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Lecanosticta acicola, causal fungus of brown spot needle blight in *Pinus thunbergii*, new to Japan

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A needle blight disease with brown spots was found on ornamental trees of *Pinus thunbergii* in Shimane Prefecture, Japan. The causal fungus was identified as *Lecanosticta acicola*, known as causal fungus of brown spot needle blight of pines recorded in the Americas, Europe, and China. *Pinus thunbergii* was heavily infected following inoculation with the fungus in June, but only slightly infected following inoculation in September. The mycelia of the fungus were raised and produced conidial masses on potato-dextrose and Waksman agars. They grew well at 20–25°C within the range of 5 to 35°C.

Key Words——brown spot needle blight; *Lecanosticta acicola*; *Pinus thunbergii*.

Yoshii and Sogawa (1955) reported on the fungus causing brown spot needle blight (kappan-byo in Japanese) in *Pinus* spp., *Septoria acicola* (Thümen) Sacc., collected probably in Shikoku, Japan. In their abstract paper, its morphology, cultural characteristics, conidial germination, and pathogenicity were briefly noted. Full paper of their study has not been published and the detailed features of the fungus and disease are unknown. Since then, no occurrence of the disease has been reported and the disease is treated as having 'no occurrence in Japan' (The Japan Phytopathological Society, 1983).

In October 1996, a serious needle blight was found on several ornamental trees of *Pinus thunbergii* Parl. in Mitoya-cho, Shimane Prefecture, Japan. The fungus on the infected needles was identified as *Lecanosticta acicola* Thümen according to its morphological characteristics, and the disease was diagnosed as brown spot needle blight, being newly named kappan-hagare-byo in Japanese (Suto and Ougi, 1997).

The purpose of this paper is to elucidate the symptoms and signs of the disease, and the morphology, pathogenicity, and some physiological characteristics of the causal fungus, as well as to examine the causal fungus taxonomically.

Etiological Studies

Symptoms and signs From early September, small circular grayish green flecks 0.5 mm in diam appear on the distal parts of infected needles. These flecks develop into spots and bands approximately 1.5 mm in length, mostly brown in color with slightly darker margins and rarely encircled with yellow margins. These spots and bands frequently coalesce and the distal parts of the needles are killed. On the other hand, the proximal parts of the needles remain green. Infected needles droop and fall by September of the next year. If the infection is severe, whole needles are killed and drop, leaving bare branches.

Small gray spots, the stromata of the fungus, form under the epidermis of the brown spots and bands. These dark olivaceous stromata become erumpent, 0.2–0.4 × 0.1–0.3 mm in size, and open by 1–2 longitudinal slits, raising a flap of the needle tissue. Dark green masses of conidia are exuded in wedge-shaped cirri under moist conditions (Fig. 1).

Two kinds of lesions were reported on needles of *P. palustris* Mill., typical brown spot lesions and 'bar spots,' consisting of a small brown area surrounded by a yellow zone (Kais, 1975); the latter was thought to be associated with resistance (Verrall, 1934). Both types of lesions were also observed on needles of *P. thunbergii* in our observation, though typical brown spots were more common.

Morphology of the fungus Acervuli dark olivaceous, subepidermal, erumpent, elongate, basal stroma shallow to deep, composed of dark brown pseudoparenchyma (textura angularis). Conidiophores simple or branched, septate, pale brown to brown, wall smooth. Conidiogenous cells holoblastic, annellidic, pale brown, cylindrical, wall smooth. Conidia subhyaline to dark brown, verrucose, thick-walled, straight to curved, 1–5-septate, fusiform to cylindrical, 20–53 × 3.3–5.0 μm, with a round apex and truncate base (Figs. 2, 3).

Pathogenicity of the fungus To confirm the pathogenicity of the fungus, inoculation experiments were conducted on 23 June and 11 September 1997. Potted 2- and 6-yr-old seedlings of *P. densiflora* Sieb. & Zucc. and *P. thunbergii* and nursery planted 4-yr-old seedlings of *P. thunbergii* were used. Monoconidial isolate La-1 (*P. thunbergii*, Mitoya-cho, Shimane Pref., 29 Nov. 1996) was used for these experiments. A conidial suspension

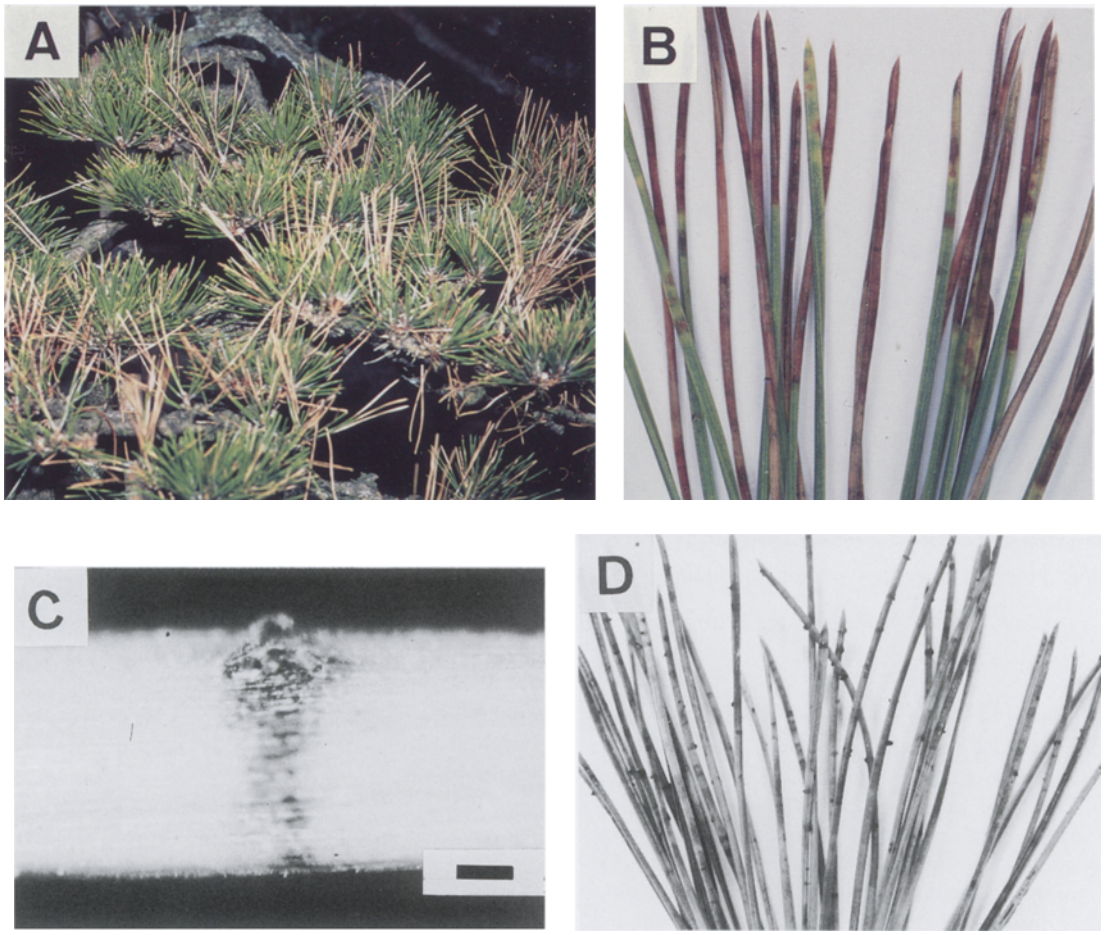

Fig. 1. Symptoms and signs of the brown spot needle blight in *Pinus thunbergii*.
A. Symptoms on *P. thunbergii* showing heavy blight. B. Brown spots and needle death in distal portion of needles. C. A stroma formed on a needle surface. D. Conidial masses exuded in wedge-shaped cirri under moist conditions. Scale bar = 1 cm.

obtained from 1-mo-old colonies on PDA (potato-dextrose agar) was used as inoculum, being sprayed onto the seedlings. As a control, sterilized distilled water was sprayed onto the seedlings instead of the inoculum suspension. Treated seedlings were each covered with polyethylene bags for 2 d, then kept in the bare ground.

The seedlings were examined 60 d after inoculation. The intensity of the infection was recorded on the current-year needles based on the following classes: 0, not infected; 0.5, a trace of infection; 1, slightly infected (one-third or less of the needles infected); 2, moderately infected (about one-half of the needles infected); 3, heavily infected (two-thirds or more of the needles infected). The infection index of each tree species and each age of seedling was then calculated according to the formula:

$$\text{Infection index} = \frac{0n_0 + 0.5n_{0.5} + 1n_1 + 2n_2 + 3n_3}{N}$$

Where N = total number of seedlings; n_0, $n_{0.5}$ $\cdots$ n_3 = number of seedlings in each infection class.

In the inoculation of late June, first symptoms occurred 20, 40, and 60 d after inoculation on 2-, 4-, and 6-yr-old seedlings of *P. thunbergii*, respectively, and 40 d on 2-yr-old seedlings of *P. densiflora*. The 2-yr-old seedlings of *P. thunbergii* were most heavily infected, 4-yr-old seedlings heavily, and 6-yr-old seedlings only slightly. By December, 9 of 12 2-yr-old seedlings and 1 of 10 4-yr-old seedlings were killed. Two-yr-old seedlings of *P. densiflora* were slightly infected, and 6-yr-old seedlings were not.

In the inoculation experiment of mid-September, the first symptom occurred 60 d after inoculation on 2-yr-old seedlings of *P. thunbergii*. The needles developing from lammas shoots were mainly infected. No symptoms of the disease were observed on 2-yr-old seedlings of *P. densiflora* and 4-yr-old seedlings of *P. thunbergii*.

The symptoms on the needles induced by inoculation with the fungus were the same as those on the naturally infected needles. Stromata of *L. acicola* were formed on the lesions 10 to 20 d after the first symptoms appeared. Production of abundant conidia was observed on the stromata under moist conditions.

All the control seedlings remained uninfected (Table 1).

As a result of these inoculation experiments, *P. thunbergii* is considered to be susceptible to *L. acicola*. Field

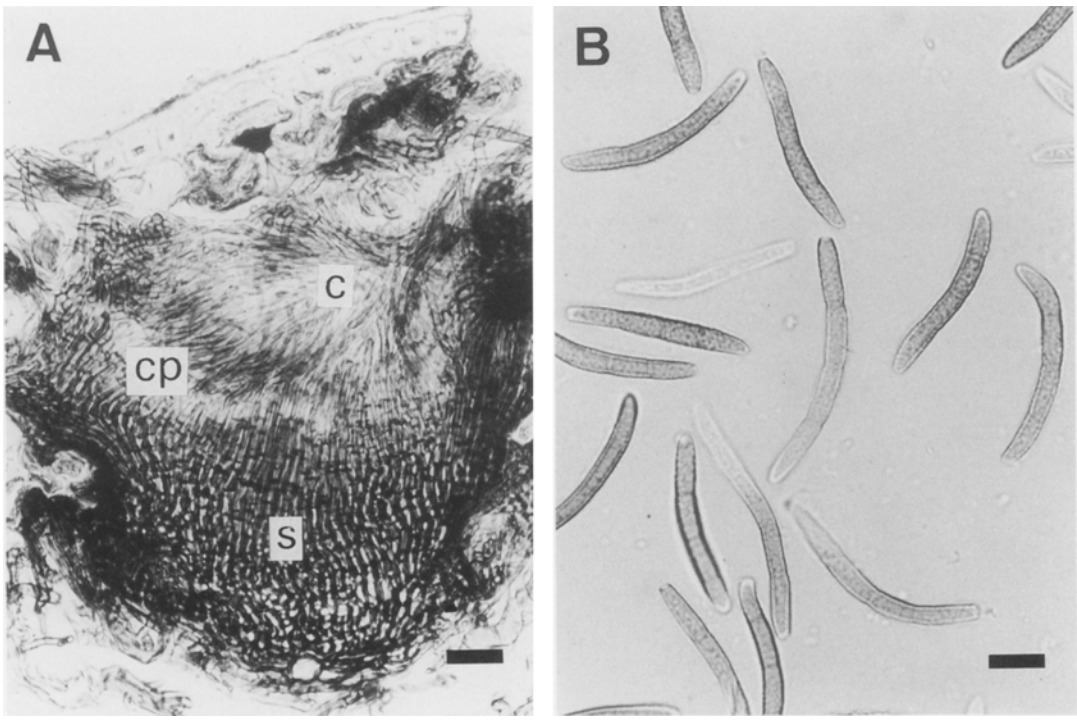

Fig. 2. *Lecanosticta acicola*.
A. Stroma (s), conidiophores (cp), and conidia (c). B. Conidia. Scale bars: A=50 μm, B=10 μm.

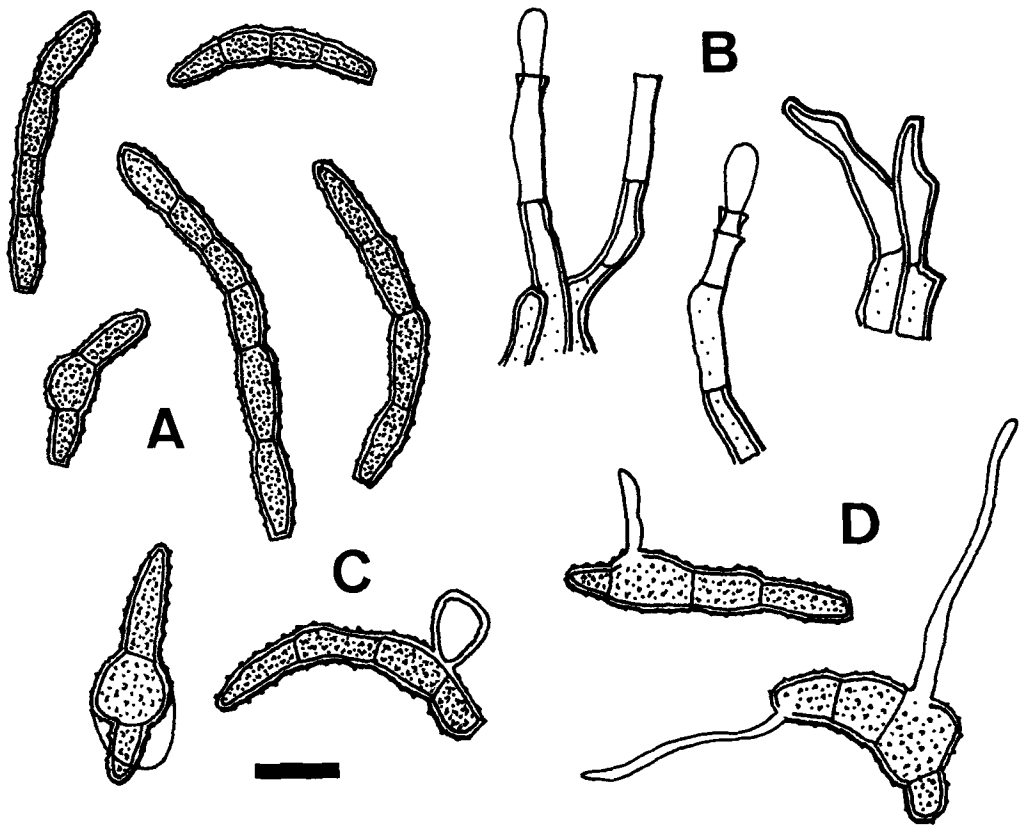

Fig. 3. Conidia, conidiophores and conidiogenous cells, and conidial germination of *Lecanosticta acicola*.
A: Conidia. B: Conidophores and conidiogenous cells. C: Endospores produced on PDA. D: Conidial germination. Scale bar: 10 μm.

observation also indicated serious infection on the ornamental trees of this pine species (Suto and Ougi, 1997). *Pinus thunbergii* was listed as a host in the southern U.S.A. (Siggers, 1944). Li et al. (1986) reported that *P. thunbergii* was as severely damaged as *P. elliottii* Engelm. and *P. taeda* L. by the brown spot needle blight in southeastern China.

In the present inoculations, the young needles of late June were susceptible to *L. acicola*, while the mature needles of mid-September were resistant. In inoculations with the fungus onto *P. palustris* (Snow, 1961; Kais, 1977), newly expanding needles were extremely susceptible to the infection.

Taxonomy of the Causal Fungus

From the morphological characteristics previously mentioned, the causal fungus seems to belong to the genus *Lecanosticta* Sydow. In the genus *Lecanosticta*, Evans (1984) recognized three species, *L. acicola*, *L. cinerea* (Dearn.) Evans, and *L. gloeospora* Evans, on needles of *Pinus* spp. The present fungus was identified as *L. acicola* by the size and the number of septa of the conidia.

Although conidial size is somewhat different among specimens, the range was within that of hitherto described sizes (Table 2).

Species epithet and synonym of the present fungus are rearranged as follows:

Lecanosticta acicola (Thümen) H. Sydow, Annls. Mycol. **22**: 400. 1924.

Basionyms: *Cryptosporium acicola* Thümen, Flora **61**: 178. 1878.

Table 1. Result of inoculation experiments with *Lecanosticta acicola* on seedlings of *Pinus densiflora* and *P. thunbergii*.

Date of inoculation	Tree species inoculated	Seedling age (yr)	Inoculation		Control	
			Number of seedlings	Infection index	Number of seedlings	Infection index
23 June 1997	*P. densiflora*	2	10	0.3	5	0
	P. densiflora	6	6	0	3	0
	P. thunbergii	2	12	3.0	5	0
	P. thunbergii	4	10	2.1	5	0
	P. thunbergii	6	6	1.1	3	0
11 Sep. 1997	*P. densiflora*	2	10	0	5	0
	P. thunbergii	2	10	0.3	5	0
	P. thunbergii	4	10	0	5	0

Table 2. Size and number of septum of conidia of *Lecanosticta* spp.

Species	Locality	Host	Size of conidia (μm)	Number of septa
Lecanosticta sp.[a]	Shimane Pref., Japan	*Pinus thunbergii* (SFH-1401)	20–44×3.3–5.0	2–4(3)
	Shimane Pref.	*P. thunbergii* (SFH-1402)	29–53×3.8–5.0	2–5(3)
	Shimane Pref.	*P. thunbergii* (SFH-1421)	18–40×2.5–3.5	1–3(3)
	Isolate La-1, on PDA		24–50×3.3–5.0	1–5(3)
	Isolate La-1, on Waksman agar		29–50×3.0–5.0	3–5(3)
L. acicola	Florida, U.S.A.[b]	*Pinus* spp.	19–32×3.5–4.5	1–3
	Louisiana, U.S.A.[c]	*P. palustris*	27–32×3.5–4	1–3
	Canada[d]	*P. contorta* var. *latifolia* and *P. banksiana*	21–44.5×2.5–3.5	(0–)3(–4)
	Central America[e]	*Pinus* spp.	(10–)12–45(–55)×2–4.5	1–5
	Germany[f]	*P. mugo*	19.2–48×2.5–5.0	0–5
	Switzerland[g]	*P. mugo* and *P. uncinata*	28–54×2.9–4.9	0–3
	China[h]	*P. elliottii*, *P. massoniana*, and *P. thunbergii*	24.5–51.0×3.4–6.3	1–6(3)
	— [i]	*Pinus* spp.	15–35×3–4	1–3
L. cinerea	Honduras[e]	*P. pseudostrobus*	(12–)14–18(–20)×(3.5–)4–5	(1–)3
L. gloeospora	Mexico[e]	*P. pseudostrobus*	(9.5–)10.5–14.5(–17)×3.5–4.5	1(–3)

a) The authors; b) Dearness (1928), as *Cryptosporium acicolum*; c) Hedgcock (1929), as *Septoria acicola*; d) Laut et al. (1966); e) Evans (1984); f) Pehl (1995); g) Holdenrieder and Sieber (1995); h) Li et al. (1986); i) Punithalingam and Gibson (1973), and Sutton (1980).

Septoria acicola (Thümen) Sacc., Syllo. Fung. **3**: 507. 1884.

Synonym: *Lecanosticta pini* H. Sydow apud Sydow & Petrak, Annls. Mycol. **20**: 211. 1922.

Specimen examined: on needles of *Pinus thunbergii* (Japanese black pine, Kuromatsu) – Mitoya, Shimane Pref., 11 Oct. 1996, R. Ouguni (SFH-1401); 5 Nov. 1996, Y. Suto and D. Ougi (SFH-1402,1403, 1404, 1405, 1406, 1407, 1408); 4 June 1997, D. Ougi and R. Ouguni (SFH-1421, 1422, 1423); 12 Dec. 1997, Y. Suto and R. Ouguni (SFH-1429, 1430, 1431). These specimens are deposited in the Herbarium of the Shimane Prefecture Forest Research Center (SFH), Shinji-cho, Yatsuka-gun, Shimane, Japan.

The present fungus has been reported from the Americas: Belize (Evans, 1984), Canada (Laut et al., 1966), Colombia (Evans, 1984), Costa Rica (Evans, 1984), Cuba (Alonso and Pérez, 1987), Guatemala (Evans, 1984), Honduras (Evans, 1984), Mexico (Evans, 1984), Nicaragua (Evans, 1984), U.S.A. (Thümen, 1978); Asia: China (Li et al., 1986); and Europe: France (Chandelier et al., 1994), Germany (Pehl, 1995), Switzerland (Holdenrieder and Sieber, 1995), Yugoslavia (Milatovic, 1976), and on a wide range of *Pinus* spp.

The teleomorph of the fungus, *Mycosphaerella dearnessii* Barr (Basionyms: *Oligostroma acicola* Dearness, *Scirrhia acicola* (Dearness) Siggers, *Systremma acicola* (Dearness) Wolf & Barbour, *Dothidea acicola* (Dearness) Morelet), and the synanamorph of the fungus, *Asteromella* sp. (Evans, 1984), were not observed on the Japanese specimens.

Physiology of the Fungus

Germination of conidia Conidial suspension was obtained from 1-mo-old colonies on PDA. The suspension was streaked over the surface of water agar plates and incubated at various temperatures. After 24 h, the germination percentage of 300 conidia and the germ tube lengths of 10 conidia were recorded. The experiment was repeated twice.

Conidia germinated between 10 and 30°C, with the optimum of 25–30°C. No conidial germination was recorded at 5 and 35°C. The frequency of conidial germination was generally low, 18.7 and 12.7%, even at 25 and 30°C, respectively. The length of the germ tubes measured 4.5–20 μm and 2.5–15 μm, at 25 and 30°C, respectively. In germination, the conidia swelled 1.5–2 times in width and produced a germ tube from each end and occasionally from the sides. The germ tube was 1 μm in width and hyaline in color (Fig. 3D).

Growth of mycelial colony Isolate La-1 was used in these experiments. Pieces of fresh mycelium were transplanted onto the center of Petri dishes containing agar media. The diameter of the colonies was measured 30 d after inoculation. Five dishes were prepared for each treatment, which was repeated twice.

Mycelial growth of the fungus was examined with six kinds of agar media at 25°C. Radial mycelial growth was rich on PDA, malt, and pine needle decoction agars, while was poor on Waksman, Richards, and Czapek agars. Colonies were raised and granulose consisting of conidial masses colored dark olivaceous, on PDA and Waksman agars, but were plane with no conidial production on malt, Richards, and Czapek agars; slight conidial production appeared on pine needle decoction agar (Table 3, Fig. 4).

Conidia produced on PDA and Waksman agar were similar to those produced on naturally infected needles, but were slightly darker and more distinctly verrucose (Table 2). A few conidia produced on the agar media swelled at one cell and formed an endospore (Evans, 1984) (Fig. 3C). Although fresh cultures of the fungus produced abundant conidia on PDA, the cultures which were transplanted several times developed a carnouse texture and produced no conidia.

The general appearance of the culture was similar to that of hitherto reported cultures (Snow, 1961; Evans, 1984; Pehl, 1995).

The mycelial growth of the fungus was examined on PDA at various temperatures. Colonies were formed at temperatures ranging from 10 to 30°C, with the optimum at 20–25°C. No mycelial growth was recorded at 5 and 35°C. When the plates of these cultures were

Table 3. Mycelial growth of *Lecanosticta acicola* on various agar media.[a)]

Agar medium	Diam of colony (cm)[b)]	Conidial production	Mycelial appearance
PDA	31	+++	raised, granulose consisting of conidial masses colored dark olivaceous; margins thin, narrow, white, entire
Malt	33	–	plane, radiately hyphal fasciate colored brownish green, centrally floccose colored black green; exudate dark brown droplets; margins thin, narrow, white, entire
Pine needle decoction	40	+	plane, thin, white spots consisting of a few conidial masses colored red gray; margins entire
Waksman	23	+++	raised, granulose consisting of conidial masses colored dark olivaceous; centrally sparse hyphae colored white; margins thin, narrow, white, entire
Richards	22	–	plane, thin, white, centrally raised with hyphal floccose colored white
Czapek	24	–	plane, thin, white, centrally raised with hyphal floccose colored gray

a) At 25°C, after 30 d.

b) Average from two replicates.

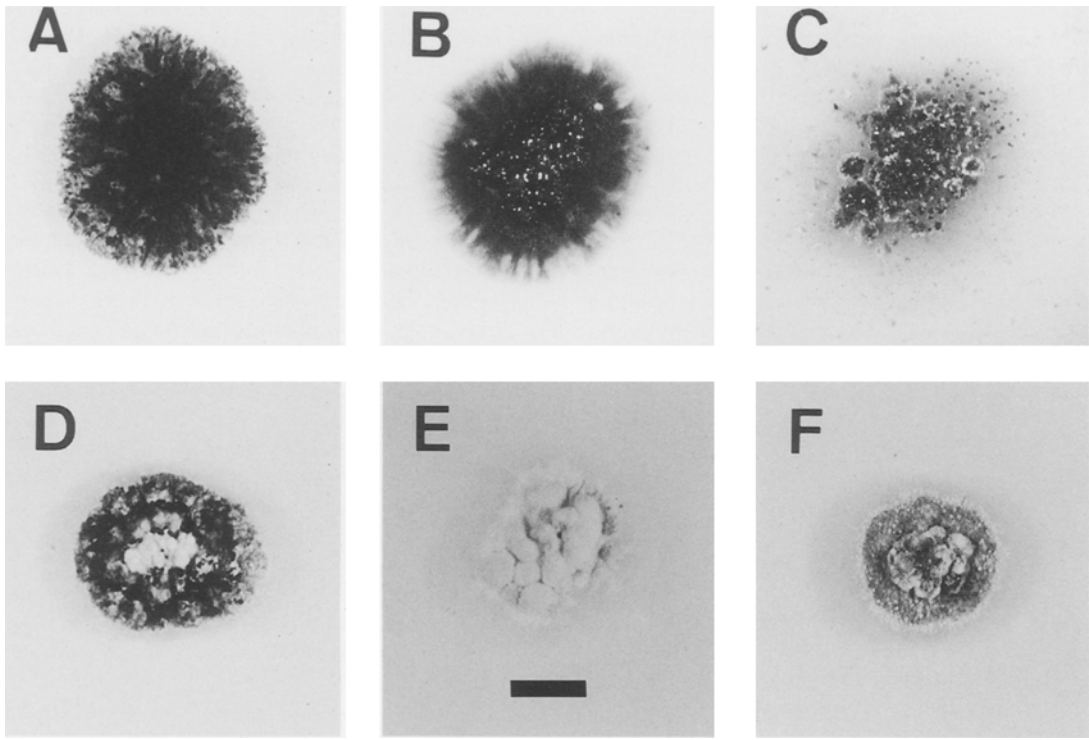

Fig. 4. Mycelial growth of *Lecanosticta acicola* on various agar media.
A: PDA. B: Malt agar. C: Pine needle decoction agar. D: Waksman agar. E: Richards agar. F: Czapek agar. At 25°C, after 30 d. Scale bar: 1 cm.

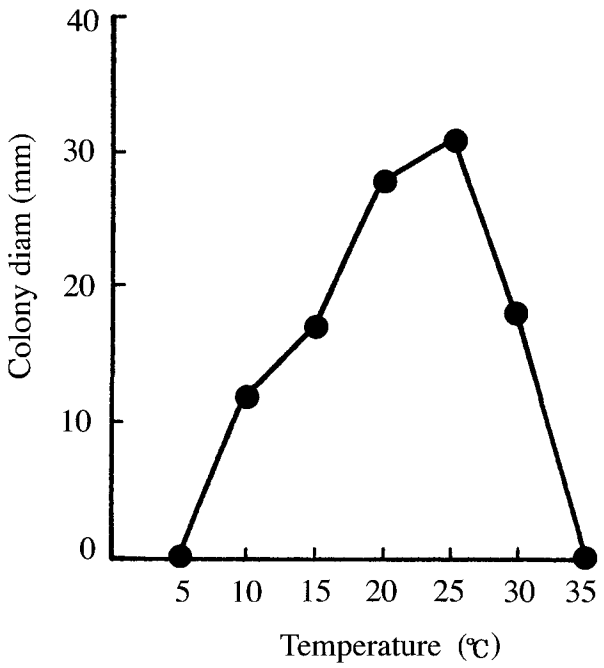

Fig. 5. Mycelial growth of *Lecanosticta acicola* at different temperatures.
On PDA, after 30 d.

transplanted to 20°C after 30 d, the colonies which had been placed at 5°C grew normally, but no growth was observed on the plates which had been placed at 35°C (Fig. 5).

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Literature cited

Alonso, R. M. and Pérez, P. 1987. Association of *Lecanosticta acicola* (*Mycosphaerella dearnessii*), *Lophodermium australe* and *Pestalozzia* sp. Rev. For. Baracoa **17**: 99–103. (In Spanish.)

Chandelier, P., Lafaurie, C. and Maugard, F. 1994. Découverte en France de *Mycosphaerella dearnessii* sur *Pinus attenuata*×*radiata*. C. R. Acad. Agric. Fr. **80**: 103–108.

Dearness, J. 1928. New and noteworthy fungi V. Mycologia **20**: 235–246.

Evans, H. C. 1984. The genus *Mycosphaerella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on pines. Mycol. Pap.**153**:1–102.

Hedgcock, G. G. 1929. *Septoria acicola* and the brown-spot disease of pine needles. Phytopathology **19**: 993–999.

Holdenrieder, O. and Sieber, T. N. 1995. First report of *Mycosphaerella dearnessii* in Switzerland. Eur. J. For. Pathol. **25**: 293–295.

Kais, A. G. 1975. Brown spot needle blight. In: Forest nursery diseases in the United States, (ed. by Peterson, G. W.

and Smith, R. S. Jr.), pp. 69–71. USDA For. Service, Washington, D. C.

Kais, A. G. 1977. Influence of needle age and inoculum spore density on susceptibility of longleaf pine to *Scirrhia acicola*. Phytopathology **67**: 686–688.

Laut, J. G., Sutton, B. C. and Lawrence, J. J. 1966. Brown spot needle blight in Canada. Plant Dis. Reptr. **50**: 208.

Li, C., Zhu, X., Han, Z., Zhang, J., Shen, B., Zhang, Z., Zheng, W., Zou, K. and Shi, F. 1986. Investigation on brown-spot needle blight of pines in China. J. Nanjing Inst. For. *1986(2): 11–18.*

Milatovic, I. 1976. Needle cast of pines caused by fungi *Scirrhia pini* Funk et Parker and *S. acicola* (Dearn.) Siggers in Yugoslavia. Poljoprivredna Znanstvena Smotra **39**: 511–513.

Pehl, L. 1995. Eine neue Kiefernkrankheit in der Bundesrepublik Deutschland. Nachrichtenbl. Deut. Pflanzenschutzd. **47**: 305–309.

Punithalingam, E. and Gibson, I. A. S. 1973. *Scirrhia acicola*. CMI Descriptions of pathogenic fungi and bacteria. No. 367. CMI, Kew, Surrey, UK.

Siggers, P. V. 1944. The brown spot needle blight of pine seedlings. U.S. Dept. Agr. Tech. Bull. No. 870.

Snow, G. A. 1961. Artificial inoculation of longleaf pine with *Scirrhia acicola*. Phytopathology **51**: 186–188.

Suto, Y. and Ougi, D. 1997. First report of the brown spot needle blight caused by *Lecanosticta acicola* on *Pinus thunbergii* in Japan. Forest Pests **46**: 206–201. (In Japanese.)

Sutton, B. C. 1980. The Coelomycetes, pp. 343–343. CMI, Kew, Surrey, UK.

The Phytopathological Society of Japan. 1983. Common names of economic plant diseases in Japan, vol. 4, 2nd ed., p. 160. Phytopathol. Soc. Japan, Tokyo. (In Japanese.)

Thümen, D. de. 1878. Fungorum americanorum triginta species novae. Flora **61**: 177–184.

Verrall, A. F. 1934. The resistance of saplings and certain seedlings on *Pinus palustris* to *Septoria acicola*. Phytopathology **24**: 1262–1264.

Yoshii, K. and Sogawa, S. 1955. Two fungal species causing brown needle blight diseases in *Pinus* spp. Ann. Phytopathol. Soc. Japan **20**: 116. (In Japanese.)