Infection and Colonization of Grapevines by *Ganoderma lucidum*

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ABSTRACT

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Isolates of *Ganoderma lucidum* were shown to be pathogenic to *Vitis champini* 'Dog Ridge' in greenhouse studies and to *V. vinifera* 'Robin' in field studies. One-year-old grapevines growing in the greenhouse were inoculated with the fungus by placing infested wood blocks adjacent to wounded or unwounded roots. Reisolations after 24 mo from the inoculated grapevines yielded only *G. lucidum* from the dead and declining plants. The fungus initially invaded the heartwood and in later stages infected the sapwood. Six infected plants developed symptoms of leaf wilting, yellowing, and dying, similar to symptoms reported for naturally infected grapevines. Control grapevines remained healthy with no wood decay, and *G. lucidum* was not isolated from these plants. When grape wood chips infested with the fungus were placed in trunk tissue of 17-yr-old grapevines growing in the field, white-rot decay columns, limited to the heartwood, extended to 42 cm after 17 mo.

Northern Mexico has about 40,000 ha of commercial vineyards in the states of Sonora, Durango, Coahuila, and Baja California. In 1979, a report from northern Mexico implicated but did not prove that a *Ganoderma* species was the cause of a disease of grapevines (*Vitis vinifera* L.) called *colapso* (12). Symptoms reported by Teliz (12) included yellowing and wilting of leaves and collapse of vines within 3 days of onset of symptoms. Fruiting bodies of the fungus were found on the trunks of collapsed grapevines and also on adjacent trellis posts. Inoculum for the disease was considered to be mycelium growing from the infested posts. *Ganoderma lucidum* (W. Curt.: Fr.) Karst. is known to be indigenous to the Sonoran Desert region, occurring on mesquite and palo verde (3). The fungus is also known to cause a white-rot wood decay of many species of trees in North America and in the tropics including oak, sycamore, honeylocust, ash, sassafras, maple, white mulberry, *Dalbergia* sp., *Acacia* sp., and *Casuarina* sp. (1,2,4–6,10).

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The objectives of this research were to determine if *G. lucidum* was a pathogen of grape and to follow the development of the fungus from infection to establishment and colonization of the host.

MATERIALS AND METHODS

Greenhouse studies. *V. champini* Planchon softwood cuttings of the rootstock cultivar Dog Ridge were made following the technique of Winkler et al (15). Forty-eight cuttings were planted in sand without root hormone and supplied with bottom-heating (23–26 C). Cuttings were misted automatically for 30 sec every 90 sec for 12 hr a day. After 2 wk, cuttings were transplanted into 8-L pots containing a peat-sand-loam (1:1:2 ratio) pasteurized mix and watered two to four times per week throughout the 2-yr study. Summer temperatures were 32–35 C (day) and 24–27 C (night), and winter temperatures were 24–27 C (day) and 21–24 C (night). Grape plants were pruned and fertilized with Osmocote 19-6-12 (Sierra Chemical Co., Milpitas, CA) four times per year. The greenhouse was fumigated with insecticides periodically, but no fungicides were used.

Basidiocarps of *G. lucidum* were collected from a variety of hosts in Arizona, Ohio, and Louisiana. Isolates were obtained from basidiocarp context tissue and grown on 2% malt extract agar (MEA).

Sterilized silverleaf oak (*Quercus hypoleucoides* A. Camus) wood blocks (about 6 × 6 × 10 cm) that had been colonized by *G. lucidum* were used for root inoculations. The colonized wood blocks were placed about 1 cm below the soil surface within 1–2 cm of the grapevine stem. Each 1-yr-old plant was inoculated with one wood block. Cuttings were divided into wounded and unwounded treatments. Vines for the wounded treatment were injured with a grafting knife by exposing 2 cm of xylem tissue in a tangential cut of the stem 4 cm below the soil surface on the side facing the inoculum block. Controls consisted of grapevines with noninfested wood blocks and grapevines without wood blocks. All treatments were replicated six times during the 2-yr experiment. At the end of 24 mo, the plants were harvested, cut in half longitudinally, and reisolations attempted. For this, several pieces of grapevine wood were plated onto MEA. Each reisolation was repeated twice.

Field studies. Fifteen 17-yr-old cultivar Robin grapevines at the University of Arizona Campbell Ave. Field Station, Tucson, were either inoculated with *G. lucidum* or maintained as controls. The vines were about 15 cm in base diameter, 125 cm tall, and were grown on a "T" trellis system. Inoculum consisted of sterilized wood chips infested with one of two isolates of *G. lucidum* from Arizona or Louisiana. Inoculations were accomplished by drilling a 1-cm-diameter hole to the center of the heartwood at the base of each trunk and inserting the wood-chip inoculum into each cavity, which was then plugged. Five dormant grapevines were inoculated in January and another five in March. Five actively growing grapevines were inoculated in June. Controls were three uninoculated vines that were not drilled and vines inoculated with noninfested wood chips. Two years after the first inoculations, grapevines (roots and trunks) were removed from the field and cut into transverse sections 10–14 cm long. The sections were cut in half longitudinally, decayed tissue was measured, and samples were plated on MEA.

RESULTS

Identification of cultures of *G. lucidum*. Isolates of *G. lucidum* had cultural characteristics as described by Nobles (8). Chlamydospores, cuticular cells, staghorn hyphae, and clamp connections were readily identified in culture.

Infection and development of *G. lucidum* in vines of *V. champini* in the greenhouse. Fruiting bodies of *G. lucidum* developed on the soil surface from belowground wood-block inoculum 3 mo after inoculation; most fruiting occurred within 16 mo. Single fruiting bodies developed from 24 of the 36 infested wood-block inocula. Fruiting bodies typically were stipitate (3–6 cm) with an eccentric pileus (2–4 cm). Occasionally, aberrant fruiting bodies developed with tubes on the upper surface.

Initial disease symptoms appeared 12–18 mo after inoculation. Leaves wilted, turned yellow, and shoot development was retarded after pruning. After 24 mo, four of the 12 inoculated cultivar Dog Ridge plants in the wound-inoculation treatment were infected. One of these plants was dead, and two fruiting bodies of *G. lucidum* developed on the stem. Only one plant was infected in the unwounded inoculation treatment. The

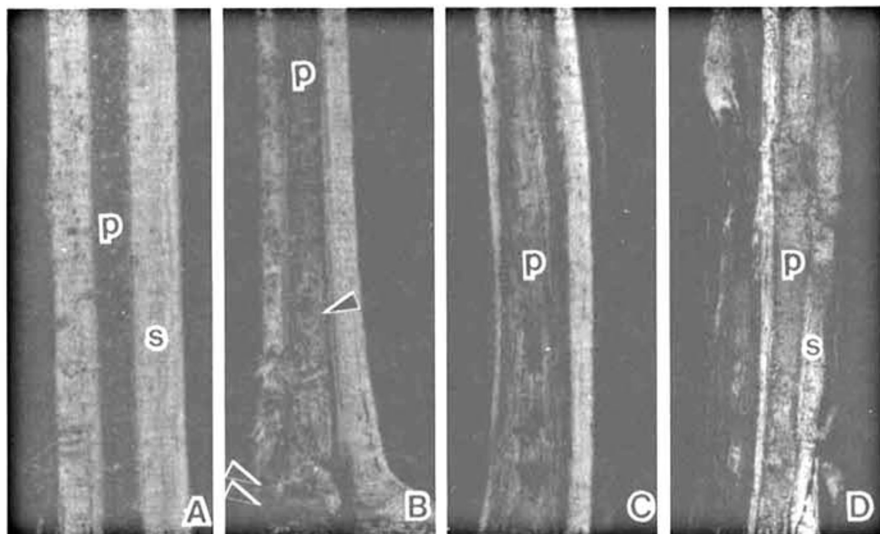

Fig. 1. Stages of decay in the development of *Ganoderma lucidum* after 24 mo within 3-yr-old cultivar Dog Ridge grapevines grown and inoculated in the greenhouse. Longitudinal sections of grapevine stems 1.0–1.5 cm in diameter are shown. **(A)** Section of control grapevine stem with dark pith (p) and lighter colored sapwood (s). **(B)** Early stage of decay limited to the pith (arrow) and entering at wound-callus area (double arrow), upper pith (p) uninfected. **(C)** Pith (p) completely decayed as white rot. **(D)** Sapwood (s) and pith (p) at an advanced stage of decay.

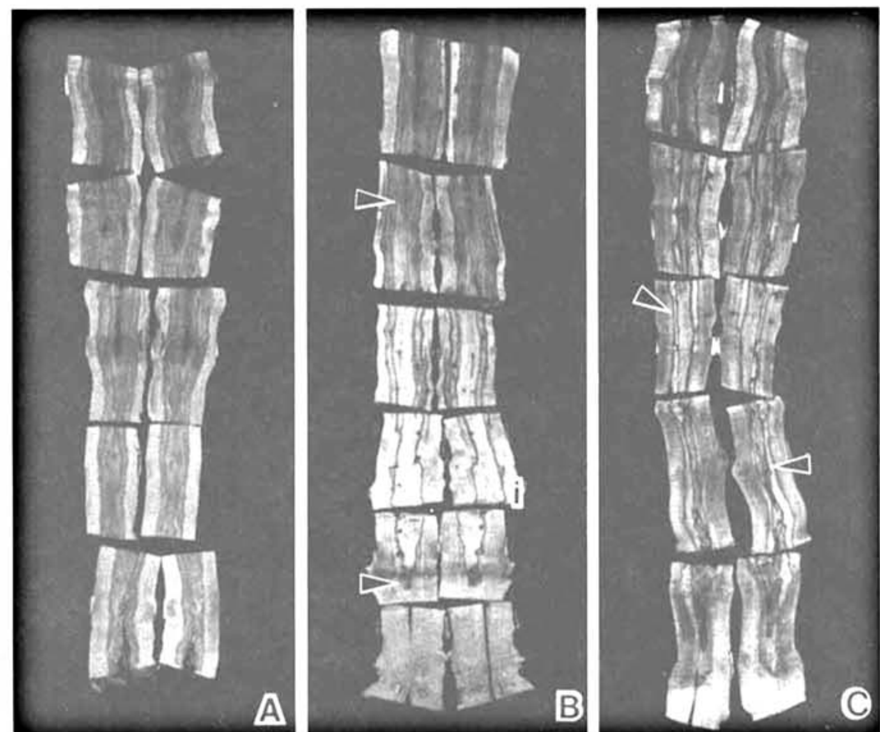

Fig. 2. Nondecayed and decayed trunks of 17-yr-old cultivar Robin grapevines inoculated with *Ganoderma lucidum* under vineyard conditions. Trunks were cut transversely into segments 10–15 cm and then cut radially. Base of the trunk is the bottom wood segment of each photo. **(A)** Control vine with no decay. **(B)** Grapevine inoculated with isolate JEA 141. Decay column limited to the heartwood (arrows indicate upper and lower limits of decay; i = inoculation point). **(C)** Grapevine with mixed infection of *G. lucidum* and *Phellinus texanus*. Zone lines (arrows) separate the two decay columns.

five infected grapevines were sectioned longitudinally and areas of decay identified. Decayed wood, which ranged from the lower pith of the grapevine stem to the entire pith and sapwood, was bleached and spongy and was characteristic of white rots (Fig. 1). *G. lucidum* was isolated from decayed areas. No other basidiomycetes were isolated. Primary and secondary roots were not infected by the fungus. No control grapevines were infected, and the plants with noninfested wood blocks showed no symptoms of decline.

Development of *G. lucidum* in grapevines in the field. *G. lucidum* was reisolated at the end of the study from three of nine inoculated vines. Two of these three vines developed decay columns 25 and 42 cm long from the initial point of inoculation within 17 mo. The fungus grew mostly upward in the heartwood, with some growth downward (5–10 cm) (Fig. 2). The fungus was not isolated from the sapwood. Susceptibility could not be related either to time of inoculation or to the isolate of *G. lucidum*. *G. lucidum* was not reisolated from the other six inoculated plants. Two of these remained healthy; the other four had advanced decay columns of *Phellinus texanus* (Murr.) A. Ames, *P. badius* (Berk.) G. H. Cunn., or *Peniophora albobadia* (Schw.: Fr.) Boidin. Fruiting bodies of the three species were found on the upper branches of the grapevines. Cultures from decay columns were identified by comparison with cultures from basidiocarps.

Two control plants were decayed by *P. texanus* and one control plant by *P. badius* in the upper trunk. Another control plant had a small decay column, about 8 cm, that originated from a soil-line shoot injury. Isolations from the decayed area yielded *G. lucidum*. The remaining control plants were disease-free.

DISCUSSION

G. lucidum infected living grapevines grown in the greenhouse when inoculum was placed adjacent to stem and root tissue. Infection occurred in the callus tissue of the cut ends of the grape stem cuttings. Our results indicate that infested wood blocks could function as inoculum for *G. lucidum* in grapevines grown in the greenhouse. Support posts could also function as inoculum for this fungus as suggested by Teliz (12). Although Bakshi et al (2) considered it necessary for host tissue infected with *G. lucidum* to be in direct contact with a healthy root for infection to occur, our study suggests that *G. lucidum* can grow 1–2 cm through the soil from an inoculum source to cause infection. The environmental conditions of the treatments in the greenhouse-grown grapevines were conducive for plant growth. Evidence that conditions were conducive for fungal growth were fruiting body production and decay of inoculum blocks. Control plants had well-developed root systems and healthy top growth.

Many reports suggest that *G. lucidum* is either a wound pathogen or an organism infecting weakened plants (9–11) but other investigators have reported it to be pathogenic on unwounded plants (7,13). In our studies, *G. lucidum* was found to be pathogenic regardless of the treatment. Wounding favored infection, suggesting the possibility of transmission of the fungus through wounds created by management practices such as cultivation or pruning. Basidiospores may also be a source of inoculum. Although Bakshi et al (1) concluded that basidiospores were not involved in infection of stumps of khair, Turner (14) and Adaskaveg and Gilbertson (*unpublished*) have found different genotypes present in a natural population of *G. lucidum* from closely distributed hosts in a specific area. This would indicate the possible involvement of basidiospores in the distribution of the fungus.

The decay caused by *G. lucidum* in our greenhouse-grown grapevines was limited to the heartwood, with lateral development into the sapwood during later stages. Stages of decay could be recognized because multiple infections did not occur. Field-inoculated vines had decay columns only in the heartwood after 17 mo. These studies suggest that the sapwood is decayed by the fungus only during the later stages of the disease. The ability of *G. lucidum* to colonize grapevine heartwood and later grow into the sapwood and decay active xylem tissue may explain the wilting and yellowing leaf symptoms of grapevines reported by Teliz (12) and also seen by us. Teliz also noted that plants died within 3 days of the first symptoms. Only one plant in our greenhouse test died but not until 5 mo after symptoms were first noted. Other reports indicate that *G. lucidum* may develop for 7–60 mo before killing the specific host studied (7,13). Infected plants probably die only during advanced stages of heartwood colonization.

Several other wood decay fungi, *Phellinus badius*, *P. texanus*, and *Peniophora albobadia*, were isolated from some of the 17-yr-old field-grown grapevines. These fungi have not been recorded before on grape. *Phellinus texanus* causes a heartrot in numerous living desert plants, whereas *P. badius* decays living heartwood of mesquite and Acacia spp. (4). Both species occur on exotic ornamentals in Arizona and are associated with pruning wounds. The presence of fruiting bodies and decay of the grape wood indicated that these three wood decay fungi were established within the field-grown grapevines before this study.

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