A NECTRIA DISEASE OF COFFEE IN WESTERN GUATEMALA[1]

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INTRODUCTION

Although the *Nectria* canker of *Coffea* probably existed in Guatemala more than a decade ago, little importance was attached to it since only an occasional tree died and had to be removed. In 1935 the trunks of all the coffee trees at La Soledad, a large finca near Tumbador, Depto. de San Marcos, were vigorously rubbed with coffee sacking in order to remove the accumulation of algae, mosses, and lichens. That year the crop production was the highest in the history of the finca, but the following year the *Nectria* canker assumed alarming proportions. This fact suggests that the disease had probably existed before and was spread by the rubbings. It is now to be found in scattered spots throughout the finca.

The study of this disease has been in the direction of a definite determination of its specific causative agent and an attempt to cultivate it artificially and to clarify its relationship to the imperfect *Fusarium* stage.[2]

THE PERFECT STAGE

The morphological details of the fruiting bodies were studied from a preserved specimen of the diseased bark. To facilitate the identification of the organism, the specimen was embedded according to the method prescribed by Koneff and Lyons[3]. Sections 7.5, 10, and 15 μ in thickness were made and stained with Heidenhain's iron haematoxylin[4] and safranin.

The organism was discovered to be a species of *Nectria*, a genus which was founded in 1846 by Fries[5] and which was placed in the family Hypocreaceae by Saccardo[6]. It is a genus comprising over 500 species and is divided by Saccardo into ten sections based upon the following characteristics:

[1] An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of Master of Science.

[2] The author wishes to express her appreciation to Dr. C. W. Dodge who, in addition to suggesting and directing the problem, supplied the specimens examined throughout the course of this study and the notes of his field observations. The author also wishes to thank Dr. George T. Moore, Director, for the facilities offered by the Missouri Botanical Garden, members of the Botany department faculty, and members of the library staff, for their assistance.

[3] Koneff, A. A., and W. R. Lyons. Rapid embedding with hot low-viscosity nitrocellulose. Stain Technol. **12**:57-59. 1937.

[4] Johansen, D. A. Plant microtechnique. p. 50. 1940.

[5] Fries, E. Summa Vegetabilium Scandinaviae. p. 387. 1846.

[6] Saccardo, P. A. Sylloge fungorum **2**:479-511. 1883.

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I. Eu-Nectria—Perithecia typically smooth, caespitose, and with a stroma.
II. Dialonectria—Perithecia almost separate and smooth.
III. Hyphonectria—Perithecia smooth but seated upon a cottony subiculum.
IV. Lepidonectria—Perithecia scaly.
V. Lasionectria—Perithecia hairy.
VI. Cryphonectria—Perithecia somewhat brittle, almost immersed within a stroma.
VII. Cosmospora—Spores warted, reddish.
VIII. Phaeonectria[7]—Spores yellow-brown, slightly striate.
IX. Zimmermania[8]—Ostiole with a toothed crown.
X. Lichenonectria[9]—Parasitic upon lichens.

The species, which falls within the section Dialonectria, may be described as follows:

Nectria Dodgei Heiser, sp. nov.

Perithecia solitaria vel 2–4 aggregata, 140–270 μ lata, 160–250 μ alta, globosa vel ovoidea, laevia, aurantiaca; ostiolum non papillatum; asci ca. 50 x 5 μ; ascosporae hyalinae, uniseptatae, ellipsoideae, 7.6 x 3 μ.

Hab: in cortice *Coffeae arabicae* var. *maragogipes* vivantis in Guatemala.

Type: in Missouri Botanical Garden.

Perithecia solitary or 2–4 aggregate, 140–270 μ broad, 160–250 μ high, globose or ovoid, smooth, orange; perithecial wall pseudoparenchymatous, about 32 μ thick at the apex and 20 μ at the base, composed of 3 to 5 layers of cells; cells at the apex very nearly isodiametric, averaging 15 x 17 μ, cells at the base compressed dorsally, averaging 4 x 13 μ; ostiole not papillate, the canal lined with numerous periphyses; asci about 50 x 5 μ; ascospores hyaline, uniseptate, ellipsoid, not constricted, 7.6 x 3 μ.

Hab: On bark of living *Coffea arabica* var. *maragogipes*, in Guatemala.

SYMPTOMS

The disease which is produced by *Nectria Dodgei* usually appears under good conditions of culture and manifests itself through the following symptoms:

Trunk.—Circular or elliptic cankers occur at the base of the tree and rarely as high as two feet above the soil. Yellow-orange perithecia turning to bright red-brown with age appear on the cankers. The bark becomes blackened, and there is a partial destruction of the tissue which may extend to the cambium. However, below the uninfested areas of the trunk the bark is not blackened, and the lateral roots show no rhizomorphs. Sometimes the canker is near an old machete cut, but not enough trees were examined to know whether this is significant or not.

[7] Op. cit. 11:359. 1895.
[8] Op. cit. 17:787. 1905.
[9] Op. cit. 17:797. 1905.

Roots.—The blackening on the trunk extends down below the soil and along the tap-root to about one foot below the second laterals. Below that the bark appears normal, nearly white. The wood is normal but the cambium is discolored. The outer layers of bark on the upper laterals show cracking and scaling, with embedded black to dark brown rhizomorphs which change to white as the root becomes smaller; the small rootlets are usually unifected. The second laterals, being near the lower limits of the blackened bark, show white rhizomorphs near the trunk and little or no infection near the outer ends. Neither the third whorl of laterals nor the lower portion of the tap-root show any infection.

Leaves.—The leaves above the canker show irregular mottling at first, as in some mosaics, and then become greenish yellow. They are often spotted like *ojo de gallo,* and tend to fall prematurely.

Twigs.—There is frequently some dieback of the twigs as a more or less secondary infection.

Fruit.—The fruit is not directly affected. In fact, the last season before the death of the infected side of the tree, a very heavy crop of fruit is set. However, whether or not it matures depends upon the survival of sufficient leaf surface.

ISOLATION OF THE FUNGUS

Sabouraud's agar inoculated with the *Nectria* ascospores produced only the imperfect *Fusarium* stage. Numerous attempts were subsequently made to induce the production of the perfect stage on various types of media, but only the repeated production of the *Fusarium* resulted.

Since the *Fusarium* species, in general, show great variability in conidial septation and color reactions under different environmental conditions, the study of the cultural characteristics was restricted to cultures grown only on those types of media used by Reinking and Wollenweber[10] in their work on tropical *Fusaria.* By so doing, the results can be compared more easily with those of the above-mentioned authors, who are among the foremost workers on this difficult group of fungi. The microscopical details were studied from smear mounts (lacto phenol acid fuchsin), hanging drop cultures, and celloidin sections.

CULTURAL CHARACTERISTICS

Aerial mycelium white, usually not well developed, smoothly but distinctly warted upon certain media; microconidia[11] straight or allantoid, unicellular, rarely 1-septate, borne in false heads upon simple, occasionally branched conidiophores; macroconidia slightly sickle-shaped, somewhat pedicellate, usually borne in sporodochia; sclerotia and chlamydospores absent. The average measurements of conidia are as follows:

[10] Reinking, O. A., and H. W. Wollenweber. Philippine Jour. Sci. **32**:103-253. 1927.
[11] This term is here used to designate 1- and 2-celled conidia.

0-septate	2	x	8.5 μ
1-septate	3	x	14 μ
2-septate	3.5	x	24 μ
3-septate	3.8	x	34.8 μ
4-septate	4	x	41 μ
5-septate	4.5	x	43 μ

The septation of the conidia varies considerably with the type of medium used. For example, on oat agar from 90 to 100 per cent of the conidia are microconidia, but on *Alnus* stems, rice, and potatoes, the number of septa ranges from 0 to 5. Even on these last three, the conidia borne on the mycelium are predominantly 0- to 2-septate, whereas those borne in sporodochia are almost exclusively 3- to 5- (mostly 5-) septate. Although the septation varies greatly with the medium, the size of a particular conidial form is essentially constant; that is, unicellular conidia from Substrate A will have approximately the same dimensions as those from Substrate B even though they may be present in greatly different percentages upon the two media.

The following observations were all made of cultures one month old:

Hard potato agar.—Cultures characterized by a medium growth of white to pale pinkish buff[12] woolly mycelium, often with a dull blackish green line in the agar along the line of inoculation, and a raw sienna ring around the base. A cream buff and water green pionnotal mass may be present.

Potato-agar plate, 5 per cent dextrose.—Rather scant, woolly, loosely matted, grayish white aerial mycelium produced over the plate, the agar becoming citrine or vetiver green and sometimes grayish blue green in part.

Oat agar.—Fine cottony pinkish buff mycelium produced at the tip of the slant; the rest of the slant showing little aerial mycelium and being rather powdery in appearance. The agar appears much the same as on the potato-agar plates, and is dark Delft blue near the base.

Rice.—Fine cottony white mycelium produced on top of the medium; mycelium at bottom of the tube light grayish vineaceous. Color of the rice ranges from white through sayal, pecan, and wood brown to liver brown. Heaps of light ochraceous buff pionnotes, which become almost black with age, appear throughout the rice. No benzoic odor noticed.

Potato-tuber plug.—Cultures characterized by white to cinnamon buff felty mycelium that may be honey yellow, forest green, deep grayish olive, and dark Delft blue in patches. Sporodochia may or may not be present.

Alnus stem.—A tuft of fine white mycelium occurs at the point of inoculation, the growth being rather scarce over the remainder of the stem. A number of white and tawny sporodochia are present on the lower portion.

[12] All color terms mentioned in this section are according to: Ridgway, R. Color standards and color nomenclature. 1912.

In addition to the various media mentioned in the preceding paragraphs, a number of others were also inoculated, with the primary object of promoting the growth of the *Nectria* stage. Although in no instance was the *Nectria* produced, some supplementary observations were able to be made concerning the growth of the imperfect stage. Prune agar, coffee-dextrose agar, and potato-dextrose agar (with and without 1 per cent glycerin) all produced much more abundant mycelial growth than any of the media mentioned heretofore. Growth was rather sparse upon Sabouraud's agar and coffee agar, and practically nil upon corn-meal agar. Autoclaved coffee twigs (in a tube having one inch of glass wool covered with 1 per cent glycerin) produced a medium amount of mycelial growth and sporodochia at the base.

Upon prune agar and Sabouraud's agar the mycelium had rather rounded warts, a character not apparent on the other media.

Since several different isolations were made from the diseased coffee trees, cross-inoculations were made upon autoclaved coffee twigs, Sabouraud's, and potato-dextrose agar plus 1 per cent glycerin, in an attempt to discover possible existence of different physiological strains. Here again the results were negative.

COMPARISON WITH OTHER NECTRIAS DESCRIBED ON COFFEA

In order to show how *N. Dodgei* differs from the other species of *Nectria* described as growing on *Coffea*, a key based upon morphological differences, followed by a discussion of physiological differences, is given:[13]

I. Perithecia yellow and clothed with well-developed hairs ..... *N. luteopilosa*
II. Perithecia not clothed with well-developed hairs.
  A. Perithecia reddish brown.
    B. Perithecia caespitose or subcongested; spores sub-fusiform ..... *(N. saccharina)*
    BB. Perithecia densely gregarious; spores ellipsoid ..... *(N. coffeicola)*
  AA. Perithecia yellow, orange, red, or purple.
    B. Spores less than 10 μ long ..... *N. Dodgei*
    BB. Spores more than 10 μ long.
      C. Perithecia over 250 μ in diameter.
        D. Spores ellipsoid or fusoid, 14–15 μ long ..... *(N. anisophila)*
        DD. Spores ovoid, 8–12 μ long ..... *N. tropica*
      CC. Perithecia less than 250 μ in diameter.
        D. Spores 17–20 μ long ..... *(N. coccidophthora)*
        DD. Spores 10–13 μ long.
          E. Spores constricted.
            F. Perithecia sparse to sub-aggregate, red ..... *(N. Behnickiana)*
            FF. Perithecia densely aggregate, yellow ..... *N. fructicola*
          EE. Spores not constricted ..... *N. coffeigena*

Of the ten species listed in the key one has subsequently been transferred to another species of *Nectria*, and four have been transferred to the genus *Hypomyces*. This is readily understandable since the two genera are closely related. Both are in the Hyalodidymae section of the Hypocreaceae, the chief difference being that

[13] *Nectria coccidophthora* has been transferred to *N. coccophila*, and the other species enclosed in parentheses have been transferred to the genus *Hypomyces* by Wollenweber (in Reinking, O. A. and H. W. Wollenweber, Die Fusarien. pp. 34, 132-133, and 159. 1935).

the perithecia of *Hypomyces* are somewhat immersed while those of *Nectria* are more or less superficial.

Probably only four of the species are parasitic on *Coffea.* The remaining six are most likely only saprophytic since they were described from dead leaves, twigs, and fruit, and no indication was given in the original descriptions that they were the cause of any diseases. In addition, none of them has since been reported as the cause of any disease of *Coffea.*

Although *Hypomyces ipomeae (N. coffeicola)*[14] was found on stumps of dying coffee trees, it is doubtful if that fungus was the cause of death because: (1) no mycelium was found in the cambium; (2) the species was also reported on *Melia Azedarach* and dead fruits of *Theobroma Cacao;* and (3) wound inoculations of living trees failed to give positive results. *Nectria luteopilosa* and *N. fructicola*[15] were both found on blackened fruits of *C. liberica* in Java. *Hypomyces ipomeae (N. saccharina),*[16] *H. ipomeae* var. *major (N. Behnickiana),*[17] and *N. coccophila (N. coccidophthora)*[18] were found on dead coffee twigs, the last two being reported upon hosts among other genera also.

The chief problem lies, then, in distinguishing *Nectria Dodgei* from *N. tropica,*[19] *H. haematococcus (N. anisophila),*[20] and *N. coffeigena,*[21] all of which cause diseases of *Coffea.* In addition to the differences in morphological details indicated above, these four organisms may also be distinguished on the basis of their pathological effects upon the host.

The disease caused by *N. coffeigena* is characterized by cankers localized principally at the top of the trunk as contrasted with the basal cankers of *N. Dodgei.* The disease caused by *H. haematococcus (N. anisophila)* was reported from Costa Rica, and although some of the symptoms it produces overlap those produced by *N. Dodgei,* such as blackening of the stem, defoliation, and failure of the fruit to mature, there are several important differences. In *H. haematococcus (N. anisophila)* a large number of rootlets become blackened and partially or totally necrotic and "often the injury extends to the primary and secondary roots."[22] In *N. Dodgei* the root infection is mainly on the first and second laterals and upper portion of the tap-root, while the small rootlets usually remain uninfected. Also the blackening occurs on the basal portion of the trunk below the canker, whereas in *H. haematococcus* it is the young shoots which become blackened.

The other organism which must be distinguished from *N. Dodgei* is *N. tropica.*

[14] Zimmerman, A. Centralbl. für Bakt. II, 7:101-106. 1901.
[15] *Ibid.* 8:182. 1902.
[16] Berkeley, M. J., and M. A. Curtis. Jour. Linn. Soc. Bot. **10**:378. 1869.
[17] Hennings, P. Hedwigia **44**:172. 1905.
[18] Zimmerman, A. Centralbl. für Bakt. II, 7:872. 1901.
[19] Toro, R. A. Phytopath. **19**:969-970. 1929.
[20] Picado T., C. Jour. Dept. Agric. Puerto Rico **16**:389-400. 1932.
[21] Pascalet, M. Ann. Cryptogam. Exot. 7:21-22. 1934.
[22] Picado T., C. *op. cit.*

It was originally described as *N. coccinea* var. *tropica* by Wollenweber[23] from Brazilian coffee collections and later was given specific rank by Toro,[24] who reported it from Colombia. His basis for giving it specific rank was the "contextu radiati-fibrato peritheciorum" character which was entirely wanting in *N. coccinea.* In addition, the spores were much smaller than in *N. coccinea.* This organism causes a disease known under the common name of "llaga" (canker). Toro reports that there are two distinct sets of symptoms. The one is characterized by a dry rot on the upper surface of the roots and the base of the trunk. The bark turns black and partially disintegrates, thus exposing the wood. In the second form, which is said to be rarer, the bark often remains attached and acquires a greenish color. Sometimes it turns soft and gives off a pungent odor. In a few cases the perithecia of *N. tropica* were found on the roots and stumps of coffee trees which had died from this second form. The connection of these two sets of symptoms with the same disease was established upon the fact that in some cases of the second form "the mycelial strands of the first form were also present."[25]

According to Alvarado,[26] who reported the same disease from Guatemala, the canker is more or less extensive over the shoots and roots, and after the death of the tree the fungus forms a single gangrene covering the whole tap-root. From the fact that the fungus gains entrance to the roots through wounds, he concludes that insect wounds and careless cultural practices are predisposing factors of the disease.

Nectria Dodgei, in contrast with *N. tropica,* causes a canker of much more limited extent, and there is no such characteristic gangrene in connection with which the perithecia of *N. tropica* are produced. Furthermore, there is some evidence to indicate that *N. Dodgei* is wind-disseminated, because the infected trees seem to form a linear pattern which corresponds to the direction of the prevailing winds. At the same time, evidence shows that *N. Dodgei* is not spread through the soil, since when clods of soil adhering to an infected stem and upper lateral were carefully scraped off and examined with a hand lens no rhizomorphs were found.

Judging from the three accounts of *N. tropica,* some confusion seems to exist as to the conidial stage, thus raising the question of whether the authors were dealing with the same organism. As Wollenweber[27] described the fungus, the conidia were 5–7-septate. Toro,[28] who reported a *Fusarium* associated with his specimen of *N. tropica* (although the relationship was not proved), could very well have been dealing with the same organism. Alvarado,[29] while apparently dealing with the same organism on the basis of pathological effects upon the host,

[23] Wollenweber, H. W. Angew. Bot. 8:191. 1926.
[24] Toro, R. A. *op. cit.*
[25] *Ibid.*
[26] Alvarado, J. A. Tratado de caficultura practica. 1:319-320. 1935.
[27] Wollenweber, H. W. *op. cit.*
[28] Toro, R. A. *op. cit.*
[29] Alvarado, J. A. *op. cit.*

states quite definitely that the conidial stage is *Tubercularia vulgaris*. Since *T. vulgaris* is classed among the Amerosporae, it cannot possibly agree with the conidial stage described by Wollenweber. In spite of the fact that Toro and Alvarado may have been dealing with different organisms, either organism in question may be distinguished from *N. Dodgei* on a pathological basis. Furthermore, Toro's account, which deals with the morphological as well as the physiological aspect, shows that *N. tropica* can be distinguished from *N. Dodgei* on a morphological basis alone, by its much larger perithecium, 380–450 μ, its purple color, and its "contextu radiati-fibrato peritheciorum."[30].

CONTROL MEASURES

In April part of the coffee tree trunks at La Soledad were painted to a height of three feet with lime sulphur or a type of Bordeaux mixture. By the end of May, except in a few cases where normal perithecia were found in the crevices, which evidently had not been completely covered by the brush, the "Bordinette" was effective. It also killed most of the algae, mosses, and lichens expect a species of *Isidium* which remained healthy-looking. Lime sulphur appeared more efficient, however, in the control of both parasite and epiphyte, since it killed everything except *Isidium* and that looked quite sick.

INOCULATION OF COFFEE PLANTS

On March 11, 1944, in the Washington University greenhouse, three 3- to 5-year-old plants of *C. arabica* were inoculated with a spore suspension of a 5-day-old potato-dextrose agar culture of the *Fusarium*. Three plants of *C. excelsa* were also inoculated, and a control was kept of each species. One plant of both species was sprayed in an attempt to learn if the organisms were able to penetrate uninjured tissue; one of each was wound-inoculated near the base of the stem; and the soil in the flower pots containing the remaining two plants was inoculated to learn if the organisms could gain entrance through the roots. After a lapse of one year none of the symptoms characteristic of the disease appeared in any of the plants, but the negative results cannot be accepted as conclusive until the experiment is repeated and revised in such a manner as to eliminate the following possible causes of failure: (1) The only plants available for inoculation were not of the same variety as the ones from which the disease was described and were probably more resistant. (2) Some factor in the environment may have made the test plants more resistant to infection, although every attempt was made to simulate as closely as possible the natural environment of the coffee plants. (3) The prolonged period of culture upon artificial media may very likely have resulted in the loss of virulence of the *Nectria*. (4) The age of the coffee plants tested may also have been a factor in their increased resistance over older plants.

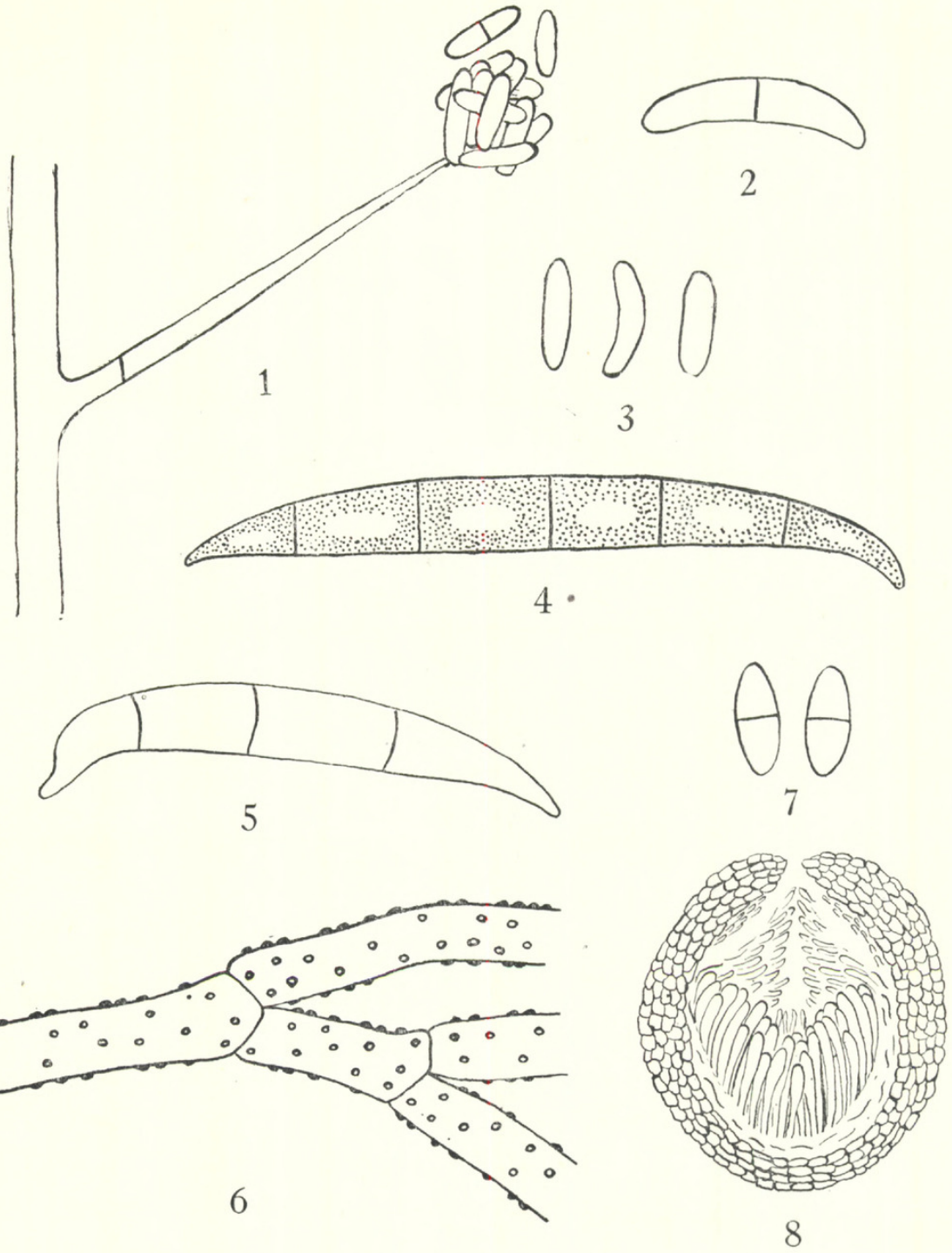
[30] Toro, R. A. Phytopath. **19**:969-970. 1929.

EXPLANATION OF PLATE

PLATE I

Nectria Dodgei

Fig. 1. Microconidia borne in a false head, from a 6-day Sabouraud's agar culture, X 755.
Fig. 2. Two-celled microconidium from a 30-day potato-plug culture, X 1620.
Fig. 3. Unicellular microconidia from a 30-day oat agar culture, X 1320.
Fig. 4. Six-celled macroconidium from a 21-day hard potato-agar culture, X 1500.
Fig. 5. Four-celled macroconidium from a 30-day potato-plug culture, X 1500.
Fig. 6. Warted mycelium from a 7-day prune-agar culture, X 1580.
Fig. 7. Ascospores, X 1500.
Fig. 8. Perithecium, X 175.



HEISER—NECTRIA DODGEI