

Leaf Blight of *Ailanthus altissiman* Caused by *Phytophthora boehmeriae*

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A leaf blight disease was found on *Ailanthus altissiman* growing in the Manchon Mountain Park in Daegu city. When isolated, the causal fungus readily formed sex organs, being homothallic. Oogonia were spherical, 19.5-42.9 μm in diameter with an average of $29.4 \pm 4.2 \mu\text{m}$. Antheridia were amphigynous, round to ovoid, and measured 11.3-15.0 μm long and 12.5-14.5 μm wide. Oospores in the oogonia were spherical, 26.1-29.0 μm in diameter. Sporangia that formed in water were spherical to sub-spherical with a conspicuous papilla and measured 19.5-56.6 \times 15.6-44.9 μm with an average of $44.0 \pm 8.7 \times 32.7 \pm 6.3 \mu\text{m}$. The mean length/breadth (l/b) ratio was 1.35. Papillae were 3.9-11.7 μm high and 3.9-9.8 μm wide. Sporangia formed slowly on V8 juice agar medium when cultured under fluorescent light at 12-hour alternation. The sporangia that formed on the agar medium were more spherical and measured 26.5-39.0 \times 23.4-35.1 μm with an average of $33.6 \pm 3.4 \times 28.2 \pm 3.2 \mu\text{m}$ and l/b ratio of 1.19. Disease symptom was reproduced by artificial inoculation of the healthy plants with the isolate. The causal organism was identified as *Phytophthora boehmeriae* on the basis of its morphological characteristics.

Keywords : tree of heaven, taxonomy, forest, woody plant disease

Ailanthus altissiman Swingle (tree of heaven) is a deciduous woody plant growing wild in the mountains or near dwellings in Korea. A leaf blight disease was observed on *Ailanthus altissiman* growing in the Manchon Mountain Park in Daegu city during the rainy summer season in 1993 (Kim and Kim, 1993). Major symptoms were water-soaking and subsequent blighting of leaflets, petiolules, and rachises of pinnate leaves and young shoots of plants growing in shade under taller and more aggressive trees such as acacia (*Robinia pseudo-acacia* L.) (Fig. 1).

A species of *Phytophthora* was consistently isolated from the diseased plant parts and sporangia were even found on fresh lesions on young rachises or shoots. Sporangia first observed on infected plant tissues were globose to ovoid,

with a conspicuous papilla and a pedicel trace, and measured 40.6-46.4 \times 40.6-43.5 μm in dimension with papilla 4-6 μm high and 8-9 μm wide (Fig. 2A). When small pieces of plant tissue taken from the edge of the lesions were placed on water agar plates for pure isolation of the causal organism, mycelia grew out from the infected plant tissue to produce globose or spherical sporangia with conspicuous papilla within 24 hours. Sporangia were produced on a simple sympodial sporangiophore. Sporangia commonly observed were 35.1-42.9 \times 27.3-41.0 μm in dimension. Pure isolates were obtained by transferring the hyphal tips of the fungus to V8 juice agar or PDA plates. The fungus grew, forming white smooth colonies with some aerial mycelia. Mycelia were hyaline, coenocytic and 2.5-7.5 μm thick (Table 1).

Sex organs readily formed in a single culture prior to sporangia on agar medium. Oogonia were globose and measured 19.5-42.9 μm in diameter with an average of $29.4 \pm 4.2 \mu\text{m}$. Antheria were amphigynous, round to ovoid, and measured 11.3-15.0 μm long and 12.5-14.5 μm wide (Fig. 2E, F). Oogonia were mostly plerotic but occasionally aplerotic ones were also observed. Oospores in the oogonia were spherical and 26.1-29.0 μm in diameter. Sporangia formed abundantly on mycelial blocks 6-7 days after being placed in water. Sporangia that formed in water 7 days after immersion of mycelial pieces were spherical to sub-spherical and measured 19.5-56.6 \times 15.6-44.9 μm in range with an average of $44.0 \pm 8.7 \times 32.7 \pm 6.3 \mu\text{m}$ (Fig. 2B). The length/breadth ratio was 1.35. Papillae were 3.9-11.7 μm high and 3.9-9.8 μm wide with an average of $7.1 \times 6.6 \mu\text{m}$. Papillae of some sporangia were so big that some of them looked like beaks. Sporangia slowly formed on V8 juice agar medium also when cultured under 12-hour fluorescent light for 2 weeks in a temperature range of 20-28°C. Sporangia that formed on the agar medium were mostly spherical, 26.5-39.0 \times 23.4-35.1 μm in range, $33.6 \pm 3.4 \times 28.2 \pm 3.2 \mu\text{m}$ in average with l/b ratio 1.19 (Fig. 2C). Papillae were 4.1 μm high and 5.7 μm wide at the base. Sporangia readily released zoospore in water (Fig. 2D). In empty sporangia, openings of papillae were 4.6-6.0 μm , and trace of pedicels and septum plugs were visible.

Pathogenicity of the isolate was tested by inoculating *Ailanthus altissiman* plants growing in shade under taller

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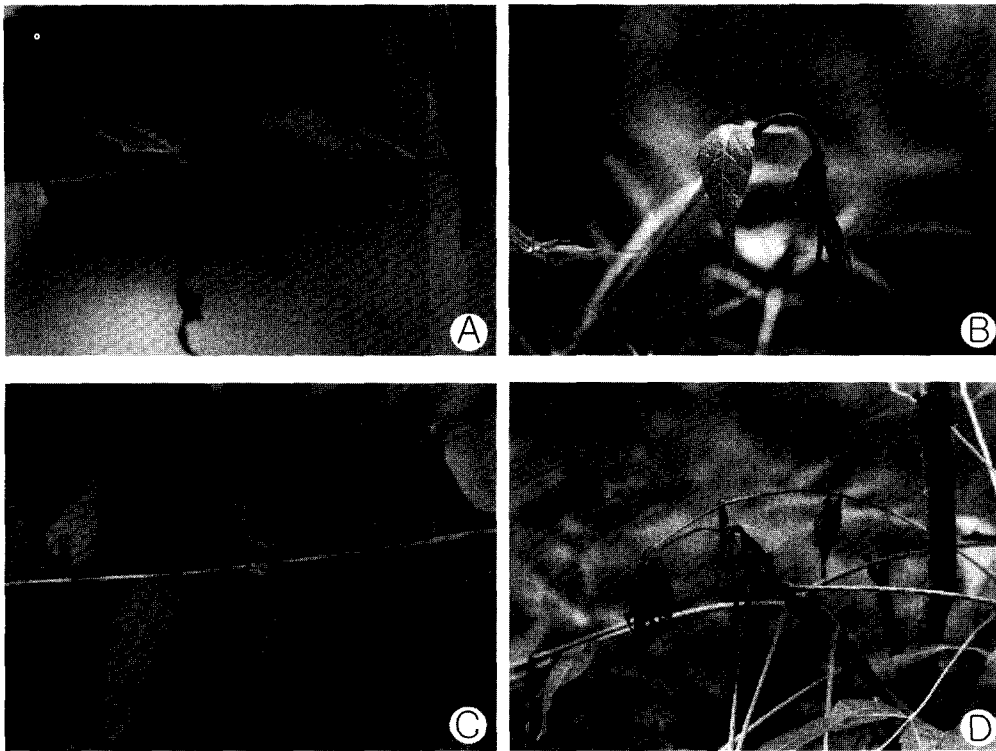


Fig. 1. Symptoms on *Ailanthus altissiman* caused by *Phytophthora boehmeriae*. (A-C) Blight of leaflets, petiolules, and rachises; (D) Shoot blight.

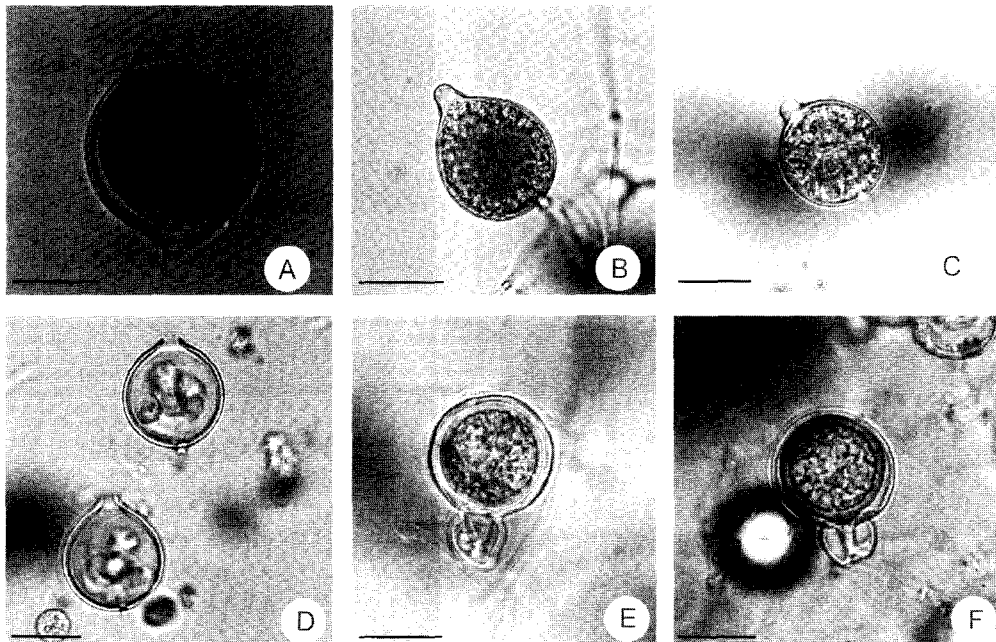


Fig. 2. (A) Sporangium taken from fresh infected tissue; (B) Sporangium formed in water; (C) Sporangium produced on V8 juice agar; (D) Sporangia formed on agar medium and indirectly germinated; (E) Oogonium and antheridium with aplerotic oospore; (F) Oogonium with more conspicuously amphigynous antheridium. Scale bars = 20 μ m.

trees in the Kyungpook National University campus with mycelial pieces with sporangia and oospores. The isolate

was initially cultured on V8 juice agar plates. Mycelial pieces with agar were cut out from the fresh mycelial mat

Table 1. Characteristics of *Phytophthora* sp. causing leaf blight of tree of heaven plants

Organ	Characteristic
Mycelium	Hyaline, coenocytic, 2.5-7.5 μm thick Maximum growth at 25°C and similar growth at 20 and 30°C as at 25°C, no growth at 35°C
Sporangium	Formed in water and on V8 juice agar medium Spherical to sub-spherical, lightly caducous Formed on simple or sympodial sporangiophores Formed in water: 19.5-56.6 \times 15.6-44.9 μm , average 44.0 \pm 8.7 \times 32.7 \pm 6.3 μm . L/b ratio 1.35, Papilla 3.9-11.7 μm , average 7.1 μm high Formed on V8 juice agar: 26.5-39.0 \times 23.4-35.1 μm , average 33.6 \pm 3.4 \times 28.2 \pm 3.2 μm . L/b ratio 1.19 Papilla 3.1-5.9 μm , average 4.1 \pm 0.7 μm high Pedicel visible, septum plug visible after release of zoospores
Oogonium	Globose, 19.5-42.9 μm with an average of 29.2 \pm 4.0 μm in diameter
Oospore	Light orange brown when mature, plerotic in oogonium Common in 26.1-29.0 μm
Antheridium	Globose to ovoid, 12.5-14.5 \times 11.3-15.0 μm

and placed in water. Seven days after immersion in water, the mycelial pieces with sporangia and oospores were cut into even smaller pieces with a scalpel. Later in the afternoon, the mixture of mycelia, sporangia, oospores, and water were placed on young fresh leaflets of *Ailanthus altissima* using a spoon. The leaves were further wetted by spraying sterile distilled water and were covered with a plastic bag as a humidity chamber for incubation. The plastic bag was removed next morning. Symptoms similar to natural infection appeared 3 days after inoculation. The same fungus was isolated again from the diseased leaves.

The isolate was identified as *Phytophthora boehmeriae* Sawada on the basis of its morphological characteristics observed according to dichotomous keys (Ho, 1992; Ho et al., 1995; Katsura, 1972; Waterhouse, 1963). Characteristics of the causal fungus matched well with *P. boehmeriae* Sawada in other types of keys (Ho, 1981; Leonian, 1934; Newhook et al., 1978; Stamps et al., 1990) and in other descriptions (Jee et al., 2000; Katsura, 1972; Waterhouse, 1970). *P. boehmeriae* was first isolated from leaves of the Chinese silk tree, ramie, *Boehmeria nivea* (L.) Gaud-Beau, by Sawada (1926) in Taiwan, and was established as a new species of *Phytophthora*. However, it has not been reported in Taiwan anymore. Hosts so far reported are *Boehmeria nivea* (L.) Gaud-Beau, *Broussonetia papyrifera* L'Her, *Cedrus deodara* (G. Don) D. Don, *Citrus sinensis* (L) Osbeck, *Eucalyptus pilularis* Sm., *Gossypium* spp., *Gossypium hirsutum* L., and *Pinus patula* Schlechtend and Cham (Erwin and Ribeiro, 1996).

To determine the optimum temperature for cultivation of the fungus, PDA plates were inoculated with mycelial plugs 5 mm in diameter cut out from the edge of an actively growing mycelial mat on PDA and incubated in six different temperature regimes (10, 15, 20, 25, 30, and 35°C). Colony diameter measured 7 days after inoculation

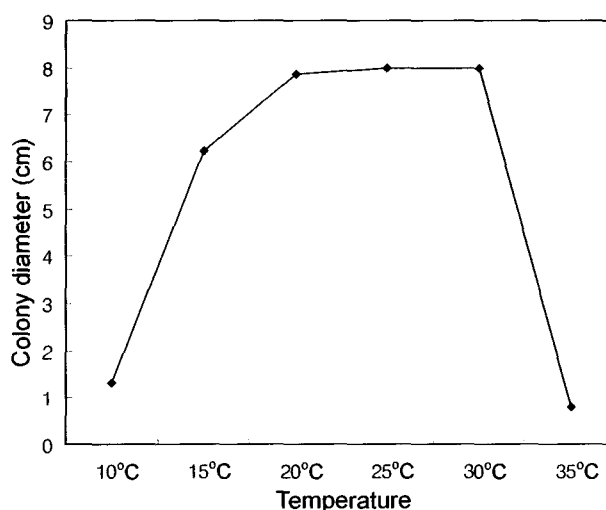


Fig. 3. Effect of temperature on mycelial growth of *Phytophthora boehmeriae* 7 days after transplanting to PDA.

is shown in Fig. 3. Mycelial growth was best at 25-30°C, but very limited at 10°C; the fungus did not grow at 35°C. The high optimum temperature for mycelial growth was consistent with the occurrence of the disease in the hot and humid summer season.

The causal fungus produced abundant oospores but very few sporangia on the agar media. Abundant sporangia formed once the mycelia were placed in water. Therefore, six different temperature regimes were tested for sporangial sporulation in distilled water and soil filtrate. Soil filtrate was prepared as follows. Fifty (50) mL of soil sampled from the mountain where the disease was found was placed in 1 liter of distilled water. The soil water was stirred well, let settle overnight, and the supernatant was filtered through a sheet of Whatman No. 2 filter paper. Mycelial plugs were cut out from marginal areas of actively growing mycelial

Table 2. Effect of temperature on oospore and sporangium formation in water and soil filtrate of *Phytophthora boehmeriae*

Spore production	10°C		15°C		20°C		25°C		30°C		35°C	
	DW ^a	SW ^a	DW	SW	DW	SW	DW	SW	DW	SW	DW	SW
Oospores	+++ ^b	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sporangia	- ^b	-	+	-	+	+	+++	+++	+++	+++	+	+

^aDW= distilled water; SW = soil filtrate.

^b'-' = none; '+' = a little; '+++ = abundant.

mats on V8 juice agar plates and placed in sterile distilled water and soil filtrate in Petri dishes. The Petri dishes with the mycelial plugs were incubated in six different temperature regimes (10, 15, 20, 25, 30, and 30°C). The results 7 days after immersion are presented in Table 2. Oospores were already formed in mycelial plugs at the time of immersion so that oospores were found in all temperature regimes. Abundant sporangia were formed at 25 and 30°C in both distilled water and soil filtrate, but only a few formed at 15, 20, and 35°C. Thus, soil filtrate was not better than distilled water in inducing sporangium formation. Sporangia did not form at 10°C at all either in water or soil filtrate. It has been reported that soil extracts or bacteria may stimulate sporangium formation in some species of *Phytophthora* (Erwin et al., 1983), but it did not happen in this experiment.

Occurrence of the disease was confined to *Ailanthus altissiman* plants growing in shade under tall trees in the hot and humid summer. The disease was never found on plants growing in sunny area. Thus, the disease was thriving on dwarfed *Ailanthus altissiman* plants in humid shade under the taller tree species. Similar symptoms were also found on *Ailanthus altissiman* plants growing in similar conditions in Sangju in Gyeongbuk province in August 2003. Thus, the disease may occur on *Ailanthus altissiman* plants growing in shade in other areas of Korea. Leaf blight caused by *Phytophthora boehmeriae* of *Ailanthus altissiman* has not been recorded so far anywhere in the world.

References

Erwin, D. C. and Ribeiro, O. K. 1996. *Phytophthora diseases*

- worldwide*. APS Press. St. Paul, Minnesota, USA.
- Erwin, D. C., Bartnicki-Garcia, S. and Tsao, P. H. 1983. *Phytophthora, its biology, taxonomy, ecology, and pathology*. APS Press, St. Paul, Minnesota, USA.
- Ho, H. H. 1981. Synoptic keys to the species of *Phytophthora*. *Mycologia* 73:705-714.
- Ho, H. H. 1992. Keys to the species of *Phytophthora* in Taiwan. *Plant Pathol. Bull. (Taiwan)* 1:104-109.
- Ho, H. H., Ahh, F. J. and Chang, H. S. 1995. *The genus Phytophthora in Taiwan*. Institute of Botany, Academia Sinica. Monograph Series 15. 86pp.
- Jee, H. J., Cho, W. D. and Kim, C. H. 2000. *Phytophthora diseases in Korea*. National Inst. Agr. Sci. Tech., RDA, Suwon, Korea.
- Katsura, K. 1972. *Phytophthora diseases of plants, Theory and Practice*. Yokendo, Tokyo, Japan.
- Kim, J. S. and Kim, B. S. 1993. *Phytophthora blight of tree of heaven caused by Phytophthora boehmeriae*. *Korean J. Plant Pathol.* 9:327 (Abstr.).
- Leonian, L. H. 1934. *Identification of Phytophthora species*. W. Va. Univ. Agric. Exp. Stn. Bull. 262. 36p.
- Newhook, F. J., Waterhouse, G. M. and Stamps, D. J. 1978. *Tabular key to the species of Phytophthora de Bary*. Mycol. Pap. 143. Commonw. Mycol. Inst. Kew, Surrey, U.K. 20p.
- Stamps, D. J., Waterhouse, G. M., Newhook, F. J. and Hall, G. S. 1990. *Revised tabular key to the species of Phytophthora*. Mycol. Pap. 162. Commonw. Agric. Bur. Int. Mycol. Inst. Kew, Surrey, U.K. p 28.
- Waterhouse, G. M. 1963. *Key to the species of Phytophthora de Bary*. Mycol. Pap. 92. Commonw. Mycol. Inst. Kew, Surrey, U.K. 22p.
- Waterhouse, G. M. 1970. *The genus Phytophthora de Bary*. Mycol. Pap. 122. Commonw. Mycol. Inst. Kew, Surrey, U.K. 104p.