion is based exclusively upon a study of American specimens. Two of the exsiccati which he lists (E. Bartholomew, Fungi Columbiani 2043, 2238) appear in this work under the reference numbers *45* and *40* respectively. These have been relegated to Group III by the writer since the asci of both specimens are truncate and provided with a pore. Therefore what was said about that group applies also to these specimens. Another specimen which he lists (Ellis & Everh., N. Amer. Fungi 161) is the same as that appearing here under the number 38 (E. & E. Fungi Columbiani 618). Seaver had more than one species in the collection on which he based his description of *Creonectria coccinea* (Pers.) Seaver, for this specimen (38) has asci which are rounded at the apex and which are without a pore. It was, therefore, referred to Group II. (See Table 4).

Reduced to the simplest terms, the point at issue is whether the *Nectria coccinea* of Fries is the same organism as *Sphæria coccinea* Pers., and not whether the latter is synonymous with *N. ditissima* Tul. It appears therefore that taxonomy is obligated to retain the specific name *ditissima* until proof that Fries's *N. coccinea* is identical with *Sphæria coccinea* Pers. is forthcoming. Hence the specimens listed in Group II have been referred to as *Nectria ditissima* Tul.

The specimens in Group I cannot be separated from one another by any method known to the writer, and therefore they are all considered as variations of one species. It appears probable that all the varieties of *Nectria galligena* and *N. ditissima*, as those names are used by Wollenweber, are but variations of this same species.

It may be that among this group a certain amount of physiological specialization or host adaptation occurs, and that the group for this reason may be said to be constituted of a number of "strains." The inoculation experiments of the several workers (*45*), (*34*), (*25*) indicate that the pathogenicity of the various isolants used by them varies both qualitatively and quantitatively. But nowhere in this is there any proof

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that the variations in the behavior of different isolants are manifestations of fixed differences. On the contrary, abundant evidence is available to suggest the instability of such variations. The results of the inoculations made by the writer in the spring of 1932 show that pathogenicity may be entirely lost for a given time. Westerdijk and Luijk noted the frequent loss of virulence by isolants which had been kept for a lengthy period in pure culture. That this change in the fungus is not due entirely to culturing is borne out by the following quotation from their publication: "Anderseits trifft man wieder jahrelang kultivierte Staemme, die eine frische Virulenz haben, an." Leonian (24) has shown that different dissociants from a single monosporous culture of *Fusarium moniliforme* vary tremendously in appearance and in their virulence towards corn. Sleeth (36) has found the same to be true with dissociants of *Fusarium niveum*. These dissociations are not irreversible, however, but any segregate may revert at any time to the original or change to other types.

A temporary adaptation of this Nectria to a particular host or mode of living may be and probably is accompanied by slight changes in size or color of the structures of the fruit-bodies. For example, the spores of specimens collected from cankers on beech are rather consistently somewhat smaller than those from apple. This may be nothing more than a response of the fungus to a different nutritional condition, and not a manifestation of an inherent difference.

For the convenience of any future investigators, a description of the fungus as it appears on black walnut is included. It need only be mentioned that this description is not intended to delimit the species. It is intended to describe the fungus only in so far as it has been seen by the writer, who holds no belief that he has seen all the variations of which this species is capable on that host.

Stroma poorly developed, on bark erumpent, on bare wood may be lacking; perithecia distinct, spherical to egg- or pear-shaped, isolated or in small groups (2-5) on a common stroma, sometimes in large, irregular clusters formed by the crowding together of a number of separate stromata, usually occurring in fissures on the bark, brownish-orange when mature and frequently darker around the ostiolum, brighter orange when young and darker (brick-red to wine-colored) in age, usually not collapsing, 260-450 x 230-390 microns; asci broadly clavate, eight-spored, usually biseriate at the enlarged apical end, monoseriate below, elliptically rounded at the apical end to which the topmost spore is usually appressed; ascospores hyaline, smooth 1-septate variable in size and shape, oval, elliptical, spindle-shaped, or constricted at the septum, mean size 15.6 x 7.0, normal variation 14.1-16.6 x 6.4-80 microns; paraphyses delicate, longer than asci, septate, branched, basal cells greatly distended between septa, where hourglass-like constrictions occur, apically terminated by a whip-like filament. Conidial stage a Cylindrocarpon Wr. Conidia subcylindrical, straight, or slightly curved, somewhat clavate usually, but sometimes tapering slightly towards both ends, 0.9 septate, but 5- and 6-septate most common, apical cell elliptically rounded, basal cell spherically rounded or slightly flattened, size

0- septate 6.6 — 20.4 x 3.3 — 6.5 microns,

5- septate 56.1 x 6.1 microns (52.6 — 68.0 x 5.6 — 6.5 microns),
absolute range regardless of number of septations

6.6 — 151.3 x 3.3 — 6.8 microns.

In this description the mean size of the ascospores is the average of the means of the 8 specimens from black walnut given in Table 5. The normal variation is the range of those 8 means. The figures given for the 5-septate conidia were similarly determined.

TEMPERATURE STUDIES

During the course of this investigation, cankers have been found which for a number of years had ceased to enlarge. In such cases, the healing process of the tree had been uninterrupted for the same period, and many of these cankers were well on the way to a complete recovery. More often, however, one part of the periphery of the canker would be healing over, while other parts still continued to recede. This inactivation of the fungus, together with what had been learned of the distribution of the disease in West Virginia and the observed fact that the canker is most destructive on more protected parts of the tree, suggested that some environmental factor exerted a deleterious effect upon the fungus.

A preliminary test to determine the approximate optimum temperature for growth of the fungus suggested the probable nature of this factor. The fungus was favored most by temperatures ranging between 15°-23° C., but at 30° growth was practically at a standstill. This test had been made in liquid medium, and since the solubility of oxygen decreases rapidly with increased temperature, it was thought that perhaps the effect observed might be due partly to lack of oxygen in the medium. Therefore the experiment was repeated at the higher temperatures.

In this and all subsequent experiments the medium used was made up according to the following formula:

KH_2PO_4 _____	1	gr.
$Mg\ SO_4$ _____	1	gr.
Malt extract_____	5	gr.
Dextrose _____	10	gr.
Agar agar_____	20	gr.
Water (tap) _____	1000	c.c.

Petri dishes containing 15 c.c. of this medium were autoclaved for 20 min. at 15 lbs. pressure. After cooling, the plates were inoculated with small discs of agar cut from the margin of previously grown petri-dish cultures by means of a sterile cork-borer. When the cultures had reached a size of approximately 30 mm. the limits of each culture were marked on the bottom of the petri dish with a wax pencil, and triplicate cultures of each isolant were transferred to incubators regulated at 31°, 32.5°, 34°, 35°, and 36° C. These incubators fluctuated no more than 0.5 of a degree in any instance. The cultures were incubated at these temperatures for 5 days and then transferred to an incubator the temperature of which fluctuated between 15°-20° C. At the end of eleven days the increase in the size of each colony was measured. These measurements are given in Table 11. The figures are the averages of the triplicate determinations,

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except in a few cases. Since none of the cultures grew in the incubators at any of the above temperatures, the figures given indicate the amount of growth made by each isolant after the temperature treatment.

TABLE 11—*Growth increase after temperature treatment for five days*

Reference number	31°C	32.5°C	34°C.	35°C.	36°C.
1A	35*	31	14	0	0
2J	42	33	15	0	0
2L	42	31	20	0	0
3	31	29	17	0	0
4L	37	35	22	0	0
5	44	37	21	—	—
6	43	29	0	0	0
7	33	29	17	0	0
8	31	25	18	0	0
1C	—	—	—	0	0
2K	—	—	—	0	0
3S	—	—	—	0	0
4A	—	—	—	0	0

* Figures indicate millimeters of growth in eleven days at 15°-20°C. after removal from incubator.

The table shows that even a temperature as low as 35° C. if continued for 5 days kills all the isolants used. One was killed at 34°. Those plates in which the cultures failed to grow were re-inoculated with the same fungus, and in a few days vigorous colonies resulted. This shows that the failure of the fungus to grow was not due to changes in the medium. The injurious effect of temperatures even below 34° can be perceived in the decrease in growth as the temperature rises. The amount of growth made in 11 days by the cultures which had been incubated at 32.5° was in every case less than that made in the same period by the cultures incubated at 31° (Pl. VII, fig. 1).

It might be supposed that this decrease was occasioned by a greater loss of water from the medium which had been subjected to the higher temperatures. To guard against just such a contingency, each of the incubators had been supplied with an evaporating pan filled with water. This provision kept the air in the incubator near the saturation point, and little water was lost from any of the culture dishes. Even at the end of the expermient, the medium appeared as fresh as at the beginning.

An attempt was now made to determine the shortest time in which temperatures 35° and 36° C. would kill the different isolants. A duplicate series of cultures prepared as described above was transferred to the 35° and 36° incubators each day for 4 successive days. On the fifth day all the cultures from both incubators were removed and placed in the incubator at a temperature favorable for growth (15-20°). The results, which are given in the first 8 columns of Table 12, were read at the end of 11 days. The letter *G* indicates that the cultures grew after the temperature treatment; *K* indicates that no growth occurred.

A similar experiment was run at temperatures 38° and 40° C., but the time was decreased to a matter of hours. The results of this experiment are given in columns 9-16 of Table 12.

Since the fungus in nature is subjected, probably only very rarely, to temperatures above 40° C., it was thought inadvisable to raise the temperature above this point. Instead, an attempt was made to determine whether the injurious effects of shorter treatments at this temperature might be cumulative if repeated on successive days. Cultures of the different isolants were prepared as above. On successive days these cultures were placed in the 40-degree incubator, where they remained for 4 hours. They were removed at the end of this period and placed in

TABLE 12—*Effect of various temperature treatments on the canker organism*

Ref. No.	35°C. Days				36°C. Days				38°C. Hours				40°C. Hours				(40°C.) ⇌ (20°C.) (4 Hrs.) 4 Days	(20°C.) (20 Hrs.) 6 Days
	1	2	3	4	1	2	3	4	2	4	6	16	2	4	6	24		
1A	G	K	K	K	G	K	K	K	G	G	G	G	G	G	G	K	3	2
1A₁	–	–	–	–	–	–	–	–	G	G	G	G	–	G	G	K	3	2
1C	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	3	3
2J	G	G	G	G	G	K	K	K	G	G	G	G	G	G	G	K	3	3
2L	G	G	K	K	G	K	K	K	G	G	G	G	G	G	G	K	3	2
3	G	–	–	K	G	K	K	K	–	G	G	G	G	G	G	K	2	1
4L	G	G	G	K	G	K	K	K	G	G	G	G	G	G	G	K	3	2
5	G	K	K	K	G	K	K	K	G	G	G	G	G	G	G	K	2	1
6	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	2	1
7	G	G	K	K	G	K	K	K	–	–	–	–	G	G	G	K	3	3
8	G	G	G	G	G	G	G	K	G	G	G	G	G	G	G	K	3	3
9	–	–	–	–	–	–	–	–	–	G	G	G	G	G	G	K	3	3
10	–	–	–	–	–	–	–	–	–	G	G	G	G	G	G	K	–	3
11	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	3	3
13	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	3	3
14	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	3	3
15	–	–	–	–	–	–	–	–	G	G	G	G	G	G	G	K	2	1
16	–	–	–	–	–	–	–	–	–	G	G	G	G	G	G	K	3	3
17	–	–	–	–	–	–	–	–	–	G	G	G	G	G	G	K	3	3
19	–	–	–	–	–	–	–	–	–	–	G	G	G	G	G	K	3	3
24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3
27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	2
30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	2

G indicates that cultures grew after treatment.
K indicates that cultures did not grow after treatment.
1 indicates that cultures did not grow after treatment.
2 indicates that points from which new growth occurred were isolated or discontinuous.
3 indicates that points of origin of new growth were continuous..

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the low-temperature incubator. One series of cultures was subjected to this alternate treatment for 4 days, and another series for 6 days. After the final treatment the cultures were allowed to remain in the low-temperature incubator for 11 days.

In the last-mentioned studies it had been noted that although many of the cultures grew after the temperature treatment, the new growth did not originate with the young marginal hyphæ of the colony, but sprang from the older parts of the mycelium. In many cases life had persisted at only a few isolated points of the colony. These points became the centers of new colonies which grew out over the old. In order to indicate these differences, the writer was compelled to employ a more subjective method of appraisal than that used heretofore. The results of this last experiment which are given in the last two columns of Table 12 are therefore indicated by numbers. These numbers represent varying degrees of injury. The number (1) indicates that no growth occurred; (2) indicates that the points from which the new growth arose were isolated or discontinuous; and (3) indicates that points of origin of the new growth were continuous.

The fact that successive, daily, 4-hour treatments at 40° C. for six days killed 4 of the cultures, whereas one 6-hour treatment failed to kill any, shows that the injury of short daily treatments is cumulative.

DISCUSSION

So far no other method of infection has been discovered except through wounds which penetrate to the living cells. Even in the case of such wounds, the time of year at which the wound is present influences the chances of infection. During the dormant period of the host, a wound barely penetrating the corky outer layer would furnish an avenue through which successful infection might occur. This same wound, however, would offer no opportunity to the fungus during the growing season. The reason for this is to be found in the behavior of the host. Before the fungus could penetrate to the wood, the infected area would be excluded by the formation of a periderm and eventually sloughed off completely. For successful infection during the growing season, therefore, wounds must penetrate into the neighborhood of the cambium, or even to the wood.

This fact should have some bearing on the question of insect transmission of the disease. Since sucking insects do not ordinarily wound deeper than the phloem, it appears extremely doubtful that they can be considered as important vectors of the parasite on black walnut. This may not apply to the undifferentiated tissues of the growing points, for in these parts all tissues probably are equally favored by the insect.

It seems more than probable that a direct relationship exists between the distribution of the disease in West Virginia, the occasional healing of cankers of several years' growth, and the effect of temperature on the parasite. The fact that short daily exposures to temperatures as low as 40° C. can cause the death of the fungus makes plausible the hypothesis

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that higher temperatures may at times kill the parasite in the tissue of the host, and limit the spread of the canker to the cooler sections of the state. Even within those sections, the disease is more destructive in shaded and therefore cooler habitats.

It is not necessary to suppose that temperatures even as high as 40° C. are required to prove fatal to the fungus. All but one culture among those tried were killed by a 48-hour exposure to 36° C. Temperatures in that range and even higher were of almost daily occurrence in parts of West Virginia during the summers of 1930 and 1931. The treatment to which the cultures were subjected in the last temperature experiment was probably mild in comparison to that experienced in nature. In the experiment, after a 4-hour treatment at 40° C., the fungus was furnished for 20 hrs. with optimum conditions for recovery, whereas temperatures rising to 109° F. during the day and falling to no less than 85° F. at night were experienced during those summers. But even this lower temperature is near that which totally inhibits growth of the fungus, and it is very unlikely that the restorative processes would be very active at such an unfavorable temperature.

It is even probable that when the cankered area is exposed to direct sunlight, the fungus may be subjected to temperatures higher than the surrounding atmosphere. That dry wood under such conditions may become heated to a point where it is uncomfortably hot to the touch is well known. It was shown above that the fungus was confined to the dead areas of the wood during the growing period. These areas must become rather dry when the wood is exposed by the sloughing off of the dead bark. Solar radiation of these exposed surfaces may heat the underlying wood above the temperature of the surrounding air.

METHODS OF CONTROL

The methods that have been recommended for the control of European canker can be summarized under two headings: (1) destruction of badly cankered trees, and (2) surgical treatment for trees slightly affected.

The first recommendation applies to trees for which the second treatment would prove uneconomical. Such trees should be felled, and all cankered parts burned. The remainder may be used for any purpose for which it is suitable.

The second treatment is recommended for trees that have an economic value beyond the cost of treatment. When cankers are confined to the limbs, these should be removed according to approved methods. Small cankers on the trunk can be cut out profitably, provided the wound left does not reduce too greatly the strength of the tree. Care should be exercised, however, that all diseased tissue is removed.

All wounds left by pruning or removal of infected tissue should of course be covered with some material which will protect the exposed tissue from infection by wood-destroying and parasitic organisms. A

coating of asphaltum or white lead may be used for this purpose. Zeller (56) reports excellent results from the use of a bordeaux paste.

The temperature studies described above suggest another method of control. Since the activity of the fungus is greatly reduced in positions which are exposed to the direct radiation of the sun for long periods of the day, such locations should be favored for new plantings of walnut trees. In addition the trees should be spaced in a manner which would obviate an undue amount of shading. Plantings so arranged and so located should need little attention to keep them free of canker.

The results of cross-inoculations show that canker on the hickories, oaks, walnut, and butternut trees is the work of one organism. It is, moreover, a reasonable assumption that the same organism is the cause of the cankers on a number of other deciduous trees. Cankered trees are, therefore, a menace not only to trees of the same species but also to a number of others. Hence, forestation projects which include plans for eradication of canker from very susceptible hosts, such as *Juglans nigra*, should include provisions for its eradication at the same time from all other species.

SUMMARY

The results of this investigation may be summarized briefly as follows:

A destructive disease which has been identified as ''European Canker'' is described as it appears on *Juglans nigra* L. Similar cankers have been found on *Acer rubrum* L., *Liriodendron tulipifera* L., *Juglans cinerea* L.. *Quercus rubra* L., *Q. alba* L., *Q. velutina* Lam., and *Hicoria glabra* (Mill.) Britton. The last five species have, to the writer's knowledge, not been reported hitherto as among the hosts afflicted by this disease.

Evidence of the presence of the canker on black walnut in 50 counties of West Virginia, in eight other states, and in one province of Canada is reported.

Nectrias have been found associated with cankers on all the above-mentioned hosts. The pathogenicity of the Nectrias isolated from *Juglans nigra*, *J. cinerea*, *Quercus alba*, *Q. velutina*, and *Hicoria glabra* has been proved for the respective host from which each Nectria was isolated. In addition it has been shown by cross-inoculation that all these Nectrias as well as one from *Quercus rubra* are capable of infecting black walnut, that the black-walnut organism can infect white oak, white walnut, black oak, and four species of hickory including pignut hickory, and that the organism from oak and hickory can infect hickories and oaks respectively. The development of the infections on black walnut with the fungus from that host has been followed for several years, and typical cankers resulted.

The pathological anatomy incident to the disease on black walnut has been studied and described, and the manner in which the canker is

formed, detailed. It has been shown that the growth habit of the fungus in the extracambial parts of the host is intercellular, but that the mycelium in the xylem is entirely intracellular. The primary effect of the parasite is necrotic; the secondary response of the host hyperplastic.

The conditions of the host which are necessary for successful infection have been studied and reported.

A full discussion of taxonomy of canker-causing Nectrias and closely related species is given.

It has been shown that size of spores is of no value as a means of dividing the Nectrias responsible for canker of deciduous trees into species and varieties.

Certain morphological characters by means of which the canker-causing Nectrias may be distinguished from closely related species are described. On the basis of these characters the Nectrias associated with cankers on the hosts mentioned above have all been referred to *Nectria galligena* Bres.

It has been shown that temperatures experienced under natural conditions in West Virginia, if sufficiently prolonged, are high enough to kill the fungus responsible for cankers; and that short daily exposures to such temperatures are cumulative in their effect on the parasite.

A summary of the methods proposed for the control of European Canker is given. In addition, a further method which was suggested by the effects of temperature on the fungus is described.

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