Mycoscience 41: 503–507, 2000

Short Communication

Observations on the teleomorph of the white root rot fungus, *Rosellinia necatrix*, and a related fungus, *Rosellinia aquila**

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Accepted for publication 4 July 2000

The process of teleomorph development in the white root rot fungus *Rosellinia necatrix* is described on diseased roots of Japanese pear. Stromata were also found on dead plants in nonagricultural lands such as yards and forests. The stroma of *R. aquila* is also described.

Key Words——*Rosellinia aquila*; *Rosellinia necatrix*; white root rot.

The causal fungus of white root rot of fruit trees and other crops in Japan has been referred to as *Rosellinia necatrix* Prillieux. Identification of the fungus was based solely on vegetative criteria, such as its white, fan-shaped mycelia on diseased roots and pyriform swellings adjacent to septa in the hypae (e.g., Ito and Nakamura, 1984; Kanadani et al., 1994). The only available study of its conidial stage is that of Watanabe (1992), who described its anamorph, *Dematophora necatrix* Hartig. Most studies were focused on the control and ecology of the causal fungus (e.g., Tanaka et al., 1966; Ieki et al., 1969; Umemoto and Murata, 1986; Fukushima, 1998), and knowledge of its basic biology is largely lacking, including the description of its sexual stage. Only two papers (Watanabe, 1963; Ezuka et al., 1973) and a note (Zinno, 1977) are available on the teleomoph of the white root rot fungus in Japan, and these as well as reports from U.S.A. (Hansen et al., 1937) and Portugal (Teixeira de Sousa and Whalley, 1991) deal only briefly with the process of teleomorph development.

On starting a project to control white root rot of fruit trees with double-stranded RNA (Matsumoto, 1998), we needed to confirm the identity of our isolates of the white root rot fungus as *R. necatrix*, since the *Rosellinia* stage had not been found since the report of Ezuka et al. (1973), who further suggested the involvement of more than two species in white root rot. Our effort to produce stromata on diseased roots of Japanese pear (*Pyrus montana* Nakai) was successful, and the process of their development is described in this paper. We also found stromata produced on dead trees in natural vegetation and in the yard. A sample of *R. aquila* (Fr.: Fr.) De Not. was found among them, and its description is also presented.

Materials and Methods

Roots (3–6 cm diam) were detached from Japanese pear plants killed by white root rot in March 1999 in Chiba and cut into small pieces (ca. 15 cm long). These were placed in hollows on the ground surface in the shade of trees and covered with rice straw at Natl. Inst. Agro-Environmental Sci., Tsukuba. The development of stromata on root segments was examined periodically with the naked eye and under the microscope. Roots with synnemata were collected from dead loquat plants [*Eriobotrya japonica* (Thunb.) Lindl.] in Chiba in July 1999, and small segments (2–3 cm diam, 10 cm long) were incubated in Petri dishes with moist unsterile soil at room temerature for subsidiary observations. Stromata produced on naturally killed plants were also collected from Mt. Tsukuba, Mt. Kaba, and the yards of Natl. Inst. Agro-Environmental Sci., Tsukuba, Ibaraki and Forestry and Forest Products Res. Inst., Kukizaki, Ibaraki in 1999.

Results

Rosellinia necatrix The process of stroma development was as follows. Dark, woolly subicula developed on the root surface, often associated with synnemata in May–June (Fig. 1). Several to more than 10 synnemata were found arising from a common base on the surface of diseased roots in May–June (Fig. 2). They were dark, setose, ca. 5–8 mm long, and tapering toward the tip, which became white to gray, enlarged with conidiopores, and of powdery appearance (Fig. 3). Conidiophores were geniculate (Fig. 4). Conidia were hyaline, subglobose, one-celled, and truncate at the base. Their ger-

*Research based on the program for Promotion of Basic Research Activities for Innovative Biosciences.

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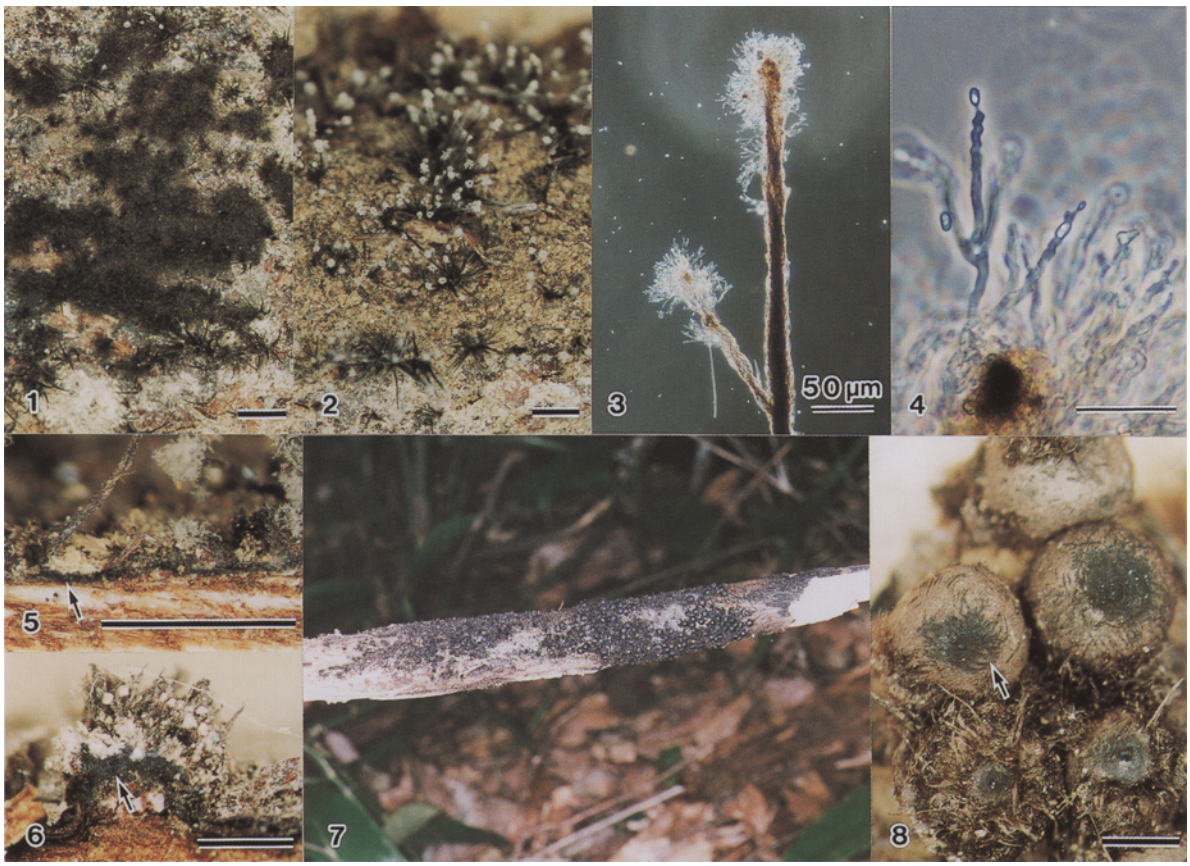
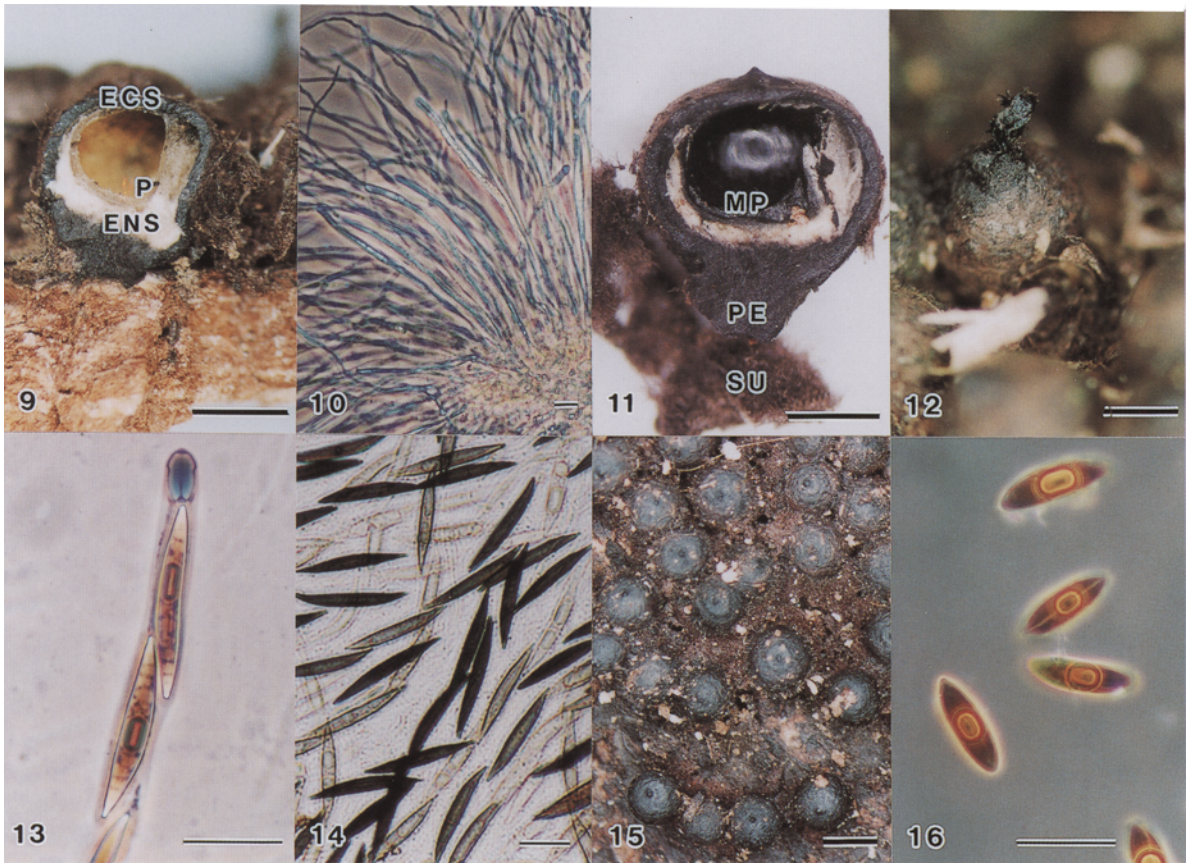


Figs. 1–8. *Rosellinia necatrix*. 1. Subiculum. 2. Synnemata arising from a common base. 3. Close-up of synnema apex. 4. Geniculate conidiophores. 5. Thin, immature ectostroma just beneath synnemata (arrow). 6. Developing stroma (arrow indicates thickened and elevated ectostroma). 7. Stromata (ca. 60% of life size). 8. Stromata surrounded by subicule with dark streaks (arrow) around the top. Thick scale bars: 1.0 mm; thin scale bars except in Fig. 3: 20 μm.

minability differed from sample to sample, but they never developed into mycelia on any media examined. Black, thin ectostroma was found just beneath synnemata (Fig. 5) and gradually became elevated and thick, producing white entostroma beneath it (Fig. 6). A bunch of synnemata often remained undetached on the stroma. Stromata became apparent by the end of August (Fig. 7), often surrounded by subicule in the lower half (Fig. 8). They were almost globose, ranging from 1.17 to 1.70 mm in diam, papillate, and usually pedicellate and copper brown to dark brown, having dark streaks on the surface which were most numerous around the top (Fig. 8). The ectostroma was dark, carbonaceous, and entostroma white and felty (Fig. 9). Each stroma had a single perithecium, containing immature asci and paraphyses (Fig. 10) in a yellow layer within the stroma (Fig. 9). When asci matured in late August–September, the yellow layer of asci turned black and the papilla became prominent (Fig. 11). A mass of ascospores glued in slime exuded when the stroma was moistened (Fig. 12). Asci were clavate to cylindrical with an amyloid plug (Fig. 13), containing 8 uniseriate ascospores. Ascospores were one-celled, dark, narrow, and asymmentrically fusiform, lacking caps (Fig. 14). A straight germ slit, ca. 10–20 μm long, was rarely found. Stromata of *R. necatrix* were also found on dead trees of *Callicarpa mollis, Neolitsea serisea*, and unidentified species in Mt. Tsukuba and in the yard of the research institutes in August–October. Spore dimensions were variable from sample to sample and ranged 44.4–52.3 × 5.4–7.1 μm with an overall average of 47.7 × 6.3 μm (Table 1).

Rosellinia aquila Stromata were found on a detached twig of unidentified hardwood in September 1999 in the yard of Natl. Inst. Agro-Environmental Sci, Tsukuba. Stromata were gregarious on some parts of the substratum and scattered on other parts, and thick, reddish brown subiculum covered a part of the twig where stromata were found partially embedded in the subicule (Fig. 15). Stromata ranged 0.99–1.41 mm in diam with an average of 1.13 mm and were globose, papillate and dark brown. Ascospores were dark brown and ellipsoidal or asymmetrically ellipsoidal, rarely with a cellular appendage at one or at both ends, and had a straight germ slit of entire spore length (Fig. 16). They averaged 22.0 × 7.5 μm, ranging 17.5–25.0 × 6.3–8.8 μm. Asci or ascal plugs were not found. Conidiomata were not present.



Figs. 9–14. *Rosellinia necatrix*. 9. Inner structure of a stroma with a yellow perithecium (P) and white entostroma (ENS); ECS: ectostroma. 10. Immature asci and paraphyses. 11. Mature stroma with a dark perithecium (MP); PE: pedicele; SU: subiculum. 12. Stroma with a mass of ascospores exuding from ostiole. 13. Amyloid ascal plug and ascospores. 14. Ascospores.
Figs. 15, 16. *Rosellinia aquila*. 15. Stroma embedded in subiculum. 16. Ascospores. Thick scale bars: 1.0 mm; thin scale bars: 20 μm.

Table 1. Comparison of ascospore dimensions of *Rosellinia necatrix* collections.

Collection	Locality	Range	Average
Japanese pear	Inzai, Chiba	37.5–52.5 × 5–8.8 μm	45.4 × 6.3 μm
Mt. Tsukuba 1	Tsukuba, Ibaraki	40–55 × 5–7.5 μm	48.0 × 6.3 μm
Mt. Tsukuba 2	Tsukuba, Ibaraki	40–50 × 5–6.3 μm	44.4 × 5.4 μm
Mt. Kaba 1	Yamato, Ibaraki	40–55 × 6.3–8.8 μm	48.3 × 7.1 μm
Mt. Kaba 2	Yamato, Ibaraki	(37.5–)47.5–60 × 5–7.5 μm	52.3 × 6.5 μm
Average			47.7 × 6.3 μm

Other authors	Range
Hansen et al. (1937)	31.1–47.6 × 5.1–7.1 μm
Khan (1959)	31–47 × 5–7 μm
Watanabe (1963)	30–46 × 5–7 μm
Sivanesan and Holliday (1972)	30–50 × 5–8 μm
Ezuka et al. (1973)	36–48 × 5.0–7.5 μm
Francis (1985)	36–46 × 5.5–6.3 μm
Teixeira de Sousa & Walley (1991)	35–44 × 5.4–7.2 μm
Gonzalez & Rogers (1995)	38–46 × 7–7.5 μm

Discussion

Stromata of *R. necatrix* have exclusively been reported on cultivated plants such as apple trees (*Mallus pumila* Mill.: Hansen et al., 1937, Teixeira de Sousa and Whalley, 1991), tea plants (*Camellia sinensis* (L.) O. Kuntze: Watanabe, 1963; Ezuka et al., 1973), and peach trees (*Prunus persica* (L.) Batsch: Araki, 1967), with an exception from tropical rain forest in Mexico (Gonzalez and Rogers, 1995). The rarity of *R. necatrix* stromata may be ascribed to the fact that farmers would prevent the pathogen developing from vegetative to sexual structures by using fungicides or by removing moribund or dead plants. We collected infected roots, which were still alive on treatment in early spring. Therefore, the roots were considered to have been infected a year before or more. Teixeira de Sausa and Whalley (1991) spent two years to obtain stromata on inoculated apple trees. This paper may represent the second report on the occurrence of *R. necatrix* in natural vegetation, as Ieki et al. (1969) had previously recovered its mycelia from the forests of Hyogo and Kyoto. *Rosellinia necatrix* appears to develop its teleomorph more frequently in non-agricultural lands where infected plants remain longer.

In the present paper, *R. necatrix* was found to produce stromata at the stem base of dead trees; however, the cause of the death may not always be ascribed to the disease. A detached stem left stabbed in the ground in the forest of Mt. Tsukuba had stromata above the soil line, suggesting saprophyitc colonization of the fungus. *Rosellinia necatrix* can grow saprophytically on plant debris in the F, H and A_1 layers of soil (Ieki et al., 1969). *Rosellinia aquila* colonized a detached branch which was more decomposed than stems with *R. necatrix*.

Ascospore dimensions of *R. necatrix* determined by other authors were more uniform and much smaller than ours, though the averages overlapped (Table 1). We identified our materials as *R. necatrix* by the unique shape of ascospores in the genus *Rosellinia* (Petrini, 1993). Ezuka et al. (1973) suspended identification of a collection with long ascospores (mean 57.6×5.9 μm) of similar shape. Petrini (1993) recognized size variation in ascospores of *R. necatrix* and suggested that this fungus was a species complex, each member differing only in ascospore size. Molecular comparative studies are necessary to confirm the range of variation to synonymize or separate taxa within isolates of *R. necatrix*. Cultures we collected so far from diseased trees all had pyriform hyphal swellings adjacent to septa, but culturing from ascospores was not successful since ascospores did not germinate. Ascospores of *R. necatrix* are hard to germinate (Hansen et al., 1937).

The ability of conidia to germiante was doubtful (Khan, 1959) or differed from sample to sample (Watanabe, 1963), as in our case. Conidia never developed into mycelia, and their role as propagules was suspect (Khan, 1955). They are borne on the apex of synnemata, which mostly disappear prior to the appearance of mature stromata. Abe (personal communication) observed anastomosis between vegetative hyphae and conidia in *Hypoxylon*, a related genus. These facts imply that conidia may act as spermatia. If this is the case, mating experiments, along with molecular studies, should reveal the genetic relationships among variable isolates of *R. necatrix* with a wide geographic distribution and a wide host range.

Thanks are due to S. Umemoto (Chiba Prefect. Agric. Exp. Stn.), Y. Ebihara and S. Uematsu (Chiba Horticultural Exp. Stn.), and H. Masuya, Forestry and Forest Products Research Institute, for helping us collect samples and to Y. Abe, Forestry and Forest Products Research Institute, for his suggestions on *Rosellinia aquila*.

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