DORMANCY OF *SCLEROTIUM CEPIVORUM* SCLEROTIA IN NEW ZEALAND SOILS

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ABSTRACT

Sclerotium cepivorum sclerotia require incubation in soil to overcome constitutive dormancy, a condition where the sclerotia will not germinate even when stimulated. In Trial 1, artificial onion extract, diallyl disulphide (DADS) was used to stimulate sclerotial germination of laboratory produced sclerotia after 1, 2 and 3 month conditioning periods when incubated in two different soil types. The results showed that soil type and fungal isolate did not affect dormancy and that approximately 16, 33 and 21% of the sclerotia germinated after 1, 2 and 3 month conditioning periods, respectively. In Trial 2, DADS significantly increased sclerotial germination compared with the control after 2, 3, 4, 5 and 6 month conditioning periods. Sclerotia required 6 months in soil before high rates of germination occurred (>89%) when stimulated. When a natural population of sclerotia (≤ 8 weeks old) (Trial 3) was exposed to DADS, 51% of the population germinated compared with 21% in the control ($P < 0.001$).

Keywords: *Sclerotium cepivorum*, sclerotia, dormancy.

INTRODUCTION

Sclerotium cepivorum Berk. is the causal agent of onion white rot, one of the most economically important disease of onions worldwide (McLean et al. 2005). *Sclerotium cepivorum* survives in soil as sclerotia, which can remain viable in the soil for a substantial length of time (Coley-Smith 1959; Coley-Smith et al. 1990). Sclerotia are subject to two forms of dormancy, exogenous and constitutive. Exogenous dormancy is brought about by an unfavourable environment and is broken by a return to conditions favouring growth. Constitutive dormancy is the inherent inability of sclerotia to germinate, which is overcome by special stimuli not required for vegetative growth (Sussman & Halvorson 1966). Before constitutive dormancy can be broken, sclerotia need to reside in non sterile soil for a length of time; this is known as a conditioning period.

Sclerotia germinate in the presence of *Allium* crops. *Allium* species exude S-alk(en)yl-L-cysteine sulfoxides, which are broken down by soil micro-organisms to form 1-propyl- and 2-propenyl (diallyl) disulphide compounds, which stimulate *S. cepivorum* sclerotia to germinate (Coley-Smith & King 1969). A synthate of one of these exudates, diallyl disulphide (DADS), has been used at 0.1–0.2 g/litre to trigger high levels of germination (90–100%) in the absence of a suitable host crop (Coley-Smith & Parfitt 1986). This reduced the inoculum density in the field and lowered the disease incidence in subsequent *Allium* crops.

This paper reports on the length of conditioning period required by sclerotia before constitutive dormancy of *S. cepivorum* sclerotia can be broken by exposure to DADS. Trial 1 examined the effect of soil type and conditioning period on sclerotial dormancy of three *S. cepivorum* isolates using a laboratory-based method. A second trial studied the effect of conditioning period on dormancy of five *S. cepivorum* isolates in the field. Trial 3 examined the response of a natural population of sclerotia to DADS exposure.

MATERIALS AND METHODS

Trial 1

Three *S. cepivorum* isolates were isolated from onion-growing land in New Zealand (SP1 and SP2 from Pukekohe, North Island, and SK1 from Kaiapoi, Canterbury, South Island). Sclerotia of each isolate were produced on onions in the laboratory (Coley-Smith 1985). When the sclerotia were mature (6 weeks after inoculation), they were collected from the onions by progressive wet-sieving (Alexander & Stewart 1994) through 850, 500 and 250 μm sieves. Only the sclerotia collected on the 250 μm sieve were retained for use as this size was the most commonly produced under natural conditions. Sclerotia were air-dried for 1 h, counted into lots of 75 and contained in nylon mesh bags (10 cm^2 , mesh size 85 μm , Scapa Filtration Ltd., Auckland) with 20 g of sand and 20 g of sieved soil (<150 μm).

The bags of sclerotia (45/soil type) were either buried 10 cm deep in Patumahoe clay loam soil (pH 5.8–6.3, 4% organic matter with a bulk density of 0.85–1.20 g/cm^3 (Alexander & Stewart 1994; Molloy et al. 1998)) or Wakanui silt loam soil (pH 6.9, 4% organic matter with a bulk density of 1.01 g/cm^3 (McLaren & Cameron 1996)) for conditioning. Each soil type was contained in five plastic bins (47 cm diameter, 53 cm deep) (9 bags/isolate/bin) with a 5 cm layer of gravel in the base of each bin to aid drainage. Drainage holes (8/bin, 1.5 cm in diameter) were drilled into the sides of the bins. The bins were buried, in a field site at Lincoln University, with only the top 1–2 cm of the bins exposed. The bins were arranged in a grid pattern with bins containing the two soils placed alternately, approximately 1 m apart.

Ten bags per isolate (1 bag from each bin for both soil types) were removed at 1, 2 and 3 months after trial set up. The sclerotia were recovered from the bags by wet sieving as previously described. Twenty-five sclerotia were randomly selected from the 75 sclerotia originally put in each bag, and placed on top of a piece of nylon mesh (9 cm diameter). An additional piece of mesh was then placed on top of the sclerotia. The sclerotia encased in the nylon mesh were placed on top of a 0.5 cm thick layer of soil, the same as that used for the conditioning period, in a Petri dish. An additional layer of soil (0.5 cm) was placed on top of the nylon mesh enclosing the sclerotia before the lid was placed on the Petri dish. For each soil type, 1 ml of 0.08% DADS (Elliott Chemicals Ltd., Auckland, New Zealand) was added to each of three Petri dishes (Brix & Zinkernagel 1992) and the remaining two dishes received 1 ml of water and acted as controls. Soil in all Petri dishes was moistened with 1 ml Tween 20 solution (Brix & Zinkernagel 1992). The dishes were sealed with plastic film and incubated at 18°C in the dark. The DADS treated dishes were incubated in a separate, identical incubator to the controls to prevent diffusion of DADS and cross contamination among the dishes. After 3 months in the incubator, sclerotial germination was assessed. For the first assessment (1 month conditioning period) germination was determined by examining each sclerotium under the stereo microscope. As this method was time consuming, a new method was developed where the sclerotia were gently squeezed between the thumb and forefinger. Germinated sclerotia collapsed when gentle pressure was applied whereas dormant sclerotia remained intact. Thus, for the second and third assessments, sclerotial germination was assessed by applying pressure to the sclerotia.

Trial 2

The methodology for Trial 1 was used with the following modifications. Five *S. cepivorum* isolates were tested (SP3 and SP4 from Pukekohe were used in addition to SP1, SP2 and SK1). The nylon bag size was decreased (5 cm^2 , with 5 g of sieved soil

and 5 g of sand) although the number of sclerotia per bag was maintained at 75. All bags were placed in one bin of Wakanui silt loam soil. Five bags of sclerotia for each isolate were removed every month for 6 months. One ml of 0.08% DADS was applied to each of three bags per isolate using a pipette, where the pipette tip was used to puncture the nylon mesh to deliver the DADS to the sclerotia. Bags were then placed in a new soil bin. The remaining two bags acted as the controls and were injected with water and placed in a separate soil bin. Scoring of germination was done 2 months after DADS was applied. Intact sclerotia recovered from the bags by wet sieving, as previously described, were classified as dormant as it was shown from preliminary trials that germinated sclerotia with hollow rinds would have disintegrated during the sieving process.

Trial 3

A natural population of sclerotia was collected from 30 infected onion bulbs in a commercial field at Pukekohe. The sclerotia were assumed to be 2 months old or less as there were no white rot symptoms observed in the collection area 2 months prior to sampling and a portion of sclerotia had half-formed rinds indicating that they were immature. Only black sclerotia with fully-formed rinds were used. There were 20 replicate bags with 100 sclerotia per bag. Half of the bags were immediately exposed to DADS as described for Trial 2 and then buried in a single bin of Wakanui silt loam soil. The remaining 10 bags acted as the control and were treated with water and buried in a separate bin of soil. Sclerotia were recovered from the bags after 2 months incubation using wet sieving and any intact sclerotia were counted and scored as dormant.

Data analysis

The percentage of germinated sclerotia was analysed using an Analysis of Variance (ANOVA) with the factors isolate, soil type, treatment and incubation period (if applicable). Where significant main or interaction effects were found these were further explored using Fisher’s protected Least Significant Difference test (LSD). Although data were initially arcsin transformed prior to analysis, ANOVA results were consistent irrespective of transformation, therefore the raw data were analysed and presented.

RESULTS

Trial 1

The application of DADS significantly increased sclerotial germination compared with the control treatment for all conditioning periods (Table 1). For each of the conditioning periods, there were no significant interactions. The main effects of isolate and soil type were not significant ($P>0.05$).

TABLE 1: Mean percentage of *Sclerotium cepivorum* sclerotial germination in response to diallyl disulphide in Trial 1 after 1, 2 or 3 months of conditioning.

Treatment	Length of conditioning period (months)		
	1	2	3
DADS	16.4	33.8	21.3
Control	3.7	2.0	1.7
LSD ($P=0.05$)	7.6	7.2	6.8

Trial 2

Sclerotium cepivorum sclerotial germination when exposed to DADS following conditioning periods of 1 to 6 months in length is shown in Figure 1. Across all treatments isolate SP2 had lower ($P=0.005$) sclerotial germination (27.6%) than the other isolates (SP1 (43.4%), SP3 (37.5%), SP4 (39.1%) and SK1 (41.2%)) but there was no differential isolate effect in response to DADS. After a 1 month conditioning period, there was no significant difference ($P=0.59$) in the number of germinated sclerotia between treatments

(Fig. 1). After a 2 month conditioning period, significantly more ($P=0.023$) sclerotia germinated when exposed to DADS compared with the control sclerotia. This trend continued for the remainder of the trial with significantly more ($P<0.001$) sclerotia germinating following 3, 4, 5 and 6 month conditioning periods when treated with DADS compared with the control. An increasing linear relationship was observed between sclerotial germination and conditioning period length when sclerotia were exposed to DADS. After a 6 month conditioning period sclerotial germination rates for all isolates when treated with DADS were 89-98%.

Trial 3

When the field population of sclerotia was exposed to DADS, 51.3% germinated compared with the control treatment where significantly less ($P<0.001$) sclerotia germinated (20.5%).

FIGURE 1: Mean percentage *Sclerotium cepivorum* sclerotial germination for five isolates with or without exposure to diallyl disulphide (DADS) following conditioning periods of 1, 2, 3, 4, 5 or 6 months.

DISCUSSION

Trial 1 results showed that a small proportion of sclerotia (16%) broke dormancy within 1 month of soil conditioning. Sclerotial germination increased to 33% after a 2 month soil conditioning period but this trend of increasing germination with increased conditioning period did not continue. The laboratory incubation method used in Trial 1 gave variable results, which were also observed in other trials (A. Stewart, unpubl. data) using this method. The literature also supports this finding. For example, Coley-Smith et al. (1987) used a laboratory-based method to study constitutive dormancy of one *S. cepivorum* isolate, and found that sclerotial germination increased to 63% after a 2 month conditioning period and then decreased to 31% after a 3 month conditioning period. The field incubation method used in Trial 2 and 3 was considered to give a more realistic assessment of sclerotial germination behaviour.

Trial 2 results indicated that dormancy broke for all isolates in a linear fashion and that increasing the length of the conditioning period resulted in an increase in sclerotial germination rather than there being a definite time period of soil incubation after which all sclerotia germinated when stimulated to do so. The literature supports this result (Coley-Smith et al. 1987; Brix & Zinkernagel 1992). Brix & Zinkernagel (1992) found that 18 and 34% of sclerotia from two *S. cepivorum* isolates, 9/85 and 15/86 respectively,

germinated after a 6 week conditioning period when exposed to DADS, which increased to 39 and 59% after 12 weeks. The progressive breakage of dormancy of *S. cepivorum* sclerotia may be a survival mechanism that enables some sclerotia to germinate and infect the current susceptible crop and the remaining proportion to wait until next season before germinating.

Some sclerotia (approximately 20%) germinated in the control treatments. Similar proportions were observed for the sclerotia produced on the onions in the laboratory (Trial 2) and the field produced sclerotia (Trial 3). While DADS treatment will stimulate *S. cepivorum* sclerotia to germinate, low temperature treatments or stress factors can also trigger sclerotial germination (Crowe & Hall 1980; Gerbrandy 1989). It is also possible that a proportion of sclerotia, irrespective of treatment, decayed and these would have been included in the germination counts because of the method of assessment used in this study. A number of factors cause sclerotial decay; these include environmental parameters, microbial attack and the physical properties of the soil (Legget et al. 1983; Legget & Rahe 1985; Coley-Smith et al. 1990; Alexander & Stewart 1994).

This research has practical applications for the integrated disease management of onion white rot. Diallyl disulphide can be used to reduce *S. cepivorum* inoculum levels in the soil. As high rates of *S. cepivorum* sclerotial germination occurred after 6 months in soil, an application of DADS in the spring would be more appropriate than an autumn treatment following a disease outbreak. This should be practical as DADS can be applied while a cover crop is in place (Tyson et al. 2000). There was no soil type or strong isolate effect detected in this study, which is consistent with the literature (Gerbrandy 1990), therefore DADS recommendations would be applicable to different soil types and onion growing regions.

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