Sydowia, Annales Mycologici Ser. II.
Vol. 38: 75–86 (1985)
Verlag Ferdinand Berger & Söhne Gesellschaft m.b.H., 3580 Horn, Austria

Rosellinia necatrix – fact or fiction?

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Summary. – A description, with synonymy, is given of *Rosellinia desmazieresii* (BERK. & BR.) SACC. and the species is compared with *R. arcuata* PETCH, *R. bothrina* (BERK. & BR.) SACC. and *R. necatrix* PRILL. *R. bothrina* is thought to be the correct name for *R. arcuata*.

Introduction

An examination of the British collections of *Rosellinia* which I began in 1978, and have continued at intervals over the past seven years, presented several problems which were usually put to one side in the hope that further studies and increasing knowledge would provide solutions.

One such problem was a collection from the soil surface around unhealthy paeonies in Peebleshire, Scotland (WATLING & al., 1964) which had lain somewhat uneasily in the folders of CMI and Kew as *R. necatrix* PRILL. A note on the packet at K stated ominously "the spores are small". The material was old and sparse and only free, overmature spores were found in the ascocarps.

In June 1979 two cultures of a *Rosellinia* were sent to CMI for determination by D. K. BARRETT, University of Oxford. The isolates came from *Salix repens* on the Ainsdale Nature Reserve, Lancashire and were thought to be the cause of a ring-dying of the *Salix*. The material was excellent and ascocarps which had been grown on sterilized twigs of *Salix*, contained asci and ascospores in all stages of development. It seemed possible that this was indeed *R. necatrix* but once again the spore size was small and very reminiscent of the paeony material.

The *Salix* isolates were found to be *R. desmazieresii* (BERK. & BR.) SACC. and it is suggested that the paeony material also belongs to this species.

Descriptions

1. *Rosellinia desmazieresii* (as "*desmazierii*") (BERK. & BR.) SACC., – Fig. 1 a–f

Michelia 1: 371. 1878.

Sphaeria desmazierii BERK. & BR., Ann. Mag. nat. Hist. ser. 2, 9: 318. 1852. – *Byssosphaeria desmazierii* (BERK. & BR.) COOKE, Grevillea 15: 122. 1887. – non *Sphaeria desmazierii* FR. in DESMAZIERES Plantes Crypt. de France., Ed. I, 1825–51,

Ser. 1, no. 563, (nomen nudum). – *Rosellinia andurnensis* CES. & DE NOT., Sfer. ital., cent. 1: 20. 1863. – *Rosellinia fusispora* KIRSCHST., Krypt. Fl. Brandenb. 7: 213. 1911. – *Hypoxylon globulare* BULL. in Fuckel, F. rhen. 1060. 1864.

Ascocarps in dense clusters on a coarse brown subiculum formed from long, straight, thick-walled hyphae. Ascocarps 0.75–1.5 mm diam, rounded to slightly elongate with a distinct cone-like papilla. When old and dry the walls of the lower half of the fruitbodies often collapse inwards to give a fluted appearance. The texture is leathery to carbonaceous. – Asci p. sp. 180–125 × 7–9 µm (from apex to base of lowest spore), rarely seen complete with mature spores, apex rounded with an apical apparatus 7–8 × 6 µm, blue in I, stalk long and tapering, spores eight, uniseriate, usually overlapping. – Ascospores 29–35 × 6–8 µm brown, fusiform, inequilateral with tapered, pointed ends, each with a small hyaline appendage about 2 µm long, one appendage often shorter and wider than the other; germ slit 14–20 µm, straight and always shorter than the spore.

Anamorph: Conidia 5–7 × 2.5–3.5 µm, ovoid to elliptical, hyaline, developing at the apices of fertile branches. – Conidiophores mononematous, hyaline to pale yellow, up to 2.5 µm wide near the base, irregularly branched except near the apex where the fertile branches are usually borne in whorls. Fertile branches with 2–5 conspicuous conidial scars on the slightly enlarged tips, rarely geniculate.

Habitat: on the ground, spreading over fallen leaves and other debris or on roots of plants at, or near, ground level.

Identification: The European species most likely to be confused with *R. desmazieresii* is *R. thelena*. *R. desmazieresii* is distinguished by having larger spores with sharply pointed ends and smaller appendages. The longitudinal germ slit is always shorter than the spore. The spores of *R. thelena* measure 24–28 × 7–8 µm and have blunter, non-tapering ends with slender hyaline appendages 6–9 µm long. The germ slit is the same length as the spore.

Specimens examined. – Herb. BERKELEY, *Sph. murina* BERK. & BR. ≡ *S. desmazierii* BERK. & BR., Aug. 1851, 23 Oct. 1851, Collyweston, nr. Kings Cliffe, (Holotype, K). *Rosellinia andurnensis*, Erbar. Crittogam. Ital. 55. 1055 a S. Giovanni d'Andorno nel Biellese, Settembre 1861, CESATI, (K). *Rosellinia fusispora*, KIRSCHSTEIN, auf faulenden Grasern, Rathenow Stadtforst, W. K., (Holotype, B). *Hypoxylon globulare* BULL., Fuckel, Fungi rhenani 1060, (K).

ENGLAND: Herb. C. E. BROOME, *Sphaeria desmazierii* BERK. & BR., nr. Kings Cliffe, Norths., Oct. 1851, Nov. 1851; Herb. BLOXAM, Kings Cliffe, 23 Oct. 1851, C. E. B. All specimens at K. Isol. ex *Salix repens*, Ainsdale Dunes, Lancs., comm. June 1979, BARRETT 89. M3A (239174). Scotland: on dead *Ulex*, Lochbuie, Isle of Mull, Argyll, 4 July 1966, BANKS F. 75, as *"R. aquila"* (K); on soil around paeony, Peebleshire, WATLING, comm. 20. 8. 1963 (102018).

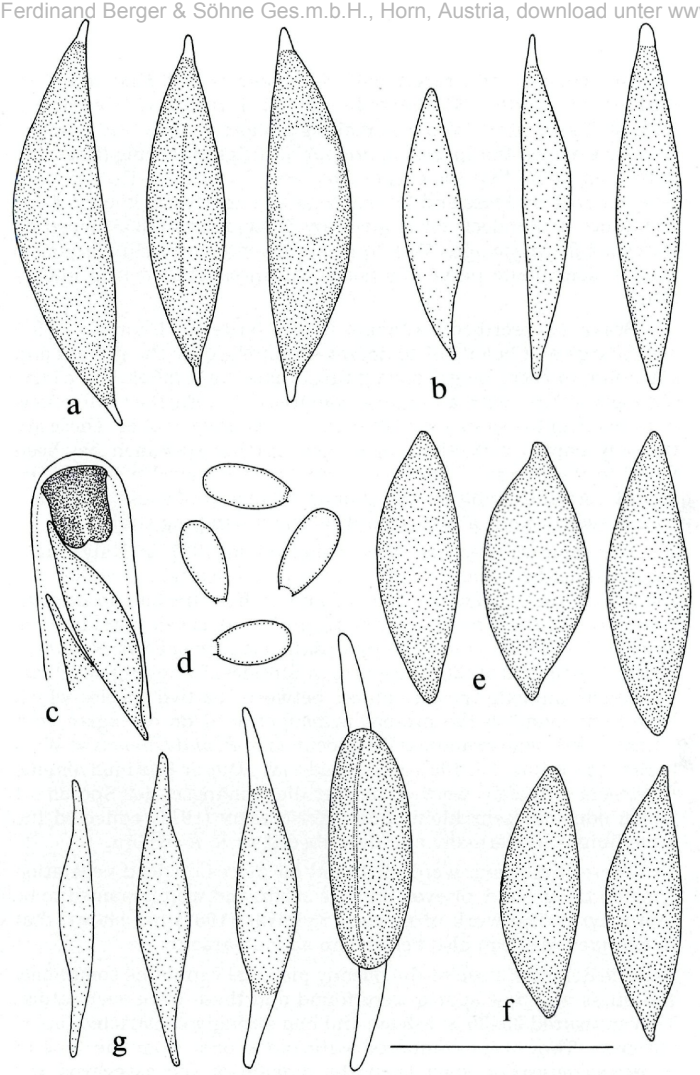

Fig. 1. *Rosellinia desmazieresii* (Holotype, K): a. Ascospores. – b. Developing ascospores. – c. Ascus apex with apical apparatus. – d. Conidia. – e. Ascospores from exterior of ascocarp. – f. Ascospores from paeony (IMI 102018). – *Rosellinia thelena* (IMI 91004): g. Ascospores, developing and mature. – bar = 20 µm.

This curious, and rarely collected, species was first found in 1851 at Collyweston, Northants by Rev. M. J. BERKELEY in company with M. DESMAZIERES who was making a short visit to this country. BERKELEY named the species in honour of his guest noting (BERKELEY & BROOME, 1852) that the earlier *Sphaeria desmazierii* FR. had been rejected both by FRIES and DESMAZIERES as imperfectly known. Confirmation of this decision is given by DESMAZIERES (1855) when he states (of *S. desmazierii* FR.) "que son inventeur (FRIES) n'a jamais décrite, sans doute parce que nos échantillons étaient trop imparfaits".

BERKELEY described his fungus in the Gardeners'Chonicle (1851) as "A large and beautiful *Sphaeria* – running over the ground and spreading to every neighbouring tuft of moss and fallen leaf covering them at first with a mouse – coloured felt". Further collections were made in the same year between August and October. These are the only named collections in K. One further specimen has been found in the *R. aquila* folder at K on a very decayed piece of *Ulex* almost certainly lying on the ground as pieces of moss and other debris have become attached to it by the developing subiculum.

The same species had been collected in 1837 in Italy but it remained undescribed as DE NOTARIS was uncertain whether the species was distinct from *R. thelena* and at that time had no adequate material of the latter for comparison. Twentyseven years later he described a further collection by CESATI as a new species, *R. andurnensis*. His figures of the ascospores in Sferiacei Italici (fig. 8, 10 tav. 17) clearly indicate the differences between the two species which have been found in the present investigation. I do not agree with PETRAK (1961) who examined the specimen of *R. andurnensis* at Wien in Erb. Critt. Ital., 55. 1055 and stated that „Dieser Pilz muß als eine *R. thelena* aufgefaßt werden, bei der die Anhängsel der Sporen oft fehlen oder nur sehr klein sind". KIRSCHSTEIN (1911) collected the same fungus in Germany and described it as *R. fusispora*.

These three taxa were all found on pieces of dead vegetation lying on the ground, or even on bare earth, and were assumed to be saprophytic. The work of BARRETT & PAYNE (1982) has shown that *R. desmazieresii* can also become an active parasite.

The determination of the paeony material cannot be completely certain as very few spores were found and these were overmature. They measured 25–30 × 7–9 µm and had strongly constricted tapering ends. They were compared with old spores from the type of *R. desmazieresii* scraped from the outside of the ascocarps and found lying on the subiculum and agreed reasonably well (fig. 1, f). The tapering ends to the spores might suggest *R. necatrix* but the spores of this species are at least 10 µm longer and also narrower.

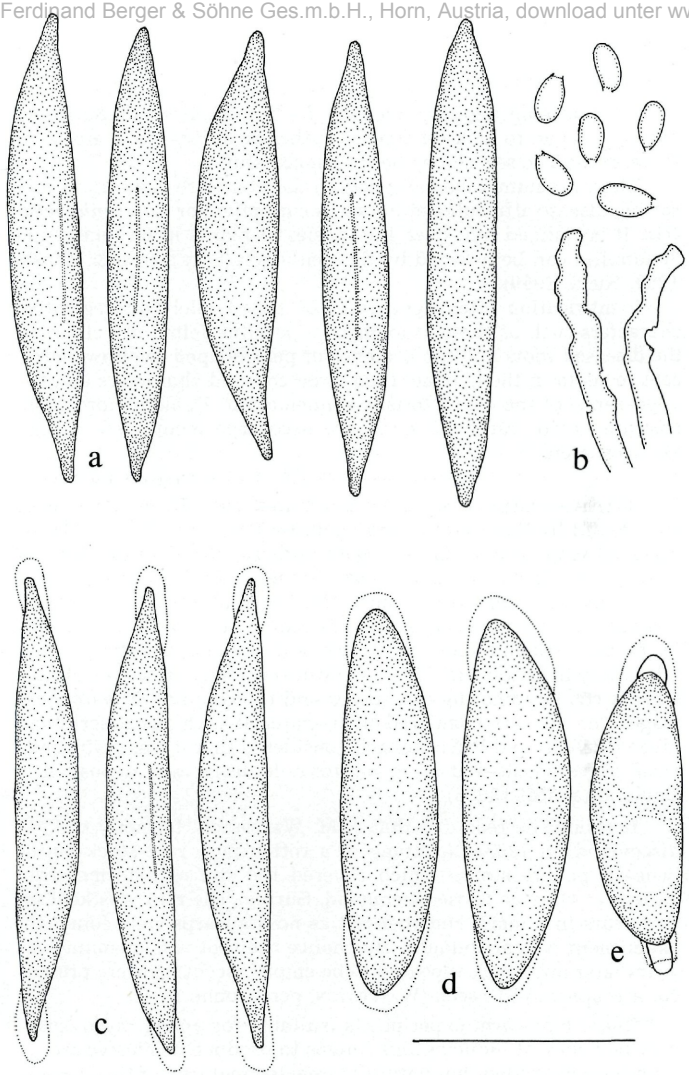

Fig. 2. *Rosellinia necatrix* (IMI 13328): a Ascospores. – b. Conidia and conidiophore tips. – *Rosellinia bothrina* (Holotype, K): c. Ascospores. – *Rosellinia* sp., "broad spore" (Ceylon 299, K): d. Ascospores. – *Rosellinia aquila* (IMI 204601): e. Ascospore. – bar = 20 µm.

2. *R. necatrix* (Fig. 2a, b)

When attempting to find a name for the *Rosellinia* on *Salix* and paeony I began to look for type or authentic ascocarpic material of *R. necatrix*. The search has been unsuccessful.

There are numerous references in the literature to a serious root rotting disease attributed either to *Dematophora* or *Rosellinia necatrix*. It is claimed that over 170 species of plants in 63 genera and 30 families can be infected by this pathogen (SZTEJNBERG & MADAR, 1980; KHAN, 1959).

Identification often appears to be based solely on vegetative characters such as the development of a white felted mycelium on the diseased roots and the presence of pear-shaped swellings adjacent to septa in the hyphae. Less often conidial characters and the appearance of the characteristic synnemata of *Dematophora necatrix* are noted; rarely, if ever, are ascocarps mentioned or, one assumes, seen.

Dematophora necatrix was described and figured by HARTIG in 1883. Ascocarps, apparently associated with *D. necatrix*, were first found by VIALA (1891) and again by PRILLIEUX (1897, 1904) on diseased vine roots in France. Both authors gave detailed descriptions which included spore measurements. No further reports of ascocarps have been traced until HANSEN & al. (1937) published a description of perithecia that had appeared on apple roots infected by *D. necatrix* and kept for two years in a moist chamber in their laboratory in California, USA. A cultural connection was established between germinating ascospores and the conidial *Dematophora* stage. The authors compared their material with the descriptions given by VIALA and PRILLIEUX and considered that it was sufficiently close to be considered as *R. necatrix*. Material was deposited in England (as IMI 13328).

It should be recorded that J. M. WATERSTON, formerly of CMI, discovered, in 1963, the stump of a rotten pear root-stock buried beneath paving stones which covered the site of a former fruit garden at Garrick Close, Richmond, Surrey. The root was kept for two years in a moist chamber but as no ascocarps were found the experiment was abandoned. By chance the root was examined six years later and what appeared to be empty ascocarps were present. No ascospores were seen (WATERSTON, pers. comm.).

These long-term experiments (suitable for young mycologists) may be a way of inducing this fungus to produce its elusive ascigerous state, provided that naturally infected material of the *Dematophora* stage is available. This behaviour is in marked contrast to *R. desmazieresii* where the *Salix* isolates produced ascocarps readily in culture.

No ascosporic material has been found at K, E or PC. At K there is a note in the folder stating that "a large specimen exists at the Ministry of Agriculture Laboratory, Harpenden, Herts". A visit and a most co-operative search by the staff failed to locate the specimen.

The material named as *R. necatrix* at PC was examined and consisted of three specimens none of which contained ascocarps. One came from the Ecole N. d'Agriculture de Montpellier, Laboratoire de Viticulture and was labelled "*Dematophora necatrix* de R. HARTIG. Pourridié Vitis, 2 Mars 89, G. VIALA". The material consisted of seven very small pieces of ? root with synnemata of the *Dematophora* amongst which were sclerotical-like bodies. A few thin sections were taken from one but the structure crumbled and had no internal contents. The second and third specimens came from the herbarium of E. MUSSAT, one labelled, "Rac. du *Pirus communis.* cult. Août 1899, Grignon" and the other "Rac. du *Prunus persica,* Sept. 1899, Grignon". The material on *Pyrus,* which was less than 1 × 0.5 cm in size, had synnemata resembling *D. necatrix* but no conidia were found. Synnemata of *Dematophora,* also without conidia, were present on the *Prunus* specimen together with sclerotial-like bodies. One fragment of what might have been an ascocarp wall was seen but no spores were present on the even smaller piece examined.

It is possible that there is ascosporic type material of *R. necatrix* in a European herbarium not discovered in my rather limited searches. At present however the American material deposited in Herb IMI in 1936 is the only specimen known to me with characters that agree essentially with the original diagnosis. The following description is given (from IMI 13328):

Ascocarps clustered on a sparse subiculum of coarse copper coloured hyphae which have an almost metallic sheen. Ascocarps 1.0–1.5 mm diam, rounded, with a slightly flattened top and small conical papilla. The walls are hard, carbonaceous and rusty-brown coloured. – Asci, p. sp. 230–290 × 7 µm, apex rounded with an apical apparatus, blue in I, spores uniseriate. – Ascospores 36–46 × 5.5–6.3 µm, brown, inequilateral with sharply tapered or somewhat apiculate ends, without appendages. A conspicuous sheath (as "epispore") was described by HANSEN & al. (1937) but was not seen clearly in this material. Germ slit 10–14 µm, always shorter than the spore.

Anamorph: Conidia 3.6–4 × 1.8–2.5 µm, ovoid to elliptical, hyaline. – Conidiophores synnematous 0.5–1.5 mm long arising in groups from the subiculum. Fertile ends sparingly branched, geniculate with 5–10 conidial scars.

Habitat: developed on roots of apple in a laboratory, California, USA, comm. 28. 9. 36, THOMAS (13328).

ARNAUD (1931) suggested that *R. desmazieresii* and *R. necatrix* were synonyms. This is not so and the ascocarps, ascospores and anamorphs of the two taxa are quite distinct. However, a herbarium note left by E. W. MASON stating that he could see little difference between the *R. necatrix* on apple from California (IMI 13328) and *R. arcuata* prompted an examination of the type material of the latter.

3. *R. arcuata* and *R. bothrina* (Fig. 2 c)

R. arcuata has to be considered together with *R. bothrina* as both names have been associated with a disease causing a root rot of tea and other plants in Ceylon (Sri Lanka).

R. bothrina (as "Sphaeria") was described by BERKELEY & BROOME (1873) from collection no. 299 sent by THWAITES from Ceylon in the 1860's. The description reads: – "*S.* (Byssisedae) *bothrina* B. & BR. Peritheciis in foveolis mycelii sitis; sporidiis fusiformibus acutissimis (no. 299). Resembling somewhat as to the form of the sporidia *S. desmazierii,* but very distinct; sporidia .0018 long". On the basis of the description PETCH (1910) assumed *S. bothrina* to be the causal agent of the root rot disease of tea in Ceylon and published an extended description together with an account of the symptoms of the disease. However, when he examined the type of *S. bothrina* at K (PETCH, 1916) he found that probably two species were present, one was immature while the other had broad spores with rounded ends quite unlike the long narrow spores of the tea parasite. He found the latter species to be present in THWAITES' collection no. 219 which BERKELEY & BROOME (1873) had named as "*S. aquila,* in part" and suggested that the packets had been confused. PETCH (1916) gave the name *R. arcuata* to the tea parasite.

The type folder of *R. bothrina* at K contains material from Herb. BERKELEY and Herb. BROME. The latter was formerly at BM and perhaps was not seen by PETCH. The material is in slightly better condition than BERKELEY's some of which appears to have been removed.

There are two specimens on the Herb. BROOME sheet each consisting of five small pieces of wood. A spore sketch and measurements are given for each specimen. The measurements for the first specimen (the upper one on the sheet) are 0.001 to 0.0012 × 0.0004 to 0.0005 and the sketch shows a wide spore with somewhat abruptly pointed ends. On four of these pieces the broad spored species is present and on the fifth, the long narrow spored species. The second specimen also has five small pieces glued to the sheet and these are all the long spored species. The spore sketch and measurement of 0.0018 agree exactly with the original diagnosis. The specimens in Herb. BERKELEY also show both species but while the broad spored

species is in good condition the material of the long narrow spored species is poor and is possibly what PETCH referred to as "the immature species". There is also an envelope labelled "sent before, no. 299, from Central Province. Dec. 1868" containing six small pieces of wood all of which bear ascocarps of the broad spored species.

THWAITES' collection 219 is also a mixture of the same two species but includes rather better material of the long spored species. It was used by PETCH as type for his *R. arcuata*. However as this same species is present in BERKELEY & BROOME's cited type 299, and clearly agrees with the description they give, their epithet of *bothrina* must have priority. The second species in these collections ("broad spore") is not *R. aquila* as BERKELEY & BROOME (1873) suggested and has not yet been matched with any of the numerous species of *Rosellinia* already described for Sri Lanka and India.

In view of the mixed contents of these collections, PETCH's suggestion that BERKELEY confused the packets seems rather unfair. A possible explanation is found in the introduction to the "Fungi of Ceylon" (PETCH & BISBY, 1950) where one reads that THWAITES, the Superintendent of the Royal Botanic Gardens Peradeniya, sent numerous collections to specialists in Europe and "in many cases the actual collecting was no doubt entrusted to his plant collectors; and that may account for the inclusion sometimes of several species under one THWAITES' number".

A description is given of *R. bothrina* and of the broad spored species (Fig. 2 c, d):

Rosellinia bothrina (BERK. & BR.) SACC., Syll. Fung. 1: 257. 1882. – Fig. 2 c

Sphaeria bothrina BERK. & BR., J. Linn. Soc. 14: 125. 1873.
Rosellinia arcuata PETCH, Ann. R. bot. Gdns Peradeniya 6: 175. 1916.

Ascocarps grouped or widely scattered on a sparse copper coloured subiculum or on bare wood and then sometimes surrounded by synnemata. Ascocarps 0.75–1.5 mm diam, spherical, walls rusty-brown coloured, sloping gradually to a small black conical papilla, carbonaceous. – Asci p. sp. 208–230 × 7 µm, apex rounded with an apical apparatus 9 × 5 µm, blue in I, eight spores, uniseriate, usually overlapping. – Ascospores 37–43 × 5–6 µm, brown, fusiform, inequilateral, ends tapered with the final 1–2 µm strongly compressed. No appendages. A sheath forms conspicuous hyaline caps around both ends of each spore. Germ slit 9–11 µm, always shorter than the spore.

Anamorph: Conidia and fertile branches were not found. Conidiophores synnematous, 1–1.5 mm high, copper coloured, usu-

ally formed in groups. PETCH collection 3145 had fertile branch endings which were geniculate but no conidia were found.

Habitat: no details given, but the ascocarps are on small pieces of wood.

Specimens examined. – *Sphaeria (Byssisedae) bothrina*, Ceylon 299, Herb. C. E. BROOME (Holotype, K). – *Sph. aquila*, Ceylon 219, G.H.K.T., Herb. BERKELEY (Holotype, *R. arcuata*, K). – *Sph. bothrina* B. & BR. ex Herb. M.J.B. in Herb. M. C. COOKE, K; as "*R. arcuata*", Hakgala, Ceylon, PETCH 3145, May. 1910, K.

4. *Rosellinia* sp. (the "broad spore" of THWAITES' collection 299) – Fig. 2d

Ascocarps scattered and almost completely immersed in a thick subiculum which forms a dense copper coloured mat on the surface of the wood. Ascocarps 1.00–1.25 mm, hard and carbonaceous, spherical with a markedly flattened black top, this, and the black conical papilla, are usually the only portion of the ascocarp that is visible above the subiculum. – Asci p. sp. 184 × 7 µm (only one spore group was seen), apex rounded with an apical apparatus 12 × 9 µm, blue in I, eight spores, uniseriate. – Ascospores 30–37 × 7.5–10.5 µm, brown, ellipsoidal, inequilateral, ends blunt, either rounded or with a slight point. No appendages. A sheath surrounds each spore and projects (1.5 µm) at each end. Germ slit 9–11 µm, always shorter than the spore.

Anamorph. – not certainly found, synnemata were present near some of the ascocarps but possibly belonging to *R. bothrina*.

Habitat. – no details given, otherwise as *R. bothrina*.

Specimens examined. – *Sphaeria bothrina* B. & BR., Central Province, Dec. 1868, No. 299, "sent before", Herb. BERKELEY, K; *Sphaeria aquila* FR., Ceylon 299, Herb. CURREY, (material included 5 pieces "broad spore" and one of *R. bothrina*) in *R. arcuata* folder, K. Only specimens where all, or most, of the pieces showed the broad spored *Rosellinia* have been cited here. Further details have been deposited with the folder at K.

Discussion

The similarity between the American specimens named as *R. necatrix* and the type material of *R. bothrina* is striking and the only morphological difference that I can find is the presence of a sheath on the spores of *R. bothrina* and its absence from spores of the *R. necatrix* material. I attribute this absence to old age as the spores are overmature and sheaths and appendages disappear in old spores. HANSEN & al. (1937) noted the presence of a hyaline epispore in their material.

The presence of pear-shaped swellings on the mycelium is sometimes cited as diagnostic for *R. necatrix* but PETCH (1923) describes their frequent occurrence in the darker hyphae of the mycelial strands of *R. bothrina* (as "*R. arcuata*").

Another apparent difference, found in the literature, is the descriptive term "White root rot" for the disease attributed to *R. necatrix* and "Black root rot" for *R. bothrina*. I do not know whether this is a valid and constant difference or dependent to some extent on the age of the material and also on temperature. *R. necatrix* is usually recorded from temperate areas of the world while *R. bothrina* occurs in the tropics. It is interesting to note in this connection that MANTELL & WHEELER (1973) suggest that the *Rosellinia* causing "White root rot" of *Narcissus* in the Scilly Isles (England) was possibly introduced on exotic ornamentals brought from the tropics and sub-tropics at the beginning of this century and planted in gardens such as the Abbey Gardens on Tresco. If the "Black" and "White root rots" are caused by the same species of *Rosellinia*, as I suspect they may be, a colour difference is scarcely deserving of specific rank and *R. bothrina,* as the earlier name, would have priority.

This paper does not offer a solution to the problem of "What is *R. necatrix*?" but attempts to state the problem. It was obvious from conversations with plant pathologists at the 4th International Congress of Plant Pathology in Melbourne, Australia, that the root rotting species of *Rosellinia* are still causing troublesome diseases. What is needed is a concerted effort by the owners of these problems to attempt production of the ascocarps of their *Rosellinia* using the "long-term method" so that ascocarp structure and disease symptoms can be correlated from as many different parts of the world as possible. We have a source of supply of the "White root rot" in England from the bulb fields of the Scilly Isles. I intend to try again and invite other workers to set up similar experiments with their *Rosellinia* material.

I thank the Directors of the Royal Botanic Gardens, Kew (K) and the Museum National d'histoire Naturelle, Paris (PC) for permission to examine material in their keeping; also B. J. COPPINS for checking the *Rosellinia* folders at the Royal Botanic Garden, Edinburgh (E). The loan of a microscope from the Scientific Investigations grant, administered by the Royal Society, greatly facilitated this work.

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