

台湾における *Macrophoma theicola* による茶樹の胴枯病

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著者名	Chen, J.S. Thseng, F.M. Ko, W.H.
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Twig Die-Back of Tea Caused by *Macrophoma theicola* in Taiwan*

Jee-song CHEN**, Fang-ming TSENG** and Wen-hsiung KO***

Abstract

Dead twigs of unknown cause standing among healthy twigs with normal green leaves in the same bush of tea have been observed frequently in Taiwan since 1955. *Macrophoma theicola* was isolated from 102 of 104 diseased twigs collected from various cultivars at different locations. Three weeks after inoculation with wheat-oat grains colonized by *M. theicola*, more than 86% of inoculated twigs showed die-back symptoms similar to those observed in nature. *Macrophoma theicola* was reisolated from all of the experimentally infected twigs. All control twigs remained healthy during the experiment. Twigs inoculated with *M. theicola* grown on potato dextrose agar failed to show any disease symptoms. *Macrophoma theicola* thrived at relatively high temperature and endured low water potential. The fungus was able to grow about 0.3 cm linearly in 2 days at 38 C and in 5 days at -71 bars.

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Key words : twig die-back, tea, *Macrophoma theicola*, Taiwan.

Introduction

Tea (*Thea sinensis* L.) is an important agricultural crop in Taiwan. Its young shoots and tender leaves are dried and prepared for both export and local use to make the popular oriental beverage of ancient origin. In 1955 twig die-back of tea was noticed in central and northern Taiwan. The disease, which is most serious during the dry summer, caused the death of more than 40% of tea bushes in certain orchards^{1,2}. A similar disease has also been reported in Japan^{2,3,5}. Since 1959, a number of plant pathologists have attempted to determine the cause of the disease without success^{2,12}. Numerous fungi including *Colletotrichum* sp., *Pestalotia* sp., *Nectria* sp., *Phomopsis* sp., *Macrophoma* sp. and *Botryodiplodia* sp. have been isolated from diseased tissues, but none of them caused the same disease in pathogenicity tests^{2,12}. We report here evidence suggesting that *Macrophoma theicola* Petch is the causal organism of twig die-back of tea in Taiwan. We also studied the effects of temperature and osmotic water potential on the growth of the pathogen.

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** Taiwan Tea Experiment Station, Yangmei, Taoyuan, Taiwan 326, R. O. C.

*** Department of Plant Pathology, University of Hawaii, Beaumont Agriculture Research Center, Hilo, Hawaii 96720, USA.

Materials and Methods

Isolation. Sections (about 5-7 mm diam. and 5-10 mm long) of infected twigs obtained from the advancing margin were washed with water containing a small amount of detergent to remove dusts, surface-sterilized by dipping in 75% ethanol for 2-3 min and 1% NaClO for 10 min, and rinsed thrice with sterile distilled water. Surface-sterilized twig sections were cut into small pieces (about 3×3×7 mm) and plated on 2% water agar.

Pathogenicity tests. The wheat-oat medium which rendered *Kretzschmaria clavus* very effective in causing disease on branches and seedling stems of macadamia^{6,7)} was used to grow *Macrophoma theicola* (isolate No. 46) for pathogenicity tests. The fungus was grown on wheat-oat medium (10 g whole wheat grains, 10 g whole oat grains and 10 ml distilled water) for 7 days at 28 C. Tea twigs (2-3 or 5-7 mm diam.) of cv. Chinhsin Oolong were surface-disinfected with 75% ethanol and scraped gently to remove the epidermis from bark tissues. Four colonized grains were placed on the scraped portion of the twig, wrapped with Parafilm, and secured with vinyl tape. Twigs similarly inoculated with autoclaved grains were used as controls. To determine the time required for infection of the host by the pathogen, the inoculum was removed after incubation for 3, 6, 9, 12, 15 and 21 days. Data were recorded 12, 15, 18 and 21 days after removal of the inoculum. Each treatment consisted of 18-30 replicates. The pathogenicity tests were done three times.

Effect of temperature and water potential. *Macrophoma theicola* was grown on potato dextrose agar (PDA) at 28 C for 2 days. Agar discs (6 mm diam.) cut from the periphery of the colony with a sterile cork borer were used to inoculate agar plates. To determine the effect of temperature on growth of the pathogen, inoculated PDA plates were incubated at 12, 16, 20, 24, 28, 32, 34, 36 and 38 C, and colonies were measured after 2 days. To determine the effect of water potential on the growth of the pathogen, various amounts of KCl were added to a basal medium consisting of 0.75 g of Na₂HPO₄, 0.75 g of KH₂PO₄, 0.12 g of NaCl, 0.4 g of NH₄NO₃, 1.8 g of glucose, 0.1 g of yeast extract, 1.0 g of malt extract, and 15 g of agar per 1,000 ml of distilled water^{10,11)}. Inoculated plates of basal medium with theoretical water potentials of -1, -9, -22, -35, -53, -71 and -90 bars were incubated at 24 and 32 C. Colonies were measured after 5 days. Five replicates were used for each treatment and experiments were done three times.

Results

Symptoms

The common sight of tea twig die-back observed in the field is one or more dead twigs with or without brown leaves standing among living twigs with normal green leaves in the same bush. In an early stage of the disease development, leaves of the affected young shoot turn light green and lose vigor. These are followed by browning and with-

Table 1. Relation between inoculation time and development of tea twig die-back caused by *Macrophoma theicola* (isolate No. 46)

Inoculation time (days)	Disease incidence (%) after			
	12	15	18	21 (days)
3	0	0	0	0
6	10	10	40	42
9	20	40	79	82
12	35	45	80	85
15	40	50	84	89
21	40	55	85	85

ering of the whole shoots and abscission of dead leaves. Browning frequently occurs on veins before the other parts of leaves turn brown. During the dry hot season, a shoot may appear light green in the morning, but drooping by noon. Affected branches turn brown and die gradually downwards. It may be several years before the disease reaches the crown and kills the whole bush. In some cases the disease appears as cankers with elongated dark patches on the wood of branches surrounded by callus.

Pathogenicity

In 1983, a fungus identified as *Macrophoma theicola* Petch^{8,9)} by Dr. R. A. Samson of Centraalbureau voor Schimmelcultures was isolated from 46 of 48 diseased tea twigs on 12 different cultivars at 15 different tea production areas on the island of Taiwan. In 1984, *M. theicola* was isolated from all 56 diseased tea twigs on 6 cultivars at 17 locations.

Three weeks after inoculation with wheat and oat grains colonized by *M. theicola*, 26 of 30 inoculated twigs showed symptoms similar to those observed in nature. *Macrophoma theicola* was reisolated from all the experimentally infected twigs. The 30 control twigs remained healthy during the experiment. Twenty nine tea twigs similarly inoculated with *M. theicola* grown on potato dextrose agar also failed to show any disease symptoms.

When inoculum was removed at different time intervals, it was found that more than 3 days were needed for successful infection of *M. theicola* on tea twigs (Table 1). None of the inoculated twigs developed die-back symptoms when the inoculum was removed on the third day. The disease incidence increased with increasing time of inoculation to a maximum at about 9-12 days.

Effect of temperature and water potential on mycelial growth

Macrophoma theicola appeared to thrive at relatively high temperature (Fig. 1). The optimum temperature for the growth of this fungus was 28 to 34 C. Even at 38 C the fungus was able to grow about 0.3 cm linearly in two days. The fungus did not grow at 12 C.

Macrophoma theicola was able to grow on basal medium at relatively low water potential caused by addition of KCl (Fig. 2). The fungus showed sparse growth at -90 bars and grew about 0.3 cm linearly at -71 bars in 5 days at 32 and 24 C. The optimum water potential for growth of *M. theicola* was -1 bar at 32 C and -9 bars at 24 C.

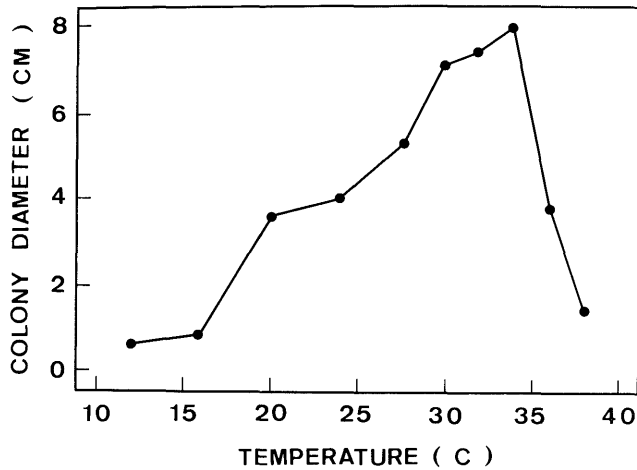


Fig. 1. Growth of *Macrophoma theicola* (isolate No. 46) on potato dextrose agar for 2 days at different temperatures.

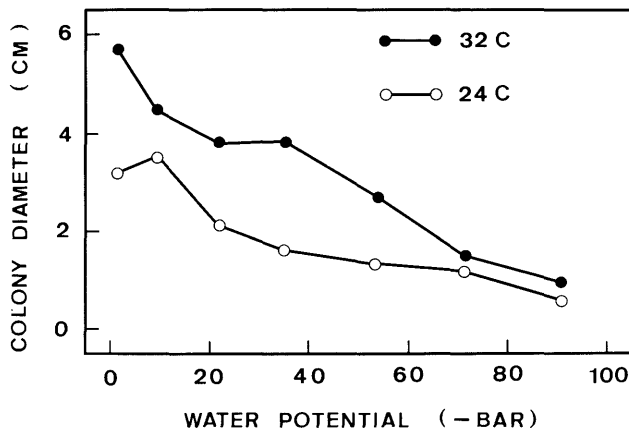


Fig. 2. Growth of *Macrophoma theicola* (isolate No. 46) on basal medium at different water potentials caused by addition of KCl at 24 and 32°C for 5 days.

Discussion

Numerous unsuccessful attempts have been made by plant pathologists in Taiwan and Japan to determine the cause of twig die-back of tea since 1957^{2,12)}. In this study, *M. theicola* which was consistently isolated from tea twigs with die-back symptoms was found to be responsible for inciting the disease. Upon inoculation of healthy twigs of tea, *M. theicola* caused symptoms of die-back similar to those occurring in nature, and the fungus was reisolated from all experimentally infected twigs.

Although *M. theicola* has been considered to be the causal agent of twig die-back of tea in Japan since 1931¹⁾, previous attempts to prove pathogenicity with *Macrophoma* sp., presumably *M. theicola*, isolated from diseased tissues had not been successful^{2,12)}. This was probably due to the usage of unsuitable media for growing the fungus. Our results showed that *M. theicola* was pathogenic to tea plants when grown in wheat-oat medium, but not when grown in potato dextrose agar. Virulence of several plant pathogenic fungi, including *Rhizoctonia solani* and *Pythium ultimum*, also has been shown to be affected by exogenous nutrients^{4,14)}. The substance in the wheat-oat medium which

is responsible for the virulence of *M. theicola* remains to be determined.

The observation that twig die-back of tea was most serious during the dry summer appears to result from the combination of the ability of *M. theicola* to tolerate high temperature and low water potential, and the stress suffered by tea plants under hot and dry conditions which may render the host more susceptible to the pathogen.

Macrophoma theicola has been reported to cause mainly cankers of tea twigs in other regions¹³⁾. This may be due to the differences in environmental conditions and varieties of tea plants existing in those areas.

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和 文 摘 要

Jee-song CHEN · Fang-ming THSENG · Wen-hsiung Ko : 台湾における *Macrophoma theicola* による茶樹の胴枯病

台湾では1955年以来、茶樹において健全枝に混じって原因不明の枯死枝がしばしば観察されてきた。異なる産地の種々の品種から収集した104本の罹病枝のうち102本から *M. theicola* が分離された。コムギーエンバク培地で生育させた *M. theicola* を接種した場合、3週間後には86%以上の接種枝で自然病徴と類似の胴枯症状が再現され、またすべての感染枝から *M. theicola* が再分離された。オートクレーブした菌および PDA 培地で生育させた同菌を接種した場合にはいずれも無病徴で健全のままであった。*M. theicola* は比較的高温下で生育良好であり、また低い水ポテンシャルでも生育可能で、38Cおよび-71パールではそれぞれ2日および5日で約0.3cmの菌糸の伸長が認められた。