On a Disease of *Tradescantia*.

BY

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With Plates IV and V.

DURING the summer of 1899 it was found that two species of *Tradescantia*, viz. *T. fluminensis* (Vell.) and *T. zebrina* (Hort.), growing in the greenhouses of the University Botanical Gardens, were suffering from an epidemic disease which showed all the symptoms of attack by a Fungus. In some of the houses the host-plants were completely killed off over considerable areas, while in others the plants, although obviously infected, nevertheless continued their growth in a more or less normal manner. It was noticed that many of the dead leaves and stems, in addition to showing dark spots and patches, were covered with long white conidiophores of a *Botryosporium* (Fig. 10), and also that dead leaves which did not show these structures speedily became covered with them when placed on damp blotting-paper under a bell jar. It appeared possible that this Fungus was the cause of the disease, and it thus became necessary to obtain pure cultures to follow out the complete

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development under the microscope by the hanging-drop method, and subsequently to attempt infection experiments on healthy host-plants.

In addition to the above symptoms, many of the still green leaves showed pale areas, as if the chlorophyll was being decolourized, and on some of these the microscope showed minute brown spots and, here and there, fungal hyphae.

Preliminary plate-cultures were made, using a food-material consisting of ten per cent. saccharose-gelatine, containing yeast-extract to supply the necessary mineral food materials. This medium was only used for one series of plates, as it was found to contain dead yeast-cells, and was consequently unsuitable for use in hanging-drops—it being difficult to determine when the drop contained a single spore of the Fungus under investigation. Subsequent cultures were made with ten per cent. saccharose-gelatine containing Klebs's solution:—

Potassium nitrate	2	grams	per	litre.
Magnesium sulphate	1	"	"	"
Potassium phosphate	2	"	"	"

which was found to be suited to the needs of the Fungus. One of the third series of plates was found to be pure, and from this hanging-drops were prepared. Some difficulty was experienced in obtaining the Fungus free from a species of *Cladosporium* or *Hormodendron*—a significant fact in view of subsequent observations—the peculiarities of which were studied later, and will be described below.

In the hanging-drops and on the plates the spores germinated in less than twenty-four hours, and in thirty hours colonies were visible with the naked eye on the first of a series of plates. In four days conidiophores were visible. The details of germination are shown in the drawings (Figs. 1-3), and those of spore-formation in Figs. 4-6.

The hyphae are septate and copiously branched, averaging $4-5\mu$ in diameter, and creeping horizontally: they throw up long conidiophores (Figs. 10, 11), each consisting of a

septate, cylindrical main axis, some 10μ or so thick and 1-3 or more mm. long, from which are put forth acropetally and at right angles, peg-like branches about 5μ thick in the middle, and 50μ or more long (Fig. 11). Each of these pedicels tapers below to its insertion in the axis, and widens above into a club-shaped head about 10μ thick. The head puts forth four or five irregularly swollen sterigmata, each about $10-12\mu$ long, whence clusters of spores (conidia) bud out, as in *Botrytis* (see Figs. 11 and 12). The conidia are ovoid or globoid, and average $4-5\mu$ in diameter. All the parts—mycelium, axis, heads, and capitula, and spores—are colourless, and glisten like silver or white floss silk in the mass, and the sum of the characters brings this Fungus into Corda's genus *Botryosporium*¹.

The fertile hyphae grow down into the air below the drop and appear in the air darker than the submerged sterile hyphae. They vary in length between wide limits, are rarely branched, and give off lateral branchlets of equal length in a racemose manner as described. Each branchlet ends in a clavate swelling, which bears three or four circular appendages, which in turn bear the conidia, the whole forming a nearly globose head of spores.

During the formation of conidia the fertile hyphae were seen to bear globular bodies resembling gas-bubbles (Figs. 4, 5), the nature of which was not determined². In many cases a head of conidia was seen to be encased in one of these 'bubbles,' and on several occasions, when such heads came in contact with the cover-slip, the 'bubble' burst, and the conidia were thrown some distance. It would thus appear as if these bodies are useful in spore-distribution. Such liberated spores were found to have a small circular body attached to one end, and it appeared probable that this structure served as an abjuncto to free the spore from the

¹ See Saccardo, *Sylloge Fungorum*, Vol. iv, p. 54, and Massee, *Brit. Fungus Flora*, Vol. iii, p. 291.

² Such gas-bubbles are very commonly found on conidiophores in the act of developing spores. The gas is probably enclosed in a slimy matrix; see Brefeld, *Unters. aus dem ges. Geb. der Mycol.*, Heft 10, p. 177 and Plate V, Fig. 42.

conidiophore. In many cases these abjunctors were seen between the conidia and conidiophores, and in such cases the conidia rapidly became free (Fig. 6).

In several instances a conidiophore fell into the film of water on the surface of the cover-slip, and at once ceased to form spores, the tip growing into an ordinary sterile hypha. Later, the tip grew down into the air, and again gave rise to branchlets and conidia, thus bearing out Klebs's observation¹ that conidia can only develop in relatively dry air, and are converted into ordinary hyphae if too wet.

After spore-formation had ceased, many of the sterile hyphae had brownish-yellow bodies, with rounded edges, attached to their surfaces (Fig. 14). These had the appearance of proteid crystals, but may have been mineral matters deposited from the concentrated and altered drop.

On the eleventh day it was found that the hyphae in the hanging-drops commenced to knot up in a remarkable manner, and on the twelfth day it was found that the hyphal contents commenced to aggregate in certain parts of the mycelium, leaving the rest empty (Fig. 7). This went on until finally thick-walled circular chlamydospore-like bodies containing granular protoplasm were found which afterwards showed budding (Fig. 8).

Cultures were next made with a food-material consisting of raisin-extract and gelatine. Vegetative growth was much more rapid and luxuriant than before, while the formation of conidiophores was delayed, especially on plate-cultures. After spore-formation had ceased chlamydospores were formed in a similar manner to that noted above (Figs. 8 and 9).

The Fungus was then grown by infecting sterilized blocks of the sap-wood of oak with spores from a pure culture. In the first instance an abundant feathery white mycelium was formed, and in seven days long white conidiophores made their appearance, and these speedily covered the wood.

The Fungus proved to be a species of *Botryosporium*, as

¹ Klebs, Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen, p. 452.

already stated, and the specific characters were found to agree most nearly with those of *B. diffusum*, Corda, occurring in rotten wood, branches, leaves, &c.¹

It now became necessary to try infection experiments with healthy host-plants in order to test the parasitic nature or otherwise of the Fungus.

Preliminary experiments were made by placing a colony of the Fungus, with attached food-material, from a pure plate-culture, on both the upper and under surfaces of the leaves of apparently healthy host-plants growing in the greenhouses, and also out of doors. No infection took place, the leaves being kept under observation for fourteen days.

Next, a large quantity of infected *Tradescantia* material was collected, ground up with sand, and the extract filtered through a Pasteur filter. The filtered extract was then collected in minute capillary tubes, sterilized when drawn, the fine sealed ends of which were then broken as they were pushed into the tissues of the plants. Healthy plants infected with this extract did not show any ill effects, although as many as six capillary tubes were placed in each.

Afterwards infection experiments were made on a large scale, using healthy leaves to which a portion of the internode was attached, floating on water. Growing hyphae, germinating spores, or spores direct from the conidiophores, were sown on the upper and under surfaces of the leaves of both species of host-plants, the epidermis being left in the normal condition in some, or pricked with a sterilized needle in others. A similar set of experiments was made, in which a drop of food material was used in addition², and in all cases blank experiments were performed to serve as a control. Only in rare instances did infection take place, and in all such cases the *entering hyphae were found to be olive-brown coloured and of a different character from the normal mycelium of the Botryosporium*. On the whole, the infection experiments demonstrated the non-parasitic nature of the Fungus

¹ See Massee, Brit. Fung. Fl., Vol. iii, p. 291.

² See Marshall Ward, A Lily Disease, Ann. Bot., Vol. ii, p. 355.

investigated, especially when it was borne in mind that plants from all the greenhouses, and also from out of doors, were used.

It was found in the case of the naturally-growing host-plants that infection started either on the upper surface of the leaf as a small semi-transparent dot or from the margin. Tangential sections of the upper epidermis containing one of these areas, and also of the infected margin, were made with a sterilized razor, and were placed in hanging-drops, with and without food-material. In all cases, the hyphae seen on the epidermis gave rise to the same species of *Cladosporium* as that mentioned above (p. 28) occurring as a weed in the early *Botryosporium* cultures (Figs. 15 and 16). From one of these drops pure plate-cultures were obtained, and the complete development was followed from a single spore in hanging-drop cultures under the microscope, as shown in Figs. 18–22.

After spore-formation the olive-brown septate mycelium appeared to be exhausted, no chlamydospore-formation being noted, as in the case of the *Botryosporium*, but any joint of the dark olive mycelium may serve as a chlamydospore-like body, and reproduce the Fungus vegetatively. The spores were liberated by a spherical abjunctor-like body similar to that noted in the case of the *Botryosporium*.

The *Cladosporium* was next grown on sterilized oak sapwood, by infection with spores from a pure culture. Dark olive patches were produced, which gradually spread over the substratum.

The spores were found to be ellipsoid in shape, simple, olive-coloured, 4 to 6 μ long and 2 to 3 μ broad. The hyphae varied in breadth from 4 to 6 μ .

As seen growing on the epidermis, or after some days in culture, the mycelium is cut up into short segments, the older of which are thick-walled and dark olive-brown in colour. These segments may be barrel-shaped or globoid, and from 5 or 6 to 10 or 12 μ in diameter or more. They tail off into the thinner colourless hyphae with segments

3 to 4μ thick and 16 to 18μ long (Fig. 15). Any of the older segments, which have oily-looking drops in them, may serve as new centres of germination, acting like chlamydo-spores, as already stated.

The conidiophores are borne in immense numbers on the well-nourished mycelium, and present an extraordinary resemblance to *Penicillium* at first sight, a feature quite in accord with the earlier name of at least one member of the genus¹. Each conidiospore consists of a pedicel of a few segments, which apparently branches in a pseudo-dichotomous manner, and bears the conidia at the tips of the ultimate branches. The totally different mode of origin from those of *Penicillium* is shown in Fig. 21.

Infection experiments were now performed on healthy host-plants and on leaves floating on water. In the first instance, a drop of food-material containing spores from a pure culture was placed on the upper surface of floating leaves. A control experiment, when no spores were used, was made at the same time. In three days all the leaves on which spores had been placed showed infection through the cuticle. No infection took place in the control leaves. Next, food-material and spores were placed on the upper and under sides of the leaves of growing host-plants, the upper epidermis being pricked with a sterile needle in one set of experiments. A control plant was used in each case. In a week all the leaves showed infection where spores had been sown, while the control plants, with the exception of one leaf, showed only negative results.

Stomatal infection was rare, the hyphae preferring to penetrate the middle lamella or even a guard cell. The points of infection were easily apparent by the dark-brown discolouration of the epidermis. These experiments were repeated on floating leaves, the result confirming the above.

Lastly, a series of experiments were made on floating leaves where spores were sown on the wounds formed by

¹ See Loew's description of *Penicillium Cladosporioides* (now *Hormodendron*, Sacc.) in Pringsh. Jahrb., Bd. vii, p. 494.

pricking the upper epidermis. No spores were sown on the control leaves. In three days infection took place, the spores sending out hyphae which passed down into the leaf. (See Fig. 23.)

As regards the systematic position of this Fungus, we are again—since no higher form of fructification could be obtained—driven to regard it as one of the Hyphomycetes or *Fungi Imperfecti*; and of the four great groups into which these are divided, the present form clearly comes into the second one, viz. the *Dematieae*, characterized by the dark-coloured hyphae and conidia, and the absence of any fasciculate stipes or ‘sporodochium’¹.

Now appears a difficulty, however. If we follow the classification logically, the present Fungus falls naturally into section I, *Amerosporeae*, because the elliptical or oblong conidia are continuous, i. e. non-septate; but it is evident on comparing it with *Cladosporium* (which Saccardo puts into section II, the *Didymosporeae* with typically uni-septate spores) that our Fungus comes into that or a closely allied genus.

Adhering to the classificatory scheme of the Sylloge, however, we place it in the second subsection (*Macronemeae*) of the *Amerosporeae*, and so get to the genus *Hormodendron*.

A good deal of light is thrown on the matter by a paper published by Schostakowitsch in 1895 on the conditions of conidia-formation in the Sooty Moulds², where he shows pretty clearly that *Hormodendron cladosporioides*, Sacc., is practically *Cladosporium*, although the author himself attempts to uphold the individuality of both. This seems hardly possible in view of his own admission, on p. 369, that on agar ‘*Cladosporium* bildet hier eine Form, die man sehr schwer von *Hormodendron* unterscheiden kann,’ and the comparison of his own figures on pp. 366 and 368 points also to the practical impossibility of maintaining the autonomy of the two genera by characters of any utility.

¹ See Saccardo, Sylloge Fungorum, Vol. iv.

² Ueber die Bedingungen der Conidienbildung bei Russthaupilzen, Flora, Bd. lxxxi, 1895, p. 362.

Nevertheless, other writers have insisted also on the autonomy of the genus *Hormodendron*, of whom Bruhne¹ is especially worth noting, since the form he describes is parasitic on Barley with symptoms almost identical with those of the present disease.

Laurent² and Costantin³ both maintain that *Hormodendron* and *Cladosporium* are merely forms of one and the same genus; Janczewsky⁴, on the other hand, failed to obtain *Hormodendron* from *Cladosporium* or vice versa.

The whole controversy—in so far as it concerns these two genera—really seems to turn on one or two trivial points of difference, as already indicated, and of which the following are further illustrations.

Schostakowitsch (l. c. p. 367) says the spores of *Hormodendron* have smooth surfaces, those of *Cladosporium* being warted: nevertheless Bruhne figures the conidia of his *Hormodendron* (l. c., Plate I, Fig. 2) with warts, and expressly states (l. c. pp. 5 and 6) that they are *smooth or warted* according to the different conditions of culture.

Again, Schostakowitsch (l. c., p. 370) says that *Cladosporium* ‘besitzt die merkwürdige Eigenschaft, bei 0–2° C. seinen vollen Entwicklungsgang zu durchlaufen . . . Die Hormodendronsporen vermögen unter solchen Bedingungen nur kurze Keimschläuche zu bilden.’ Nevertheless Bruhne (l. c., p. 31) shows that cultures of *Hormodendron* produced spores abundantly at temperatures ranging from 5.5° at mid-day to ‘einige Grade unter 0° C.’ at night, and the vessels were frozen.

It is obvious, therefore, that much remains to be done before we can regard this question as settled. Meanwhile, it is interesting to note that the course of events in the life-history of this Fungus on *Tradescantia*, and in the parasitism it displays, are extremely similar to those above referred to.

¹ *Hormodendron Hordei*, Zopf's Beitr. zur Phys. u. Morph. niederer Organismen, Heft 4, 1894, p. 1.

² Recherches sur le polymorphisme du *Cladosporium herbarum*, Ann. de l'Inst. Pasteur, Vol. ii, 1888.

³ Sur les variations des *Alternaria* et des *Cladosporium*, Rev. gén. de Bot., 1889.

⁴ Zur Entwicklungsgeschichte von *Penicillium*, Pringsh. Jahrb., Bd. vii.

Cladosporium is by no means unknown as a parasite, although its easy culture as a pronounced saprophyte suggests that special conditions either of the host or the Fungus are necessary before it can attack living plants with such energy as to cause an epidemic.

Lopriore¹ has described an epidemic on Wheat caused by *C. herbarum*, and collected the literature to date.

Frank² quotes yet other cases where various Monocotyledons, fruit trees, olives, tomatoes, cucumbers, &c., are injured by other species of *Cladosporium*, and also brings the literature up to date, showing at the same time what enormous confusion exists as to the connexion between this and other genera.

The difficulties of confusion are increased when we find that Brefeld³ describes as the conidial form of *Microsphaerella* a Fungus of essentially the same type as that under discussion, and it is evident that nothing final can be done before we trace the more perfect stages.

Brefeld refers to this type as the *Ramularia* form, and quotes Tulasne's Fig. 7, Table XXXI, in the *Carpologia*, which certainly bears out his comparison. The doubt arises, however, whether Tulasne did not draw more than one Fungus in his figure. Tulasne⁴ also refers this type to *Pleospora*.

Hence it is evident that a long research will be necessary to clear up the numerous difficulties accumulated around the Fungus *Cladosporium* and its allies.

In conclusion, I wish to thank Professor Marshall Ward, under whose constant supervision this work has been carried out, for much kind assistance and advice.

CAMBRIDGE BOTANICAL LABORATORY,
September 4, 1899.

¹ Die Schwärze des Getreides, Ber. d. deutsch. bot. Ges., 1892, Bd. x, p. 72.

² Die Krankheiten der Pflanzen, 2nd ed., 1896, p. 315, &c.

³ Unters. aus dem ges. Gebiete, &c., Heft 8, p. 213, and Plate VI, Figs. 38-42.

⁴ *Carpologia*, Vol. ii, Plate XXXII, Figs. 13 and 14.

EXPLANATION OF FIGURES IN PLATES IV AND V.

Illustrating Mr. Howard's paper on a Disease of *Tradescantia*.

Fig. 1. Three spores of *Botryosporium* germinating in a hanging-drop of nutrient gelatine. The sowing was made July 29 at 1 p.m., temp. = $21-22^{\circ}\text{C}$. throughout. a = at 12.15 a.m. July 30; b = 12.35 p.m. July 30; c = 12.25 p.m. July 30.

Fig. 2. The same a few hours later. a' = 2.30 p.m.; b' = 3.15 p.m.; c' = 3.25 p.m. July 30.

Fig. 3. The same later still. a'' = 6.40 p.m.; b'' = 7.45 p.m.; c'' = 7 p.m. July 30.

Fig. 4. Three stages in the development of the conidiophore and conidia from a mycelium six days old. a = Aug. 4, 10 a.m.; b = 11.45 a.m.; c = 2.30 p.m. same day. The $\times$ is in a gas bubble which remained throughout. $t = 22^{\circ}\text{C}$. Zeiss BB.

Fig. 5. Similar stages of an older conidiophore under $\frac{1}{6}$, showing the development of the conidia. a = 11 a.m.; b = 12.40 p.m.; c = 6.20 p.m.

Fig. 6. Two heads of the conidiophore, showing the abjunction of the spores, each of which has a minute drop-like body at its proximal end—the 'disjunctors'?

Fig. 7. Stages in the formation of chlamydospore-like segments. a , A piece of the mycelium of a hanging-drop culture several days old, on Aug. 10 at 10.15 a.m., showing the accumulation of the protoplasm in certain segments, leaving others empty. The segments marked x are the same throughout the series. b , The same on Aug. 11 at 10.45 a.m.; c , at 10.40 a.m. next day; d , at 11.15 a.m. Aug. 13; e , at 8.50 p.m. same day; f , at 11.30 a.m. Aug. 14. The parts with firmer walls and filled with protoplasm now pass over to a resting condition, the intermediate empty portions shrivelling up.

Fig. 8. Chlamydospore formation in a coiled piece of mycelium several days old. a , on Aug. 10 at 10 a.m.; b , twenty-four hours later; c , forty-eight hours later still. The lower part of the coil (at $\times$) is drawn separately. d , twenty-four hours later than c . In addition to forming the chlamydospore-like gemmae, some of the segments in c and d are putting forth bud-like processes.

Fig. 9. Gemmation or budding of old mycelium. a , A piece drawn at 10 a.m. Aug. 21; b , the part marked $\times$ a week later; c , the same Aug. 31, showing slow budding.

Fig. 10. Piece of the Fungus (*Botryosporium*) under low power to show habit.

Fig. 11. Portion of a conidiophore, showing septa, spicules, &c., under $\frac{1}{6}$.

Fig. 12. Terminal portion of spicules and spores under higher power.

Fig. 13. Portion of conidiophore under still higher power, showing septum with pit.

Fig. 14. Portion of mycelium, showing crystalline accretions, or possibly excretions of apparently proteid nature. $\frac{1}{6}$.

Fig. 15. Portion of a mycelium as found growing on the epidermis under $\frac{1}{4}$.

Fig. 16. Low power view of *Cladosporium* (*Hormodendron* form) with conidiophores to show habit.

Fig. 17. One of the conidiophores of last, more highly magnified and showing apparent dichotomous branching.

Fig. 18. Germination of spore of *Cladosporium* in a hanging-drop (made Aug. 25 at 9 p.m.) at 10.30 p.m. Aug. 26. $t = 22.5^{\circ}\text{C.}$ $\frac{1}{8}$.

Fig. 19. The same next day, Aug. 27, at 11.30 a.m. $t = 22.5^{\circ}\text{C.}$ $\frac{1}{8}$.

Fig. 20. The same at 3.30 p.m. under low power. At 6.15 p.m. aerial hyphae were emerging from the drop to form conidiophores.

Fig. 21. Stages in the development of one of the above conidiophores. a = at 6.15 p.m. Aug. 28, $t = 23^{\circ}\text{C.}$; b = at 10.30 p.m., $t = 22^{\circ}\text{C.}$, several minute gas-bubbles have appeared on the elongated hyphae; c = at 12 p.m., $t = 22^{\circ}\text{C.}$, the tip has grown forwards and been cut off by a septum; d = at 12.20 a.m., $t = 21^{\circ}\text{C.}$, the terminal segment is budding out another segment at its apex; e = 12.35 a.m., $t = 21^{\circ}\text{C.}$, another bud appears below the first septum ($\times$).

This point ($\times$) is marked as a fixed point throughout. f , the last-named bud is now (12.55 a.m., $t = 22^{\circ}\text{C.}$) budding at its apex, and minute gas-bubbles appear at the surface of the older buds; g = at 1.10 a.m., $t = 23.5^{\circ}\text{C.}$ [N.B. The rise in temperature was due to the heat radiated from the lamp.] h = at 9.30 a.m., $t = 21.5^{\circ}\text{C.}$

The conidiophore is now complete, and any segment acts as a spore. At the * is another branch, the fate of which was further followed. i = this branch at 9.45 a.m., $t = 21.5$. The large gas-bubble at the right hand grows larger. k = at 3.15 p.m., $t = 21.5$. All under $\frac{1}{8}$.

Fig. 22. Stages in growth of a branch from a mycelium twenty-four hours old in hanging-drop. a , at 9.30 a.m., Aug. 27, $t = 21^{\circ}\text{C.}$; b , at noon same day, $t = 22.5$. The vacuolation, beginning in a , is now more pronounced, and several septa have formed. c = at 2.45 p.m., $t = 22.5^{\circ}\text{C.}$; d , the distal moiety of same at 4 p.m., $t = 23$; e , at 5.45 p.m., $t = 22.5$. Next day the walls begin to thicken and turn brown, and in a day or two the short swollen segments (as in Fig. 13) predominate. $\frac{1}{8}$.

Fig. 23. Portion of epidermis, seen from within, showing penetration of infecting hypha. The part in paler outline is the germinating spore on the outside: the portions inside are running in the middle lamella.

Howard, Albert. 1900. "On a disease of Tradescantia." *Annals of botany* 14, 27–38. <https://doi.org/10.1093/oxfordjournals.aob.a088770>.

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