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A leaf disease of Walnuts.

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(With 1 Photogr. and 6 Textfigures.)

The production of Persian or English Walnuts in Alabama is in no wise to be regarded as an important industry. The reason for this is not to be sought in the liability of the trees to disease, for, when grown under favorable conditions, they are subject to comparatively few insect pests and fungoid maladies. The most serious walnut trouble, perhaps, which affects this industry in the United States, occurs in California, to which state the commercial production of the crop is largely confined. It is known as the walnut blight or bacteriosis of walnuts¹⁾. Fortunately, so far as has been observed, this disease does not occur in Alabama. There has been under observation, however, at Auburn, Ala., during the past two years a heretofore unknown leaf disease of English walnuts. This disease manifests itself by the presence of numerous, irregular, circular to angular, dry spots, varying in size from minute specks to large, brown areas, 5 mm in diameter. These spots are, for the most part, brown in color and with a grayish center, marking the initial point of infection. Some may be uniformly brown on both leaf-surfaces and others may be quite grayish, due to the elevation of the cuticle and the consequent entrance of air. Frequently tissues adjacent to those invaded by the fungus are chlorotic.

A single leaf may have 500—1000 or more centers of infection remaining quite distinct or in other cases coalescing so as to involve the leaf margins or tips or even the entire leaf, causing them to become dry and brown (s. Photogr.). During the summer of 1912, this disease was so severe, in a small grove in which the trouble has been studied, that the trees were completely defoliated twice prior to the time when the leaves should normally have fallen.

The organism which causes this disease is one of the imperfect fungi belonging to the genus *Cylindrosporium*. The acervuli or fruit bodies of this form are borne on the lower surface of the leaves. They are very minute and inconspicuous, measuring only 75—100 microns in diameter. They are initially subepidermal, but break through the cuticle at

1) SMITH, R. E., SMITH, C. O. and RAMSEY, H. J., Walnut culture in California. Walnut blight [in pars] (Cal. Agr. Exp. Stat. Bul. 231, 320—371, figs. 78 to 90, 1912).

maturity, liberating the conidia (fig. 2). The conidia are curved, tapering at the upper end and pluriseptate. There are typically no constrictions at the septa although they have been observed to occur. They vary in



Photograph of English Walnut leaves affected by *Cylindrosporium Juglandis*.

size, being $20-50 \times 3-3,5$ microns and are hyaline with refringent granules and oil droplets. Conidia taken from dried material which has been in the laboratory for a year, had shrunken and measured $16-28 \times 2,5-3$ microns.

The fungus has several times been isolated in pure culture by the dilution plate method and has been successfully grown for over a year upon various kinds of nutrient media. The conidia were found to germinate more readily in water than in nutrient agar. In the process of germination the cells first become distended from the water which they have absorbed, and consequently are more or less constricted at the septa. From each cell, when cow-pea agar is used, one or more germ tubes arise, which rather tardily develop into a much branched, septate mycelium. Within 48 hours the hyphae will not have reached a greater length than about 100 microns (fig. 6) and the colonies may be visible to the eye only at the end of four days. Growth proceeds slowly and the colonies, which are dark olivaceous in color, will not have attained a size greater than half the area of a pin's head within three weeks or a month. Conidia are readily formed upon this medium, however, and have been observed eight days after the plates were poured. They are successively abstricted from short mycelial branches (fig. 5), but are indistinguishable from conidia formed in acervuli (fig. 4). In culture the mycelium becomes divided into chains of rounded, thick walled cells as shown in fig. 3.

When conidia are germinated in drops of water, in VAN TIEGHEM cells or on glass slides which have been placed in moist chambers, the hyphae quite commonly anastomose, as represented in fig. 1. This phenomenon is not uncommon with germinating spores of various fungi, and has been noted by several investigators, the most extensive observations having probably been made by BREFELD¹). It has been observed by JARIUS²), while investigating the conidial stage of *Sphaerella pinoides* (BERK. et BLOX) NISSEL³) and by STONE while investigating the ascogenous stage⁴). Not uncommonly too, one notes that secondary conidia as shown in fig. 1, may be formed in water culture.

Thus far attempts to induce this fungus to develop an ascigerous stage in culture have been fruitless. Even when affected leaves were

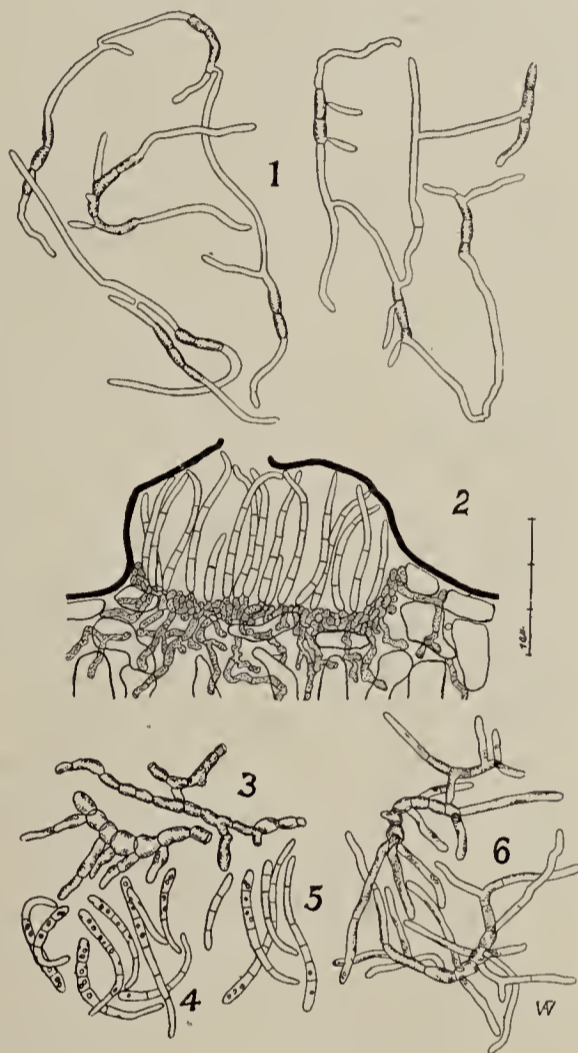


Fig. 1. Conidia of *Cylindrosporium Juglandis* anastomosing and forming secondary conidia, in water, culture, 24 hours old.

Fig. 2. An acervulus in vertical section.

Fig. 3. Mycelium in old culture.

Fig. 4. Conidia from an acervulus.

Fig. 5. Conidia formed in pure culture on cow-pea agar.

Fig. 6. Germination on cow-pea agar, 48 hours old.

1) BREFELD, O., Untersuchungen usw., 4, 116, pl. 9, fig. 14.

2) JARIUS, M., *Ascochyta Pisi* bei parasitischer und saprophytischer Ernährung (Bibl. Bot. 1896, 34, 1-45, pl. 1).

3) According to SACCARDO, P. A. (Syll. Fung. 1913, 22, 120).

4) STONE, R. E., The life history of *Ascochyta* on some leguminous plants (Ann. Mycol. 1912, 10, Nr. 6, 564-592, pl. 19-20).

permitted to overwinter in wire baskets out of doors no evidence has been found of the occurrence, on them, of an ascigerous stage. On the contrary acervuli, bearing conidia which germinated within 24 hours in water culture, were still present April 24, 1913, on diseased leaves which had been collected during the previous autumn and had been exposed to conditions comparable to those presented to fallen leaves in the grove. Since the fungus remains viable in the conidial stage upon fallen leaves until the trees are again in full leaf it seems quite probable, at any rate, that no ascigerous stage exists, at least in this vicinity, for this species of *Cylindrosporium*. It is not improbable also that under certain environmental conditions a perfect stage might develop since some species of this genus are known¹⁾, to possess an ascomycetous form.

That this disease may be quite easily controlled has been demonstrated by some experimentation conducted during the past summer. The diseased fallen leaves had been gathered up, from beneath the trees upon which the fungicides were to be used, and destroyed, during the autumn, in order to lessen the chances of infection from this source. Certain of the trees were sprayed with self boiled lime sulphur, 8—8—50, the first application being made May 3rd, at a time when the newly formed leaves had almost attained their mature size. A second application was made on July 2nd. By July 29th, a considerable amount of spotting, comparable to that on the untreated trees, had developed, indicating the ineffectiveness of this fungicide. Several trees, however, were sprayed with Bordeaux mixture, 4—4—50, the first application having been made on July 31st, and a second on Aug. 27th. It is to be noted that leaf spot had already appeared on these trees when the first spray was applied so that it was only hoped to check the disease. This end was very satisfactorily attained and these trees retained their foliage until the frosts caused them to fall. As a result of this work it may be recommended that a satisfactory control of walnut leaf spot may be obtained by the destruction of diseased leaves during the trees' dormancy, and by the application of Bordeaux mixture, as a supplementary measure, at the inception of the disease.

While searching available literature for the identity of the species of *Cylindrosporium* under investigation, it was found that one form only has previously been reported upon *Juglans*. This species, *C. nanum* COOKE²⁾, has been reported from New Zealand. It has been impossible to secure specimens of *C. nanum* for comparison with the form upon English walnut, but the two are most certainly not identical, and, judging from the original description of *C. nanum*, are not to be referred to the same species.

There has also been described from London, Canada, upon *Carya amara*, a form, *Cylindrosporium caryogenum* E. et E.³⁾ which is very similar morphologically to the form upon the leaves of English walnut.

The two are, however, very different⁴⁾ in macroscopic characters. Because of this structural similarity and the possibility that the macroscopic

1) HIGGINS, B. B., The perfect stage of *Cylindrosporium* on *Prunus avium* (Science N. S. 1913, 37, Nr. 956, 637—638).

2) COOKE, M. C. (Grevillea, 14, 130).

3) ELLIS, J. B. and EVERHART, B. M., New species of North American fungi (Proc. Acad. Sci. Philad. 1893, p. 168).

4) Comparisons with the type material, N. A. F. 2451, collected Sept. 1889, have been made by Prof. G. F. ATKINSON of Cornell University and Mrs. FLORA W. PAT-

differences might be due only to certain differences in hosts, inoculations were made both with pure cultures and with conidia from the leaves of the English walnut upon *Carya illinoensis* (WANG.) K. KOCH., *C. ovata* (MILL.) K. KOCH., *C. cordiformis* (WANG.) K. KOCH. (which includes *C. amara* NUTT.), and *Juglans nigra* L. In all cases the leaves were placed in moist chambers lined with moist filter papers to render the atmosphere as humid as possible. The conidia from the living leaves of English walnut were placed in small drops of water upon both surfaces of the leaves. A portion of a colony together with adhering agar was applied in case pure cultures were employed in making inoculations. These inoculations were several times repeated during the summer and fall using leaves of different ages but in no case were infections secured. That the conidia used for inoculations were viable is known since some were removed from the drops of water on the leaves, examined microscopically, and observed to have germinated. It has been noted, too, that pecans and black walnuts growing near the diseased English walnuts, at distances of less than 100 feet, have never been observed to be attacked by *Cylindrosporium*. From this evidence it would seem that the species of *Cylindrosporium* is distinct from *C. caryogenum* and probably occurs only on the English walnut. SMITH (l. c. p. 320) reports, too, that the natural occurrence of bacteriosis of the walnut or walnut blight is probably confined to the English walnut or to its hybrids with the black walnut, which might be regarded as evidence of the possible restriction of the leaf spot organism to the same host. Even though the microscopic differences are insignificant, it seems advisable, to regard this only as a coincidence, in view of the failure to secure infections upon a closely related species, *J. nigra*, and upon a closely related genus, *Carya*, one species of which is the type host for *C. caryogenum*. On this account the name *Cylindrosporium juglandis* is proposed for the form causing a leaf disease of *Juglans regia* and the following Latin diagnosis is given:

Cylindrosporium Juglandis: Maculis foliicolis amphigenis, numerosis, irregulariter rotundatis v. angulosis, minutis, demum 5 mm diam.; brunneolis, centro albicanto, quandoque confluentibus, arescendo-marginatis; acervulis hypohyllis, minutis, subcutaneis, erumpentibus, pallidis, 75—100 mm diam.; conidiis clavatis, curvulis, pluriseptatis, guttulatis, granulosis, hyalinis, 20—50 \times 3—3,8 μ .

Hab. in foliis vivis *Juglandis regiae*, quae desiderere faciunt.

TERSON, of Washington, D. C.; Prof. ATKINSON reports that the two "are closely related, although the spots are very different. This may be due to physiological conditions of the host in relation to the parasite". — Mrs. PATTERSON is of the same opinion in that she states that "according to conidial characters it does not differ essentially from the description of *C. caryogenum* E. et E. Macroscopically the differences are considerable. Inoculations from your material upon *Carya* spp. might be advisable, as thereby the macroscopic effect of the parasite upon a closely related genus might be demonstrated". Thanks are herewith tendered to each for this advice and help so kindly given. Thanks are also due Prof. ATKINSON for suggestions and criticism in the preparation of this manuscript.

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