

## INOCULATION OF *PINUS CARIBAEA* VAR. *HONDURENSIS* SEEDLINGS WITH *GANODERMA LUCIDUM* IN FIJI

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### ABSTRACT

Woodblock cultures of two isolates of *Ganoderma lucidum* sensu lato from a basidiocarp on a dead tree of *Pinus caribaea* var. *hondurensis* Barr. & Golf. (Caribbean pine) were used to inoculate 98 potted Caribbean pine seedlings in Fiji. Although the inoculum remained active for 22 months in direct contact with seedling roots, pathogenicity was not demonstrated using these isolates.

### INTRODUCTION

Species of *Ganoderma* Karst. having affinity with *G. lucidum* (Curtis ex Fr.) Karst. (i.e., characterised by a shiny, varnished, frequently stipitate basidiocarp; Karsten 1881) have been recorded on a wide range of angiospermous tree species (Browne 1968; Bakshi *et al.* 1972; Steyaert 1967, 1972). Some members of this group are responsible for root diseases of palms and broadleaf trees in Asia (Petch 1910; Bryce 1924; Venkatarayan 1936; Wijbrans 1955; Turner 1965; Bakshi *et al.* 1976; Mahmood 1971; Peries 1974), Africa (Bull 1954), and America (Pirone 1957; Toole 1966; Téliz 1979). Reports of occurrence on coniferous hosts have been less frequent (Browne 1968; Bolland, in Gibson 1979).

Recently a member of the *G. lucidum* "complex" (Steyaert 1972, p. 94; *see* Patouillard 1889, p. 70; Parham 1953) was found in stands of Caribbean pine established on grassland areas in Fiji, and this represents a potential risk to future pine plantings (Singh 1978). An inoculation experiment was conducted as part of a study designed to assess the behaviour of the Fijian *Ganoderma* species in Caribbean pine plantations and complementary observations were also made in the field.

Descriptive features of the Fijian fungus are close to those of *Elfvigina tornata* (Persoon) Murrill given by Cunningham (1965), and basidiocarps match collections so determined from Fiji and other South Pacific Islands and which are housed at Plant Diseases Division, Auckland (e.g., 3499, 6584, 32771, 34952, 38663, 38665). Although *Elfvigina* Karst. is now generally considered a synonym of *Ganoderma* (Donk 1974,

p. 217), the Fijian fungus differs from *G. tornatum* (Persoon) Bresadola as described by Steyaert (1967), and without examination of herbarium material is not assigned to any of the other species monographed by him (Steyaert 1961, 1962, 1967, 1972). Throughout this article the Fijian *Ganoderma* species is therefore referred to as *G. lucidum* sensu lato.

## METHOD

Inoculations were made using two dikaryotic (clamped) isolates of *G. lucidum* from the pore region of a young sessile fructification taken from a large, dead, Caribbean pine tree at Nadarivatu, Viti Levu. Inoculum was prepared by growing the isolate on untreated wood blocks (10 × 5 × 5 cm) of poplar (*Populus* species) and radiata pine (*Pinus radiata* D. Don). Each freshly cut block was partly buried, with one end face exposed, in moist coarsely sieved topsoil held in a 1-l-capacity preserving jar. Jars and contents were sterilised by autoclaving for 30 minutes at 103 kPa after being sealed with a screw cap lid (pierced by a round opening plugged with a cotton wool bung to permit aeration). Wood cultures were started by aseptically placing one half of a 7- to 15-day-old 3% malt agar plate culture of the isolate on to the exposed end of each wood block. Lids were replaced and sealed with transparent film permeable to air but not water vapour. Cultures were incubated at ambient temperature in Lautoka for 6 months before being used as inoculum.

Each of 98, approximately 9-month-old, container-grown seedlings of Caribbean pine, 35–50 cm tall, was inoculated in May 1980 by potting up with one poplar and one pine woodblock culture in a plastic bucket. Blocks were removed from jars and buried vertically, face to face, with root systems held closely between. Block faces in direct contact with roots were scraped free from soil prior to potting up, and roots were not wounded. A further 34 control seedlings were potted up in the same way after the woodblock cultures had been killed by autoclaving. Treatment and control buckets were arranged together in random order, partly buried to conserve soil moisture, and watered as required. A tarpaulin sheltered the seedlings during part of the experimental period in order to prevent excessive wetting of the heavy potting soil by rain. After 22 months seedlings were uplifted, and roots and woodblocks were washed and examined.

To complement the inoculation experiment a series of field observations was made in second-rotation plantations of Caribbean pine established on grassland areas in Viti Levu. Searches were made for first-crop stumps colonised by *G. lucidum*, two of which were partly excavated, and the health of nearby second-crop trees was noted. These were mostly 2 years old and of height 1.4 m or greater.

## RESULTS

At the end of the inoculation experiment all seedlings were healthy and vigorous, with the exception of four inoculated plants that had died. Mean seedling heights were 97 cm (treatment seedlings) and 93 cm (controls), and mean stem diameters at ground level were 19 mm and 18 mm, respectively. Seedling roots were healthy with active mycorrhizas present, and foliage was green.

Inoculum in all treatment buckets was still viable at the end of the experiment. Extensive, white mycelial growth was present on woodblocks at the time of inoculation and several cultures had formed rudimentary basidiocarps while still within the jars. The presence of zone lines and wood discoloration also indicated that blocks were well colonised at this time. Three months after inoculation fresh mycelial growth was observed within the soil in three out of four randomly selected treatment buckets. Basidiocarps formed in 29 buckets during the first hot, wet season (September–February 1980–81) and at the end of the experiment fructifications were present in 87% of treatment buckets and in no controls. Basidiocarps were bright red, laccate, varnished, stipitate, up to 7 cm long, and many had produced a white pore zone on the lower surface. In 25 buckets basidiocarps grew in contact with seedling stems, and some were intimately attached to the bark (Fig. 1). No associated diseased tissue was present in such seedlings, except on one in which a narrow lesion 8 mm long was present in the cambium/outer-xylem region beneath the point of attachment, accompanied by slight resinosis. With one exception *G. lucidum* was re-isolated from at least one woodblock in each treatment bucket that did not contain a sporophore. A white streaky decay, characteristic of this *Ganoderma* species, was present in 90% of all treatment blocks at the end of the experiment, but in none of the control blocks. Both poplar and pine appeared equally effective as culture substrates.

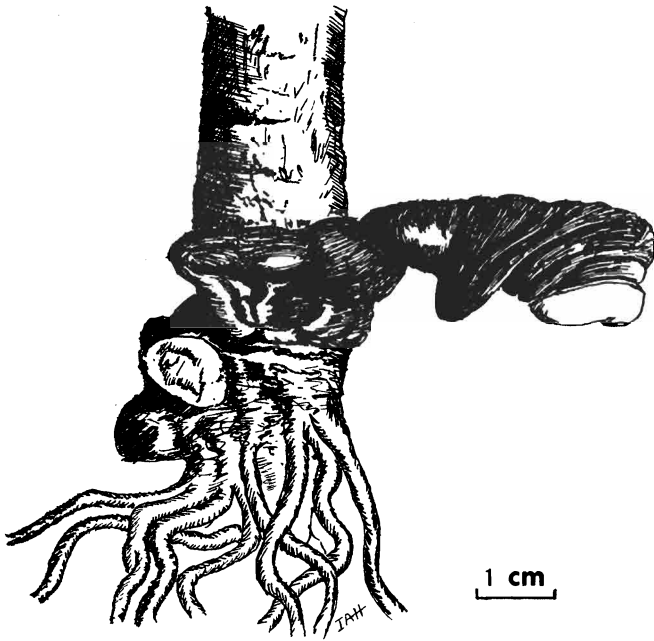


FIG. 1.—Basidiocarp of *Ganoderma lucidum* sensu lato attached to a healthy seedling of Caribbean pine 22 months after inoculation. No diseased tissue was present on the host plant.

Pine stumps with basidiocarps of *G. lucidum* were commonly seen in young second-rotation plantations. Fructifications were also present on hurricane-caused snags in an older first-crop stand. Counts in five higher-incidence areas ranging in size between 0.07 and 0.35 ha yielded densities of between three and eight colonised stumps or snags per 0.1 ha. Basidiocarps were stipitate or sessile (narrowly attached), sometimes lobed, and up to 15 cm or more across. Younger fructifications were white pored and varnished reddish-brown above, while older basidiocarps were black and less glossy. No diseased or dying second-crop pines were found associated with 44 colonised pine stumps. Excavated roots of two plants were disease free, despite contacts with roots decayed by *G. lucidum* on two colonised stumps. The contact region between one root pair extended for 18 cm. A basidiocarp was present just above ground level on the stem of one young pine adjacent to a colonised stump. Although bark penetration had occurred, cambium, wood, and bark tissues were undiseased, roots and foliage were healthy, and growth appeared good.

## DISCUSSION

The inoculation experiment indicated that the two Fijian isolates of *G. lucidum* were not pathogenic to Caribbean pine seedlings, even when actively present in direct contact with roots for 22 months. The cause of death of four treatment seedlings is not known, as they had been dead for some time when examined, but there was no sign of resinosis in roots or stems. It is known that one died shortly after accidental root breakage while being moved with its bucket. Observations in pine plantations were compatible with results of the inoculation experiment which suggests that *G. lucidum* is unlikely to become a serious problem in stands of Caribbean pine in Fiji.

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