INFECTION SITES OF *SCLEROTIUM CEPIVORUM* ON ONION ROOTS

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ABSTRACT

Onion (*Allium cepa*) root infection by *Sclerotium cepivorum* was examined in two glasshouse bioassays. In the first bioassay, the effect of sclerotial depth was examined. Sclerotia placed at 1, 10 and 20 cm depths in soil filled planter bags germinated and infected onion roots at 5, 7 and 13 weeks after planting, respectively. The second bioassay determined that the severity of infection varied when sclerotia were placed at the stem base, along the length of a root and at the root tip. After 22 weeks, a greater ($P < 0.05$) number of stem base infections led to total plant collapse compared to infections occurring along the length of the root or at the root tip.

Keywords: *Sclerotium cepivorum*, sclerotia, infection, *Allium cepa*, onion.

INTRODUCTION

Sclerotium cepivorum Berk. is the causal agent of onion white rot disease. The fungus survives in the soil as sclerotia which are stimulated to germinate by the alkyl-cysteine sulphoxide compounds released by the roots of *Allium* species (Coley-Smith and King 1969). The roots are directly penetrated and the hyphae grow within and between the epidermal cell walls (Stewart *et al.* 1989). Sclerotia are mostly restricted to the top 25 cm of soil as a result of ploughing (Adams 1981; Crowe and Hall 1980) although sclerotia as deep as 30 cm can infect garlic bulbs (Crowe and Hall 1980). In this study, root infection by *S. cepivorum* was examined in two bioassays. Firstly, sclerotia were banded at 1, 10 and 20 cm depths and secondly, different root positions were examined to determine preferential infection sites. Understanding where sclerotia infect onion roots will enable better targeting of biological control agents.

METHODS

Sclerotia of *S. cepivorum* (isolate SC3, isolated from an infected onion, Pukekohe, Auckland, N.Z. in November 1996) were produced on whole wheat grains (Alexander and Stewart 1994) and harvested by wet sieving through 850 and 500 mm sieves. Sclerotia retained on the 500 mm sieve were air dried for 24 h and viability was determined (McLean and Stewart 2000) before use in both experiments.

The effect of sclerotial depth on infection

Planter bags (25 x 5 cm) were filled with field soil (Wakanui silt loam soil; -0.3 bars matric potential; Lincoln University field research area) to 3, 13 and 22 cm. Thirty sclerotia were placed on top of the soil in each bag and covered with additional soil to fill the bags to 23 cm (2 cm from the top of the bag). A control treatment consisted of field soil only (23 cm). For each treatment, 33 replicate planter bags were set up. Three thiram (Nufarm Ltd, 0.8 g ai/100 g seed) treated onion seeds (Regular Pukekohe Long Keeper, May and Ryan®) were planted in each bag at a depth of 1 cm. The bags were randomly positioned in a glasshouse with an air temperature range of -1.5 to 32.1 °C (recorded with a Tiny Tag temperature logger with internal sensor, Gemini Dataloggers, Chichester, UK) for the length of the trial (27 May 1999 - 2 December 1999). The bags were watered as required. Three weeks after planting, the seedlings were thinned

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manually to one seedling/bag. At 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 weeks, three bags/treatment were randomly selected. Any above ground white rot disease symptoms on the onion seedlings such as yellowing of the outer leaves, leaf tip die back, wilting and collapse of the stem base were recorded. The bags were cut open and the roots were carefully examined for signs of infection with a 10× hand lens. A pair of tweezers and a spatula were used to remove soil from the roots. Presence of root infection was recorded if mycelium was visible, the roots were brown instead of white, the roots felt soft instead of firm and/or had collapsed when touched with tweezers.

The effect of root position on infection

Infection sites were identified on onion roots using a split tube method (Ahmad and Baker 1987) adapted as follows. Poly-vinyl chloride open ended tubes (40 x 8 cm) were cut longitudinally into two halves. Each tube half was sealed at both ends with half a Petri dish and packed with Wakanui silt loam soil (-0.3 bars matric potential). The Petri dishes were removed from the tube ends and the tubes were incubated in plastic bags for 48 h before use. Two thiram treated onion seeds were placed on one tube half, 1.5 cm down from the top end of the tube in the middle of the open face of the soil. The other soil filled tube half was wrapped in muslin cloth, placed on top of the first tube half and secured with two rubber bands. The tubes were placed vertically into large plastic bins (64 x 41 cm) with the top end upwards. Soil was added to the bins to surround the tubes. The bins were placed in a glasshouse with a temperature range of 5.4 to 34.8°C for the length of the trial (3 August 1999 - 14 January 2000). The tubes and surrounding soil were watered as required and care was taken not to dislodge the soil. Three weeks after planting, seedlings were thinned manually to one/tube. A 2-factorial design was used to determine the effect of inoculum position on disease severity. The roots were inoculated at three sites: 1 - the stem base, 2 - along the length of a root and 3 - the root tip. Each site was randomly assigned twelve tubes. The sclerotia were positioned in the tubes at different times to determine whether the age of the seedling influenced sclerotial infection. At 6, 8, 10 and 12 weeks after planting, three tubes for each site were opened and a location for sclerotial placement was chosen. The location for the sclerotia was at approximately the same depth between the tubes for each treatment. Sclerotial placement was never greater than 8 cm below the stem base. Ten sclerotia were placed at each location to ensure infection. Twenty two weeks after seed sowing, the tubes were opened and the roots in the vicinity of the sclerotia were carefully examined using a hand lens to detect infection. The number and severity of infections was recorded as follows: severe - total plant collapse, moderate - root only infection and healthy - no infection. The disease severity of the seedlings was analysed using analysis of variance (ANOVA) with the position of the sclerotia and the time of sclerotial placement as variables.

RESULTS

Sclerotial viability was recorded as > 96% for both bioassays.

The effect of sclerotial depth on infection

No root or above ground plant part infections were recorded in the control treatment. Roots were white and firm to the touch and above ground foliage was bright green. Sclerotia placed 1, 10 and 20 cm below the soil surface caused plant infections from 5, 7 and 13 weeks, respectively after planting until trial completion. For all treatments, the number of plants infected increased over time (Table 1). Sclerotia placed at 1 cm caused severe plant infections, with collapse of above ground parts and the majority of roots breaking away from the stem base when touched with tweezers. White mycelium was present on the roots and radiated outward from infected roots a distance of 1-2 cm. Any uncolonised roots were brown and soft or collapsed. Infections progressed throughout the trial with some root disintegration. New sclerotia were produced on the collapsed onion bulb. Infections from sclerotia placed at 10 and 20 cm were moderate. Above ground plant parts remained healthy, although the roots were infected. Mycelium was visible on the infected roots but only in the area where

the sclerotia were positioned. The remainder of the roots appeared healthy and were attached to the stem base although some had softened. Mycelium was not always present on infected roots, however brown colouration and softening of the roots indicated infection.

TABLE 1: Number of onion seedlings infected by *Sclerotium cepivorum* sclerotia positioned at 1, 10 and 20 cm depths in soil at two weekly intervals.

Treatment	Weeks after planting										
	5	7	9	11	13	15	17	19	21	23	25
Control	0 ¹	0	0	0	0	0	0	0	0	0	0
Sclerotia at 1 cm	1	2	2	3	3	3	3	3	3	3	3
Sclerotia at 10 cm	0	1	2	2	3	3	3	3	3	3	3
Sclerotia at 20 cm	0	0	0	0	2	2	2	3	3	3	3

¹There were three replicate plants for each treatment at each assessment.

The effect of root position on infection

There was no difference ($P < 0.05$) in the number of infected seedlings when the sclerotia were positioned on the onion stem base, along the length of the root or at the root tip at 6, 8, 10 and 12 weeks after planting. There was no significant interaction between the number of infected seedlings and the time the sclerotia were positioned on the onion roots. The position of the sclerotia on the onion roots significantly ($P < 0.05$) influenced the severity of the infection. Sclerotia positioned at the stem base caused more ($P < 0.05$) severe infections (77%) than sclerotia positioned along the length of the root (23%), at the root tip (0%) and the control (0%). For severe infections, above ground plant parts collapsed, roots rotted away from the stem base and sclerotia formed on the collapsed bulb. Sclerotia positioned at the root tip caused a greater ($P < 0.05$) number of moderate plant infections (65%) than sclerotia positioned along the length of the root (29%), at the stem base (6%) and the control (0%). For moderate infections, above-ground plant parts remained healthy for all treatments, although the roots were infected. The majority of the infected roots were brown and soft, but mycelium was not present.

DISCUSSION

Infection did not occur during the first 4 weeks as sclerotia produced *in vitro* need a period of at least 1 month to break constitutive dormancy before germination can occur (Coley-Smith 1960).

Germinating sclerotia infected onion roots when placed at 1, 10 and 20 cm depths in the soil and grew outward from infected roots. These results confirm an earlier report, where mycelium spread outwards from *S. cepivorum* infected garlic roots grown in glass tubes (Crowe and Hall 1980). Sclerotia positioned at 10 and 20 cm deep in the soil caused root infection only and above-ground plant parts remained healthy for the 6 month period of the trial. Other research has reported the presence of above-ground disease symptoms in garlic after just 4 months (Crowe and Hall 1980). The differences in results could be due to variations in disease progression between the two *Allium* species or to differences in isolate virulence. One *S. cepivorum* isolate was tested in this study. It would be valid to test a number of isolates to determine whether disease development is consistent between isolates.

When sclerotia were placed at specific sites on the roots, the severity of infection depended on the position of the sclerotia but not the time after planting that the sclerotia and the root came into contact. However, the rate of disease development early in the trial may not have been detected as only one disease assessment was made at 22 weeks after sowing. Sclerotia positioned at the stem base caused severe infections. The production of root exudates which stimulate sclerotia to germinate (Coley-Smith and King 1969) would be greatest at the stem base due to the abundance

of roots. Infection of the root tip occurred more commonly than along the length of the root.

Infections arising from sclerotia in the top 1 – 2 cm closest to the stem base probably contribute most to yield loss. Infections initiated at lower depths will progress upwards within the root towards the stem base, but are unlikely to contribute to significant yield losses as bulbs would be harvested before bulb infection occurs.

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