EVALUATION OF BIO-START™ SOIL CONDITIONERS FOR CONTROL OF SCLEROTIA OF *CIBORINIA* *CAMELLIAE*

R.F. VAN TOOR, M.V. JASPERS and A. STEWART

Soil, Plant and Ecological Sciences Division, PO Box 84, Lincoln University,
Canterbury

Corresponding author: vantoorr@lincoln.ac.nz

ABSTRACT

Bio-Start™ soil conditioners and nitrogen were assessed for their effectiveness at enhancing microbial decay of soil-borne sclerotia of *Ciborinia camelliae*. In a laboratory assay, Mycorrcin.nz and Digester.nz were applied alone or combined at 8 and 2.5 l/ha and 10 x these rates, with and without 50 kg/ha nitrogen as urea. These treatments had no significant effect on the viability of sclerotia embedded in soil after 3 months, although nitrogen increased the proportion of parasitised sclerotia from 4% to 8%. In a field trial, an application of 10.5 litres/ha Mycorrcin.nz:Digester.nz (8:2.5) in 1000 litres water/ha plus 50 kg nitrogen/ha as urea in February and June, had no significant effect on apothecia production during the following spring or on the number of viable sclerotia.

Keywords: *Ciborinia camelliae*, sclerotia, Bio-Start™, Mycorrcin.nz, Digester.nz.

INTRODUCTION

Camellia flower blight is caused by the fungus *Ciborinia camelliae* (Kohn), and results in pre-mature browning and drop of flowers. The disease is becoming common throughout the world in temperate regions wherever camellias are grown, and has been found in most regions throughout New Zealand except south of Christchurch (Taylor and Long 2000).

A number of control strategies have been tried, but none are sufficiently effective or practical for ready adoption by camellia growers. Controlling ascospore infection in flowers with frequent applications of fungicides has not been successful (Taylor & Long 2000). Raking up and burning fallen camellia flowers because they might contain developing sclerotia was found to be effective but labour intensive. Repeated application of fungicides to soil under camellia bushes during spring did suppress apothecial production from over-wintering sclerotia, thereby preventing ascospore discharge and subsequent infection of flowers (Fullerton et al. 1998). However, since many growers of camellias have expressed reluctance to rely solely on fungicides, and the wide dispersal of *C. camelliae* ascospores means that good control of the pathogen requires a universally acceptable solution, the potential for biological control was investigated.

The feasibility of using microbial antagonists to control the soil-borne sclerotia as an alternative to chemical options was tested in laboratory and field experiments. Mycoparasites found to be effective against *Sclerotinia sclerotiorum* (Rabeendran et al. 1999), a pathogen closely related to *C. camelliae*, were not able to parasitise sclerotia (R.F. van Toor, unpubl. data). However, two isolates of *Trichoderma* sp. recovered from natural *C. camelliae* sclerotia demonstrated a degree of pathogenicity when applied to healthy sclerotia (R.F. van Toor, unpubl. data). This suggested that microbial antagonists were present in the soil. There is a possibility that their activity could be enhanced with soil amendments, an approach that has been used successfully by Dhingra & Sinclair

New Zealand Plant Protection 55:146-149 (2002)

(1975). They found that the density of *Macrophomina phaseolina* sclerotia declined rapidly in wet soils amended with glucose and NaNO_3 in C:N ratios of 10:20, and that the decline was significantly correlated with an increase in the population of soil microbes.

In this study, the Bio-Start™ soil conditioners Mycorrcin.nz and Digester.nz, which contain fermentation extracts and selected minerals, were considered to have potential for enhancing degradation and decay of *C. camelliae* sclerotia by stimulating microbial activity. Bio-Start Ltd reported that Mycorrcin.nz could increase fungal biomass. At Blenheim, three applications of 2 litres/ha, increased active fungal biomass from 1 to 32 µg/g soil, and at Katikati, applications of 15 litres/ha at one year and 10 litres/ha for another two years, increased the fungal biomass from 0.5 to 5.8 µg/g soil (Bio-Start™ 2001). This paper reports on a laboratory assay and a field trial that evaluated mixtures of Bio-Start™ Mycorrcin.nz and Digester.nz with nitrogen as urea, for control of soil-borne sclerotia of *C. camelliae*.

METHODS

Laboratory assay

Soil containing at least 20 sclerotia/m² was taken from under 10 year old *Camellia japonica* trees and used for the assay since this soil was likely to contain micro-antagonists that had been exposed to *C. camelliae*. The soil, collected to a depth of 50 mm on 29 January 2001, was screened through a 4 mm sieve, and 30 g (70 ml) placed into each of 84 mm diameter x 20 mm deep Petri dishes. *C. camelliae* sclerotia, collected from the Wellington Botanical Gardens during November 2000 and stored at 10°C, were washed under tap water, sorted into small, medium and large sizes, air-dried and weighed. Sclerotia averaged 228 (SEM = 7), 477 (SEM = 11) and 911 (SEM = 17) mg for the three sizes, respectively. For each sclerotium size, four sclerotia were pressed into the soil in three replicate dishes, equidistantly across the area, and the dishes shaken so that the soil covered the sclerotia.

The soil treatments comprised an untreated control, and Bio-Start™ products applied at the recommended field rate: Mycorrcin.nz at 8 litres/ha (4.43 mg/dish), Digester.nz at 2.5 litres/ha (1.39 mg/dish), and Mycorrcin.nz at 8 litres/ha plus Digester.nz at 2.5 litres/ha. These treatments were also tested at 10 times the recommended field rate. In addition, the untreated control and all Bio-Start™ treatments were compared with and without urea (46% N) at 50 kg N/ha (60 mg N/dish). All the ingredients were applied to the soil in the same volume of water (554 µl) per dish on 1 February 2001, followed by 5 ml of tap water dribbled evenly over the soil in each dish, to bring the moisture content to 82% of field capacity. Field capacity was measured by the tension method, using a Buchner funnel connected to a hanging water column (Cameron & Buchan 2001). The treatments were arranged in a randomised block design and incubated at 22°C with a 12 h diurnal light regime. Tap water, sufficient to replace moisture lost due to evaporation, was added weekly to each dish. After 12 weeks incubation, sclerotia were extracted from the soil, and assessed for viability.

Field trial

On 23 February 2001, a solution of Mycorrcin.nz plus Digester.nz at 8 and 2.5 litres/ha, respectively, was applied in 1000 litres water/ha with a hand-held garden sprayer, to soil under camellia bushes in the camellia garden at the Wellington Botanic Gardens. Immediately afterwards, 109 kg urea prills/ha (50 kg N/ha) were broadcast onto these plots by hand. At this time, new sclerotia were developing in infected flowers that had dropped to the ground. The treatment was reapplied on 1 June 2001, prior to apothecia production by existing soil-borne sclerotia. The Bio-Start™/urea treatment was compared to an untreated control. All treatments had six replicate plots (1.0 x 1.0 m), which were arranged in a randomised block design.

To ensure there were sufficient numbers of young healthy sclerotia for assessing treatment effects, sclerotia were collected from infected flowers during spring 2000 and seeded into each plot prior to treatment application in February 2001. Eighty medium-sized sclerotia were placed into four 200 mm long x 25 mm diameter onion bags (mesh

size of 5 x 5 mm), positioned equidistantly in the centre of the plot, 200 mm in from each of the corners, and buried level with the soil surface. The number of apothecia produced in the onion bags and across the plot from naturally occurring sclerotia, was recorded on 16 August, 3 and 19 September, and 10 and 19 October 2001. The number of sclerotia remaining in the soil of each plot was estimated on 14 November 2001 by counting all the sclerotia found within a 36 cm diameter soil core to 5 cm depth. Sclerotia remaining in the onion bags were also counted.

Determining sclerotial viability by the firmness test

Sclerotia were rinsed using a jet of water over a 4 mm sieve to remove adhering soil. Sclerotia that disintegrated and passed through the mesh were recorded as not viable. Viability of the remaining sclerotia was assessed by the compression test described by van Toor et al. (1999), which was modified to increase speed of assessment. Intact sclerotia were placed onto a Mettler PE3600 balance and tared. A steel round-ended probe with a 2.8 mm² surface area was pressed vertically down until the balance displayed 1 kg, giving an indentation pressure of 1.6 MPa. Sclerotia were considered viable if there was no indentation of the rind, and not viable (soft) if the rod penetrated the rind into the medulla. Soft sclerotia were considered to be parasitised by other micro-organisms, and recorded as diseased. The validity of this assumption was tested by compressing 104 sclerotia, and relating their firmness to the presence of micro-organisms growing from them. The sclerotia had been surface-sterilised without antibiotics in 13.5% NaOCl/ethanol (83/17, v/v) for 3 min and re-washed without ethanol (van Toor et al. 1999), bisected on PDA, and incubated at 20°C under 12 h diurnal light for 8 days.

Statistical analysis

The proportion of sclerotia recovered and parasitised in the laboratory assay, and the proportion of firm or soft parasitised sclerotia in the firmness test, were analysed by generalised linear model with a binomial logit link. Results are presented as percentages, followed by low and high 95% confidence limits in brackets. In the field trial, apothecial production and density of surviving sclerotia were analysed by analysis of variance on log_e-transformed data.

RESULTS AND DISCUSSION

Firmness test

Of the 52 sclerotia that tested firm, 94% (90-97%) yielded actively growing *C. camelliae* mycelium and were deemed viable, with the remaining 6% exhibiting parasitic micro-organisms. In contrast, all of the 52 sclerotia that tested soft, failed to produce active mycelium and were parasitised by other micro-organisms, demonstrating the method to be a reliable indicator of sclerotial viability.

Laboratory assay

Mycorrcin.nz or Digester.nz had no effect ($P>0.05$) on the proportion of sclerotia recovered within any size category, which averaged 98% (95-99%), or on the proportion of recovered sclerotia that were recorded soft in the firmness test and considered parasitised, which averaged 5% (3-9%). Only the nitrogen treatment had a significant effect ($P<0.05$) on the proportion of parasitised sclerotia: 4% (2-6%) for non nitrogen treatments and 8% (5-11%) for the nitrogen treatments. The fact that addition of nitrogen resulted in a two-fold increase in the proportion of diseased sclerotia suggests that this treatment may enhance microbial parasitism. Thus, application of nitrogen to soils under camellia bushes might be a useful strategy for enhancing microbial decay of *C. camelliae* sclerotia.

Field trial

The Bio-Start™ and urea treatment had no effect ($P<0.05$) on the numbers of naturally occurring sclerotia recovered from field plots or on the extent of apothecial production (Table 1). There was also no effect on the number of sclerotia recovered from the onion bags, since 100% were recovered and viable for all treatments.

TABLE 1: Density (no./m²) of apothecia during spring and natural soil-borne sclerotia in November 2001 at Wellington Botanic Gardens¹.

Treatment	Apothecia		Sclerotia	
Untreated control	3.49	(32)	5.69	(294)
Bio-Start TM and urea	3.08	(21)	4.66	(105)
LSD (P=0.05)	1.56		1.25	

¹Data are log_e+0.5 transformed means, with back transformed means in brackets.

For any treatment to be effective, it would need to reduce sclerotial or apothecial densities to negligible levels, as only one apothecium is required to release millions of ascospores to infect a new crop of camellia flowers and continue the life cycle. Although the Bio-StartTM/urea treatments did not result in a significant reduction of sclerotia 9 months after application, sclerotial densities were a third lower than in the untreated control. The effect of repeated yearly applications of Bio-StartTM/urea may compound and result in significantly lower numbers of sclerotia in subsequent years. However, any control of sclerotia would be offset by the next generation of sclerotia developing in the newly infected flowers. This low reduction in sclerotia is in contrast to the dramatic reduction in *Macrophomina* sclerotia after application of glucose and NaNO₃ found by Dhingra & Sinclair (1975), leading them to postulate that the increased microbial activity destroyed the germinating sclerotial mycelium. Since sclerotia of *C. camelliae* do not generally germinate vegetatively in soil, potential micro-antagonists would need to be able to attack or penetrate the protective rind to degrade the sclerotia. In this study, either micro-antagonists were not present in the soil under camellia bushes, or the Bio-StartTM/urea treatment did not enhance the pathogenicity of potential micro-antagonists.

ACKNOWLEDGMENTS

Martin Dench, Manager of the Camellia Garden at the Wellington Botanic Gardens, for technical assistance; Bio-Start Limited for supply of their Bio-StartTM products; Brian Mason Scientific and Technical Trust and the New Zealand Camellia Society for financial assistance.

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