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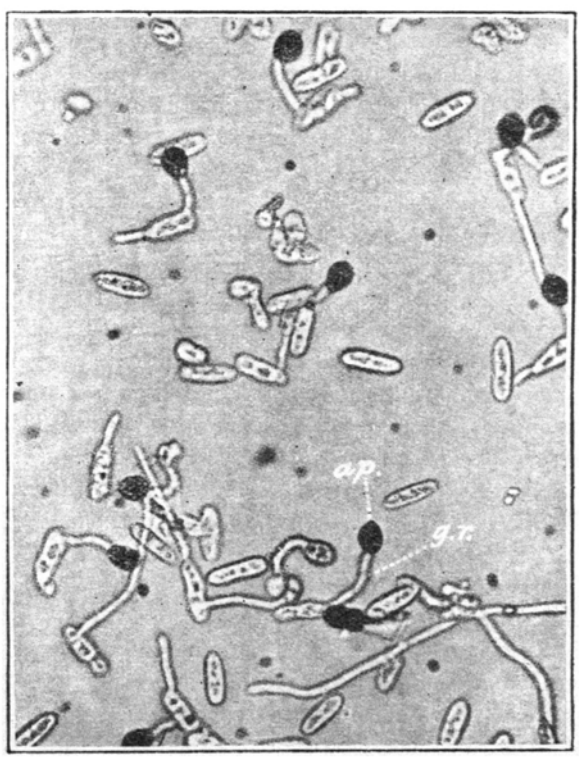
INFECTION BY *COLLETOTRICHUM GLŒOSPORIOIDES*, PENZ.

By H. CHAUDHURI, *Botany Department, Panjab University, Lahore.*

(*Read January 8, 1935*)

The method of infection by *Colletotrichum glœosporioides*, Penz. spores has been studied on leaves of malta (*Citrus sinensis* Grove).

A thick suspension of spores was made in sterilized water in a test tube from acervuli formed on a maize-agar slant which were just bursting and giving out pinkish masses of spores. The suspension was milky white. Small drops from this suspension were placed on young leaves of malta (*Citrus sinensis* Grove) which were first washed with a solution of mercuric chloride (1 in 1,000), and then in several changes of distilled water. The leaves were



TEXT FIG. I.

Photomicrograph of a hanging drop containing a large number of germinating spores showing the formation of appressoria or rather chlamydospore-like structures.

placed over a pad of moist cotton wool in glass basins with lids and incubated at 25°C.

Small bits of the leaves under the infection drops were cut out every five hours and fixed, some in Formol acetic alcohol and others in Carnoy's fluid. The former fixative proved more suitable.

Paraffin blocks were made and sections 6μ thick were cut. These were stained in Heidenhain's iron-alum hæmatoxylin and orange G in clove oil. Though the spores usually germinate in 6–8 hours' time, nothing could be seen in sections of these leaves till the appressoria were formed which fixed the germinating spores to the leaf-surface. Otherwise they are all washed away in the process of fixation and washing. The germ-tubes swell up at the tips producing a thick-walled dark adhesive organ called appressorium. The nature of the appressorium has been discussed in a previous paper by Chaudhuri and Gopal Singh (1930), and it was suggested that the appressoria should be looked upon as chlamydospore-like structures. The figure reproduced on p. 71 is a micro-photograph of appressoria (*ap.*) produced by the germinating spores on a glass surface. When a large number of spores are crowded together, these, on germinating, produce appressoria quickly when in contact with a hard surface. Hasselbring (1906) found that the appressoria of *Glœosporium fructigenum* germinated after prolonged drying and the present author has found the same thing with *Colletotrichum glœosporioides*. But Dey (1933) mentions that the appressoria of *C. glœosporioides* are unable to withstand drying. He has sketched appressoria which collapsed—their walls falling into folds when allowed to dry. A cursory examination of the photomicrograph (text fig. 1) will show, however, that the appressoria are very thick-walled structures and are very regular in shape and size. In Dey's figures (1933) they are represented as very irregular in shape and size and not so thick-walled, and it is, therefore, doubtful whether the latter structures are really appressoria. The present author found that the appressoria are almost spherical in shape and they were chocolate-brown to dark black in colour. These appressoria were seen in sections fixed after 20–25 hours. The germ-tube (*g.r.*), as it grows out in a drop of water, sooner or later touches the surface of the leaf and produces the adhesive organ (Plate I, figs. 1, 2). In some cases the germ-tube may be seen branching before touching the surface of the leaf, and in these cases, instead of a single appressorium, two appressoria may be formed (Plate I, fig. 3). Sections from materials fixed after 35 to 45 hours show the germination of the appressorium. Dey (1919), studying the penetration in *Colletotrichum lindemuthianum*, found that a peg-like infection hypha was given out from the adpressed surface of the appressorium which penetrated the cuticle by mechanical pressure as found by Blackman and Welsford (1916) in *Botrytis cinerea*. Recently Dey (1933) has studied it in *Colletotrichum glœosporioides* isolated from anthracnose spots on the leaves of *Citrus medica acida*, and has found it to be similar to *C. lindemuthianum*. In the last paper Dey has also figured one very fine peg-like infection hypha produced from the adpressed

side of the appressorium. Though a very large number of properly stained microtomic slides have been examined, in no cases, however, have such fine pegs as sketched by Dey been observed. The infection hypha produced from the adpressed surface is a regular germ-tube-like structure (Plate I, figs. 4–14). It may be a narrow one (Plate I, fig. 10), but never anything like the fine peg as mentioned by Dey. The infection hypha, as it enters the epidermal cell, may swell and branch (Plate I, fig. 8), or more usually it grows on till it passes through the epidermal layer into the cells below (Plate I, figs. 12–14). When it reaches the sub-epidermal layer it begins to branch profusely (Plate I, fig. 14); here it was found that a vesicle-like structure is formed before branching. It was noticed in almost all cases that the spots with infection drops get injected with water before the infection tubes penetrate. To determine whether it was due to some sort of excretions given out by the germinating spores, which made the cuticle and the plasma membrane permeable as a result of which they get injected with water, the following experiment was done. A thick spore-suspension was made in distilled water (p_H—7·0) in a hard glass test tube which was placed in an incubator at 25°C. After 24 hours this spore-suspension was filtered through double filter papers. The p_H was found to have become considerably alkaline (8·4). (A water control in a similar hard glass test tube was incubated at 25°C., but no change in p_H value took place.) Drops of filtered liquid containing no spores were placed on the upper surface of malta leaves which had been properly washed and dried by rubbing with absorbent cotton wool. Drops of water were also placed on the leaves as control. The leaves were placed inside large glass basins with lids containing soaked cotton pads. In about 30–40 hours the spots with the filtered liquid, in which the spores had germinated, were noticed to have become somewhat transluscent apparently due to injection. The spots with plain drops of water showed no injection or any change. The leaves were now removed and washed thoroughly and dried by rubbing with dry absorbent cotton wool. Now the upper surface of the leaf was flooded with alcoholic solution of erythrosine[1] for about 2 minutes, after which the leaves were thoroughly washed again. The spots, where the filtered drops were previously placed and showed injections, were found to have absorbed the red dye. Spots with plain water drops did not absorb any dye. This shows conclusively that when a thick suspension of *Colletotrichum glœosporioides* spore is placed on the leaf, the excretions given out by the germinating spores react on the cuticle and the plasma membrane and the cells below get injected. When infection drops are placed and the spots become injected, the infection tubes from the appressoria easily penetrate through the cuticle

[1] ·1% solution of erythrosine in 10% alcohol.

Alcoholic solution was used because it is absorbed very quickly by the injected spots. A ·1% solution in water may also be used, but it has to be kept for 20 minutes or more before it is fully absorbed.

and enter the epidermal and sub-epidermal layers. It is very likely that the nutrient substances, which diffuse out from the injected cells, stimulate the appressoria to germinate.

Before concluding it may be mentioned that one often finds very thick suspension of spores—almost slimy masses, trickling down the twigs and leaves of infected plants when the acervuli burst under very moist conditions. Hence, placing of thick spore-suspensions in the above experiments was not anything abnormal. It is just what happens normally in nature.

Summary

The germination of spores of *Colletotrichum glœosporioides*, Penz. isolated from malta (*Citrus sinensis*, Grove) twigs showing 'Wither-tip' has been studied and its mode of penetration when placed as infection drops on malta leaves has been followed.

On the hard upper surface of the leaves, the spores on germination produce appressoria-like structures which stick fast to the surface of the leaves. These appressoria are very regular in shape and size and are able to withstand desiccation. When the spores germinate they give out certain products which react on the cuticle and the plasma membrane and the cells below get injected before actual penetration takes place. The stimulus to germination of the appressoria might be supplied by nutrient substances diffusing out when cells get injected. Injection has been produced by placing filtered drops in which spores were germinating for 30–40 hours and when later the leaf was flooded with a dilute solution of erythrosine, the dye was absorbed by the injected spots only.

No fine peg-like infection hyphæ have been noticed, but instead ordinary germ-tube-like structures have been seen to penetrate.

In conclusion, the author expresses his sincere thanks to Professors V. H. Blackman and W. Brown for valuable criticism and suggestions.

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EXPLANATION OF PLATE I.

Figs. 1, 2. Showing appressoria produced by the tips of the germ tubes when in contact with the surface of the leaf.

Fig. 3. Showing the branching of a germ-tube and production of two appressoria.

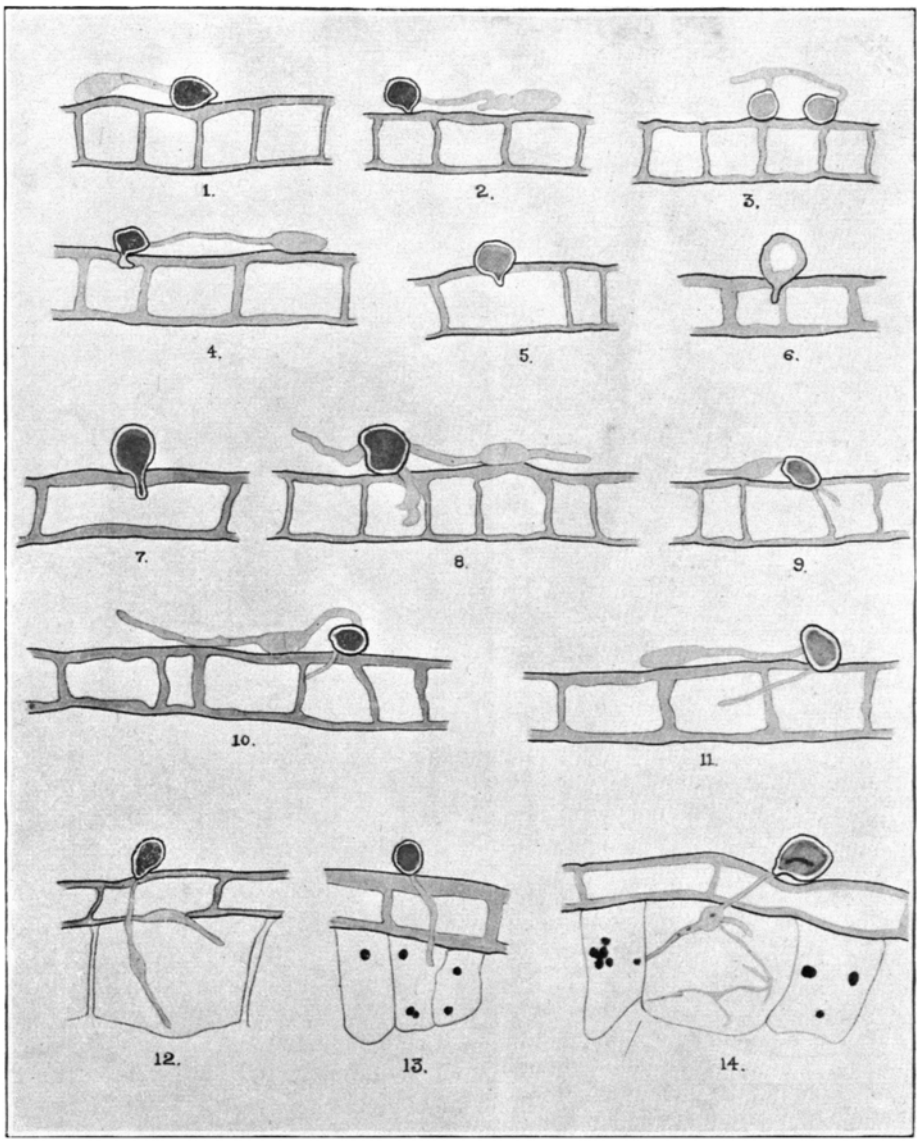
Figs. 4–7. Showing actual penetration by the germ-tube-like infection hyphæ.

Fig. 8. Showing swelling and branching of infection hypha on entering the epidermal cell.

Figs. 9–14. Showing progress of the infection hyphæ.

In Figs. 12, 14 the branching takes place in the sub-epidermal cells. Note the vesicle-like structure in Fig. 14 produced by the infection hypha before branching.

N.B.—All magnification 1,300.



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