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SHORT COMMUNICATION

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Conidioma production of the white root rot fungus in axenic culture under near-ultraviolet light radiation

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Abstract Conidiomata of the white root rot fungus were produced in axenic culture under near-ultraviolet light radiation. Pieces of sterilized Japanese pear twigs were placed on 7-day-old oatmeal agar culture in plates. The plates were further incubated for 5 days and then illuminated by near-ultraviolet light. Synnemata developed on the twigs within 5 weeks in 19 of 20 isolates tested, and conidia were observed in 12 of the 19 isolates. The synnemata and conidia produced were morphologically identical to those of *Dematophora necatrix*.

Key words *Dematophora necatrix* · Identification · Near-ultraviolet light · *Rosellinia necatrix* · White root rot

Rosellinia necatrix Prillieux (anamorph *Dematophora necatrix* Hartig) causes white root rot of many woody and herbaceous plants (Khan 1959; Watanabe 1963; Sztejnberg and Madar 1980; Ito and Nakamura 1984). The fungus does not produce a teleomorph on artificial media. Consequently, the white root rot fungus is practically identified on the basis of morphological characteristics of vegetative hyphae, i.e., the presence of pear-shaped swellings adjacent to septa in the hyphae (Watanabe 1963; Francis 1985; Teixeira de Sousa and Whalley 1991). To control white root rot using the hypovirulence factor (Matsumoto 1998), isolates collected required more precise identification based on the anamorph. However, production of conidiomata on media has been rarely reported (Hansen et al. 1937). In this study, formation of the conidiomata of the white root rot fungus was induced by near-ultraviolet (NUV) light radiation, and the method to obtain the conidiomata in culture is described.

In a preliminary experiment, an isolate (w340; see Table 3) was cultured on water agar (WA), potato dextrose agar (PDA), or oatmeal agar (OA, Difco, Detroit, USA) poured to 3 mm deep in Petri dishes (5 cm diameter) for a week at 20°C in the dark. Four autoclaved twig segments obtained from the Japanese pear (*Pyrus pyrifolia* var. *culuta*) (segments 3–4 cm long, 8–12 mm diameter, longitudinally cut into halves). Two of the four segments were placed with cutsurface up and the others with bark surface up. The plates were incubated in the dark for 5 days at 20°C and then illuminated continuously 20 cm from NUV light (peak at 352 nm; FL20S·BLB-A, Toshiba, Tokyo, Japan). The plates were observed every week for 2 months after incubation under NUV light radiation.

Isolate w340 developed synnemata on WA, PDA, and OA after 3–4 weeks under NUV light radiation (Fig. 1A,B; Table 1). Synnemata were observed exclusively on autoclaved twig segments but not on agar regardless of culture media. The number of synnemata on twigs was greater on OA than on WA or PDA (Table 1). No synnema was observed when incubated in the dark.

To confirm the identity of the anamorph produced naturally and in culture, dimensions of synnemata and conidia of isolate w340 and its original specimen, which was a bunch of Japanese pear twigs used as a bait, were compared. The bunch was buried in a Japanese pear orchard in Daiwa, Hiroshima, and retrieved from the soil 2 months later on 19 July 1999 by H. Nitta, Hiroshima Prefectural Agricultural Research Center. The voucher specimen (NIAES135101) was deposited to the herbarium of National Institute for Agro-Environmental Sciences, Tsukuba. Conidiophores of both samples were geniculate with scars after removal of conidia (Fig. 1C). There was only a slight difference in the length of synnemata. The conidial state of these samples was identical to that of the previous descriptions on *D.*

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necatrix, except for the length of synnemata (Table 2), which was not regarded to be important as a taxonomic criterion (Ezuka et al. 1973; Francis 1985; Petrini 1992; Watanabe 1992).

Twenty isolates collected from various localities in Japan, including isolate w340, were compared for conidioma production (Table 3). The experiments were conducted by the procedure described here with 12 twig disks (3–5 mm thick, 6–10 mm diameter) in each OA plate instead of twig segments. After incubation for 2–5 weeks under NUV light radiation, 19 of 20 isolates developed synnemata and 12 formed conidia on the synnemata. These isolates varied in the frequency of synnema production, and 7 isolates produced synnemata on every disk in the plate (Table 3). The frequency of synnema production did not correlate with host plant or locality in these isolates. The presence of dsRNA is known to reduce conidial production in *Cryphonectria parasitica* (Murrill) Barr (Nuss and Koltin 1990), and we determined the presence or absence of dsRNA following the method of Arakawa et al. (2002); this was not the case with the white root rot fungus (Table 3).

Anamorphs of *Rosellinia* species are assigned to three genera, i.e., *Dematophora*, *Geniculosporium*, and *Nodulisporium*. *Dematophora* and *Geniculosporium* have geniculate conidiophores, and the former is synnematous, although Watanabe (1992) reported mononematous conidiophores of a mutated *D. necatrix* isolate in axenic culture. Some species of *Rosellinia* have a *Dematophora* morph (Francis 1985; Petrini 1992). Of these, *Rosellinia bothrina* (Berk. & Br.) Sacc., a root rot pathogen in the tropics, is most closely related to *R. necatrix*, but its conidia have not been found (Francis 1985). *Rosellinia buxi* H. Fabre is mor-

Table 1. Synnema production by the white root rot fungus under near-ultraviolet (NUV) light radiation

Medium used[a]	NUV light radiation	No. synnemata/plate[b]
WA	+	101.0 ± 103.7
WA	−[c]	0.0
PDA	+	297.3 ± 190.0
PDA	−	0.0
OA	+	1302.7 ± 376.5
OA	−	0.0

WA, water agar; PDA, potato dextrose agar; OA, oatmeal agar
[a] Four twig segments of Japanese pear were placed on a plate culture of isolate w340
[b] Average number of synnemata with standard deviation of three plates, determined 2 months after NUV light radiation
[c] Incubated in the dark

Table 2. Dimension of synnemata and conidia of the white root rot fungus

	Synnemata		Conidia	
	Length (mm)	Width at base (μm)	Length (μm)	Width (μm)
Produced on bait twigs in the laboratory[a]	1.3–3.7[b] (2.5 ± 0.6)[c]	12.5–38.0 (21.8 ± 7.2)	2.7–4.5 (3.6 ± 0.4)	1.6–2.5 (2.0 ± 0.2)
Produced in axenic culture[d]	1.3–2.3 (1.7 ± 0.3)	15.0–40.5 (23.8 ± 6.3)	3.0–4.5 (3.8 ± 0.5)	1.8–2.8 (2.3 ± 0.3)
Ezuka et al. (1973)			3.0–5.0	2.0–3.4
Francis (1985)	0.5–1.5		3.6–4	1.8–2.5
Petrini (1993)	0.5–1.5	<110	3–5	2.5–3
Watanabe (1992)[e]			3.5–5.5	1.6–2.3

[a] Synnemata and conidia were produced on a bunch of Japanese pear twigs used as a bait, from which isolate w340 was obtained
[b] Range
[c] Average with standard deviation ($n = 30$)
[d] Synnemata and conidia of isolate w340 were produced on twig segments of Japanese pear on oatmeal agar
[e] Description on anamorph with mononematous conidiophores in axenic culture

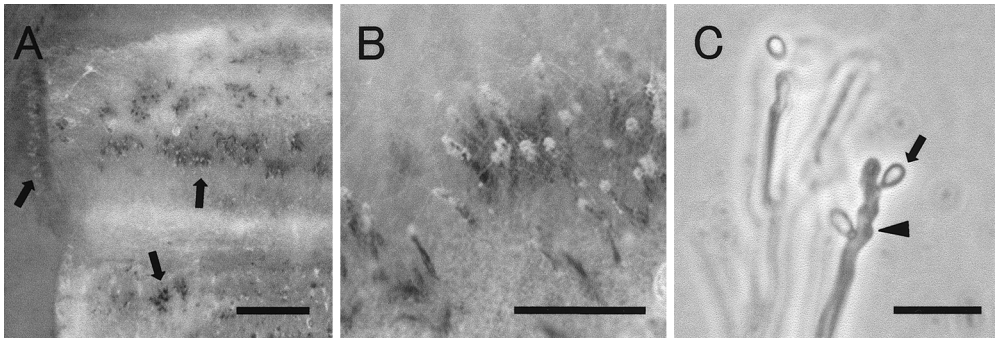

Fig. 1. Synnemata of the white root rot fungus isolate w340 produced under near-ultraviolet light radiation. **A** Synnemata on twig pieces of Japanese pear cultured on oatmeal agar. *Arrows*, clusters of synnemata. **B** Close-up of **A**: synnemata with conidia. **C** Apical part of conidiophore on a synnema. *Arrow*, conidium; *arrowhead*, scar after removal of conidium. *Bars* **A** 5 mm; **B** 3 mm; **C** 10 μm

Table 3. Isolates of the white root rot fungus used in this study and production of synnemata and conidia under near-ultraviolet light radiation

Isolate no.	Host plant	Locality	dsRNA	Frequency of synnema production[a]	Conidium production on synnema	Time required for synnema production (weeks)
w118	Japanese pear	Saga	+	6/12[b]	+	4
w135	Japanese pear	Fukuoka	+	3/12	−	4
w153	Japanese pear	Chiba	−	12/12	+	3
w233	Japanese pear	Chiba	−	4/12	+	4
w238	Loquat	Chiba	−	2/12	−	5
w281	Japanese pear	Tottori	−	12/12	+	4
w340	Buried twigs of Japanese pear	Hiroshima	+	12/12	+	3
w382	Japanese pear	Hiroshima	+	1/12	−	5
w389	Grapevine	Hiroshima	−	11/12	+	3
w422	Japanese pear	Mie	+	12/12	+	2
w430	Japanese pear	Kyoto	−	3/12	−	4
w536	Unknown (forest tree)	Akita	−	3/12	−	3
w588	*Prunus mume*	Wakayama	−	0/12	−	
w607	*Spiraea thunbergii*	Ibaraki	−	12/12	+	2
w645	*Aucuba japonica*	Miyagi	−	2/12	+	4
w652	Unknown (forest tree)	Kanagawa	−	12/12	+	3
w655	*Callicarpa japonica*	Tokyo	−	3/12	−	4
w665	Apple	Gunma	+	5/12	+	4
w672	*Illicium anisatum*	Miyazaki	+	12/12	+	2
w674	Unknown (forest tree)	Aichi	−	3/12	−	5

[a] Twelve twig disks of Japanese pear were placed on oatmeal agar culture in each plate with cut surface up; observations were made up to 2 months
[b] No. of disks of Japanese pear twig with synnemata/no. of disks placed in a plate

phologically similar to *R. necatrix* but has larger conidia than that of *D. necatrix* (Petrini 1992); its pathogenicity has not been demonstrated, either. Thus, we identified the anamorph of the white root rot fungus in Japan as *D. necatrix*.

NUV light radiation was essential for the development of conidiomata in the white root rot fungus as is true for other fungi (Leach 1962; Calpouzos and Lapis 1970). This method is considered to be useful for identifying the anamorph of white root rot fungus.

Since Nakamura et al. (2000) observed that stromata developed at the base of synnemata following conidioma production, we continued further observations for teleomorph development. However, stromata were not recognized in any isolates. Conidia were considered to be involved in mating events (Nakamura et al. 2000) because conidia never developed into mycelia. Production of stromata under axenic condition may require additional conditions such as fertilization by other strains in the white root rot fungus.

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References

Arakawa M, Nakamura H, Uetake Y, Matsumoto M (2002) Presence and distribution of double-stranded RNA elements in the white root rot fungus *Rosellinia necatrix*. Mycoscience 43:21–26

Calpouzos L, Lapis DB (1970) Effects of light on pycnidium formation, sporulation, and tropism by *Septoria nodorum*. Phytopathology 60:791–794

Ezuka A, Kasai K, Kibushi H (1973) Notes on the perithecial stage of *Rosellinia* parasitic on tea plant (in Japanese). Chagyo Kenkyu Hokoku 40:26–30

Francis SM (1985) *Rosellinia necatrix* – fact or fiction? Sydowia 38:75–86

Hansen HN, Thomas HE, Thomas HE (1937) The connection between *Dematophora necatrix* and *Rosellinia nacatrix*. Hilgardia 10:561–565

Ito S, Nakamura N (1984) An outbreak of white root-rot and its environmental conditions in the experimental arboretum (in Japanese with English summary). J Jpn For Soc 66:262–267

Khan AH (1959) Biology and pathogenicity of *Rosellinia necatrix* (Hart.) Berl. Biologia 5:199–245

Leach CM (1962) Sporulation of diverse species of fungi under near-ultraviolet radiation. Can J Bot 40:151–161

Matsumoto N (1998) Biological control of root diseases with dsRNA based on population structure of pathogen. JARQ 32:31–35

Nakamura H, Uetake Y, Arakawa M, Okabe I, Matsumoto N (2000) Observation on the teleomorph of the white root rot fungus, *Rosellinia necatrix*, and a related fungus, *Rosellinia aquila*. Mycoscience 41:503–507

Nuss DL, Koltin Y (1990) Significance of dsRNA genetic elements in plant pathogenic fungi. Annu Rev Phytopathol 28:37–58

Petrini LE (1993) *Rosellinia* species of the temperate zones. Sydowia 44:169–281

Sztejnberg A, Madar Z (1980) Host range of *Dematophora necatrix*, the cause of white root rot disease in fruit trees. Plant Dis 64:662–664

Teixeira de Sousa AJ, Whalley AJS (1991) Induction of mature stromata in *Rosellinia nacatrix* and its taxonomic implications. Sydowia 43:281–290

Watanabe B (1963) Studies on the ecology and control of white root rot disease caused by *Rosellinia necatrix* (Hart.) Berl. Appointed experiment (plant disease and insect pest). Bulletin 3, (in Japanese with English summary). Agric Forest & Fish Res Council, Ministry of Agric & Forest, and Ibaraki Agric Exp Stn, Japan

Watanabe T (1992) Sporulation of *Dematophora necatrix* in vitro and its pathogenicity. Annu Phytopathol Soc Jpn 58:65–71