SCLEROTIUM POPULATIONS AFTER INUNDATIVE APPLICATION OF *SCLEROTINIA SCLEROTIUM* TO CALIFORNIAN THISTLE

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SUMMARY

In autumn, 11 months after inoculation of a pasture population of Californian thistle (*Cirsium arvense*) with a mycelium-on-wheat preparation of *Sclerotinia sclerotiorum*, sclerotia of this pathogen and other fungi were extracted and identified from soil to a depth of 50 mm. No viable sclerotia of *S. sclerotiorum* were recovered from untreated plots, whereas 119 sclerotia/m² were recovered from the treated plots. Viability of the sclerotia of *S. sclerotiorum* was 78%, assessed both on agar or carrot discs. Several organisms were isolated from the non-viable sclerotia of *S. sclerotiorum*, including the hyperparasite *Coniothyrium minitans*. Nineteen months after inoculation, Californian thistle stems from an additional site were assessed for the presence of sclerotia. None were found in juxtaposed untreated plots, and 3.1% of the stems in the treated plots contained sclerotia. These results indicate that when *S. sclerotiorum* was used as a mycoherbicide in pasture, numbers of sclerotia added to the soil were similar to those contributed by a susceptible crop epidemic, but that subsequent pathogen carry-over and spread were minimal.

Keywords: *Sclerotinia sclerotiorum*, sclerotia, mycoherbicide, *Cirsium arvense*, Californian thistle.

INTRODUCTION

The plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary has been investigated as a mycoherbicide because of its ability to effectively kill weeds such as Californian thistle (*Cirsium arvense* L.(Scop.)) in pasture (Brosten and Sands 1986), spotted knapweed (*Centaurea maculosa* L.)(Sands *et al.* 1990) and several flat weeds in turf (Riddle *et al.* 1991). The pathogen has a wide host range both in New Zealand (Pennycook 1989) and throughout the world (Purdy 1979). It can persist in moist soil as sclerotia for 3-4 years (Adams and Ayers 1979) and longer in dry soil (Brown and Butler 1936).

The carry-over of these propagules to cause disease in subsequent seasons could cause problems in the use of *S. sclerotiorum* as a mycoherbicide. Myceliogenic germination of sclerotia may cause infection to either the residual target host or to subsequently sown hosts at the treatment location. Carpogenic germination can spread the pathogen to other locations through the production of apothecia and airborne ascospores (LeTourneau 1979). Thus, assessments of sclerotium production and their addition to the soil after *S. sclerotiorum* treatment are a necessary part of determining the risk of using this pathogen as a mycoherbicide in pastures that are destined for subsequent cropping or are juxtaposed to susceptible crops.

This paper reports on the recovery, identification, and viability assessment of sclerotia from the soil in treated and untreated plots in the spring 10 to 11 months after application of *S. sclerotiorum* to Californian thistle, and on the recovery of sclerotia from stems 19 months after application.

MATERIALS AND METHODS

Plots of Californian thistle were treated at Templeton, Canterbury with two strains of *S. sclerotiorum* (S9 isolated from Californian thistle in New Zealand and S1 isolated from spotted knapweed in Montana USA). Isolate S9 was applied on 11 to 14 October, 1991, while isolate S1 was applied on 15 November. Application was by hand using a shaker jar containing a mycelial preparation of the pathogen on a kibbled wheat culture medium/carrier at the rate of 50 g/m². The trial layout was as described by Bourdôt *et al.* (1993).

The plots were rated for disease 55 to 57 (S9) and 21 (S1) days after treatment using a 0 - 5 scale (0 = no detectable symptoms of infection from *S. sclerotiorum*; 5 = all plants exhibiting extensive lesioning and necrosis).

Eighteen 100 mm diameter soil cores were taken to a depth of 50 mm from each plot in March 1992, 346 to 349 (S9) and 314 (S1) days after treatment. The cores from each plot were bulked and stored at 4°C. Extraction of sclerotia was carried out by soaking the cores in 20% w/v sodium hexametaphosphate for 2-3 h, and then washing through 2.0 and 0.85 mm screens. All bodies that resembled sclerotia were picked out from the wet sieved material under x4 magnification, placed in vials and stored at 4°C. Then, under x10 magnification, all sclerotia were cut to remove a sliver of rind to reveal the medulla. The nature of the medulla and other criteria were used to identify recovered sclerotia as follows:

Sclerotia round to oblong and of varying size with a black rind and white medulla; yielding mostly vegetative white mycelium in culture with attendant black sclerotia (Mordue and Holliday 1976) - *S. sclerotiorum*

Sclerotia striated, elongated with tapered ends and a dark purple rind grading to a purple/grey medulla (Dennis 1968) - *Claviceps purpurea* (Fr.) Tul.

Sclerotia cylindric to irregular in shape with no rind and dark brown to black throughout; brown mycelium attached; yielding no known sclerotium producing fungi in culture (Sneh *et al.* 1991) - probably sterile *Rhizoctonia solani* Kuehn

All sclerotia that had white medullae, and a sample of those with black or brown medullae, were washed in sodium hypochlorite with 1% free chlorine, and then washed twice in sterile distilled water. The sclerotia with white medullae were cut in two. One half of each sclerotium was placed on the surface of a carrot root slice under humid conditions while the other half was plated out onto malt extract agar. After incubation at 22°C for 5 days, the number of sclerotia that induced tissue softening on the discs was recorded, while the cultures that produced white mycelium with attended sclerotia were recorded as *S. sclerotiorum*. All other organisms that grew out of sclerotia either together with *S. sclerotiorum*, or independently, were identified and recorded.

Sclerotia in Californian thistles stems were counted on plots in an additional trial area in May 1993, 19 months after application of *S. sclerotiorum* as described above. All stems in the four replicates of the 3 x 1.3 m plots, both standing and on the ground were split open and assessed for the presence of sclerotia. The thistles in the plots were inspected for general health at regular intervals over the 6 months prior to assessment.

RESULTS

Table 1 gives the numbers of the different sclerotia recovered per m² in each strain. There was an increase in the number of sclerotia of *S. sclerotiorum* in the plots following treatment with an inundative application of the pathogen. This treatment induced disease in the plots within 3 to 8 weeks (Table 1). The number of *S. sclerotiorum* sclerotia in the individual treated replicate plots varied from 21 to 233/m²; of *C. purpurea* sclerotia from 0 to 70/m², and of *R. solani* from 0 to 189/m². The large LSD values reflect the wide variation in the numbers of sclerotia recovered from each plot.

TABLE 1: Population density and viability of sclerotia extracted from soil in March 1992 from plots of *Cirsium arvense* treated with *Sclerotinia sclerotiorum* (S.sc.) 4-5 months earlier.

Treatment	Disease Rating (0-5)	No./m ²			% Viability of S.sc.	
		S.sc.	C.purpurea	R.solani	on agar	on carrot
S9 (Oct)	2.3	120	27	85	72.9	70.4
S1 (Nov)	3.5	118	30	83	85.8	83.2
Nil	[0] ¹	[2]	[7]	[12]	[0]	[0]
LSD (5%)	1.3	277	65	138	23.0	38.7

¹ Square brackets indicate exclusion from statistical analysis.

The viability of *S. sclerotiorum* sclerotia, as determined by isolation and identification in pure culture and by carrot disc softening, is given in Table 1. Both methods gave similar results. The single sclerotium isolated from the untreated plots that appeared to be *S. sclerotiorum*, proved to be non-viable. The following microorganisms were isolated from non-viable *S. sclerotiorum* sclerotia: *Coniothyrium minitans* Campbell (4.4% incidence), a *Trichoderma* sp. (6.6% incidence) a bacterium (11.7%), *Fusarium* spp. (5.9%) and a *Penicillium* sp. (2.2%). Sclerotia classed as *R. solani* yielded only saprophytic fungi, and thus proved to be non-viable. No further processing was carried out on the sclerotia classed as *C. purpurea* as their identity was certain.

Table 2 gives the Californian thistle stem population densities and sclerotium recovery details from stems on plots 19 months after treatment. No sclerotia were found in the untreated plots, whereas they were detected in the stems in the treated plots. No obvious disease symptoms caused by *S. sclerotiorum* was detected throughout the season in the treated or untreated plots, and no apothecia of the fungus were detected in detailed ground searches.

TABLE 2: Stem population densities and recovery of sclerotia from stems of Californian thistles in May 1993, 19 months after *Sclerotinia sclerotiorum* application.

Treatment	Stems/m ²	Percentage of stems with sclerotia	Sclerotia per infected stem	Sclerotia /m ²
S9 (Oct)	18.4	3.1 ^a	1.4	0.8
Nil	26.2	0.0	-	0.0
LSD(5%)	5.7	-	-	-
Significance	*	**	-	-

^aPercentages compared using a chi-square test.

DISCUSSION

The large variation in the sclerotium population densities in this study and the large LSDs probably reflect variation between plots in the population density of Californian thistle stems, and density variations of stems within plots. Thus plots with low stem numbers would produce few sclerotia, and contiguous infected stems would produce clusters of sclerotia in the soil.

The numbers of *S. sclerotiorum* sclerotia recorded were calculated on an area basis as this was considered the most suitable parameter for future comparative work in pastures. Most research on sclerotium populations record levels in cultivated soils, and

numbers are expressed per unit weight of soil at different depths (Adams and Ayers 1979). However, Henderson (1962) found 0-20 sclerotia/ft² to a depth of 2 inches which is equivalent to 0-230/m². This was calculated by Adams and Ayers (1979) to be equivalent to 0-3/kg of top soil. These numbers and those recorded by Abawi and Grogan (1975) on the soil surface of 173 and 58/m² in bean fields after epidemics and prior to cultivation are similar to those recorded in the present study.

The high numbers of *C. purpurea* and *R. solani* sclerotia in the treated plots may be a reflection of the change in botanical composition of the plots following application of the mycoherbicide. With the reduction in Californian thistle dry matter (Bourdôt *et al.* 1993), there was an increase in grass and clover content in the plots. This may have caused the increases in population of these fungi which are pathogenic to grasses and clovers and probably not to Californian thistle (Pennycook 1989).

This work confirms that sclerotium viability can be determined by either direct plating to culture or on carrot discs as demonstrated by Hoes and Huang (1975). Both methods recorded a 15 (for S1) to 30% (for S9) reduction in viability of the sclerotia over the winter, assuming that no sclerotia had degraded over winter and that all sclerotia were 100% viable when released from the host to the soil in the autumn. Subsequent culturing of fresh sclerotia from the field has shown this assumption to be correct.

Coniothyrium minitans and *Trichoderma* spp. are known to be hyperparasitic on sclerotia of *S. sclerotiorum* (Adams 1989), and bacteria are reported to be important in their natural destruction (Erviö *et al.* 1964). The rate of reduction in viability of a population of sclerotia in the soil is reported to be associated more with the microbiology of the soil than with any other factor that contributes to their demise (Adams and Ayers 1979). Goh and Lyons (1992) reported a 6% reduction in viability of buried sclerotia under kiwifruit vines over a 208 day period from the end of September (spring/summer/autumn period) and did not recover *C. minitans*. The microorganisms isolated in this study are likely to have contributed to the loss of viability recorded.

The low number of sclerotia detected in the stems of the treated plots 19 months after treatment and the failure to detect any in the untreated plots indicates two important characteristics of the pathogen when applied inundatively to Californian thistle stands. Firstly, the disease did not spread from the treated plot into the untreated plots, even though there was a resident, viable population of sclerotia in the soil in the season after application. Secondly, it shows that the pathogen was still resident and capable of causing disease in the treated plots, but that the level of attack, and subsequent addition of sclerotia to the soil was low compared to that contributed by the initial application and stem death in the first season. What is uncertain is the source of the infection in the second season stems. It either arose through the pathogen remaining viable in the diseased underground perennial roots or through the myceliogenic germination of sclerotia or both.

The failure of *S. sclerotiorum* to spread to adjacent untreated *C. arvense* plots and the low carry-over rate in treated plots to either cause disease or increase the population of soil sclerotia, implies that the risk of the pathogen spreading from areas of treated pasture may be low. However, the factors that contribute to the population level of sclerotia of *S. sclerotiorum* in the soil following inundative application of the pathogen for weed control in pastures are complex as are those that contribute to their reduction in the subsequent seasons (Adams and Ayers 1979). Further studies of these factors together with those that influence the ability of the pathogen to spread from the site of application (Abawi and Grogan 1978) are now required to allow the assessment of the risk of using this pathogen as a mycoherbicide under a variety of pasture, edaphic and climatic situations.

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