Relation of *Kretzschmaria clavus* to Hypoxyloid Stromata on Diseased Macadamia Tissues

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ABSTRACT

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Hypoxyloid stromata were observed frequently on macadamia tissues infected by *Kretzschmaria clavus*. Ascospores from hypoxyloid stromata were similar in shape and size to those from typical *K. clavus* stromata. Colonies from ascospores derived from hypoxyloid stromata were similar to colonies from ascospores derived from kretzschmarioid stromata. Isolates from typical kretzschmarioid stromata and isolates from hypoxyloid stromata produced conidia similar in shape and size on sterilized stem tissues. Anastomoses occurred between hyphae derived from propagules from these two types of stromata. Isolates from kretzschmarioid stromata or from hypoxyloid stromata produced both kretzschmarioid and hypoxyloid stromata on litchi stem tissues. Results strongly suggest that the hypoxyloid stromata on diseased macadamia tissues are morphological variants of the more familiar *K. clavus* stromata.

Additional key words: *Ustulina deusta*, *Macadamia integrifolia*.

Root rot caused by *Kretzschmaria clavus* (Fr.) Sacc. (Xylariaceae, Ascomycetes) is the most serious disease of macadamia (*Macadamia integrifolia* Maiden & Betche) in Hawaii (4). The fungus attacks roots initially and invades the main trunk in the latter stages of disease development. Infected trees usually first appear unthrifty, then lose their foliage and die. Small stipitate carbonaceous stromata of *K. clavus* are produced on the surface of diseased roots and trunks. Frequently, diseased tissues are also covered with relatively large effused carbonaceous stromata which were identified as *Hypoxylon deustum* (Hoffm. ex St. Amans) Grev. [= *Ustulina deusta* (Hoffm. ex St. Amans) Petrak] by J. A. von Arx. Presence of both types of fruiting bodies on the same diseased tissues has also been observed.

We report here the nature of the hypoxyloid stromata observed on the diseased macadamia tissues.

MATERIALS AND METHODS

Isolation. Both kretzschmarioid and hypoxyloid stromata produced on diseased macadamia tissues were collected from the field. Ascospores were obtained by crushing the brittle stromata in water with a glass rod. The spore suspension was filtered through two layers of cheesecloth and centrifuged at 1,000 *g* for 5 min. After the supernatant was drained, spores were surface sterilized with 0.8% sodium hypochlorite solution for 5 min, rinsed three times with sterile distilled water by centrifugation, and plated on acidified potato-dextrose agar. Single-colony isolations were made after incubation at 24 C for 7 days.

Anastomosis. Highly purified agarose (1) was used for anastomosis tests because mycelia of the test organisms on nutrient agar or water agar were too dense to trace their origins. Six pieces (approximately 2 × 2 × 1 mm) of agar culture obtained from the colony margin of the kretzschmarioid type isolate were placed 8 mm apart near the center of a 0.6% water agar (SeaKem HGT-P Agarose; Marine Colloid, Rockland, ME 04841) plate. They were paired with six pieces of agar culture from the hypoxyloid isolate about 8 mm away. For those isolates with poor growth on agarose medium, a piece (approximately 4 × 2 × 1.5 mm) of V-8 agar was placed 2 mm from the inoculum to stimulate mycelial growth. Plates were observed for anastomosis under the microscope at ×400 after 5–10 days of incubation at 24 C. Two plates were used for each combination and the experiments were replicated twice.

Production of fruiting bodies. The method described by Ko (3) was used to induce formation of perithecial stromata by both the kretzschmarioid and hypoxyloid isolates. Sections of litchi (*Litchi chinensis* Sonn.) branches (about 5–9 cm long and 4–6 cm in diameter) in glass jars (9 × 15 cm) containing 30 ml of distilled water were autoclaved for 15 min and inoculated with agar culture of either isolate. After incubation for 1–2 mo at 24 C, colonized tissues were placed on natural soil in pots and kept moist by placing moistened sphagnum moss around them. The pots were covered with black polyethylene sheets, placed in the greenhouse, and watered daily. For production of conidia, sterilized sections of litchi or hibiscus (*Hibiscus rosa-sinensis* L.) branches (approximately 15 × 60 mm) in test tubes (25 × 150 mm) containing 2 ml of distilled water were inoculated with an isolate of either type and incubated at 24 C.

RESULTS AND DISCUSSION

Stromata collected from diseased macadamia tissues were the source of ascospores for measurements and for obtaining cultures. Ascospores from hypoxyloid stromata were similar in shape and size to those from typical kretzschmarioid stromata. Both were medium to dark brown and inequilaterally ellipsoidal to fusoid measuring 29–44 × 7–10 μm (kretzschmarioid) or 33–47 × 7–10 μm (hypoxyloid). On potato-dextrose agar colonies from ascospores from hypoxyloid stromata were similar to colonies from kretzschmarioid stromata. Both were grayish-black with white margin, compact, and tough; both developed black sterile stromatic aggregations below the surface. These characteristics were similar to those described for *H. deustum* by Jong and Rogers (2). Based on colony morphology, cultures from both typical kretzschmarioid stromata and hypoxyloid stromata were identified as *H. deustum* by S. C. Jong.

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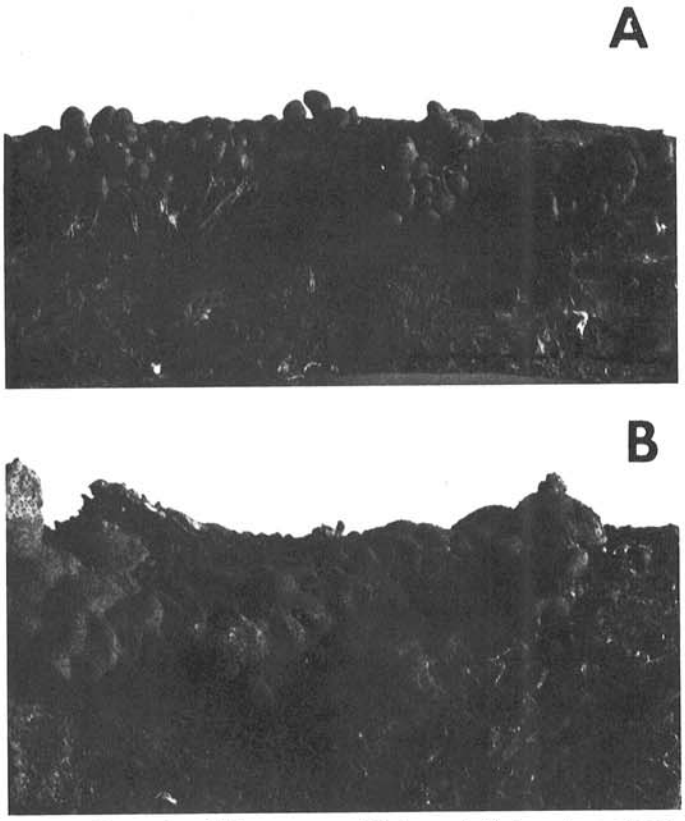

Fig. 1. A, Kretzschmarioid stromata and **B,** hypoxyloid stromata produced by *Kretzschmaria clavus*.

Isolates from neither kretzschmarioid stromata nor hypoxyloid stromata produced conidia or perithecial stromata on various nutrient media tested. These included potato-dextrose agar with or without addition of 0.5% yeast extract, V-8 agar, malt extract agar, and lima bean agar. However, some conidia were produced in pure cultures on litchi and hibiscus tissues after incubation for 3 mo. Conidia derived from cultures of kretzschmarioid isolates and hypoxyloid isolates were similar in shape and size. Both types were hyaline, ovate to elliptical and 5–11 × 3–4 μm (kretzschmarioid) or 6–11 × 3–4 μm (hypoxyloid).

It was observed recently that mycelia from the same colony derived from either kretzschmarioid stromata or hypoxyloid stromata fused readily. Since successful anastomosis can be considered as evidence that these two mycelia represent the same species (5), ability to form anastomoses between these two types were tested using two isolates from each stromatal type. In addition to anastomosis between isolates of the same type, hyphae of kretzschmarioid isolates formed anastomoses with hyphae of hypoxyloid isolates, thus confirming that these two types of stromata were produced by the same species.

When colonized litchi stem sections were incubated on natural soil for 5–8 mo, both kretzschmarioid and hypoxyloid isolates produced perithecial stromata. Kretzschmaroid isolates produced small kretzschmarioid stromata first and hypoxyloid stromata later on the same or different sections (Fig. 1). Hypoxyloid isolates produced mainly large effused hypoxyloid stromata, and occasionally small kretzschmarioid stromata.

Our results strongly suggest that on diseased macadamia tissues *K. clavus* produces stromata of both the hypoxyloid and kretzschmarioid types. *K. clavus* and *H. deustum* are closely related (6). Whether they are conspecific remains to be determined. *H. deustum* is encountered commonly in temperate regions on a wide variety of angiospermous hosts and also has been widely reported from the subtropics and tropics, along with *Ustulina zonata* (Lev.) Sacc., *U. brasiliensis* Speg., and some other taxa suspected of being variants of *H. deustum* (J. B. Rogers, *personal communication*). Thus, the relationships among these fungi, and with *K. clavus*, are complex and not likely to be elucidated rapidly. For the present, we consider our fungus to be well encompassed by the current concept of *K. clavus*.

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