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Stimulation of germination of sclerotia of Sclerotium cepivorum at different depths in soil by artificial onion oil

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Abstract

The influence of depth of inoculum on white rot and plant growth, and the response of sclerotia of *Sclerotium cepivorum* Berk. to artificial onion oil, at various depths in soil, was investigated.

Field tests showed that effects of depth of inoculum were apparent 12 weeks after sowing and by week 14, sclerotia buried at 0-2, 10-12 and 18-20 cm reduced onion growth by 75, 56 and 37% in comparison with controls. The results indicate the importance of correct placement of treatments, such as onion oil, in soil to achieve maximum levels of disease control. In vitro tests in closed containers at 15°C showed that between 50-70% of sclerotia of *S. cepivorum* germinated on soil treated with artificial onion oil. Production of secondary sclerotia was restricted to 0.5% of those which germinated. Application of oil at 10 cm depths in columns of soil caused germination of 60-70% of sclerotia at 3, 6, 14 and 17 cm over 4 weeks at 15°C. Germination at the soil surface was reduced. In field plots onion oil and one of its components, diallyl disulphide, reduced recovery of introduced sclerotia by 70%.

Additional keywords: diallyl disulphide, white rot (onion)

Introduction

Field tests (Merriman and Sutherland, 1978; Merriman et al., 1980), in which artificial onion oil was injected in soil before sowing, have demonstrated the potential of this method for control of white rot in dry bulb onions. This approach to disease control was based on research by Coley-Smith and his co-workers (Coley-Smith and Cooke, 1971). They demonstrated that natural extracts of *Allium* spp. and certain alkyl sulphides, including those found in onion oil, stimulated germination of sclerotia of *Sclerotium cepivorum* Berk. under laboratory conditions. The data from the field tests indicated a similar stimulatory response of sclerotia to onion oil and a subsequent reduction of sclerotial numbers, which appeared to be due to death of germinated sclerotia in soil free from roots of *Allium* spp.

One objective of this project was to investigate the effect of depth of inoculum on

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growth of onions, to provide information on the minimum layer of soil requiring treatment with compounds such as onion oil. A second objective was to investigate in more detail the response of sclerotia to artificial onion oil on and, at various depths, in soil. Particular attention was paid to the proportion of sclerotia which germinated and to the production of secondary sclerotia.

Materials and methods

Soils for laboratory experiments were collected from fields used for growing onions, near Steenbergen; from a field used for growing cereals near Lienden and from an experimental site in the botanic garden at Baarn. Samples were taken to a depth of 10 cm, air dried, sieved (4 mm mesh) and then rewetted to the appropriate moisture level. The Steenbergen and Lienden soils were sandy loams of pH 7.48 and 7.29, respectively. The botanic garden soil was a sand of pH 5.75.

For all experiments, except the one involving depth of inoculum and plant growth, sclerotia were extracted from a soil, near Steenbergen, with a population of approximately 500 sclerotia/kg of soil. Extraction from soil followed a wet sieving method (Merriman and Isaacs, 1977). Fine forceps were used to select firm sclerotia, which were stored in moist Lienden at 15 °C prior to use. In the experiment on depth of inoculum, sclerotia from diseased onions, which had been stored at room temperature for 6 months, were used. These sclerotia were placed on moist filter paper and after 15 min firm ones were selected for experimental purposes. When assessing effects of treatments on population of sclerotia in soil, soft as well as firm sclerotia were recorded as viable, because *S. cepivorum* could be isolated from soft sclerotia.

Soils were treated with either artificial onion oil or diallyl disulphide at various concentrations in water. Before being diluted, the concentrated chemicals were mixed with small quantities of the wetting agent Tween 80, so as to improve emulsification in water. Artificial onion oil (Bush Boake Allen, London, England, commodity code C7713) is synthesised from diallyl disulphide, di-isopropyl disulphide, allyl isothiocyanate, allyl alcohol, garlic oil and nut oil. The exact formulation remains undisclosed.

Experiments and results

Effect of depth of inoculum on growth of onions. Groups of 112 selected sclerotia were mixed with 15 samples each of 330 g of moist soil from the botanic garden (= 336 sclerotia/kg). Plastic drainage pipes (10 cm diameter × 20 cm high) were placed in soil after the removal of 10 cm core samples to a depth of 20 cm. The soil cores were then replaced in the pipes with the inoculum at either 0-2, 10-12 or 18-20 cm. For the 18-20 and 0-2 cm treatments, the inoculum was added before and after replacing the soil core, respectively. For the 10-12 cm treatment the soil core was replaced to 10 cm and then partitioned to allow addition of the inoculum before replacing the remainder of the soil core. For control treatments, soil cores were removed and replaced without the addition of inoculum. Each 'inoculum depth' treatment and controls were replicated five times, so that the experiment included a total of 20 pipes.

Onions c.v. Rivato were sown at a depth of 2 cm in soil in pipes 6 weeks after the addition of inoculum, and seedlings were thinned to two plants per pipe. Measurements of plant growth were commenced at 6 weeks after sowing and continued for 8 weeks. Plants from pipes were washed free of soil by soaking in water and the roots were examined for evidence of infection. Root segments bearing mycelium were transferred to potato dextrose agar to be checked for infection by *S. cepivorum*. The dry weights of plant tops and roots were then determined.

Results. Weekly increases in height of plants were similar from weeks 6 to 11, and therefore only data for week 11, and not the preceding weeks, are presented (Table 1). Differences between the increase in height of the controls and of the treatment with inoculum at 0-2 cm were apparent by week 12, and by week 14 the increase was less than one third of the controls (Table 1). The effects of the inoculum at 10-12 cm were apparent by week 13 and at week 14 the increase was less than one half of the controls. There was no effect of the inoculum at 18-20 cm on increase in height when compared with the controls. Inoculum at 0-2 and 10-12 cm reduced the dry weight of both tops and roots compared with the controls (Table 1) but inoculum at 18-20 only affected dry weight of roots.

S. cepivorum was isolated from roots of plants form all treatments where sclerotia had been added to soil, but not from roots of plants from uninoculated treatments. When the experiment was terminated, typical symptoms of white rot were beginning to show on the basal plates of bulbs from the treatment with inoculum at 0-2 cm.

Direct observations of the effect of artificial onion oil on sclerotia at the soil surface. Tests were made in 8 cm diameter Conway dishes, in which 2 μl drops of a solution of onion oil in water (at concentrations of 5%, 1% and 0.1%) were placed at equal distances in the outer ring of each dish. Fifteen grams of Steenbergen soil, at 42% of field capacity, were placed over the onion oil. The central well of the dish

Table 1. Effect of depth of inoculum on weekly increases in height of onions and dry weight of plant tops and roots.

Depth inoculum (cm)	Increase in plant height (cm)				Dry weights (g)	
	week 11	week 12	week 13	week 14	tops	roots
Nil (control)	3.6[1]	5.5	4.2	5.7	8.60	1.147
0- 2	3.2	2.9*	1.8**	1.5**	3.67**	0.629**
10-12	2.6	3.0	2.3*	2.5*	3.93**	0.444**
18-20	4.2	5.9	4.1	3.6	6.61	0.735*

Significantly different from controls: *P = 0.05; ** = 0.01.
[1] Figures are mean of 5 replicates.

Tabel 1. De invloed van de diepte van het inoculum in grond op de wekelijkse toename in hoogte van uieplanten en op het drooggewicht van bovengrondse plantedelen en van het wortelstelsel.

contained 2 ml of tap water. Twenty sclerotia per dish were pressed into the soil at regular intervals so that approximately half of the area of each sclerotium was in contact with soil. The lids were replaced, sealed and dishes kept at a constant temperature of 15 °C. Control dishes were prepared in the same way but without the addition of onion oil. Treatments were replicated five times.

Dishes were examined under a dissecting microscope, where ever possible at 2 day intervals, for germinating sclerotia. The observations were made through a transparent perspex sheet which was used as a temporary replacement for the lids. At the end of the experiment an examination was made for production of secondary sclerotia either on mycelium or within the shells of germinated sclerotia.

Results. Germination of sclerotia occurred over 40 days, with the highest activity between days 8 and 26 (Fig. 1). At the end of the experiment 19% of sclerotia had germinated in the dishes without onion oil, compared with between 51 and 68% (P = 0.01) for onion oil treatments. Differences between the three concentrations of oil were not significant.

Mature secondary sclerotia and immature ones without rinds, were produced from 0.5 and 6% of germinated sclerotia, respectively.
Observations over an additional 20 days showed that no further development of immature sclerotia occurred.

Effect of the application of artificial onion oil at 10 cm depth on sclerotia at five depths in soil. Twenty sclerotia were placed in small (1.0-1.5 cm diameter) bags of nylon mesh (250 μm) together with approximately 2 g of air dried Lienden soil. Sandy loam from Steenbergen with moisture adjusted to either 27 or 37% of the field capacity was added to plastic drainage pipes (10 cm diameter × 20 cm high), a few centimeters at a time, and compacted as uniformly as possible. During this procedure bags with sclerotia were placed in the centre of pipes at levels of 0, 3, 6, 14 and 17 cm (1 bag at each level). After compaction was completed artificial onion oil (0.5 ml of a 5% concentration in water) was injected by a syringe at a depth of 10 cm in pipes. A temperature of 15 °C was maintained during the experiment and mesh bags were sampled at 1, 2, 4 and 6 weeks after application of onion oil to determine

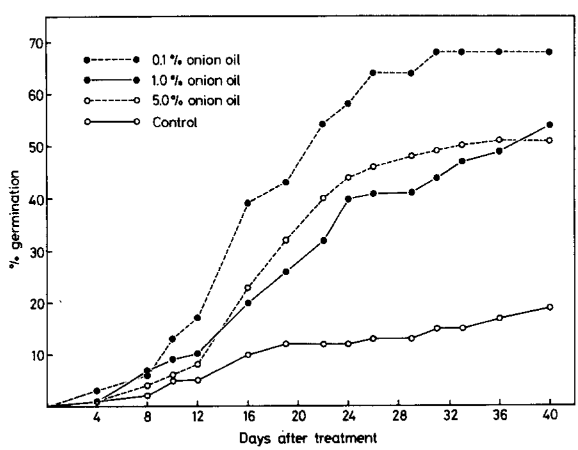

Fig. 1. Effect of three concentrations of artificial onion oil on germination of sclerotia at the soil surface in Conway dishes at 15 °C. Controls contained no onion oil.

Fig. 1. De invloed van drie concentraties synthetische uienolie op de kieming van sclerotiën op het grondoppervlak in Conway-schalen bij 15 °C. De controles bevatten geen uienolie.

the proportion of germinated sclerotia. Treatments were replicated five times and a total of 80 pipes was prepared for the experiment. Destructive sampling methods were used so that at each sampling date bags were recovered by removing soil from 20 pipes.

Moisture levels in the wetter soil were controlled by placing the pipes on a fine sand held at a suction tension equivalent to 40 cm of water (Van Vuurde and Schippers, 1980). Pipes with the drier soil were placed on a substrate of air dried potting mix. A thin layer of moist cotton-wool was placed on the soil surface of pipes to prevent excessive moisture loss. By these methods the moisture content of soils remained constant. Moisture levels in soil were checked at each sampling time using a duplicate set of pipes with soil but no sclerotia. Sclerotia were recovered during microscopic examination of the opened bags in water. The number of empty, germinated and non-germinated sclerotia from each bag was recorded. Presence of empty sclerotia indicated germination at an earlier stage.

Results. The results (Table 2) demonstrate that the treatments stimulated germination at 3 cm by week 1, at 0, 3, 6 and 17 cm by week 2 and at all depths by weeks 4 and 6. In pipes treated with oil, germination of sclerotia was less at the surface than at 3 cm (P = 0.05; weeks 4 & 6). The results of a duplicate experiment carried out with the same soil held at 37% of field capacity were similar to those in Table 2 but not presented. The only difference between the experiments was an increase (P = 0.01) in the number of germinating sclerotia in control treatments at week 4 in the wetter soil.

The results from both experiments suggest that volatiles diffuse from the onion oil in both an upward and downward direction.

Effect of two concentrations of artificial onion oil and diallyl disulphide on recovery of sclerotia. This experiment was made at a site near Steenbergen, where onions

Table 2. Effect of the application of onion oil at 10 cm on germination of sclerotia at 5 different depths in soil at 27% of field capacity.

Depth (cm)	% empty and germinated sclerotia							
	week 1		week 2		week 4		week 6	
	nil (Control)	oil	nil	oil	nil	oil	nil	oil
0	17[1]	10	10	36**	11	36**	18	40*
3	18	51**	15	48**	24	67**	16	63**
6	24	29	14	41**	6	64**	32	63**
14	15	29	13	24	18	69**	28	69**
17	10	23	11	38**	15	71**	24	64**

Significantly different from controls: *P = 0.05; **P = 0.01.

[1] Figures are the mean of 5 replicates.

Tabel 2. De invloed van uienolie op 10 cm diepte in grond op de kieming van sclerotiën op 5 verschillende diepten in grond met een vochtgehalte van 27% van de veldcapaciteit.

Table 3. Effect of two concentrations of artificial onion oil and diallyl disulphide on recovery of sclerotia from soil cores and bags sampled from plots at Steenbergen.

Treatments	%reduction in number of sclerotia recovered		
	from soil cores	from bags at 1 cm	from bags at 10 cm
Nil (control)	12[1]	28	40
0.1% Oil	45	69**	75**
5% Oil	42	56*	51
0.1% Diallyl disulphide	65	66**	75**
5% Diallyl disulphide	55	67**	70**

Significantly different from controls: *P = 0.05; **P = 0.01.
[1] Figures are the mean of 5 or 6 replicates (see text).

Table 3. De invloed van twee concentraties van synthetische uienolie en van diallyl-disulphide op de terugwinning van sclerotiën uit grond en uit in grond begraven nylonzakjes, in proefvelden bij Steenbergen.

were affected by white rot during the previous season. The population of sclerotia was assessed in five 10 cm diameter cores of soil sampled to a depth of 10 cm from plots of 1.0 × 3.5 m. In addition the effect of treatments was determined on known numbers of sclerotia in nylon mesh bags, where each plot contained a bag buried at 1 and 10 cm. Sclerotia were placed with Lienden soil in nylon mesh bags (20 sclerotia per bag) as described in the previous experiment. Artificial onion oil and diallyl disulphide were applied by injecting 2.5 ml every 25 cm at a depth of 10 cm at either 0.1 or 5% concentration in water. Control plots remained untreated. Onion oil treatments and controls were replicated six times and diallyl disulphide treatments five times, so that the experimental site incorporated 28 plots.

Soil core samples were taken immediately before and also 6 weeks after treatment, whereas bags with sclerotia were added to plots prior to treatment and sampled 6 weeks later. The soil samples were stored at 15 °C prior to the extraction of sclerotia by the wet sieving technique, and assessment of sclerotia in bags was as described for the previous experiment.

Results. The results from the mesh bags (Table 3) demonstrate the effects of the onion oil treatment and indicate that onion oil at 0.1% and diallyl disulphide at 0.1 and 5% were the most effective treatments. Similar effects were not apparent when sclerotia were extracted from soil cores, and this was thought to be due to the uneven distribution of sclerotia in plots and insensitivity of the sampling method.

Discussion

Although the mechanism of action of artificial onion oil on sclerotia has yet to be determined, the experiments in this study (Fig. 1 and Table 2) confirmed that the treatment stimulated germination of sclerotia. The question of whether the effect is direct or indirect involving mycostatis (Coley-Smith et al., 1967; Allen and Young, 1968; Keyworth and Milne, 1969) remains unresolved, but mycostasis may explain some unusual effects of the onion oil treatment. Data from previous experiments (Merriman et al., 1980) and from this study suggest a dose response relationship, because artificial onion oil appears more effective at low (0.1%) than high (5%) concentrations in water. This is unexpected with a volatile chemical because, in theory, high concentrations should eventually be as effective as lower ones due to dilution effects when gases diffuse from soil. One explanation of this situation is that different microfloras are established at different concentrations of onion oil and these in turn affect the maintenance of mycostatis. An examination of the microflora associated with sclerotia after treatment with different concentrations of onion oil might clarify this. A second possibility is that the reduced effectiveness of onion oil at higher concentrations is associated with germination inhibiting compounds which are more effective at higher concentrations of the oil.

The experiments in Conway dishes also demonstrated that secondary sclerotia are produced after germination and this confirms the findings of others (Entwistle and Munasinghe, 1978). However the results indicate that the frequency of production is low, and therefore production of secondary sclerotia is unlikely to be a significant factor in the replenishment of populations of sclerotia depleted by onion oil.
Two important aspects which require further investigation are associated with sclerotia at the soil surface and with the residual population of sclerotia after treatment. The experiment where sclerotia were placed at different levels in soil (Table 1) illustrated not only the subclinical effects of white rot, but also the importance of treating the surface layers of soil for disease control. In this context the results from the experiment with sclerotia at different depths in columns of soil are relevant, because the effect of the onion oil was reduced at the soil surface. This "surface" effect may be associated with increased dilution of volatiles at the soil-air interface, and treatments including covering soil with plastic sheeting, a surface spray of onion oil and rolling and wetting soil deserve investigation.

The residual population of sclerotia after treatment is likely to be a problem in soils with high populations of *S. cepivorum*. For example, a previous field experiment with dry bulb onions (Merriman et al., 1980) showed that, after treating a soil with 190 sclerotia/kg soil, the reduction in sclerotial numbers to 90/kg soil was insufficient to affect incidence of the disease. References to whole population levels of sclerotia however can be misleading because observations indicate that in many soils the distribution of sclerotia is uneven, and numbers may vary by 100 times. Therefore, from a practical point of view it seems that infested soils should be treated more than once to account for pockets of high numbers of sclerotia. The effect of subsequent applications of onion oil are unknown, but if each eliminates 60% of sclerotia, then after three treatments numbers of sclerotia varying from 50-300/kg soil should be expected to fall to 3 and 19/kg respectively. Experience in Victoria indicates that at these levels incidence of white rot in dry bulb onions is low.

The introduction of this type of disease control into commercial practice will inevitably require improvements in the methods and timing of the application of treatments. For example, one problem is associated with the retention of the onion oil-water mixture as an emulsion and, consequently, intermittent shaking of the mixture is required during application to soil. This may be overcome by use of more suitable wetting agents and preliminary tests indicate that Teric 17A3 (ICI, Australia) is more suitable than Tween 80. Another improvement may be associated with the use of diallyl disulphide as a soil treatment instead of artificial onion oil.

This should be considered not only because diallyl disulphide appears to be equally as effective as onion oil (Table 3) but also because there may be fewer variables, such as germination inhibiting substances, associated with the use of an individual chemical than with a complex formulation such as onion oil.

One of the benefits of this method of disease control is its flexibility, because treatments may be applied while cultivating soil during intercropping periods or possibly when sowing other crops. However before recommending this procedure tests are required to determine whether the treatment has adverse effects on soil microflora or other crops. At concentrations of 0.1% in water and a rate of 300-400 litres/ha, the cost of the chemical is unlikely to be restrictive. Therefore onion oil and related compounds are considered to have significant potential for treatment of white rot in dry bulb onions.

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Samenvatting

Stimulatie van de kieming van sclerotiën van Sclerotium cepivorum op verschillende diepten in grond door synthetische uienolie

Het effect van de diepte in grond waarop het inoculum van *Sclerotium cepivorum* Berk. zich bevindt op het optreden van witrot in ui en op de ontwikkeling van de uieplant werd onderzocht. Ook werd de uitwerking van in grond geïnjecteerde uienolie op de op verschillende diepten in grond geplaatste sclerotiën bestudeerd.

In veldexperimenten werd na 12 weken een duidelijk effect van de diepte van het inoculum op de ontwikkeling van de planten waarneembaar. Na 14 weken bleken sclerotiën die op 0-2, 10-12 en 18-20 cm diepte in grond waren geplaatst de groei van de uieplanten met respectievelijk 75, 56 en 37% te hebben verminderd ten opzichte van de controleplanten. Deze resultaten wijzen er op, dat voor een zo goed mogelijke bestrijding van witrot, de diepte in grond waar middelen zoals uienolie moeten worden toegediend, van grote betekenis kan zijn.

Bij in vitro toetsen in gesloten Conway-schalen bij 15 °C kiemde 50-70% van de sclerotiën van *S. cepivorum* op het oppervlak van grond die behandeld was met synthetische uienolie. De vorming van secundaire sclerotiën beperkte zich tot 0,5% van het aantal gekiemde sclerotiën. Toediening van uienolie op 10 cm diepte in grondkolommen veroorzaakte de kieming van 60-70% van de sclerotiën op 3, 6, 14 en 17 cm diepte over een periode van 4 weken bij 15 °C. Bij het grondoppervlak was de kieming lager. In veldexperimenten werd na behandeling van grond met uienolie en met een bestanddeel daarvan, diallyl-disulfide, het aantal in grond geïntroduceerde sclerotiën dat kon worden teruggevonden, met 70% gereduceerd.

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