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Root rot of macadamia caused by *Ganoderma lucidum* and *Kretzschmaria clavus in Taiwan*

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Abstract : About 10% of macadamia trees at Puli showed various degrees of decline due to extensive root decay. The decay also extended to the main trunk and eventually caused the death of tree s. Infected tissues of most diseased trees turned soft. This was found to be caused by *Ganoderma lucidum*. Other declining trees were resulted from invasion by *Kretzschmaria clavus* which formed black lines on infected tissues. *Ganoderma lucidum* grew faster and appeared to thrive at relatively high temperature in compars-ion with *K. clavus*.

Key words: Tree decline, macada mia, root rot, *Ganoderma lucidum*, *Kretzschmaria clavus*.

Introduction

Slow decline of macadamia trees (*Macadamia integrifolia* Maiden & Betche) indicated by dieback of branches (Fig. 1-A) was noticed at puli in central Taiwan in 1982. About 80 out of 840 macadamia trees in the orchard showed various degrees of decline. Similar problem was reported in Hawaii in 1973 (7). The disease in Hawaii was originally thought to be due to deficiency of certain essential elements, but was subsequently found to be mainly due to root rot caused by *Kretzschmaria clavus* (Fr.) Sacc. of Xylariaceae (5). We report here the factors causing the macadamia decline in Taiwan.

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Materials and Methods

Isolation

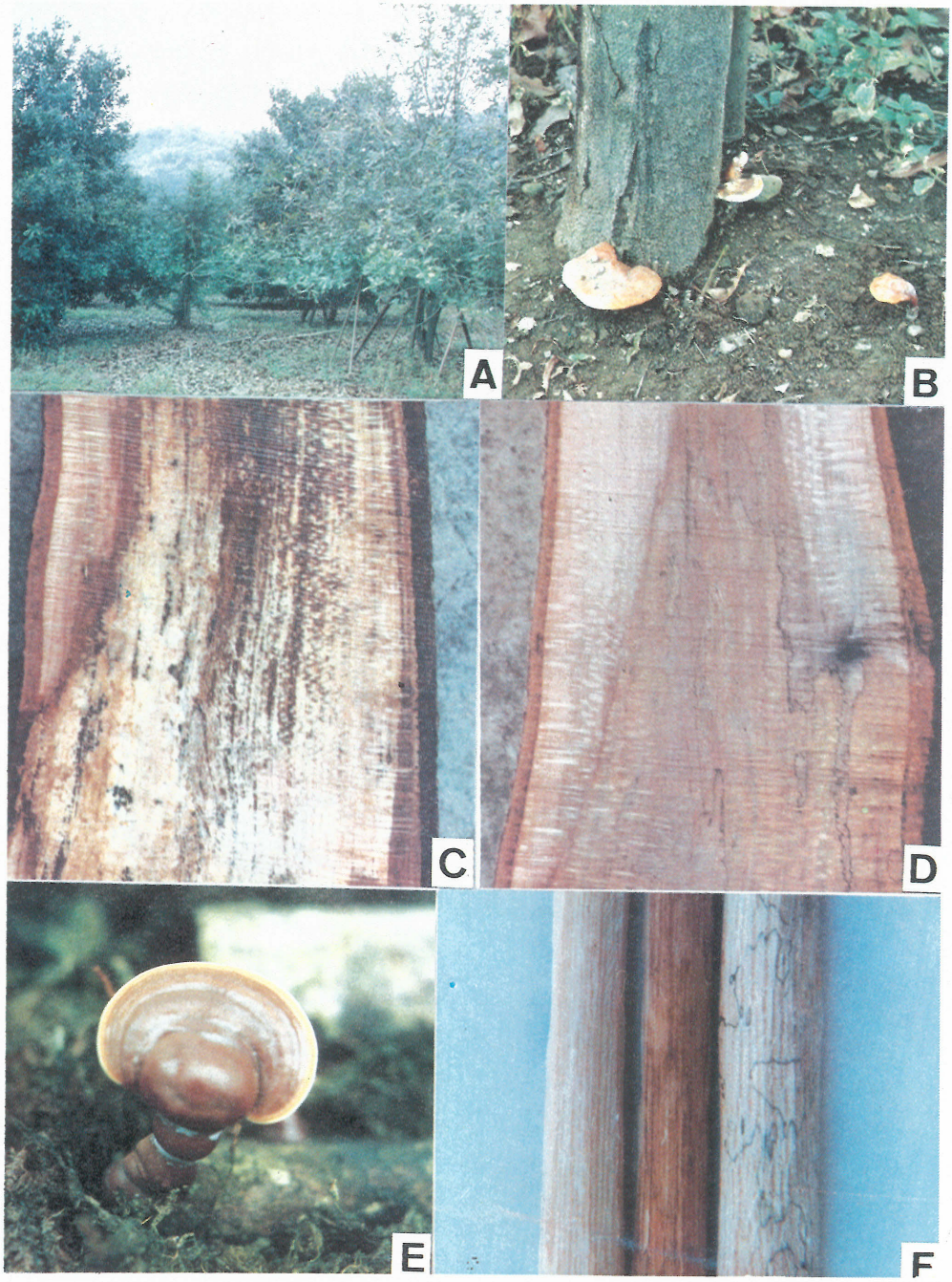

Fig. 1. Root rot of macadamia. (A). Unthrifty appearance of root rot affected tree (right). (B). Fruiting bodies of *Ganoderma lucidum* from infected root and trunk of macadamia tree. (C). Symptoms on macadami atrunk infected by *G. lucidum*. Note the whitis h and dark brown appearance in infected area. (D). Symptoms on macadamia trunk infected by *Kretzschmaria clavus*. Note the black lines ini nfected area. (E). A fruiting body of *G. lucidum* produced on an autoclaved litchi stem section. (F). Macadamia stems artificially infected with *G. lucidum* (center) and *K. clavus* (right). Stem on left is a control.

Discolored trunk tissue samples (ca. 5×2×1mm) taken immediately adjacent to healthy tissues were surface-sterilized with 0.5% sodium hypochloride solution for 3 min and placed on potato dextrose agar (PDA). Six pieces of tissues were placed on each plate and incubated at 24 C. The fungus growing from the diseased tissues were transferred to water agar (2% Bacto agar). Single hyphal tips obtained from the fungus growing on water agar were cultured and maintained on PDA.

Pathogenicity tests

The wheat-oat medium which rendered *K. clavus* very effective in causing disease on branches and seedling stems of macadamia (4, 6) was used to grow isolated fungi for pathogenicity tests. The fungi were grown on wheat-oat medium (10g whole wheat grains, 10g whole oat grains and 10ml distilled water) for 1 month at 24C. Macadamia branches (10-15mm diam.) were surface disinfected with 75% ethanol and scraped gently to remove the epidermis from bark tissues. About 10g of colonized grains were placed on the scraped portion of the branch, wrapped with transparent polyethylene plastic sheet and secured with vinyl tape. Scraped branches similarly i noculated with autoclaved grains were used as controls. Each treatment consisted of 25 branches from 5 macadamia trees. Data were recorded 3 and 6 months after inoculation.

Pboductuon of fruiting bodies

The method developed by Ko (2) was used for production of peritheical stromata by *K. clavus* and basidiocarps by *Ganoderma lucidum* (W. Curt. ex Fr.) Karst. Autoclaved sections of macadamia or litchi branches (ca. 10cm long and 3-4cm in diam.) in glass jars were inoculated with culture of *K. clavus* or *G. lucidum*. After incubation for 1-2 months at 24C, colonized tissues were placed on natural soil in plastic containers (24×18×5cm) and maintained moist by placing moistened sphagnum moss around them. Plastic containers were placed in the greenhouse and watered daily.

Gbowth of fungi

G. lucidum and *K. clavus* were grown on PDA for 3-5 days at 24C. Agar discs (5 mm in diam.) cut from the periphery of the colonies with a sterile cork borer were used to inoculate plates. To determine the effect of temperature on growth of fungi, inoculated PDA plates were incubated at 10, 15, 20, 25, 30 and 35C in darkness. Colonies were measured daily until the fungus reached the edge of the plate or 10 days after inoculation. Three plates were used for each treatment and the experiment was done two times.

Results

Symptoms

The disease is evidenced by slow decline, indicated by fading and dropping of leaves from branches (Fig. 1-A). The infected trees died eventually. About 80% of the diseased macadamia trees were associated with fruiting bodies produced by the fungus identified as *G. lucidum* of the Polyporaceae by T. T. Chang. Brown basidiocaps with stipe appeared on trunk near the soil line or on the ground near the trunk of a diseased tree (Fig. 1-B). Most of roots were decayed and the decay extended into the main

trunk. The color of infected tissue changed from light brown to dark brown as the disease progressed. Eventually the texture of the diseased tissue became soft and the color turned whitish (Fig. 1-C).

The other 20% of the declining macadamia trees were not associated with fungal fruiting bodies. However, most of the roots of these trees were also decayed and the decay extended into the main trunk in the later stage of the disease development. The disseased tissue turned brown, showed distinct black lines, and remained firm and hard (Fig. 1-D) similar to that caused by *K. clavus* (5).

Pathogenicity tests

A fungus producing colony which appeared whitish at the begining and turned yellowish later on PDA, was consistently isolated from tissues of diseased plants associated with fungal fruiting bodies. The fungus was identified as *G. lucidum* because it produced fruiting bodies indistinguishable from those produced by *G. lucidum* (Fig. 1-E) on colonized litchi and macadamia branches after incubation on soil for 1 month. The percentage of macadamia branches killed by *G. lucidum* was 40 and 80% after inoculation for 3 and 6 months, respectively (Table 1). Infected tissues turned brown (Fig. 1-F) just like those observed in nature. *G. lucidum* was reisolated from all the artificially infected branches. Control branches remained healthy. The fungus also did not infect branches without wounding.

Table 1. Pathogenicity of *Ganoderma lucidum* and *Kretzschmaria clavus* on macadamia branches.

Fungus	Disease incidence (%) after			
	3 months		6 months	
	Inoculated	Control	Inoculated	Control
G. lucidum	40	0	80	0
K. clavus	20	0	60	0

The fungus consistently isolated from diseased tissues with black lines was very slow in growth. Colonies produced by the fungus on PDA were grayish black on the surface behind the white advancing margin. The fungus developed blackish sterile stromatic aggregates below the surface causing the colonies to crack frequently. These characteristics were similar to those expressed by *K. clavus* (5). The fungus produced relatively large effused carbonaceous stromata on colonized branches of litchi and macadamia after incubation on soil for 2 months. The fungus was identified as *K. clavus* because these perithecial stromata were indistinguishable from hypoxyoid stromata produced by *K. clavus* (3). The percentage of macadamia branches killed by *K. clavus* was 20 and 60% after inoculation for 3 and 6 months, respectively (Table 1). The fungus produced black lines on the infected tissues similar to those observed in nature. *K. clavus* was reisolated from all artificially infected branches. The fungus also did not infect branches without wounding.

Growth of fungi

The growth rate of *G. lucidum* was much higher than that of *K. clavus* at 25C, *G. lucidum* grew 8.6mm/day, while *K. clavus* grew only 1.7mm/day (Fig. 2). *G. lucidum* appeared to thrive at relatively high temperature in comparison with *K. clavus*. The optimum temperatures for *G. lucidum* and *K. clavus* were 25-30C and 20-25C, respectively. The minimum temperature for both fungi was about 10C. *K. clavus* was not able to grow at 30C. However, even at 35C *G. lucidum* grew about 3 mm/day.

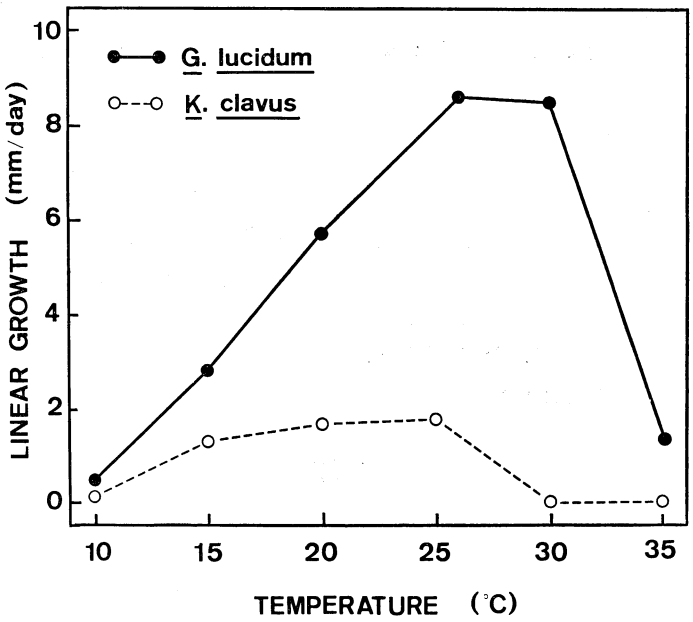

Fig. 2. Growth rate of *Ganoderma lucidum* and *Kretzschmaria clavus* on potato dextrose agar.

Discussion

Our data show that root rot caused by *G. lucidum* is the major cause of decline of macadamia trees in central Taiwan. Only about 20% of the declining macadamia trees in this area were caused by *K. clavus* root rot. This is quite different from that occurring in Hawaii where *K. clavus* root rot is the major cause of macadamia decline. A macadamia tree infected by both *G. lucidum* and *K. clavus* had not been observed at Puli.

G. lucidum has a relatively wide range of temperature suitable for its growth. The fungus also has a very broad geographical distribution (1). Therefore, *G. lucidum* root rot may occur frequently when macadamia cultivation is further expanded world wide in the future.

In Hawaii, *K. clavus* also attacked trees in the forest by the macadamia orchard with the decline problem (6). This appeared to be the main source of inoculum. The sources of inoculum of *G. lucidum* and *K. clavus* for macadamia orchard at Puli are still unknown.

Acknowledgments

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Ganoderma lucidum 及 *Kretzschmaria clavus* 在臺灣引起之澳洲胡桃根腐病[1]

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摘　要

在埔里地區，由於根腐病之蔓延，約有10%之澳洲胡桃顯現不同程度之枯萎。而且這種組織腐敗擴展至主幹，最後引起樹木死亡。

大部份染病的澳洲胡桃組織軟化，證實系由靈芝 *Ganoderma lucidum* 感染所致。另有部份衰敗的植株，則因 *Kretzschmaria clavus* 侵入之故，病菌並在木材上形成黑色線紋，但組織並不軟化。與*K. clavus*相較，*G. lucidum*生長較快速，且偏好高溫。

1.臺灣省農業試驗所　研究報告第 1436 號及夏威夷熱帶農業暨人力資源研究所期刊第 3261 號。

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