

## THE EFFECT OF *CONIOTHYRIUM MINITANS* ON SCLEROTIAL VIABILITY OF *SCLEROTINIA SCLEROTIUM* AND *CIBORINIA CAMELLIAE*

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### ABSTRACT

The effect of *Coniothyrium minitans* on *Sclerotinia sclerotiorum* and *Ciborinia camelliae* sclerotial viability was determined on three different substrates: sand, soil and sawdust using fully factorial, repeat experiments (Trials 1 and 2). In Trial 1, *C. minitans* significantly reduced the number of viable *S. sclerotiorum* sclerotia in sand (48%) and sawdust (0%) but not in soil (60%) compared with the untreated sclerotia (92, 64 and 88%, respectively) after 8 weeks. Although *C. minitans* had no effect on *C. camelliae* sclerotial viability, the sawdust only treatment reduced viability to 0% after 4 weeks. In the repeat experiment (Trial 2), *C. minitans* had no effect on *S. sclerotiorum* or *C. camelliae* sclerotial viability, although *C. camelliae* sclerotial viability was again significantly reduced in the sawdust control treatment (8-12%) compared with the sand and soil control treatments (>84%). *Coniothyrium minitans* has some potential for biocontrol of *S. sclerotiorum*, but not of *C. camelliae*. Sawdust may be an option for use as an under plant mulch for control of *C. camelliae*.

**Keywords:** *Coniothyrium minitans*, *Sclerotinia sclerotiorum*, *Ciborinia camelliae*, sclerotial viability, biological control.

### INTRODUCTION

*Sclerotinia* and *Ciborinia* are closely related genera (Jones & Stewart 1995). When *Ciborinia camelliae* Kohn was first isolated in 1919 it was named *Sclerotinia camelliae* Hara, but in 1984 the fungus was reclassified as *C. camelliae* (Kohn & Nagasawa 1984). Both genera belong to the Sclerotiniaceae, a family of discomycetes, which infect plants by producing apothecia from stoma or sclerotia (Holst-Jensen et al. 1997). While *Sclerotinia* species infect over 400 plant species, *S. sclerotiorum* (Lib.) de Bary is the most economically important species as it attacks many vegetable crops, including bean, pea, cabbage and lettuce (Boland & Hall 1994).

*Ciborinia camelliae* is a host specific pathogen limited to *Camellia* species and causes flower or petal blight, the most destructive disease of camellias. Holcomb (1983) indicated that successful control of *C. camelliae* would most likely be achieved using methods shown to give successful control of *Sclerotinia* diseases.

Biological control of *S. sclerotiorum* has focused on the use of *Coniothyrium minitans* Campbell, which has been shown to be an effective sclerotial parasite (de Vrije et al. 2001). *Coniothyrium minitans* penetrates the sclerotia either intercellularly or via cracks in the rind surface and continues inter and intracellularly through the unpigmented internal tissues of the cortex and medulla, thereby inhibiting myceliogenic and carpogenic germination (Whipps & Gerlagh 1992). Within New Zealand, *C. minitans* isolate A69 has given promising activity against *Sclerotinia* diseases in glasshouse and field crops (Jones & Stewart 2000). *Ciborinia camelliae* disease control work has also focused on

biological applications (van Toor 2002), with pine sawdust mulch being effective in reducing *C. camelliae* sclerotial viability.

This paper reports the ability of *C. minitans* A69 to reduce sclerotial viability of *S. sclerotiorum* and *C. camelliae*. Trials were conducted in sand, soil and pine sawdust media.

## MATERIALS AND METHODS

*Coniothyrium minitans* (A69) was isolated from a parasitised *Sclerotium cepivorum* Berkeley sclerotium recovered from Pukekohe, South Auckland in 1994 and stored at 4°C in the dark on potato dextrose agar (PDA, Life Technologies/GIBCO BRL) slopes. When required, *Coniothyrium minitans* was grown at 20°C on PDA plates for 4 weeks in a 12:12 h light:dark cycle incubator. A spore suspension was prepared by adding 10 ml of 0.01% Tween 80 in sterile distilled water (SDW) to each Petri dish, and rubbing the surface with a sterile glass spreader. The resulting spore suspension was adjusted to give  $1 \times 10^6$  spores/ml using a haemocytometer.

*Ciborinia camelliae* sclerotia were collected from a privately owned garden in Christchurch, two weeks before Trial 1 was set up. Sclerotia were stored at 4°C in the dark until required. Sclerotia of *S. sclerotiorum* (LU459), originally isolated from cabbage at Lincoln in 1996 and stored at 4°C in the dark on PDA slopes, were produced on whole wheat grains (Alexander & Stewart 1994) and harvested from the grain using progressive wet sieving (Kay & Stewart 1994). Average sized sclerotia (400-800 mm diameter for *C. camelliae* and 200-600 mm diameter for *S. sclerotiorum*) were chosen for the assays. Prior to use, sclerotial viability was determined by surface sterilising 50 sclerotia of each species for 3 min in a 3% sodium hypochlorite (NaOCl) solution. The sclerotia were rinsed in SDW, bisected, placed cut side down onto PDA discs (2 cm diameter) and incubated at 20°C. *Sclerotinia sclerotiorum* was incubated for 3-4 days and *C. camelliae* for 10 days before viability was assessed.

Three different substrates were used in each trial: sand (sterile environment), soil (natural environment) and sawdust (carrier substrate that may provide additional nutrition for *C. minitans*). For the sand medium, silica sand was sterilised by rinsing the sand five times in tap water and drying in a 160°C oven for 24 h. Eighty grams of dry sand was transferred to each of 40 Petri dishes (90 mm diameter) and 20 ml of SDW was added to each dish. For the soil medium, Wakanui silt loam soil was collected from the Horticulture Research Area, Lincoln University, 5 days before use and unified through a 4 mm sieve before air-drying. Fifty grams of soil was transferred to each of 40 Petri dishes and 3.5 ml of SDW was added to give an 80% field capacity water content. For the sawdust medium, fresh pine sawdust was amended with rye flour (4:1 v/v) and sterilised by autoclaving at 121°C and 103.35 kPa for 30 min on 3 consecutive days (van Toor 2002). Twenty grams of sawdust was transferred to each of 40 Petri dishes with 40 ml of SDW. The volume of water added to each substrate ensured moist conditions for the sclerotia.

### Trial 1

*Sclerotinia sclerotiorum* and *C. camelliae* sclerotia were surface sterilised prior to use by agitating in 3% NaOCl for 3 and 5 min, respectively, then rinsed twice in SDW. Four different treatments were set up on each substrate: (1) five untreated sclerotia of *S. sclerotiorum*, (2) five sclerotia of *S. sclerotiorum* each inoculated with 100 ml of A69-spore suspension using a pipette, (3) five untreated sclerotia of *C. camelliae*, (4) five sclerotia of *C. camelliae* each treated with 200 ml of A69-spore suspension using a pipette. A smaller volume of spore suspension was applied to the *S. sclerotiorum* sclerotia compared to *C. camelliae* since the *S. sclerotiorum* sclerotia were smaller and a reduced volume was required to cover the upper surface of each sclerotium.

There were 12 treatments with ten replicate dishes per treatment. Petri dishes were sealed with cling film and arranged in a randomised block design in an incubator at 20°C under a 12:12 h light:dark regime. After 4 and 8 weeks of incubation, five replicate dishes for each treatment were randomly selected and the sclerotia were recovered from the substrate by washing in tap water. Sclerotial viability was determined using the

method developed by van Toor (2002). Briefly, all the sclerotia from each replicate plate were soaked in ethanol and 17% NaOCl (v/v). Following 3 min agitation, the sclerotia were rinsed twice for 15 min in SDW. The sclerotia were held at room temperature for 24 h to allow contaminants to germinate before the surface sterilisation process was repeated. The sclerotia were bisected and both halves were placed onto a single PDA disc (2 cm diameter) containing 50 µl/litre penicillin and streptomycin sulfate. Sclerotia were incubated at 20°C under a 12:12 h light:dark regime. Sclerotial viability was determined by recording mycelial growth every second day for 10 days.

### Trial 2

The same 12 treatments with ten replicates dishes were repeated in Trial 2 with the following modifications: only 20 ml water was added to the sawdust substrate to reduce the amount of free water in the Petri dishes as observed in Trial 1. *Coniothyrium minitans* spore suspension was not added separately to each sclerotium, as in Trial 1. Instead, 60 sclerotia were placed in 10 ml of spore suspension in a glass vial and shaken gently using an orbital shaker for 10 min. After leaving to stand for a further 10 min, the sclerotia were removed (Whipps & Budge 1990) and placed on the substrate as described for Trial 1.

### Statistical analysis

The trials were analysed as multifactorial trials with *C. minitans* inoculation (+, -), substrate (soil, sand, sawdust), incubation period (4, 8 weeks) and sclerotial type (*S. sclerotiorum*, *C. camelliae*) used as factors. The proportion of viable sclerotia was analysed by generalised linear model with a binomial distribution using the GenStat 5 – release 4.2, statistical software. Treatment means were compared at  $P=0.05$ .

## RESULTS

Sclerotial viability at the start of Trial 1 was 100% for both *S. sclerotiorum* and *C. camelliae*. Sclerotial viability at the start of Trial 2 was 80% for *S. sclerotiorum* and 95% for *C. camelliae*.

In Trial 1, *C. minitans* significantly reduced ( $P<0.05$ ) *S. sclerotiorum* sclerotial viability in the sand and sawdust substrate but not the soil treatment after 8 weeks (Table 1). *Coniothyrium minitans* had no effect ( $P>0.05$ ) on *C. camelliae* sclerotial viability after 4 or 8 weeks on any substrate. There was a significant substrate effect with *C. camelliae*. Sclerotial viability was significantly less ( $P<0.05$ ) in the sawdust treatment (0%) compared with the sand and soil treatments (>92%), irrespective of *C. minitans* (Table 1).

**TABLE 1: Sclerotial viability (%) of sclerotia of *Sclerotinia sclerotiorum* and *Ciborinia camelliae* at 4 and 8 weeks after treatment with *Coniothyrium minitans* on three different substrates in Trial 1.**

Pathogen	Substrate	4 weeks		8 weeks	
		- <i>C. minitans</i>	+ <i>C. minitans</i>	- <i>C. minitans</i>	+ <i>C. minitans</i>
<i>S. sclerotiorum</i>	sand	100 (-) <sup>1</sup>	76 (54-89)	92 (71-98)	48 (28-68)
	soil	96 (74-100)	80 (58-92)	88 (67-96)	60 (39-78)
	sawdust	80 (58-92)	64 (43-81)	64 (43-81)	0 (-)
<i>C. camelliae</i>	sand	100 (-)	100 (-)	96 (74-100)	96 (74-100)
	soil	96 (74-100)	96 (74-100)	92 (71-98)	92 (71-98)
	sawdust	0 (-)	0 (-)	0 (-)	0 (-)

<sup>1</sup>Numbers in brackets indicate the 95% confidence limits; (-) cannot be calculated.

In Trial 2, *C. minitans* had no significant effect ( $P>0.05$ ) on *S. sclerotiorum* or *C. camelliae* sclerotial viability. However, *C. camelliae* sclerotial viability was significantly less ( $P<0.05$ ) in the sawdust treatment (8-12%) compared with the soil and sand treatments (84-100%) (Table 2).

**TABLE 2: Viability of sclerotia (%) of *Sclerotinia sclerotiorum* and *Ciborinia camelliae* at 4 and 8 weeks after treatment with *Coniothyrium minitans* on three different substrates in Trial 2.**

Pathogen	Substrate	4 weeks		8 weeks	
		- <i>C. minitans</i>	+ <i>C. minitans</i>	- <i>C. minitans</i>	+ <i>C. minitans</i>
<i>S. sclerotiorum</i>	sand	64 (46-79) <sup>1</sup>	52 (35-69)	60 (39-78)	68 (46-84)
	soil	76 (58-88)	56 (39-72)	32 (16-54)	12 (4-34)
<i>C. camelliae</i>	sawdust	36 (21-54)	32 (18-50)	48 (28-68)	8 (2-30)
	sand	92 (76-98)	92 (76-98)	96 (73-100)	84 (62-94)
	soil	92 (76-98)	100 (-)	92 (70-98)	84 (62-94)
	sawdust	12 (4-29)	12 (4-29)	12 (4-34)	8 (2-30)

<sup>1</sup>Numbers in brackets indicate the 95% confidence limits; (-) cannot be calculated.

### DISCUSSION

The reduction in *S. sclerotiorum* sclerotial viability observed in Trial 1 indicated that *C. minitans* had a detrimental effect on sclerotial health. However, the results from the present study were not as positive as those previously reported (Whipps & Budge 1990; Jones & Stewart 1995). For example, the same isolate of *C. minitans* (A69) tested in a previous trial, reduced *S. sclerotiorum* viability to 0% in sand after 4 weeks and to 17% in soil after 2 weeks (Jones & Stewart 2000). It is possible that the use of artificially produced sclerotia rather than natural sclerotia may have influenced the effect of *C. minitans*, since Trutmann et al. (1982) reported greater parasitism of natural rather than artificially produced sclerotia of *S. sclerotiorum* by *C. minitans*. In addition, it is possible that the lower sclerotial viability noted at the start of Trial 2 (80%) compared with Trial 1 (100%), made it difficult to detect significant differences when *C. minitans* was applied. Reasons for the reduced sclerotial viability between trials are unclear. Loss in *C. minitans* A69 virulence may also be a contributing factor to the reduced sclerotial parasitism observed in the present study.

*Coniothyrium minitans* had no effect on the viability of *C. camelliae* sclerotia. In both trials, there was some reduction in sclerotial viability in soil but this was not statistically significant. In other studies, *C. minitans* failed to parasitise sclerotia of *Rhizoctonia tuliparum*, *Sclerotium delphinii* and *Typhula incarnata* (Whipps et al. 1991). Those authors postulated that antibiotic compounds released by the sclerotia prevented *C. minitans* from penetrating the sclerotial rind (Whipps et al. 1991). This may also have occurred with *C. camelliae*. In addition, *C. camelliae* has a thicker, more melanised rind compared with *S. sclerotiorum* (Huang 1983; Kohn & Nagasawa, 1984), which may also have prevented hyphal penetration physically. Further research is warranted to understand these interactions.

A strong substrate effect was apparent in both trials. Sclerotial viability of *C. camelliae* on sawdust was significantly reduced in Trial 1 and completely inhibited in Trial 2. *Sclerotinia sclerotiorum* sclerotial viability was also less in the sawdust substrate but the effect was not statistically significant. It is postulated that the pine sawdust may have produced toxic compounds, which caused the loss in sclerotial viability (van Toor 2002).

This study confirmed the potential of *C. minitans* to reduce sclerotial viability of *S. sclerotiorum* but the results indicate that further definition of optimum conditions is required. Although *C. minitans* did not reduce the viability of *C. camelliae* sclerotia, the results do not exclude the possibility that *C. minitans* may inhibit apothecial production since this could not be assessed in these trials. This work has demonstrated that sawdust can reduce the viability of *C. camelliae* sclerotia. Sawdust may have potential for reducing *S. sclerotiorum* sclerotia viability but would require a longer exposure period to the sawdust.

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