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THE CONNECTION BETWEEN DEMATOPHORA NECATRIX AND ROSELLINIA NECATRIX[1, 2]

H. N. HANSEN,[3] HAROLD E. THOMAS,[4] AND H. EARL THOMAS[5]

SINCE THE FIRST THOROUGH STUDY of *Dematophora necatrix* (Hart.) by Hartig,[(1)6] who suggested its relationship to the genus *Rosellinia,* there has been a reasonable doubt as to the reality of that relationship in spite of the finding of an associated *Rosellinia* stage by Viala[(6)] and later by Prillieux.[(4)] The reason for this doubt is made clear by Viala, who says:

"Nous avons essayé, par tous les precedes, d'obtinir la germination de ces sporidies sans jamais pouvoir y parvinir. La démonstration expérimentale de la relation des péritheces et des autres formes du *D. necatrix* manque donc."[(6)] (p. 82.)

PRODUCTION OF PERITHECIA

Recently,[(5)] we reported on the occurrence of a highly destructive fungus on apple roots in California that so precisely resembled *Dematophora necatrix,* according to Hartig's description, that we did not hesitate to name it such. From time to time since late 1933, we have collected roots from apple trees killed by this fungus and kept such material in containers under various environmental conditions in an effort to produce the ascigerous stage reported by previous workers. Late in 1935, almost two years to a day after collecting the first material, mature perithecia were observed on four pieces of root that had been kept in moist cham-

[1] Received for publication December 16, 1936.

[2] We wish to express our appreciation to Mr. S. F. Ashby, Director of the Imperial Mycological Institute, and to Mr. E. W. Mason, of the same institution, for sending specimens of *Rosellinia arcuata* and *R. buxi,* and for other courtesies rendered.

[3] Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

[4] Assistant Plant Pathologist in the Experiment Station.

[5] Associate Plant Pathologist in the Experiment Station.

[6] Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

bers in the laboratory. In appearance, these perithecia (plate 1, *A, B, E*) are typical of the genus *Rosellinia* and agree closely with the descriptions of those studied by Viala[6] and by Prillieux.[4]

These investigators describe the perithecia as lacking ostioles, whereas in our fungus a definite round pore, or ostiole, is found at the apex of the papilla. The pore is not discernible in old perithecia that have ceased to discharge spores; for in such, the opening is filled with the dried gelatinous material in which the spores are exuded. The structure is readily demonstrated in young perithecia. The ostiole is illustrated by Hartig[2] in *R. quercina,* and we have observed it in *R. aquila* (Fr.) De Not., *R. linderae* Pk., *R. arcuata* Petch., and *R. buxi* H. Fabre.

In spore measurements, also, there is a slight discrepancy: Viala[6] gives the mean size of ascospores as $40 \times 7\mu$, Prillieux the range 43 to $47.5 \times 7\mu$, whereas our measurements, based on 300 spores are: range 31.1 to 47.6×5.1 to 7.1μ, average $37.1 \times 6.3\mu$. We do not, however, consider these differences to be significant. From general observations on size ranges in spores of other species of *Rosellinia,* and for that matter in spores of most fungi, Prillieux's range seems unduly small, which suggests that it was probably based on the measurement of very few spores.

In general, the ascospores are as described by Viala,[6] including the hyaline epispore. This is readily seen in unstained material and very noticeable during early stages of germination, when it often becomes greatly distended just before the germ tubes break through (plate 1, *C*). The spore is typically dorsi-ventral; and in the middle of the ventral side, a slit or suture is seen running parallel to the long axis of the spore and about one-third its length (plate 1, *D*). This slit is not mentioned in the literature as occurring in *Rosellinia necatrix* (Hart.) Berl. nor does Massee[3] mention its presence in *R. radiciperda* Mass. Hartig[2] shows it in *R. quercina* Hart., and we have observed it in the five species of *Rosellinia* examined by us.

GERMINATION OF SPORES, AND PATHOGENICITY

The abundant production of conidia in *Rosellinia necatrix* would lead one to suspect this spore form to be the principal agent of dispersal. Viala[6] reports ready germination of conidia, whereas Hartig[1] made many attempts but succeeded in only one; and in that case the culture was lost before it could be adequately studied. Though we used a large number of media and treated the conidia in various ways to stimulate germination, all our efforts resulted negatively.

At first we had difficulty with the ascospores also, but germination was finally obtained by the following method: Spores were suspended in 2 cc

of 5 per cent lactic acid. After standing for 15 minutes, 10 cc of water was added to reduce the concentration of acid, and the mixture was then poured over the surface of hard potato-dextrose agar (3 per cent agar) in petri dishes at the rate of about 1 cc per dish and incubated at room temperature (22–24° C).

Only about 3 per cent of the spores germinated, and all of those within 24 hours. Subsequent germination tests all gave the same small percentage, with no additional spores germinating after 24 hours. Germination in all cases was through the ventral slit or suture (plate 1, *G*).

Fifty of the germinated spores were transferred singly to potato-dextrose agar, where they continued to grow and eventually (within 24 days) produced the coremial stage of *Dematophora necatrix*. Some of these cultures were used to inoculate eight young apple trees by placing the inoculum in contact with their roots. Within six weeks, all the inoculated trees were dead, whereas the controls remained healthy.

The germination of ascospores, with subsequent production in culture of the coremial stage and the demonstration of pathogenicity, are considered to constitute adequate proof of the genetic relation of *Dematophora necatrix* to *Rosellinia necatrix*. Specimens bearing perithecia have been sent to the Imperial Mycological Institute at Kew and the New York Botanical Gardens.

ASSOCIATED FUNGI

Viala[6] describes in detail a pycnidial fungus which he considers to be a stage in the life cycle of *Rosellinia necatrix*. Massee[3] also describes a pycnidial stage in *R. radiciperda*, and Hartig[2] found pycnidia associated with *R. quercina* but states that he was unable to prove the relationship.

We have found constantly associated with *Rosellinia necatrix*, on apple roots, a pycnidial fungus, which upon culture proved to be a species of the form genus *Phomopsis* and in no way related to the true pathogene. The constancy of its presence, however, might easily lead one astray unless culturing is resorted to.

In apple orchards where the root rot is prevalent, and also in orchards where it has not yet been observed, we find another species of *Rosellinia*, tentatively identified as *R. aquila*. This fungus fruits abundantly on old apple prunings left in the orchards from year to year. It is, however, readily distinguished from *R. necatrix*, even in the field, because of its distinct conidial (*Sporotrichum*) stage. In the laboratory, the marked difference in size and shape of ascospores makes differentiation a routine matter.

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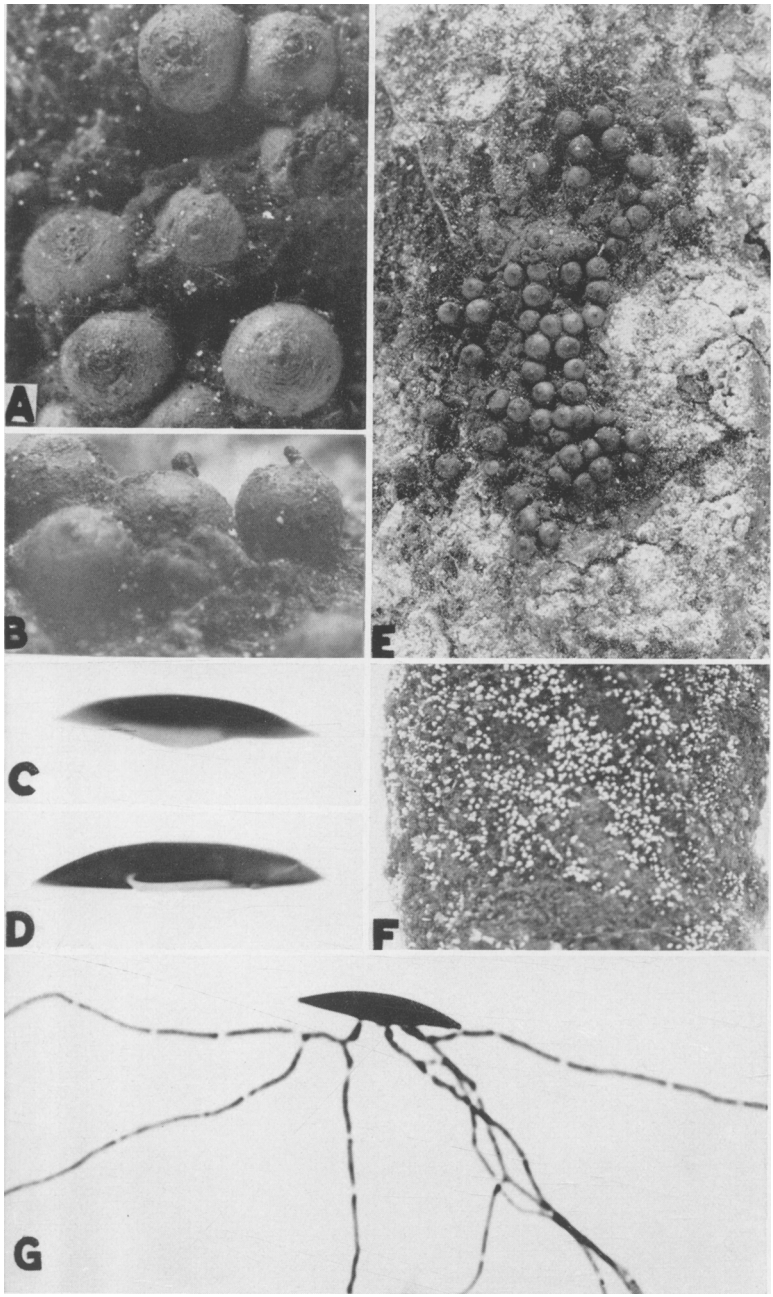

Plate 1.—*Rosellinia necatrix: A* and *B*, perithecia showing freshly exuded spore masses (× 7); *C*, spore, showing the distended epispore just prior to germination (× 900); *D*, spore, showing the ventral slit partly broken (× 900); *E*, perithecia on apple root (× 1.5); *F*, *Dematophora* or coremial stage on apple root (× 1); *G*, germinating ascospore (× 500).