Plant Pathology Bulletin 1: 153-155, 1992

Scientific Notes

A New Disease of *Cinnamomum osmophloeum* Caused by *Calonectria theae*

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Accepted for publication: September 3, 1992

Chang, T. T. 1992. A new disease of *Cinnamomum osmophloeum* caused by *Calonectria theae*. Plant Pathol. Bull. 1:153-155.

Cinnamomum osmophloeum Kaneh. is native only to Taiwan and originally grows in the broad-leaved forests at altitudes between 400 and 1200 m in central and southern Taiwan (6). Leaves, and barks of stems and roots of *C. osmophloeum* contain a high percentage of essential oils in certain clones (11) which are used for food flavor and medicine. It is potentially considered as one of the candidate species for agroforest plantations due to its high quality and quantity of essential leaf oils. Since seeds of *C. osmophloeum* are very difficult to obtain from the natural forests, cuttings are mainly used as the source of propagation for plantations. Root rot, stem rot, leaf blight (Fig. 1), and death of the cuttings were observed in the greenhouse and in the fields. The reddish ascocarps were usually found on dead tissues. The author reports here the isolation of the pathogen and its relation to the disease.

Pieces of infected rootlets (*ca.* 5 mm long), twigs (*ca.* 5 mm long) and leaves (*ca.* 3 × 3 mm) cut from the advancing margins of the tissues were placed on 10% V-8 juice agar (V8JA: 10% V-8 juice, 0.02% $CaCO_3$ and 2% Bacto agar) in petri plates and incubated at 24 C followed by surface sterilized with 0.5% NaClO for 1–2 min. Single hyphal tips obtained from the fungus on the medium were cultured and maintained on V8JA. The reddish ascocarps on the diseased tissues were surface-sterilized with 0.5% NaClO for 30 sec and cracked in a sterile mortar and pestle with a few drops of sterile distilled water. About 100 ascospores were smeared on water agar (2% Bacto agar) in a petri plate (9 cm diam.). After incubation at 24 C for 2 days, colonies originating from single ascospores were transferred onto V8JA. A fungus was consistently isolated from the diseased tissues of *C. osmophloeum* and the single ascospores.

The fungus grew and sporulated well on MEA (2% malt-extract and 2% Bacto agar) and V8JA. Aerial mycelium was white to orange. Colonies were white to yellowish orange (Fig. 2). Ascocarps were solitary or in small clusters, superficial on substrate, without a stroma, firmly attached to substrate. Ascocarps were orange to scarlet, turning red to blood color in KOH, globose to ovoid, 300–580 μm high and 300–440 μm diam., without papilla. Ascospores were 50–100 × 4–4.85 μm, narrowly fusiform with rounded ends, often curved or sigmoid,

Fig. 1. Leaf blight of *Cinnamomum osmophloeum* cutting.

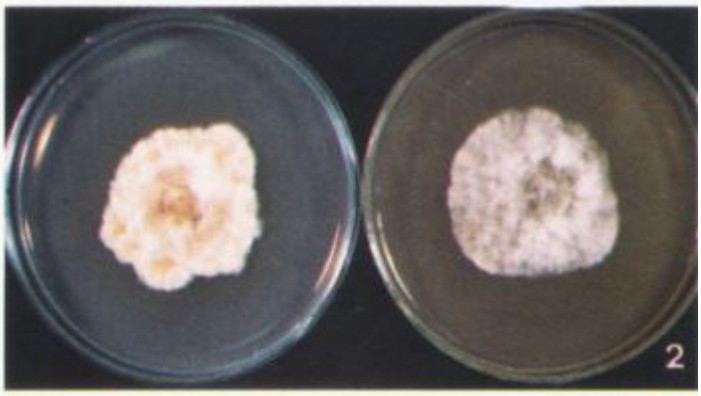

Fig. 2. Culture of *Calonectria theae* on MEA (left) and V8JA (right).

mostly 3 septate, often slightly constricted at each septum, hyaline, smooth, multiseriate (Fig. 3). Asci were unitunicate, 65–130 × 17–30 μm, broadly obovate to clavate, eight-spored, without specialized apical discharge mechanism (Fig. 4). Conidiophores were developed from both aerial and submerged hyphae, long-stalked with penicillate branching at the apex to form a head of conidiogenous cell; each head was with on to three long stalks measuring 60–280 × 2–10 μm; each was bearing an apical vesicle (Fig. 5). Conidiogenous cells were considered as phialdic but only one conidium formed on each conidiogenous cell, monoblastic, integrated, solitary, terminal, without collarette or flared opening. Conidia were cylindric, hyaline, smooth mostly 3 septate with a size of 40–120 × 4–8 μm (Fig. 6). Apical vesicle was narrowly clavate to broadly clavate or globose with a diameter of 7–9 μm (Fig. 5). Vegetative hyphae were hyaline to pale luteous, septate, smooth to slightly rounded with a diameter of 2–6 μm. Chlamydospores were forming in chains of 2–10 cells; cells measuring 5–15 μm in diameter were globose, filled with guttules, walls luteous. The fungus was identified as *Calonectria theae* Loos (anamorph: *Cylindrocladium theae* (Petch) Alfieri & Sob.) (8). The single ascospore isolates and isolates obtained from diseased tissues were able to produce sexual structures on MEA and V8JA indicating that the fungus is homothallic.

To determine the effect of temperature on the growth of the fungus, agar discs (5 mm diam.) cut with a sterile cork borer from the periphery of a colony were each on V8JA in a petri plate. Cultures were incubated at 10, 15, 20, 25, 30 or 35 C in darkness. Colonies were measured one week after inoculation. Three petri plates were used for each treatment and the experiment was done twice. Optimum temperature for fungal growth was 20–30 C (Fig. 7). Temperature above 35 C completely inhibited the fungal growth.

The conidial suspension for pathogenicity tests was obtained by adding ten ml sterile distilled water to a two-week-old culture growing on V8JA. The conidial suspension used as inoculum was adjusted to 10^4 to 10^5 conidia/ml with a microliter syinge method (5). Ten milliliters of conidial suspension was added to the soil around the roots of a cutting or the conidial suspension was sprayed on the foliage of cuttings. Controls were inoculated with sterile distilled water. The inoculated cuttings were covered with plastic bags for two days in order to keep high moisture. Five cuttings (about six-month-old) were used for each treatment. The experiment was repeated once. Five days after inoculation, leaf blight started to appear on the leaves which the inoculum was sprayed on the foliage of cuttings. Stem lesion was present on both the foliage and the root inoculated cuttings two weeks after inoculation. One month after inoculation, the root inoculated cuttings killed due to root rot and stem lesion were 60% in the first test and 80% in the second test. The same fungus was reisolated from all diseased tissues of artificially inoculated cuttings. All control cuttings remained healthy during the experiments.

It was originally reported that *C. theae* caused a severe disease on tea in Sri Lanka (9). Subsequently, *C. theae* was also reported to cause a severe tea disease in Ceylon (3) and India (10). In addition, it also caused disease on other tree species such as *Medicago sativa* Linn. and *Eucalyptus* spp. (4,7). It has been pointed out that the disease incidence on tea caused by *C. theae* was enhanced by wet conditions (8). This result shows that disease of *C. osmophloeum* in Taiwan is also caused by *C. theae*. The pathogen and disease were first observed in Taiwan and the disease has become common on cuttings of *C. osmophloeum* in the greenhouse and in the fields in

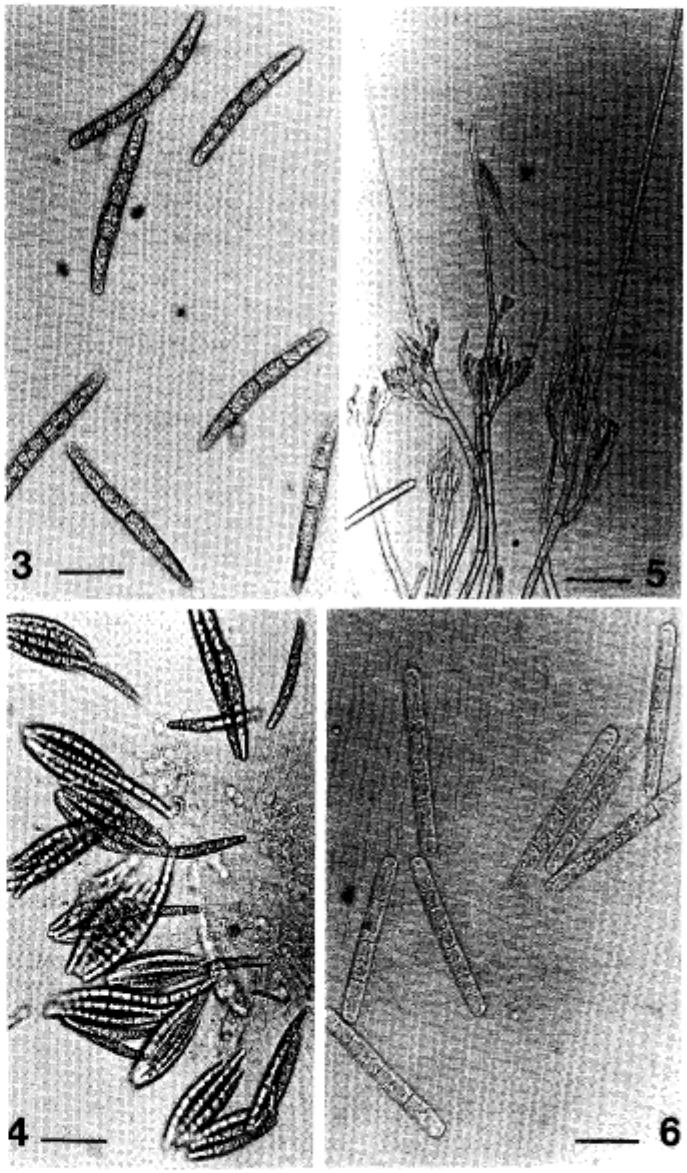

Fig. 3. Ascospores of *Calonectria theae*. Bar = 15 μm.
Fig. 4. Eight ascospores in each ascus of *Calonectria theae*. Bar = 30 μm.
Fig. 5. Penicillate-like branches on the top of conidiophores and apical vesicles of *Calonectria theae*. Bar = 30 μm.
Fig. 6. Conidia with three septates of *Calonectria theae*. Bar = 15 μm.

Taiwan. *Calonectria* and its anamorph *Cylindrocladium* are classified as soil-borne fungi usually causing diseases on the woody plants. It has been reported that *Calonectria kyotensis* Terashita and *Cylindrocladium clavatum* Hodges & May have caused black rot and leaf blight of *Cinnamomum kanehirai* Hay. cuttings in Taiwan, respectively (1,2).

Key words: *Cinnamomum osmophloeum, Calonectria theae.*

ACKNOWLEDGEMENT

This work was supported by a grant (81 Nung Chien-12.2-Lin-15 (12)) from the Council of Agriculture, Executive Yuan, Republic of China.

LITERATURE CITED

1. Chang, T. T. 1991. Black rot of *Cinnamomum kanehirai* cuttings caused by *Calonectria kyotensis.* Q. J. Chin. For. 24:111-120 (In Chinese).
2. Chang, T. T. 1992. Two new diseases on *Cinnamomum kanehirai* cuttings. Bull. Taiwan For. Res. Inst. New Series 7 (In press, in Chinese).
3. Gadd, C. H. 1927. Report of the mycologist for 1926. Bulletin, Tea Research Institute of Ceylon 2:7-18.
4. Hiremath, R. V., and Anahosur, K. H. 1976. Root and crown rot of *Medicago sativa.* Plant Dis. Reptr 60:71-72.
5. Ko, W. H., Chase, L., and Kunimoto, R. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63:1206-1207.
6. Liu, Y. C., Lu, F. Y., and Ou, C. H. 1988. Tree of Taiwan. Monographic Publication No.7. College of Agriculture, National Chung-shing University, 1019pp. (In Chinese).
7. Mohanan, C., and Sharma, J. K. 1986. Epidemiology of *Cylindrocladium* diseases of *Eucalyptus. in Eucalyptus* in India. Past, Present and Future. Proceedings of the national seminar held at Kerala Forest Research Institute. Kerala, India, 285pp.
8. Peerally, A. 1991. The classification and phytopathology of *Cylindrocladium* species. Mycotaxon 40:323-366.
9. Petch, T. 1921. Report of the Botanist/Mycologist. Tropical Agriculturist 57:318-319.
10. Venata Ram, C. S. 1962. Report of the Plant Pathologist for 1960-1961. Report, Tea Scientific Department, Planters' Association of Southern India, pp 31-45.
11. Yin, H. W. 1991. Yield and composition variation of essential oils from leaves of different *Cinnamomum osmophloeum* Kanehira clones in Taiwan. Q. J. Chin. For. 24:83-104. (In Chinese).

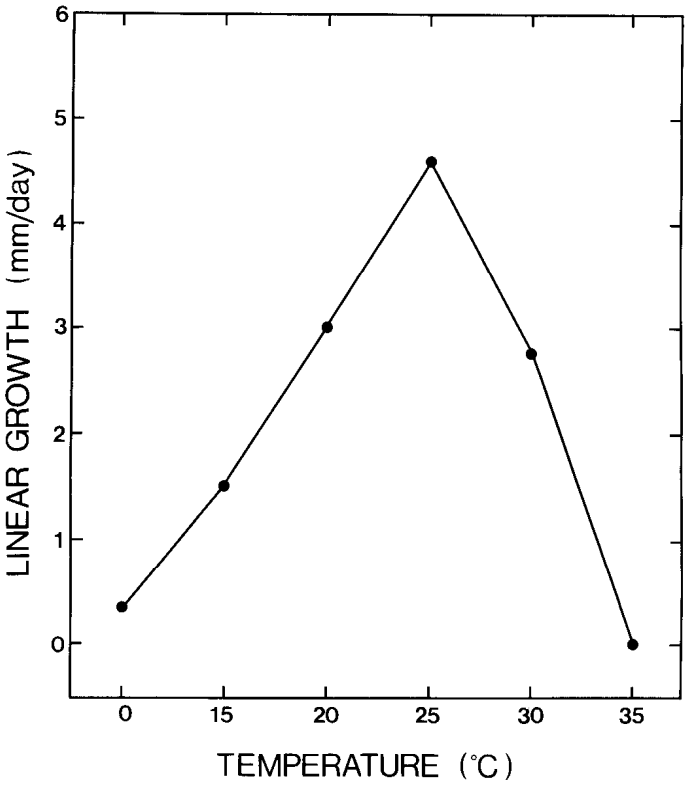

Fig. 7. Linear growth of *Calonectria theae* on V8JA.

摘　　要

張東柱. 1992. *Calonectria theae*引起土肉桂一種新病害. 植病會刊 1:153-155. (台北市　台灣省林業試驗所森林保護系)

在溫室及田間，土肉桂(*Cinnamomum osmophloeum*)扦插苗普遍存在根腐、莖腐及葉枯的現象，因而常導致全株死亡。受害組織常有紅色子囊果出現。經組織及單子囊孢子分離均可獲得 *Calonectria theae*之菌株。將 *C. theae*的分生孢子接種於土肉桂扦插苗的根部及地上部，可出現與自然界相同的病徵；且由人工接種之病組織分離，也可再獲得相同的病原菌。本病害與病原菌在台灣首次發現。

關鍵字：土肉桂、*Calonectria theae*。