SEEDLING INFECTION ASSAY FOR RESISTANCE TO *SCLEROTIUM CEPIVORUM* IN *ALLIUM* SPECIES

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ABSTRACT

Allium white rot (AWR), caused by the soil-borne fungus *Sclerotium cepivorum*, is the most devastating disease in onion crops worldwide. Integrated pest management strategies involving cultural practices, biocontrol agents, genetic engineering and selective breeding are being investigated to control this disease. A major obstacle to evaluating these different methods is the lack of a simple, accurate, rapid assessment technique for AWR pathogenesis. An assay for quantifying AWR tissue resistance in *Allium* species has been developed. Lesion development on *Allium* seedlings was used as an indicator of susceptibility to infection. A difference in AWR resistance between leek and onion seedlings was detected using this assessment technique, with infection scores of 13% and 54-56%, respectively. Leek seedlings exhibited less frequent and less extensive lesions than the onion seedlings. Engineered germplasm, onion cultivars and other related *Allium* species with varying degrees of susceptibility to AWR will be screened using this assay to calibrate levels of AWR resistance.

Keywords: *Allium cepa*, *Sclerotium cepivorum*, tissue resistance, seedling infection assay.

INTRODUCTION

Onion (*Allium cepa* L.) is the world's third most economically important vegetable crop after potatoes and tomatoes. *Allium* white rot (AWR), a fungal disease caused by *Sclerotium cepivorum* Berk., is the predominant disease of onion crops worldwide. The fungus produces long-lived survival structures (sclerotia), which are specifically induced to germinate by the presence of *Allium* root exudates, in particular alkyl cysteine sulphoxides (ACSOs; Coley-Smith et al. 1990).

Current control methods involve intensive fungicide application and crop production on uninfested land (Stewart & Fullerton 1999). However, this approach is becoming less practical as soil microbes become more efficient at degrading the active fungicidal ingredients reducing the efficacy of many fungicides. This, combined with heavy infestation, has rendered onion production unsustainable in some of New Zealand's most productive areas (e.g. Pukekohe; Webber et al. 1999). Several alternative strategies for AWR control are now being utilised as part of an integrated control programme. These include cultural practices such as soil solarisation (McLean et al. 2001) and biological control (McLean & Stewart 2000a). Unfortunately, fungicide application is still required for effective management with these systems.

Successful, stable, widespread resistance to AWR in elite cultivars has not been achieved through conventional breeding methods. Germplasm modification through biotechnology is now being investigated to combat AWR. This approach, when incorporated into an integrated disease management strategy, will reduce the need for fungicides. Possible routes to produce such cultivars include engineering onions to either prevent ACSO production in their roots or to produce compounds that will counter the

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AWR infection of roots. New breeding lines and engineered germplasm resistant to AWR will play a pivotal role in future AWR management strategies.

A major universal hurdle to research into AWR resistant *Allium* species has been the assessment of levels of AWR resistance. Field evaluation has proved extremely difficult due to numerous environmental variables and the costs involved. A rapid assessment method for AWR resistance is, therefore, essential for screening breeding lines and engineered germplasm. We present preliminary results on the development of an *in vitro* infection protocol that can detect differences in white rot susceptibility in *Allium* seedlings. This protocol was based on an assay by Stewart et al. (1989) for generating infection structures on onion roots. Leek (*A. porrum* L.), an *Allium* species considered to be less susceptible to AWR in the field (Coley-Smith & Esler 1983), and Cron19, an onion breeding line considered to be highly susceptible to AWR, were assayed.

METHODS

Sclerotia of *S. cepivorum* were produced on whole wheat grains using the technique described by Alexander & Stewart (1994). Sclerotia, 200-500 µm in diameter, were recovered from the wheat grains by progressive wet sieving (Kay & Stewart 1994), air dried for 24 h then stored in an air-tight container at 4°C. Sclerotia were surface sterilised (1 min wash in 0.5% NaOCl followed by three washes in sterile distilled water) and placed onto sterilised 9 mm circles of seed germination paper (Anchor Paper Limited, Minnesota, USA) on top of potato dextrose agar (PDA) plates amended with 0.02% diallyl disulphide (DADS; Elliott Chemicals Limited, Auckland), a specific germination stimulant. Plates were sealed with polythene wrap and incubated in an airtight container at room temperature for 5 days.

Watkins Welsh Wonder leek seeds (*A. porrum*) and Crop & Food Research Cron19 onion seeds (*A. cepa*) were germinated on moist, sterile seed germination paper folded in half length-wise. The seeds were positioned approximately 3 mm from the top of the paper at 0.5-1.0 cm intervals. The paper was folded firmly around the seeds to encourage geotropic growth, wrapped in a loosely taped plastic bag and incubated at room temperature in natural light for 5 days.

Two experiments were performed. Each experiment comprised two treatments (leek and onion) with five replicates per treatment. Leek and onion seedlings were assayed in separate replicates. A replicate consisted of 11 seedlings, 3-5 cm in length, aligned at 2 cm intervals along the top of a double layer of sterile germination paper. Newly germinated sclerotia (showing a small plug of mycelium protruding from the rind) were placed directly adjacent to the middle portion of the primary root of ten seedlings, with the additional seedling serving as a negative control. The germination paper was then folded firmly around the seedlings and placed into a loosely taped plastic bag. The seedlings were incubated upright for 4 days in indirect sunlight at room temperature to allow infection to develop in susceptible seedlings. Seedling roots were stained with 1:50 Jensen's neutral red stain to improve the resolution between healthy and diseased tissue. The seedlings were examined using a hand lens (10 X magnification). Lesion length, expressed as a percentage of the total seedling root length, was measured in millimetres using calipers. The following equation was used to determine an infection score for each replicate:

$$\text{Infection score} = \frac{(T_L / T_R) \times 100}{N_s}$$

Where: T_L = Total lesion length of inoculated seedlings in replicate (mm)
 T_R = Total root length of inoculated seedlings in replicate (mm)
 N_s = Number of inoculated seedlings in replicate

If a sclerotium was observed through the hand lens to have failed to make contact with a developing root, no result was taken. The mean infection score for each treatment was calculated. Treatment means for each experiment were analysed using a one-way analysis of variance (ANOVA) with seedling type as a factor. Significant differences between treatments were further analysed using Fisher's least significant difference (LSD) test.

RESULTS

A difference in AWR susceptibility between leek and onion seedlings was detected using this assessment technique for experiments 1 and 2 (Table 1). The mean number of seedlings infected in each replicate was significantly lower in leek than in onion in both experiments ($P<0.05$). Leek also had a significantly lower mean infection score than onion in each experiment ($P<0.05$). None of the uninoculated control seedlings were infected.

TABLE 1: Mean number of infected seedlings and mean infection scores in leek and onion challenged with *Sclerotium cepivorum* in an *in vitro* assay. Data are the mean of five replicates.

	Seedling type	No. of seedlings infected ¹	Infection score
Experiment 1	Leek	3.8	13
	Onion	8.8	54
	LSD	1.22	5.98
Experiment 2	Leek	4.4	13
	Onion	9.2	56
	LSD	2.92	7.31

¹No. infected out of a total of 10 seedlings.

DISCUSSION

These preliminary results indicate that a rapid, robust assay for host susceptibility to AWR is possible. Greater tissue resistance was detected in the leek seedlings (infection score 13) than in the onion seedlings (infection score 54-56). Lesions occurred more frequently on the onion roots and extended over a greater portion of the total root length. The short incubation time of 4 days was important, as lesions had to be restricted to the length of the root. Sensitivity in the assay is lost if lesion size cannot be measured as a proportion of the total root length, since differences in the rate of hyphal colonisation are no longer detectable. Similarly, the short incubation time limits the spread of infection beyond the root preventing cross contamination between seedlings in a replicate. Current work is focussed on evaluating whether differences between resistant and susceptible onion breeding lines can be detected using this assay.

Ultimately, digital image analysis of lesion size will be implemented in the *in vitro* assay to improve sensitivity and to increase the rate of sample throughput. Digital images of the stained root tissue in each replicate will be assessed using a computer program capable of detecting colour differences between diseased and healthy root tissue. In this way, the infection score for each replicate can be calculated from a single image rather than measuring each seedling manually.

AWR resistance assessment techniques have been developed by other researchers. A bulb scale assay, reported by McDonald et al. (1996), used lesion size produced by pre-germinated sclerotia placed on onion bulb scales as an indicator of susceptibility to AWR. Utkede & Rahe (1978) used field trials in infected soil to determine susceptibility of different onion cultivars to AWR. However, both of these assays require extended periods of time to produce the necessary plant material for testing and, therefore, are not useful for rapid and extensive screening of new breeding lines.

A second assay is also being developed to compliment the *in vitro* assay. This *in vivo* assay quantifies the potential of *Allium* species to stimulate sclerotia to germinate and is based on an existing spilt tube soil assay (Ahmad & Baker 1987; McLean & Stewart 2000b). The two assays will be used in combination to screen onion germplasm, developed through conventional breeding or genetic modification, for AWR resistance. The assays

will also assist with determining the nature of any resistance detected, for example, whether it is tissue resistance or a reduction in sclerotial germination.

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