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New canker diseases of *Abies veitchii* and *Acer crataegifolium* caused by *Neonectria castaneicola*

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Abstract New perennial canker diseases of *Abies veitchii* and *Acer crataegifolium* are described. Pathogenicity of the causal fungus was confirmed on stems of young *Abies* and *Acer* trees using two monoascospore isolates from the perithecia from the two tree species. The causal fungus, producing *Nectria*-state-teleomorph and *Cylindrocarpon*-anamorph, was identified as *Nectria castaneicola*. It was then transferred to the genus *Neonectria*, as *N. castaneicola* (W. Yamam. et Oyasu) Tak. Kobay. et Hirooka, comb. nov. followed by the recent concept of Nectriaceae.

Key words *Abies veitchii* · *Acer crataegifolium* · *Cylindrocarpon*-anamorph · *Neonectria castaneicola* · New perennial canker

Occurrence and symptoms. New perennial canker diseases were found on a trunk of *Abies veitchii* Lindl. (Japanese name: Shirabe) in 1990 in Gunma Prefecture (Fig. 1a) and on a trunk of *Acer crataegifolium* Sieb. et Zucc. (Japanese name: Urikaede) in 1995 in Kanagawa Prefecture (Kobayashi et al. 2003). The diseases start with a flow of white resin on the bark of trunks. The original sites with flowing resin enlarge annually, forming concave lesions. Many reddish nectrioid perithecia were observed on these lesions on the bark surfaces (Fig. 1b). On old lesions, active resin flow is limited to the margin of the lesion, and perith-

ecia are usually formed to the inside of the active margin. Perithecia are produced throughout the year but more abundantly in late summer to early autumn.

Isolation and culture. Monoascospore isolates from these two materials were obtained easily on Weitzman-Silva-Hunter agar (WSH) (Udagawa et al. 1978) or potato-sucrose agar (PSA) slants. They grew similar colonies and produced the identical *Cylindrocarpon*-anamorph on PSA slants. Colonies grew at 10°–35°C with an optimum at 25°–30°C.

Pathogenicity. The pathogenicity of two isolates from *Abies* and *Acer* was confirmed on potted seedlings of *Abies veitchii* (5 years old) and *Acer crataegifolium* (2 years old) after inoculation with mycelium grown for a month on rice bran/wheat bran/water medium (1:1:2) (RWBM). The mycelium were placed on intact, wounded, or burned parts of the bark, which was then bound with vinyl tape. On the control plots, RWBM without fungal culture was used. Each treatment consisted of three replications. The inoculation test was carried out twice from 2001 to 2002. Success of inoculation was judged by the development of concave lesions around inoculated areas in two tree species and by the flow of white resin from inoculated sites in *Abies*. Results 3 or 6 months after inoculation are shown in Table 1 and Fig. 1g,h. The isolated fungus was recovered from the necrotic lesions that developed.

Morphology. *Teleomorph on host* (Fig. 1c–e): Perithecia are solitary to caespitose 5–40, globose, nonpapillate, red-orange, warted, 250–380µm, stained uniformly dark red in KOH and yellow in lactic acid. Perithecial walls are composed of two layers 40–80µm thick. Asci are clavate, without apical structure, clavate, four-spored in a row, 50–80 × 6.5–11.0µm. Ascospores are hyaline to yellowish brown, narrowly ellipsoid to fusiform, equally two-celled, not constricted, finely striate, 18–28 × 6.0–8.7µm. *Anamorph on Schneider-Nirenberg agar (SNA)* (Nirenberg 1976) and *Carnation-Leaf agar (CLA)* (Fischer et al. 1982) (Fig. 1f): Microconidia are formed in false heads on short

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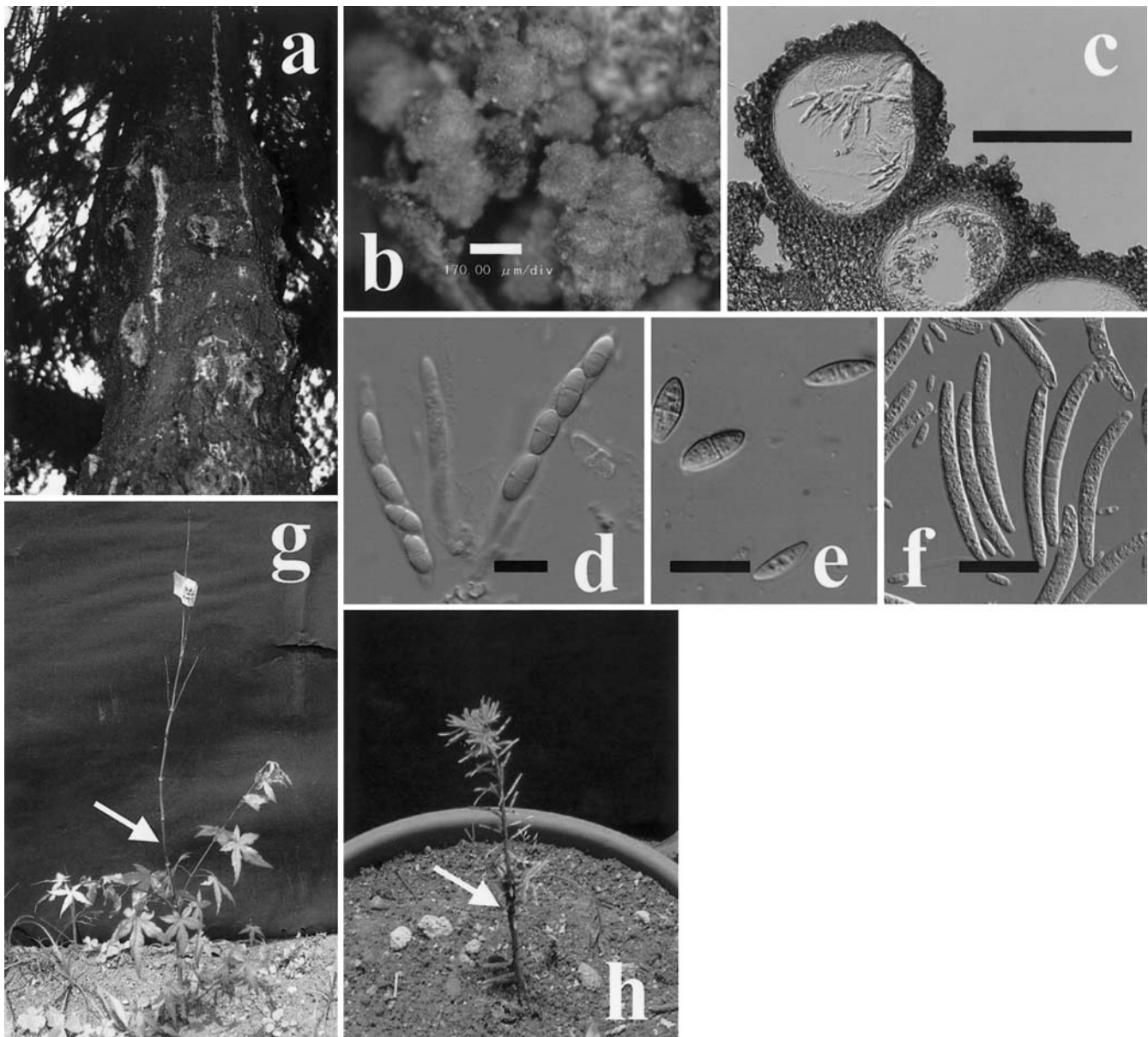


Fig. 1. Photographs and micrographs of *Neonectria castaneicola*. **a** Stem canker of *Abies veitchii*. **b** Perithecia on *A. veitchii*. **c** Vertical section of perithecia. **d** Asci having four ascospores. **e** Ascospores. **f** Macroconidia and microconidia on monoascospore isolate. **g** Dead stem of young tree of *Acer crataegifolium* inoculated with monoascospore isolate. **h** Canker with resin flow on stem of *Abies veitchii* inoculated with monoascospore isolate. **g, h** Arrows show the inoculation sites. Bars: **b** 170 µm; **c** 300 µm; **d, e** 20 µm; **f** 40 µm

Table 1. Susceptibility of *Abies veitchii* and *Acer crataegifolium* to *Neonectria castaneicola*

Inoculated plant and source of inoculum ^a	No wound		Wound without burn ^c				Burned wound ^d	
	Control ^b	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
<i>Ab. veitchii</i>								
<i>Ab. veitchii</i>	0	0	0	1	0	0	3	3
<i>Ac. crataegifolium</i>	0	0	0	0	0	0	0	2
<i>Ac. crataegifolium</i>								
<i>Ab. veitchii</i>	0	0	0	1	0	0	0	3
<i>Ac. crataegifolium</i>	0	0	0	0	0	0	0	3

^aFirst test October 18, 2001 to April 10, 2002; second test April 10, 2002 to July 16, 2002

^bLeft, number of artificially produced lesions in the first test; right, same as second test

^cWound was produced with a knife

^dBurn was made with a heated knife

conidiophores; cylindrical with rounded ends; $3\text{--}15 \times 2.5\text{--}5.0\ \mu\text{m}$ (0 septate conidia) and $15\text{--}29 \times 5\ \mu\text{m}$ (1 septate conidium). Macroconidia are formed as monophialidic on conidiophores; cylindrical with rounded ends to often slightly curved; hyaline; $63\text{--}70 \times 5\text{--}7.5\ \mu\text{m}$ (3–4 septate conidia); $60\text{--}85 \times 5\text{--}10\ \mu\text{m}$ (5–6 septate conidia); $75\text{--}92 \times 5\text{--}10\ \mu\text{m}$ (7–8 septate conidia); $80\text{--}95 \times 7.5\text{--}10\ \mu\text{m}$ (9 septate conidia).

Taxonomy. Morphological characteristics of these two nectrioid materials agree with those of *Nectria castaneicola* W. Yamam. et Oyasu [Anamorph: *Fusarium* (*Cylindrocarpon*) *castaneicola* W. Yamam., nom. inval.] described on *Castanea crenata* Sieb. et Zucc. (Yamamoto 1958, 1962; Yamamoto and Oyasu 1958; Yamamoto et al. 1957). The number of ascospores in an ascus is consistently four in both the present fungus and *N. castaneicola*. *Nectria rugulosa* Samuels et Brayford (1994), a similar species of *Nectria* having the *Cylindrocarpon*-anamorph, always has eight ascospores in an ascus. Therefore, the present fungus was identified as *Nectria castaneicola*.

Recently, the genus *Nectria* was divided into several genera based on the reaction of their perithecia to KOH or lactic acid and on their anamorph states (Brayford and Samuels 1993; Rossman et al. 1999; Samuels and Brayford 1994). Species of *Nectria* that have a *Cylindrocarpon* anamorph and perithecia that stain dark red by KOH and as yellow by lactic acid were transferred to the genus *Neonectria*. Hence, *Nectria castaneicola* has also been transferred to *Neonectria* as an independent species as follows because a species of *Neonectria* identical to this fungus was not found.

***Neonectria castaneicola* (W. Yamam. et Oyasu) Tak. Kobay. et Hirooka, comb. nov.**

Basionym: *Nectria castaneicola* W. Yamam. et Oyasu (Yamamoto 1962; Yamamoto and Oyasu 1958; Yamamoto et al. 1957).

Specimen examined: On *Abies veitchii* Lindl. (Japanese name: Shirabe): Gunma Prefecture Forestry Research Laboratory, Shinto; Kitagunma, Gunma Prefecture, August 2, 1990, by T. Kobayashi and Y. Kawashima (TFM*: FPH-7347; isolate: MAFF† 235731); September, 1996, by T. Kobayashi and C. Nomi (isolate: MAFF 237518). On *Acer crataegifolium* Sieb. et Zucc. (Japanese name: Urikaede): Kanagawa Prefecture Flower Garden, Okamoto, Kamakura, Kanagawa Prefecture July 1995, by T. Kobayashi and K. Ushiyama (isolate: MAFF 237284).

Anamorph: *Cylindrocarpon castaneicola* Tak. Kobay. et Hirooka, sp. nov. [synonym: *Fusarium* (*Cylindrocarpon*) *castaneicola* W. Yamam., nom. inval., Trans Mycol Soc Jpn 3:114, 1962].

Microconidiis et macroconidiis formatis in colonia exsotus ascosporis. Microconidiis hyalinis, cylindricis, utrinque rotundatis, 0–1 septatis, $3\text{--}29 \times 2.5\text{--}5\ \mu\text{m}$, cephaloideis in brevi conidiophoris. Macroconidiis formatis in conidiophoris brevi erectis exsotus hyphis, hyalinis, cylindricis, leniter curvatis, utrinque rotundatis, 3–9 septatis, $60\text{--}95 \times 5\text{--}10\ \mu\text{m}$, laevis.

Holotypus: colonia desiccatus in SNA exsotus ascosporis de TFM: FPH-7349 (MAFF 235731); July 3, 2004 (TFM: FPH-7649).

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* Abbreviation of herbarium

† Culture collection registered internationally