THE PROBABLE NON-VALIDITY OF THE GENERA BOTRYODIPLODIA, DIPLODIELLA, CHAETO-DIPLODIA, AND LASIODIPLODIA

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In the course of my investigations on the diseases of the sweet potato (25) the name Java black rot was applied to a disease caused by the fungus *Lasiodiplodia tubericola* E. & E. The investigation plainly showed that the above fungus behaved very much like a Diplodia. Experiments were undertaken to prove the pathogenicity of the fungus, its relationship to the genera Diplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella.

The genus Diplodia founded by Fries (9) in 1849 shows the following characteristics: Pycnidia scattered, subcutaneous to erumpent, black, characteristically papillate at the mouth, spores one-septate, brown to dark. The genus Botryodiplodia founded by Saccardo (20) in 1880 is described as follows: Pycnidia caespitose (clustered) erumpent, and in a stroma; hairy or hairless, spores one-septate, dark. The genus Diplodiella founded by Karsten (14) in 1884 resembles Diplodia in every way except that the pycnidia are superficial instead of erumpent. The genus Chaetodiplodia was also founded by Karsten (15) in the same year; it resembles Diplodia in that the pycnidia are scattered. In this genus however they possess bristles, or hair; spores one-septate, dark. The genus Lasiodiplodia was created by Ellis and Everhart (6) in 1896 and described as follows: Pycnidia and subicle are enclosed in a hemispherical stroma. In addition there are paraphyses intermingled with the basidia of the sporules in the pycnidia; otherwise as in Diplodia.

From the above descriptions it is evident that the classification is without proper basis. To separate Diplodiella from Diplodia because the pycnidia are erumpent in the latter, while they are superficial in the former is not justified from a generic standpoint. The same is true for the other genera here mentioned. From what follows, it is evident that with the exception of the genus Diplodia, which was first described, the others are not valid.

Historical.—While working on a branch and trunk disease of cocoa, Jonge and Dorst (13) in 1909, carefully studied the causative fungus *Diplodia cacaoicola* Henn. They noticed that although the organism seemingly belonged to the genus Diplodia, under certain cultural conditions its pycnidia would be hairy and possess paraphyses intermingled with the basidia of the sporules. Different workers have actually placed this fungus under the different genera here mentioned, as will be seen later under its synonymy. Jonge and Dorst (13) further found that if the diseased specimens of cocoa were placed in moist surroundings, hair developed on the pycnidia, whereas dryness tended to suppress them. From these studies they concluded that the genera Chaetodiplodia and Lasiodiplodia are not valid.

In the same year (1909) Griffon and Maublanc (10), while working on a similar cocoa disease, came to conclusions similar to those of Jonge and Dorst (13). Griffon and Maublanc placed the fungus *Diplodia cacaoicola* Henn. in the genus Lasiodiplodia and named it *L. theobromae* (Patt.) Griff. & Maubl. Bancroft (2) (1911) in his work on a para rubber and cocoa disease, found that the causative fungus, *Diplodia cacaoicola* Henn. was so variable that it could easily be placed in any one of the genera Botryodiplodia, Macrophoma, and Lasiodiplodia. Bancroft, however, found the ascigerous stage of the fungus, which he named *Thyridaria tarda* Banc. That the presence or absence of paraphyses in pycnidia or perithecia have no taxonomic value has also been indicated by Shear and Wood (22). "Their presence or absence (paraphyses) does not seem to be sufficiently constant to be of much taxonomic value."

Present work.—As previously stated these investigations are an outgrowth of studies on *Lasiodiplodia tubericola* E.&E., the cause of a sweet potato disease. In order to determine the relationship of the genus Lasiodiplodia to the genera Diplodia, Diplodiella, Chaetodiplodia, and Botryodiplodia, the following experiments were carried out. A large number of apparently healthy sweet potato roots were divided into five lots. These were carefully washed in tap water, then disinfected by being plunged in a 5 percent formaldehyde solution for ten minutes. Each lot was then carefully dried with clean cheese cloth, placed in flat moist chambers, and kept one week in the laboratory. Any root which showed signs of decay because of internal infection was discarded; infection in such cases was chiefly soft rot, *Rhizopus nigricans* Ehr. At the end of the week ten roots were finally placed in each lot. Lot I was used as a check. Lot II was inoculated with a pure culture of *Lasiodiplodia tubericola* E. & E. The method of the inoculations here recorded was to insert bits of mycelium of pure culture into slits in the epidermis and cambium made with a flamed and cooled scalpel. Lot III was inoculated with a pure culture of *Diplodia gossypii* Zim. obtained from Dr. Edgerton; Lot IV was inoculated with a pure culture of *Diplodia natalensis* Pole Evans; and Lot V was inoculated with a pure culture of *Lasiodiplodia theobromae* (Patt.) Griff. and Maubl. (*Lasiodiplodia nigra* Appel & Laub.). Attempts to secure cultures of Diplodiella, Chaetodiplodia and Botryodiplodia failed. The results of the above inoculations were all positive. Inoculations with *Lasiodiplodia tubericola* on the sweet potato reproduced the typical Java black rot (figs. 7 and 8) within ten to twenty days. The period of incubation was from eight to nine days. The same was true for all the other fungi tried out. The gross symptoms produced differed so little from the typical Java black rot that it was impossible to tell them apart (figs. 1 and 2). Specimens of sweet potatoes inoculated with *Diplodia gossypii* and *Lasiodiplodia theobromae* were submitted to some of our leading mycologists without having been told of these inoculations, and they pronounced them similar to *Lasiodiplodia tubericola.* Sweet potatoes inoculated with *Diplodia natalensis* produced symptoms of Java black rot, but the fungus failed to form fertile pycnidia. While the gross symptoms were practically alike, a closer examination showed some differences. When the fungus *Lasiodiplodia tubericola* is inoculated into the sweet potato, the parasite emits from its pycnidia long strings of hyalin, one-celled Macrophoma spores, followed by the emission of black powdery heaps of dark one-septate spores of the Diplodia type (fig. 4). The fungus *Diplodia gossypii* on sweet potatoes did not emit the whitish strings of Macrophoma spores, but only produced black powdery heaps of spores of the Diplodia type. The same was true when *Lasiodiplodia theobromae* was used.

In shape, color, and measurements the pycnospores from *Lasiodiplodia tubericola* on sweet potato could hardly be distinguished from the pycnospores of *Diplodia gossypii*, and *L. theobromae* on the same host. It is evident that the hyalin one-celled Macrophoma spores of *Lasiodiplodia tubericola* are immature Diplodia spores. These turn brown with age and become one-septate, although they are capable of germination while young.

Dilution cultures carefully made of the Macrophoma spores produce a growth typical of *Lasiodiplodia tubericola*, and when inoculated on the sweet potato produces the typical Java black rot. Similar conclusions have been reached by Emerson (7) and several others. According to Jonge and Dorst (13), *Lasiodiplodia theobromae* on cocoa emits from its pycnidia white, strongly curled tendrils made up of hyaline one-celled spores of the Macrophoma type. As alreadys een, this same fungus on the sweet potato produces only the Diplodia type of spores. This clearly indicates the influence of the host as a factor in promoting or suppressing the Macrophoma stage.

In order to study further the relationship of the genera here under discussion, sweet potato material infected with the above fungi was fixed in a chrom-acetic solution of medium strength, then sectioned and stained with safranin and gentian violet. It is only through a pathological study of the host infected with these fungi that the relationship of the supposed established genera is brought to light. As already stated, when sweet potatoes are inoculated with the fungus *Lasiodiplodia tubericola*, pycnidia are produced with or without paraphyses (figs. 14 and 15). The same also holds true for *Diplodia gossypii* on the sweet potato, *i. e.*, there are many pycnidia with and without paraphyses.

A study of the sectioned material further reveals the fact that some of the pycnidia of *Lasiodiplodia tubericola* on the sweet potato are either borne singly or in groups (fig. 15), and seem to be embedded in a stroma (figs. 14 and 19). This also holds true for *Diplodia gossypii* on sweet potato. It will be remembered that Diplodia and Lasiodiplodia differ in that the latter possess paraphyses and the pycnidia are embedded in groups in a stroma; Diplodia has no paraphyses and the pycnidia are borne singly. This at once shows how artificial is the separation of these two genera. *Diplodia gossypii* in the sweet potato produces paraphyses (fig. 21) and the pycnidia are either borne singly or in groups (figs. 22 and 26), as is the case with *Lasiodiplodia tubericola*. Because of these facts we are justified in dispensing with the genus Lasiodiplodia. Jonge and Dorst (13) reached a similar conclusion in their work on the fungus *Lasiodiplodia theobromae* as did also Griffon and Maublanc (10).

It has already been stated that Chaetodiplodia is distinguished from Diplodia in that the neck of the pycnidium is hirsute, whereas in the latter it is without hair, a distinction which is very artificial.

Further microscopical study of our sectioned sweet potato material shows that the necks of nearly all the pycnidia of *Lasiodiplodia theobromae* have hairs (fig. 23). The same is true for *Lasiodiplodia tubericola* (figs. 18) and *Diplodia natalensis* (fig. 25). In the latter two, however, the hairiness is not so pronounced and sometimes it may be absent altogether. Similar observations were made by Griffon and Maublanc (10) and by others. From this it is evident that the genus Chaetodiplodia cannot stand and that Diplodia alone should be retained.

According to Jonge and Dorst (13) and others, the hairiness of the neck of the pycnidia is largely dependent upon cultural conditions. They state that lack of moisture tends to produce glabrous necks and much moisture has the opposite effect. My studies do not support these conclusions, since the inoculated sweet potatoes were kept in moist chambers. Under similar conditions *Lasiodiplodia theobromae* produced hirsute pycnidia and *L. tubericola* produced either glabrous or sparingly hirsute pycnidia. The determining factor in the production of hirsute or glabrous pycnidia is yet to be worked out.

Botryodiplodia was distinguished from Lasiodiplodia in the arrangement of the pycnidia. In the former they are cæspitose, in the latter they are borne in a stroma. It has been shown that the genus Lasiodiplodia is untenable. In further study of the sectioned material it is seen that the pycnidia in *L. tubericola* may be borne singly, in a stroma, or cespitose (fig. 14). The same also holds true for *Diplodia gossypii* (fig. 22). This therefore clearly indicates that the genus Botryodiplodia, too, should be dropped, its characteristics being included in the genus Diplodia.

The genus Diplodiella differs supposedly from the other genera here mentioned in that the pycnidia are superficial. This distinction does not hold. In further studying sectioned material, it is seen that *Diplodia gossypii* often produces a number of pycnidia which are distinctly superficial (fig. 22). Because of these facts the genus Diplodiella is not tenable. It is evident that the genus Diplodia under different hosts and climate may take on some or all of the characteristics of the more recent genera Chaetodiplodia, Lasiodiplodia, Botryodiplodia, and Diplodiella. The common fungus *Lasiodiplodia theobromae* has actually been placed by different workers in all of these genera except Diplodiella.

Griffon and Maublanc (10) suggest that *Diplodia tubericola* (E. &

E.) Taub. may possibly be the same as *L. theobromae.* From the above studies, *Diplodia tubericola* is seen to differ from *L. theobromae* in the following manner: *Diplodia tubericola* as already stated, produces pycnidia throughout all parts of the infected sweet potato (fig. 16). Moreover, the pycnidia while hirsute are often sparingly so. In *L. theobromae* there are few or no pycnidia in the interior of the host, and the pycnidial necks are strongly hirsute (fig. 23). *Diplodia gossypii* is also distinct from the above two species, as will be seen by comparing figures 20, 21, 22 with figures 14, 17, and 19. Supported by the above studies the genus Diplodia includes the characteristics upon which these other genera, viz.; Chaetodiplodia, Lasiodiplodia, Botryodiplodia, and Diplodiella are based, hence, following the rule of priority, they are not tenable, and all the species in these genera become species of Diplodia. Further work will probably show that the genera Rhynchodiplodia and Pellionellia may likewise be referred to the genus Diplodia. The following is a somewhat broadened description of the genus Diplodia:

Diplodia Fries.—Pycnidia black, subcutaneous to erumpent or superficial, scattered or in groups, cæspitose or in a stroma; hirsute or glabrous, paraphyses present or absent, spores hyaline, one-celled when young but one-septate, brown to dark when mature.

Summary

Inoculations with two species of Lasiodiplodia and two species of Diplodia have brought out the following facts: The genus Diplodia is very variable. The fungus *Diplodia gossypii* for instance, when inoculated on the sweet potato, will show all the characteristics of the supposed genera Lasiodiplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella. The same is also true when the Lasiodiplodias are inoculated on the sweet potato. From this it is therefore concluded that because of its priority the genus Diplodia alone should be retained, while the genera Lasiodiplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella are not tenable and their species should be placed in the genus Diplodia. It is very probable that further work will show the necessity of abolishing the genera Rhyncodiplodia and Pellioniella. It seems probable also that more work will further reduce the large number of species of Diplodia.

Slides of sectioned and stained material illustrating the above studies will be deposited at the Delaware Agricultural Experiment

Station; the Department of Botany, University of Pennsylvania; and the National Museum at Washington, D. C.

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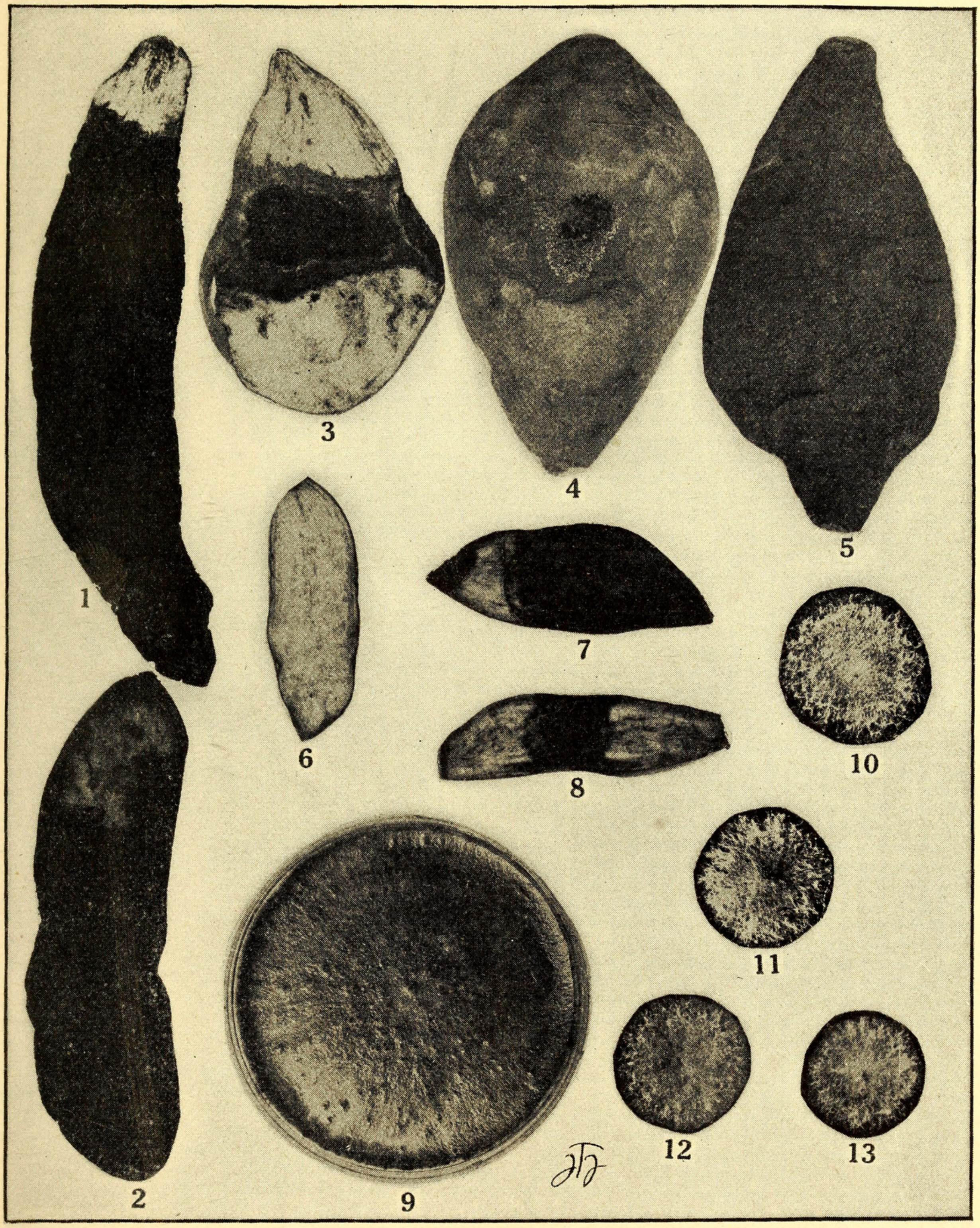

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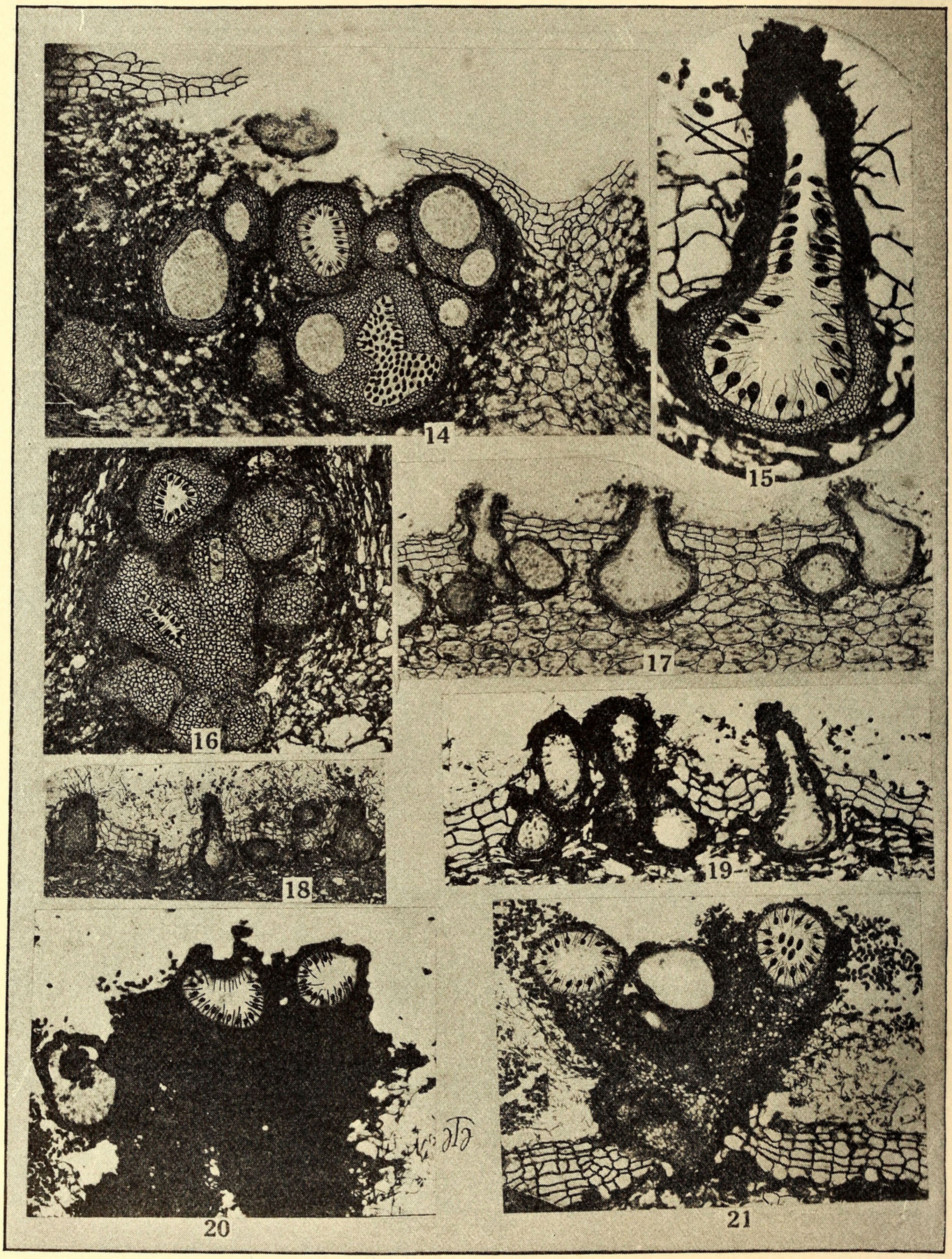

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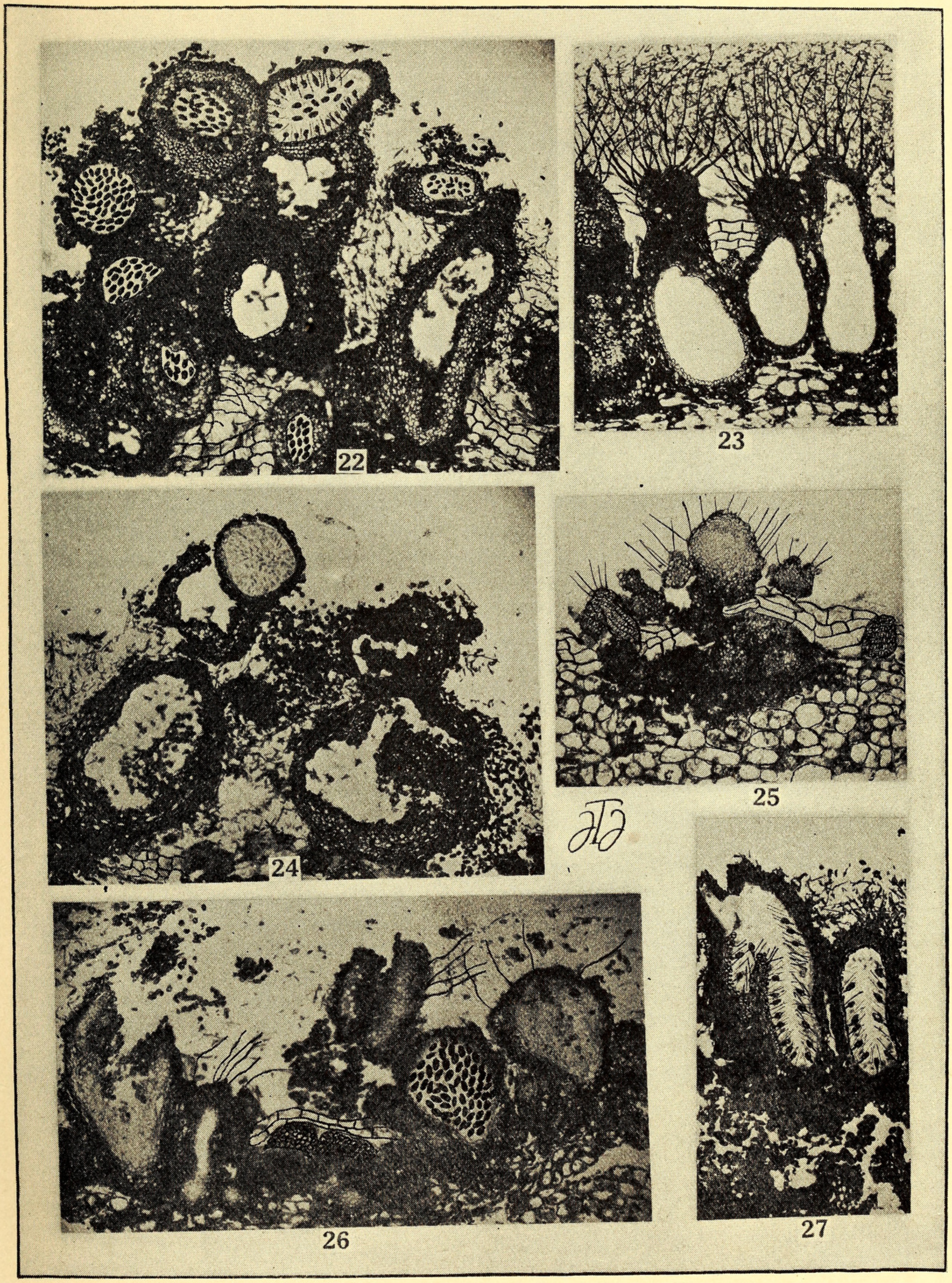

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EXPLANATION OF PLATES XII–XIV

FIG. 1. Sweet potato inoculated with Diplodia gossypii.

FIG. 2. Sweet potato inoculated with Lasiodiplodia theobromae.

FIG. 3. Cross section of sweet potato inoculated with D. tubericola.

FIG. 4. Surface view of same potato as in fig. 3.

FIG. 6. Check, healthy sweet potato.

FIGS. 7 AND 8. Sweet potatoes inoculated with L. tubericola.

FIG. 9. Pure culture of D. tubericola, showing formation of pycnidia in plate.

FIG. 10. Young culture of D. tubericola 3 days old.

FIG. 11. Young culture of D. theobromae, same age as in fig. 10.

FIG. 12. Young culture of D. natalensis, same age.

FIG. 13. Young culture of D. gossypii, same age.

FIG. 14.[2] Photomicrograph of a sweet potato section inoculated with D. tubericola showing a group of pycnidia,—with and without paraphyses.

FIG. 15. Photomicrograph of a sweet potato section inoculated with D. tubericola showing a single pycnidium with paraphyses and hair at its neck.

FIG. 16. Same material as fig. 14, showing pycnidia within the host.

FIG. 17. Same material as fig. 14, showing scattered pycnidia without hair.

FIG. 19. Same material as fig. 14, showing pycnidia single and in groups.

FIG. 18. Same material as fig. 14, showing hairy necked pycnidia.

FIGS. 20 AND 21. Photomicrograph of a section of sweet potato inoculated with Diplodia gossypii, showing pycnidia embedded in a stroma, with paraphyses in the pycnidia.

FIGS. 22 AND 24. Photomicrograph of a section of sweet potato inoculated with Diplodia gossypii, showing cespitose pycnidia borne upon the epidermis of the host as is the case with Diplodiella. In fig. 22, paraphyses are also seen in one pycnidium.

FIG. 26. Same material as fig. 22, showing sparingly hirsute pycnidia.

FIG. 27. Same material as fig. 22, showing paraphyses in the pycnidia.

FIG. 23. Photomicrograph of a section of sweet potato inoculated with L. theobromae, showing hirsute pycnidia.

FIG. 25. Photomicrograph of a section of sweet potato inoculated with Diplodia natalensis, showing hirsute sterile pycnidia.

FIGS. 14, 15, 16, 20, 21, 22, 23, 25, 26 and 27 were retouched with pen and ink in order better to show paraphyses, or hair, which the camera has failed to reproduce.

[2] Thanks are due to Dr. T. F. Manns for help rendered in making figs. 14 to 27.