NOTE

PATHOGENICITY OF *SEIRIDIUM UNICORNE* REDUCED BY SIMULTANEOUS INOCULATION WITH NORMAL AND DEGENERATE ISOLATES

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ABSTRACT

Inoculations were made into host trees using normal and degenerate strains of *Seiridium unicorne* (Cooke & Ellis) Sutton. Pathogenicity tests on rooted cuttings under glasshouse conditions showed very significant differences in pathogenicity between normal and degenerate strains. Pure normal strains were most virulent, degenerate isolates were completely impotent, while simultaneous inoculations with both types produced lesions 58–72% shorter than those with normal cultures, with a corresponding 64% reduction in associated resin bleeding and crown dieback.

Keywords: cypress canker; pathogenicity; inoculation; *Seiridium unicorne*.

INTRODUCTION

Seiridium unicorne and *S. cardinale* (Wagener) Sutton & Gibson are fungal pathogens that cause branch and stem cankers on many species in the family Cupressaceae. Cypress canker is a serious disease in many parts of the world, notably coastal California (Wagener 1939) and Kenya (Rudd-Jones 1953). The first record of *S. cardinale* in New Zealand was made by Birch (1933). *Seiridium unicorne* was identified by Fuller & Newhook (1954) who stated that much of the damage previously attributed to *S. cardinale* was in fact caused by *S. unicorne*.

In a national survey (van der Werff 1988) *S. unicorne* was found to be far more prevalent than *S. cardinale* and was distributed throughout the country except on the West Coast of the South Island. Gilmour (1966) stated that the risk of a *Seiridium* epiphytotic has been one of the main factors limiting large plantings of *Cupressus macrocarpa* Hartweg in New Zealand. The threat posed as cypresses become more widely grown in plantations in New Zealand remains difficult to quantify but should not be ignored.

Seiridium unicorne displays wide variation in pathogenicity as well as in morphology. Chou (1989) described considerable variation in cultural characteristics *in vitro*, including

a "degenerate" form which sometimes arose in subcultures as a sector from an apparently vigorous original culture. The resulting colony was morphologically different, with an associated reduction in pathogenicity in what Chou (1990) described as extreme degenerate forms.

The work reported here compared the pathogenicity of normal and degenerate forms of *S. unicorne* towards × *Cupressocyparis leylandii* (Jacks. et Dall.) Dall., and also sought to determine whether simultaneous inoculations of normal and degenerate cultures would reduce the impact of pathogenic strains of this fungus. The use of degenerate strains of pathogenic fungi as biological control agents is well recognised. A characteristic of degenerate forms of fungi is "hypovirulence", a reduction in the virulence of a pathogen, and Elliston (1982) discussed hypovirulence and its use as an agent for the control of chestnut blight.

MATERIALS AND METHODS

Plants

Rooted cuttings (1 cm diameter at root collar) of × *Cupressocyparis leylandii* cv. Leighton Green were used. This is a vegetatively propagated hybrid descended from a single tree, and so host uniformity was assured.

Fungal Inocula

(a) Normal isolates (three in number);
(b) Degenerate isolates derived from the normal isolates (three in number);
(c) Mixed normal isolates and degenerate isolates (three in number).

One of the normal isolates had been recently cultured from fresh host tissue, while the other two were from the culture collection of Chou, and were 3 years old. All pairs of normal and degenerate fungi originated from the same parent culture.

Inoculation Position

Each plant was inoculated at two points—100 mm and 300 mm above ground-level. The treatments and the number of plants in each were as follows:

- Treatment N — 3 normal isolates, 16 plants/isolate × 2 inoculation positions/plant
- Treatment D — 3 degenerate isolates, 16 plants/isolate × 2 inoculation positions/plant
- Treatment N+D — 3 normal and 3 degenerate isolates from the same parent culture simultaneously inoculated at the same positions, 16 plants/isolate × 2 inoculation positions/plant
- Treatment C — sterile agar controls, 8 plants × 2 inoculation positions/plant.

Inoculations were carried out by making incisions (10 mm long) into the stem at the chosen points and inserting agar plugs containing mycelium. The flaps were pinned down using a map pin which also served to mark the point of inoculation.

Plants were inoculated in June 1989. After inoculation, plants were kept in a glasshouse at 25° ±3°C under misting conditions (1 minute of misting every half hour) for 12 weeks to

provide favourable conditions for infection. Subsequently trees were subjected to drought stress with only occasional watering for 9 weeks, as drought brings on crown symptoms after vascular diseases.

Crown health (0 = healthy, 1 = dead), lesion length (mm), and occurrence of resin bleeding at points of inoculation (1 = resinosis present, 0 = no resinosis) were then recorded. An arbitrary upper limit of 100 mm was set when measuring lesion length. Finally, re-isolations were made from inoculation sites and the *Seiridium* cultures were identified.

An analysis of variance (ANOVA) was used to test the effect on lesion length of treatment, isolate, and point of inoculation. Because the distribution of lesion length was positively skewed, the data were transformed to log (x+1) for analysis.

The presence of both crown dieback and resin bleeding was tested against treatment, isolate, and point of inoculation using log-linear models. All analyses were performed using the SAS statistical analysis system.

RESULTS

Analysis of lesion length, crown dieback, and resin bleeding showed highly significant differences ($p<0.001$) between treatments C and D (both with no infection), and treatments N and N+D which both had significant levels of infection (Table 1).

TABLE 1–Mean lesion length, resin bleeding, and crown dieback recorded from inoculations with normal, degenerate, and mixed (normal plus degenerate) isolates of *Seiridium unicorne*, and control inoculum, by treatment.

Treatment	Mean lesion length (mm)	Cuttings with resin bleeding (%)	Cuttings wioth crown dieback (%)
Normal	37.4 a	69 a	47 a
Normal + degenerate	14.9 b	44 a	17 b
Degenerate	0.0 c	2 b	0 c
Control	0.0 c	0 b	0 c

Values in a column followed by the same letter do not differ significantly ($p = 0.05$)

An analysis of treatments N and N+D only was therefore performed to test the difference between application of normal isolates and normal + degenerate isolates. No significant differences in lesion length or resin bleeding between upper and lower points of inoculation on the stem were detected, and so these two treatments were amalgamated for analysis of other treatments.

This showed that inoculation with normal isolates produced significantly ($p<0.01$) greater lesion length and crown dieback than treatment with normal plus degenerate isolates, but there were no significant differences between the treatments in resin bleeding. The most striking result was that while inoculation with only the normal isolates resulted in 47% visible crown dieback, simultaneous inoculation with both normal and degenerate isolates caused only 17% visible crown dieback (Table 1), a decrease in incidence of 64%.

The normal isolates all showed high pathogenicity, though isolate X (the fresh isolate) was significantly ($p<0.05$) more pathogenic than the two isolates (Y and Z) from the culture collection (Table 2). Each isolate differed in length of lesions formed and amount of crown dieback.

TABLE 2–Mean lesion length, resin bleeding, and crown dieback recorded from inoculations with normal, degenerate, and mixed (normal plus degenerate) isolates of *Seiridium unicorne*, and control inoculum, by isolate.

Isolate	Mean lesion length (mm)	Cuttings with resin bleeding (%)	Cuttings wioth crown dieback (%)
X	24.5 a	27 a	34 a
Y	9.2 b	30 a	13 b
Z	6.1 c	27 a	1.5 c
Control	0.0 d	0 b	0 d

Values in a column followed by the same letter do not differ significantly ($p = 0.05$)

The analysis of variance showed no significant interaction between isolate and treatment on lesion length. This indicates that the reduced pathogenic effect associated with the N+D treatment compared with N was similar for all isolates.

Re-isolation from a sub-sample of the inoculation wounds yielded:

- 12 normal cultures from 26 normal inoculations
- one normal, one degenerate, and five mixed isolates from the mixed inoculations
- two degenerate cultures from the degenerate inoculations.

There was some difficulty in identifying re-isolated degenerate cultures as they are difficult to recognise in mixed cultures with faster-growing saprophytes, and lack any identifiable reproductive structures.

DISCUSSION

This study confirmed the results of Chou (1990), who found total loss of pathogenicity in degenerate forms of *Seiridium unicorne*, and it showed that the pathogenic effect of normal strains is reduced when abnormal strains are simultaneously inoculated, though the reasons for this reduction are not known. One possibility which merits further investigation is that the degenerate strain contains a transmissible agent such as a d-factor or mycovirus, in which case it may have potential as a biocontrol agent, as has been used in the control of chestnut blight (Elliston 1981). Degenerate strains (d-factors) were given consideration by Brasier (1986) as agents in man-managed disease control programmes. However, a preliminary trial by Chou (unpubl. data) failed to show proof of transmission of the degenerate factor. Chou was also unable to isolate double-stranded RNA, which indicates the presence of hypovirulent agents. This suggests that perhaps the reduced pathogenicity is effected by another mechanism such as induced immunity as described by Ouchi (1983).

An observed reduction in pathogenicity in the older isolates cannot be considered significant as only three isolates were tested and pathogenicity is reported to vary widely between isolates.

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