Project title:	Integrated control of Allium white rot
Project number:	FV 449a
Project leader:	John Clarkson, University of Warwick
Report:	Annual report, March 2019
Previous report:	N/A
Key staff:	Alexander McCormack, University of Warwick James Howell, VCS Erika Wedgewood, ADAS
Location of project:	University of Warwick, Wellesbourne Campus, Warwick.
Industry Representative:	Phil Langley, G's Growers
Date project commenced:	01/03/2018
Date project completed (or expected completion date):	28/02/2021

© Agriculture and Horticulture Development Board 2019. All rights reserved

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

© Agriculture and Horticulture Development Board 2019. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

All other trademarks, logos and brand names contained in this publication are the trademarks of their respective holders. No rights are granted without the prior written permission of the relevant owners.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

© Agriculture and Horticulture Development Board 2019. All rights reserved

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Alex McCormack

Researcher

Warwick Crop Centre, School of Life Sciences, University of Warwick

Signature: Alex McCormack Date: 28/03/2019

John Clarkson

Reader

Warwick Crop Centre, School of Life Sciences, University of Warwick

Signature: John Clarkson Date: 28/03/2019

© Agriculture and Horticulture Development Board 2019. All rights reserved

CONTENTS

© Agriculture and Horticulture Development Board 2019. All rights reserved

© Agriculture and Horticulture Development Board 2019. All rights reserved

© Agriculture and Horticulture Development Board 2019. All rights reserved

GROWER SUMMARY

Headline

Allium white rot disease incidence was significantly reduced by succinate dehydrogenase inhibitor (SDHI) and demethylation inhibitor (DMI) fungicides in a field trial while biological and seed treatments had no significant effect. Commercial germination stimulants based on *Allium* extracts were effective in stimulating germination of *Sclerotium cepivorum* sclerotia *in vitro*.

Background

Sclerotium cepivorum is the causal agent of *Allium* white rot (AWR) an economically important disease of onion (*A. cepa*), garlic (*A. sativum*) and other *Allium* spp. worldwide (Entwistle, 1990). The bulb onion and salad onion sectors in the UK with areas of 8,762 ha and 1571 ha respectively, were worth £134M and £25M in 2017 respectively (Defra Horticultural Statistics, 2017) and with a minimum of 2-3% bulb onions and 10-15% salad onions affected by AWR annually, this equates to losses of up to £4M for each crop. In addition to this, the heavy infestation of some sites has led growers to abandon onion growing in areas of the East and South East of England with production moved to less infested, but lower yielding areas.

The pathogen infects the root systems of plants from soil-borne sclerotia (resting structures), causing roots to collapse and decay, leading to reduced crop vigour, chlorosis and often plant death. This can result in high levels of physical and marketable yield loss, with the production of further sclerotia allowing the pathogen to proliferate and persist in soil between crops. Relatively small quantities of sclerotia are required for disease to develop with densities as low as 0.1 sclerotia L^{-1} soil leading to economic loss, whilst higher levels such as 10 sclerotia L^{-1} soil can lead to total crop loss (Crowe *et al*., 1980; Davis *et al*., 2007). In addition, sclerotia are able to survive for periods of up to 20 years in soil (Coley-Smith *et al*., 1990).

Currently management options for AWR are limited. Cultural control approaches aim to prevent infestation through practicing good equipment/field hygiene measures (although due to the small and persistent nature of sclerotia, this is challenging), whilst the use of wide rotations aims to prevent inoculum build up. Chemical control is limited in the UK to off label approvals under the HSE Extension of Authorisation for Minor Use (EAMU) scheme. At the time of writing only Signum® (Boscalid and Pyraclostrobin) and Tebuconazole are registered for use against AWR in the outdoor production of bulb/salad onion, onion sets, garlic and shallots. However other products/active substances (a.s) have shown promise elsewhere

© Agriculture and Horticulture Development Board 2019. All rights reserved

(Villata *et al*., 2004; 2005; Ferry-Abee, 2014) and were reviewed by Clarkson *et al*., 2016 in AHDB project FV 499.

Other alternative methods of AWR disease management have also been explored, such as biopesticides (Clarkson *et al*., 2002; 2004), biofumigation (Smolinska, 2000), solarisation (McLean *et al*., 2001) and sclerotial germinants (Coventry *et al*., 2006; Coley-smith *et al*., 1969) but few of these are currently practiced commercially.

Consequently, the aim of this project was to identify and test a range of treatments for the integrated control of AWR in bulb and salad onions, and generate preliminary data for the effect of selected treatments on Fusarium basal rot caused by *F. oxysporum* f.sp *cepae*. Two objectives were carried out in the current year;

1) Test fungicides and biological control agents for their effect on *Allium* white rot disease.

2) Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*.

Summary

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease.

- A good level of AWR disease control was observed for some fungicide treatments in a field trial carried out at an inoculated site at Wellesbourne (Warwickshire) but not at two commercial field sites in Cambridgeshire and Lincolnshire because of low levels of disease (<10% incidence).
- Fungicides based on SHDI and DMI chemistry gave the best levels of AWR control, with single or double applications proving to be similarly effective except for HDC 246 where two applications significantly improved control.
- Biological products were not effective in reducing AWR disease incidence at any of the sites, nor was a DMI fungicide seed treatment.
- Applications were made using a banded application and high water volume (1,000 L ha^{-1}) which likely contributed to their efficacy.

Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*.

- An *in vitro* (laboratory based) assay was developed and used to examine commercially developed and unformulated *Allium* extracts for their ability to stimulate germination of *S. cepivorum* sclerotia.

© Agriculture and Horticulture Development Board 2019. All rights reserved

- Use of pure diallyl sulphide (DAS), previously identified as a germination stimulant, promoted germination of laboratory-produced *S. cepivorum* sclerotia which had been 'conditioned' in soil, while little to no germination was observed in untreated sclerotia.
- Commercially formulated *Allium* products generally resulted in high levels of sclerotial germination, with product HDC F264 resulting in 90% germination.
- Unformulated products derived from food grade garlic granules resulted in 64-74% sclerotial germination.

Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum.

- A preliminary *in vitro* experiment showed that biofumigants were able to partially inhibit the mycelial growth of *S. cepivorum* on agar.
- Biofumigants which contained the glucosinolate sinigrin where generally more effective against *S. cepivorum* than those containing glucoraphanin or glucoerucin.
- Effects of biofumigants on *F. oxysporum* f.sp *cepae* will be tested in year 2.

Financial Benefits

None to report at this time.

Action Points

None to report at this time.

© Agriculture and Horticulture Development Board 2019. All rights reserved

SCIENCE SECTION

Introduction

Sclerotium cepivorum is the causal agent of *Allium* white rot (AWR) an economically important disease of onion (*A. cepa*), garlic (*A. sativum*) and other *Allium* spp. worldwide (Entwistle, 1990). The bulb onion and salad onion sectors in the UK with areas of 8,762 ha and 1571 ha respectively, were worth £134M and £25M in 2017 respectively (Defra Horticultural Statistics, 2017) and with a minimum of 2-3% bulb onions and 10-15% salad onions affected by AWR annually, this equates to losses of up to £4M for each crop. In addition to this, the heavy infestation of some sites has led growers to abandon onion growing in areas of the East and South East of England with production moved to less infested, but lower yielding areas.

The pathogen infects the root systems of plants from soil-borne sclerotia (resting structures), causing roots to collapse and decay, leading to reduced crop vigour, chlorosis and often plant death. This can result in high levels of physical and marketable yield loss, with the production of further sclerotia allowing the pathogen to proliferate and persist in soil between crops. Relatively small quantities of sclerotia are required for disease to develop with densities as low as 0.1 sclerotia L^{-1} soil leading to economic loss, whilst higher levels such as 10 sclerotia L^{-1} soil can lead to total crop loss (Crowe et al., 1980; Davis et al., 2007). In addition, sclerotia are able to survive for periods of up to 20 years (Coley-Smith *et al*., 1990).

Currently management options for AWR are limited. Cultural control approaches aim to prevent infestation through practicing good equipment/field hygiene measures (although due to the small and persistent nature of sclerotia, this is challenging), whilst the use of wide rotations aims to prevent inoculum build up. Chemical control is limited in the UK to off label approvals under the HSE Extension of Authorisation for Minor Use (EAMU) scheme. At the time of writing only Signum® (Boscalid and Pyraclostrobin) and Tebuconazole were registered for use against AWR in the outdoor production of bulb/salad onion, onion sets, garlic and shallots. However other products/active ingredients (a.i) have shown promise elsewhere (Villata *et al*., 2004; 2005; Ferry-Abee, 2014) and were reviewed by Clarkson *et al*., 2016 in AHDB project FV499.

Other alternative methods of disease management have also been explored, such as biopesticides (Clarkson et al., 2002; 2004), biofumigation (Smolinska, 2000), solarisation (McLean et al., 2001) and sclerotial germinants (Coventry et al., 2006; Coley-Smith et al., 1969) but few of these are currently practiced commercially.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Aims

To identify and test a range of treatments for the integrated control of AWR in bulb and salad onions, and generate preliminary data for the effect of selected treatments on Fusarium basal rot.

Objectives

Year 1.

1) Test fungicides and biological control agents for their effect on *Allium* white rot disease.

2) Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*.

3) Test biofumigants for their ability to reduce viability of *S. cepivorum*.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Materials and methods

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease

Field trial site selection

Field trials were situated at three sites, all of which had previous records of white rot infection (Table 1.0). The site at Wellesbourne has been periodically inoculated with *S. cepivorum* sclerotia over a period of approx. 40 years with the last application in 2005. However, a high disease pressure was confirmed in onion grown in this site in 2015-2017. The three field sites also allowed for the examination of other factors such as soil-type and local environmental conditions (Table 1.0).

Table 1.0. Individual locations of the field experiments in 2018 detailing; site/experiment code, host organisations address and field grid location. Soil texture was taken from the Land information system (LandIS) (Hallett *et al*., 2017), whilst Soil pH, percentage organic matter (OM%) and cation exchange capacity (CEC) (meq 100 g^{-1}) were calculated by NRM Ltd. Average annual rainfall and temperature data was obtained from Metoffice.gov.uk.

AWR001/18	AWR002/18	AWR003/18
University of Warwick	G's Growers Ltd.	JEPCO Ltd.
Wellesbourne	Redmere Fen	Norfolk House Farm
Warwick	Ely	Gedney Drove End
Warwickshire	Cambridgeshire	Lincolnshire
CV35 9EF	CB7 4SS	PE12 8BA
Grid Ref.: SP 274569	Grid Ref.: TL 640864	Grid Ref.: TF 448293
Soil texture: Loam	Soil texture: Peat	Soil texture: Loamy clay
pH: 7.3	pH: 7.6	pH: 8.1
OM%: 3.0	OM%: 38.7	OM%: 3.0
CEC: 12.0	CEC: 583.0	CEC: 63.0
Ave. rainfall: 614.8 mm	Ave. rainfall: 573.9 mm	Ave. rainfall: 610.1mm
Ave. temp.: 5.9-14.5 ºC	Ave. temp.: 5.7-14.5 ºC	Ave. temp.: 6.3-13.8 ºC

© Agriculture and Horticulture Development Board 2019. All rights reserved

Site and crop management

All cultivations, drilling and subsequent crop management were conducted by the host grower in accordance with local practice, with the exception of AWR001 which was managed by University of Warwick staff with agronomy advice from VCS.

Salad onions were chosen as the test crop due to their susceptibility to AWR and their ability to grow throughout the season without forming bulbs and senescing. Untreated seed of *Allium cepa* cv. White Lisbon (Tozer seeds Ltd., Cobham, UK) was sown across the three sites at a seed rate of 48 seeds per linear meter, with an aim to achieve 40 plants established. Four drill rows per bed were used at all of the sites, with single drill lines used at Wellesbourne and double lines at the other sites. For sowing dates see Appendix A.

Treatments and application

Active ingredients (a.i) were selected based on previous reports of efficacy against *S. cepivorum* in the literature from glasshouse and field-based trials (Clarkson *et al*., 2016, FV 499) (Table 1.1). Applications were made using hand held applicators (Vermorel 2000 HP, Berthound UK) fitted with a 110° 1.2 mm aperture flat fan nozzle (110/1.2/3; Hypro, UK) at a working pressure of 200 kPa. Total application volumes were 1,000 L ha^{-1} applied as a concentrated banded spray 0.10 – 0.15 m width centred on each drill row (Fig. 1.0), with a medium spray quality. Application timings were performed on a growth stage (GS) basis at emergence (BBCH GS 011/012 [T1]) and three to four true leaves (BBCH GS 103/104 [T2]) (Table 1.1) across all sites for consistency. Treatments comprising of seed applied products are denoted as T0. For details on treatment products, application conditions and calendar dates see Appendix B.

Trial design

The three field trials comprised of a randomised block design of 5 blocks with 11 plots each. Treatment allocation to plots was conducted separately for each site. At sites AWR002 and AWR003 an additional blocking factor (side) was added to compensate for splitting the trial vertically between sprayer wheel markings.

Individual plot sizes varied between sites comprising of; 1.83 x 6 m at AWR001, 2 x 8 m at AWR002 and 1.83 x 8 m at AWR003. Plots were separated vertically by a 1 m discard, and horizontally by the bed wheeling. The area surrounding the plots was drilled as discard/guard plots to a minimum width of 1 bed each side and one plot length top and bottom. Individual site layouts/designs are attached in Appendix C.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 1.0. Left, product application at T1, showing 0.10-0.15 m band centred on drill line. Right, whole plot view after treatment showing application bands to each of the four drill lines.

Table 1.1. List of treatments tested for AWR control including product identifier, active substance(s), time of application, active concentration and product dose.

No.	Name/Code	FRAC Group	Timing	Dose[1]
1	HDC F246	7 SDHI	T1	0.625
2	BAS 516 07 F	7 SDHI + 11 QoI	T1	1.5 *
3	HDC F247	7 SDHI + 3 DMI	T1	2.0
4	HDC F246	7 SDHI	T1 + T2	0.625
5	BAS 516 07 F	7 SDHI + 11 QoI	T1 + T2	1.5 *
6	HDC F247	7 SDHI + 3 DMI	T1 + T2	2.0
7	Prestop	*Gliocladium* spp.	T1 + T2	5.0 *
8	HDC F249	*Trichoderma* spp.	T1 + T2	2.5*+1.5 *
9	HDC F248	3 DMI + 3 DMI	T0	-
10	HDC F248 + BAS 516 07 F	3 DMI + 3 DMI, 7 SDHI + 11 QoI	T0 + T1	1.5 *
11	Untreated	-	-	-

[1](L ha^{-1} or kg ha^{-1}); *granular products; T0 = seed applied, T1 = BBCH GS 011/012 and T2 = BBCH GS 103/104.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Crop assessments

Phytotoxicity

Potential phytotoxic effects were assessed visually on a whole plot basis using a series of scores aimed at assessing the impact on onion plant establishment, seedling vigour and colouration (Table 1.2) as suggested by EPPO bulletin PP 1/135(4). Scoring was carried out before application at T1, and 2-3 weeks post T1 and T2 application.

Table 1.2. Phytotoxicity assessment indices for plant establishment, seedling vigour and colouration.

Score	0	1	2	3	4	5
Establishment	0%	<20%	21-40%	41-60%	61-80%	81-100%
Vigour	No crop	Low	Medium	Normal	Improved	-
Colour	No crop	Necrotic	Chlorotic	Yellowing	Light green	Normal

AWR incidence in the developing crop

White rot incidence was assessed in the central two rows from 4 x 0.5 m marked lengths within each plot at AWR001 and AWR002. Static markers were inserted into the soil to delineate these lengths throughout the season and spaced a minimum of 1.0-1.5 m apart within a row (Fig. 1.1). This aimed to capture the patchy nature of the pathogen and allowed for the same plants/area to be assessed over time. Due to low plant establishment at AWR003 the middle two rows of each plot were assessed, excluding 0.5 m at each end (7 m lengths total).

White rot incidence is difficult to assess non-destructively, but is commonly done so by counting the number of plants showing foliar symptoms (chlorosis, necrosis and wilting). Consequently, the number of symptomatic plants was assessed monthly from the 1st treatment application (T1). Plant death was also recorded, with this comprising of removing the whole plant and observing for the white rot fungus (white mycelium and/or sclerotia) after which the plant was recorded as white rot positive or negative and removed from the plot. Plant population counts were conducted at the first assessment timing (GS 101/102) at AWR001 and AWR002, and subsequently used to calculate white rot incidence and plant death as a proportion of the established population. Populations at AWR003 were assessed when the disease was first seen within individual plots.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 1.1. Experimental plot at site AWR001 showing the layout of the 4 x 0.5m assessment areas within the central two rows. The bar indicates a single 0.5 m assessment length, arrows denote the three other assessment areas within the plot.

AWR incidence at harvest

Trial sites were harvested in September and October 2018 (for calendar dates see Appendix A). Harvest assessments comprised of carefully lifting the plant and root system from each assessment area, or each row at AWR003, and gently removing any adhering soil. Each plant was then assessed for the presence/absence of white rot, which was confirmed by the occurrence of dense silvery/white mycelium and/or an aggregated mass of small (<2 mm)

© Agriculture and Horticulture Development Board 2019. All rights reserved

black sclerotia. Additionally, dead plants (brown and dry material/bulbs) were also assessed for the presence of these symptoms.

Statistical analysis

Statistical analysis was conducted in Rstudio (v3.4.1 [2017.06.30]) using the base functions along with additional packages where required. Analyses was conducted separately for each site, before using an F-test or other appropriate test to examine variation between sites. If no significant differences occurred between sites then the data was combined for additional statistical power.

Phytotoxicity scores were averaged (mean) across treatment replicates at each site and standard error of the mean (s.e.m) calculated. If variation was seen between treatments (as assessed by the means and representative s.e.m) then two-way analysis of variance (ANOVA) was conducted on the raw data followed by a Tukey's HSD test for treatment differences. In this situation, individual products and application number was used as the explanatory variables, and their effects examined individually and in combination.

AWR incidence and plant death were averaged across the individual 0.5 m plot assessment areas, or two 7 m rows at AWR003, and the means calculated across individual treatments for each time point. The mean cumulative incidence and initial plant count for each plot was then used to calculate the relative proportion of white rot symptoms or plant death at each time point. These proportional data were analysed using a generalised linear model (GLM) assuming a binomial distribution and logit link function, with comparison to individual products and application number separately and in combination.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*

Production and conditioning of *S. cepivorum* sclerotia

S. cepivorum sclerotia were produced using a modification of the method described by Clarkson *et al*. (2002). In brief, 192 g of silica sand (2 mm particle size) was added to 8 g of maize-meal (Oxoid) in 500 ml conical flasks. The flasks were sealed with a cotton wool bung and foil cap, before autoclaving at 121°C for 15 min. Following this, 27.5 ml of sterile distilled water (SDW) was added along with three 5 mm agar plugs taken from the leading edge of a 5 day old culture of *S. cepivorum*. Duplicate flasks were setup for each of the four isolates used (WR3, WRAR13, GS1 and WRLG11), and stored at room temperature in the dark for a minimum of 8 weeks with bi-weekly agitation to evenly distribute mycelium.

After 8 weeks, *S. cepivorum* sclerotia from one flask for each isolate were harvested for conditioning, with the other remaining in storage. Sclerotia were removed from the flasks by floatation, with tap water added to the flask and agitated. After a brief settling period, the top part of the supernatant containing maize-meal residue and any empty/immature sclerotia was discarded. Additional water was then added before again agitating to get the sclerotia into suspension, which were then captured in a funnel lined with nylon mesh (125 µm) (taking care not to decant the heavier sand portion). Sclerotia from each *S. cepivorum* isolate were then rinsed and placed into two nylon bags (50 x 100 mm) which were heat sealed. Small plastic containers (100 x 120 x 65 mm; HotFormBoxes) were filled with 200g of air-dried unsterile field soil (Dunnington Heath series), and a single nylon bag placed on top. An additional 200g of soil was then added and SDW added to obtain a total moisture content of 22% (w/w). Individual containers were used for each of the two nylon bags in case of contamination/parasitism by other soil-borne organisms. The containers were stored in an incubator (MLR-352-PE, Sanyo Panasonic Biomedical, Loughborough, UK) at 15°C for 8 weeks to condition the sclerotia for germination.

S. cepivorum sclerotia germination assay

Individual sterile petri dishes (90 mm; Sarstedt, Leicester, UK) were filled with 4.8 g of autoclaved vermiculite ensuring a level surface. The vermiculite was saturated using SDW until a fine film formed around the top most particles. A single 50 x 50 mm square of nylon mesh, previously autoclaved and dried, was placed into the centre of the dish ensuring good contact with the underlying vermiculite and water layer. *S. cepivorum* sclerotia (50) were placed on the nylon mesh using sterile forceps in a grid pattern, ensuring 5-10 mm between any two sclerotia (Fig. 1.2).

© Agriculture and Horticulture Development Board 2019. All rights reserved

Individual Petri dishes were randomly allocated to positions within a sealable plastic container (180 x 270 x 20 mm [2.7 L]; Lock'n'Lock, South Korea), with each container holding one treatment to prevent cross contamination (Fig. 1.2). Once placed into the containers, Petri dish lids were removed, and treatments were either added to the vermiculite directly (solution or powder) or to a 2 ml bijou bottle placed in the centre of the container. The individual containers were then sealed, and placed in an incubator at 15°C in the dark.

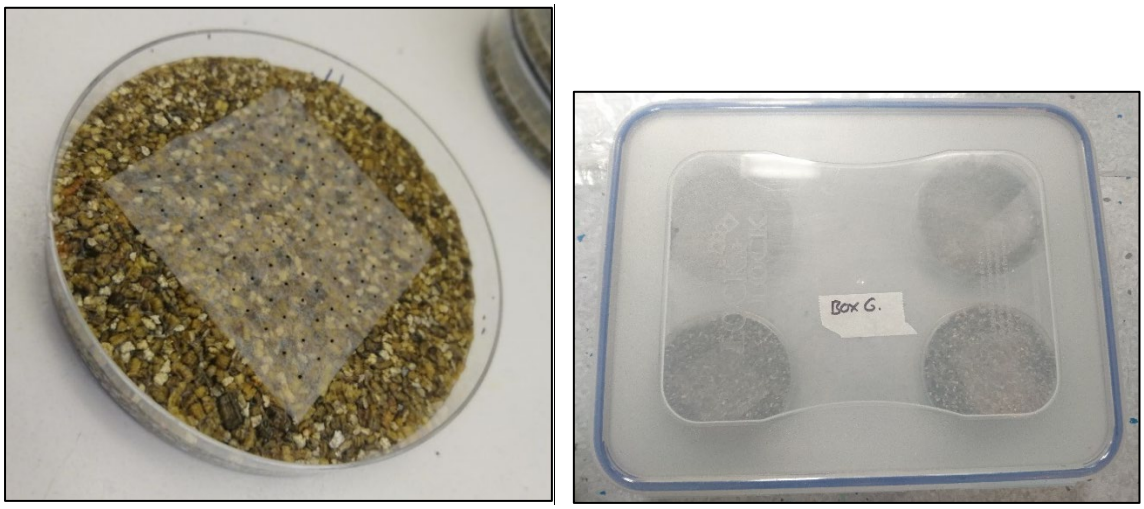

Figure 1.2. L, Petri dish containing vermiculite and nylon mesh with *S. cepivorum* sclerotia. R, Plastic container with four separate Petri dishes.

Allium product treatments

Experiments 1 and 2 examined the effect of diallyl sulphide (DAS; A35801, Sigma-Aldrich, Poole, UK) on conditioned / unconditioned *S. cepivorum* sclerotia (Table 1.3) to verify he results of previous research where this compound was identified as the major component in onion roots responsible for stimulating germination of sclerotia; however sclerotia were dried before use in Experiment 2, whilst they remained moist before use in Experiment 1. Treatments comprised of 1 ml DAS (0.5% v/v in 2% Triton X) placed into a 2 ml open bijou bottle located in the centre of each container, whilst the untreated control comprised of a 2% Triton X (1 ml) solution.

In Experiment 3, conditioned *S. cepivorum* sclerotia of isolates WRAR13 and GS1 were exposed to six treatments comprising of four commercial *Allium* products (P1-4; supplied by EcoSpray Ltd, Thetford, UK), two granule sizes of food grade garlic (Barnes Williams, Cheltenham, UK), DAS (positive control) and an untreated control. In addition, unconditioned sclerotia of each isolate were also included (Table 1.4). In this experiment, the vermiculite was saturated with 30 ml of SDW and treatments added directly through pipetting the concentrated liquid products or sprinkling the weighted dose of granule onto the entire vermiculite surface. Due to the space limitation of each container, each treatment was

© Agriculture and Horticulture Development Board 2019. All rights reserved

represented twice and the experiment repeated once. A second repeat, Experiment 4, is currently underway (Table 1.5) although the concentration of the positive control (Conditioned with DAS) was altered to 0.1% v/v based on the results of Experiment 3.

Table 1.3. *Allium* products tested for effects on germination of *S. cepivorum* sclerotia in Experiments 1 and 2.

No.	Product(s)	Active substance(s)	Isolate	Conditioned
1	DAS	Di-allyl sulphide (0.5%)	GS1	Y
2	DAS	Di-allyl sulphide (0.5%)	GS1	N
3	Control	Triton X	GS1	Y
4	Control	Triton X	GS1	N
5	DAS	Di-allyl sulphide (0.5%)	WR3	Y
6	DAS	Di-allyl sulphide (0.5%)	WR3	N
7	Control	Triton X	WR3	Y
8	Control	Triton X	WR3	N
9	DAS	Di-allyl sulphide (0.5%)	WRAR13	Y
10	DAS	Di-allyl sulphide (0.5%)	WRAR13	N
11	Control	Triton X	WRAR13	Y
12	Control	Triton X	WRAR13	N
13	DAS	Di-allyl sulphide (0.5%)	WRLG11	Y
14	DAS	Di-allyl sulphide (0.5%)	WRLG11	N
15	Control	Triton X	WRLG11	Y
16	Control	Triton X	WRLG11	N

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 1.4. *Allium* products tested for effects on germination of *S. cepivorum* sclerotia in Experiment 3.

No.	Product(s)	Active substance(s)	Application rate	Isolate	Conditioned
1	HDC F261	Unknown	6% v/v	WRAR13	Y
2	HDC F261	Unknown	6% v/v	GS1	Y
3	HDC F262	Unknown	6% v/v	WRAR13	Y
4	HDC F262	Unknown	6% v/v	GS1	Y
5	HDC F263	Unknown	6% v/v	WRAR13	Y
6	HDC F263	Unknown	6% v/v	GS1	Y
7	HDC F264	Unknown	20 kg/ha	WRAR13	Y
8	HDC F264	Unknown	20 kg/ha	GS1	Y
9	Garlic Granule	Unknown	100 kg/ha	WRAR13	Y
10	Garlic Granule	Unknown	100 kg/ha	GS1	Y
11	Garlic Powder	Unknown	100 kg/ha	WRAR13	Y
12	Garlic Powder	Unknown	100 kg/ha	GS1	Y
13	Control (-)	None	-	WRAR13	Y
14	Control (-)	None	-	WRAR13	N
15	Control (-)	None	-	GS1	Y
16	Control (-)	None	-	GS1	N
17	Control (+)	Diallyl sulphide	0.5% v/v	WRAR13	Y
18	Control (+)	Diallyl sulphide	0.5% v/v	WRAR13	N
19	Control (+)	Diallyl sulphide	0.5% v/v	GS1	Y
20	Control (+)	Diallyl sulphide	0.5% v/v	GS1	N

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 1.5. *Allium* products tested for effects on germination of *S. cepivorum* sclerotia in Experiment 4.

No.	Product(s)	Active substance(s)	Application rate	Isolate	Conditioned
1	HDC F261	Unknown	6% v/v	WRAR13	Y
2	HDC F261	Unknown	6% v/v	GS1	Y
3	HDC F262	Unknown	6% v/v	WRAR13	Y
4	HDC F262	Unknown	6% v/v	GS1	Y
5	HDC F265	Unknown	20 kg/ha	WRAR13	Y
6	HDC F265	Unknown	20 kg/ha	GS1	Y
7	HDC F264	Unknown	20 kg/ha	WRAR13	Y
8	HDC F264	Unknown	20 kg/ha	GS1	Y
9	Garlic Granule	Unknown	100 kg/ha	WRAR13	Y
10	Garlic Granule	Unknown	100 kg/ha	GS1	Y
11	Garlic Powder	Unknown	100 kg/ha	WRAR13	Y
12	Garlic Powder	Unknown	100 kg/ha	GS1	Y
13	Control (-)	None	-	WRAR13	Y
14	Control (-)	None	-	WRAR13	N
15	Control (-)	None	-	GS1	Y
16	Control (-)	None	-	GS1	N
17	Control (+)	Diallyl sulphide	0.1% v/v	WRAR13	Y
18	Control (+)	Diallyl sulphide	0.1% v/v	WRAR13	N
19	Control (+)	Diallyl sulphide	0.1% v/v	GS1	Y
20	Control (+)	Diallyl sulphide	0.1% v/v	GS1	N

© Agriculture and Horticulture Development Board 2019. All rights reserved

Assessment of germination of *S. cepivorum* sclerotia

Germination of *S. cepivorum* (mycelial or eruptive; Fig.1.3) or contamination was assessed bi-weekly, with any germinated / contaminated sclerotia then removed from the plates. Experiments were conducted over a period of 7 weeks, after which the majority of sclerotia in the positive (DAS) control had germinated. Ungerminated sclerotia which remained at the end of Experiment 1 and 2 were assessed for viability by squeezing with forceps; viable sclerotia remained tough and rigid, whilst unviable sclerotia were soft or collapsed.

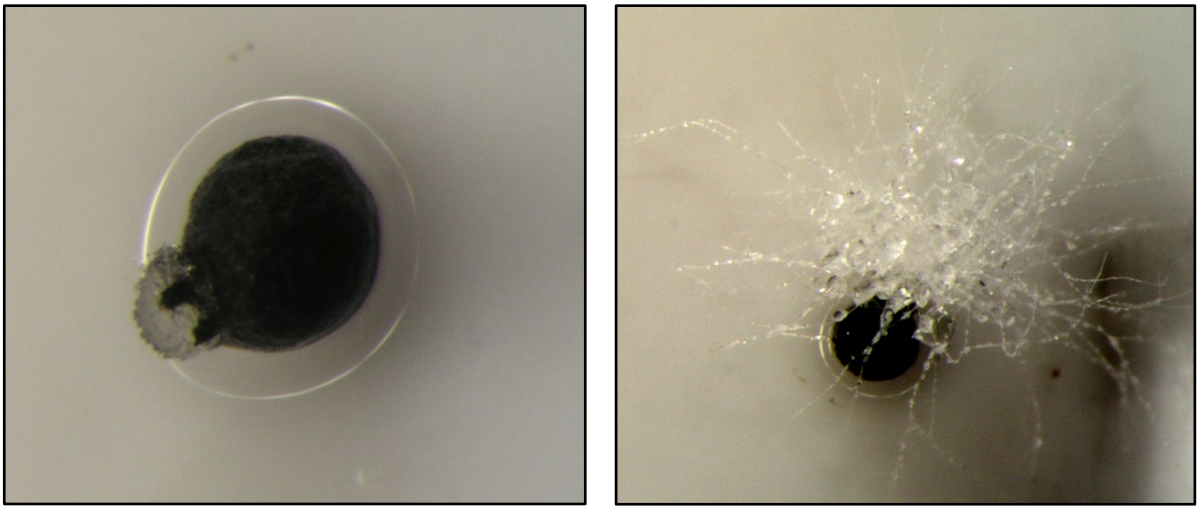

Figure 1.3. Germination of *S. cepivorum* sclerotium in response to DAS *in vitro*. Left, eruptive germination; right, mycelial growth. (Source: Clarkson *et al*., 2016 [FV 499]).

Statistical analysis

The number of germinated sclerotia was averaged (mean) across treatment replicates at each assessment time, followed by transformation to proportional data by dividing by the number of sclerotia per experimental unit. The proportional data was then analysed using a generalised linear model (GLM) assuming a binomial distribution and logit link function, with comparison to the positive control (DAS), conditioning and sampling time individually and in combination. In addition, the data from Experiment 1 and 2 were combined to examine for effects of drying sclerotia, with the proportional data analysed using a GLM as before.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum

In vitro biofumigant plant assay

An *in vitro* system was used to screen a collection of different biofumigant plant species and varieties which were grown as part of a previous PhD project (Warmington, 2015). In brief, 7.5 L pots were filled with Levington M2 compost (Scotts), sown with five seeds per species/variety, and grown under supplementary lighting in a polytunnel or glasshouse. Plants were grown on until 1-2 weeks after first flowering, when the whole plant was harvested and oven dried at 80°C for 24 hours. Following drying, plant samples were milled to a fine powder (<1 mm) and stored in sealed plastic bags at -20°C until use.

A Petri dish 'sandwich' system was used to examine the inhibitory effects of each biofumigant powder on the mycelial growth of *S. cepivorum* isolate WRAR13. Molten Potato Dextrose Agar (PDA) (23 ml) was poured into individual sterile 90 mm Petri dishes and once set, two perpendicular axes were drawn onto the reverse of each plate. A single 8 mm agar core from a 5 day-old *S. cepivorum* culture was placed mycelial side down onto the PDA at the intersection of the two lines. Two-grams of test powder was then placed into a separate clean sterile 90 mm Petri dish and 20 ml of sterile distilled water added to release the biofumigant isothiocyanate volatiles. The inoculated PDA Petri dish was then inverted and placed on top of the plate containing the biofumigant mixture, before being secured with two short pieced of masking tape and wrapped in one layer of parafilm to seal the plates. Plates were then placed into an incubator at 20°C in the dark.

Biofumigant treatments

Several *Brassica* species and varieties were selected for testing against *S. cepivorum* based on differences in glucosinolate (GLS) profile (Table 1.6). Each biofumigant powder was examined in triplicate, with one replicate tested during the current reporting period. An untreated control comprising of 20 ml of SDW was also included. Plates were arranged on trays in a complete randomised design and incubated in the dark at 20°C, until the untreated control had reached the plate edge (approx. 4-5 days).

Assessments of *S. cepivorum* growth

Mycelial growth of *S. cepivorum* was measured daily along each axis and averaged (mean) for each plate (Fig. 1.4). After 3 days the final diameter was recorded, and the percentage inhibition calculated in comparison to the untreated control.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 1.6. Biofumigant treatments tested for their effect on *S. cepivorum* in preliminary *in vitro* tests. Glucosinolate concentration was measured using HPLC by Warmington (2015) using an adapted method of Tsao *et al.* (2002).

No.	Species	Variety	Predominant GLS	Approx. GLS Conc. (*μ* mol/g DM)
1	*Brassica juncea*	Vittasso	Sinigrin	2.5
2	*Brassica juncea*	Caliente 99	Sinigrin	5.0
3	*Brassica juncea*	Pacific Gold	Sinigrin	7.0
4	*Sinapis alba*	Brisant	Sinalbin	6.0
5	*Raphanus sativus*	Terranova	Glucoraphanin	7.0
6	*Eurca sativa*	Nemat	Glucoerucin	17.0
7	Untreated Control	-	-	-

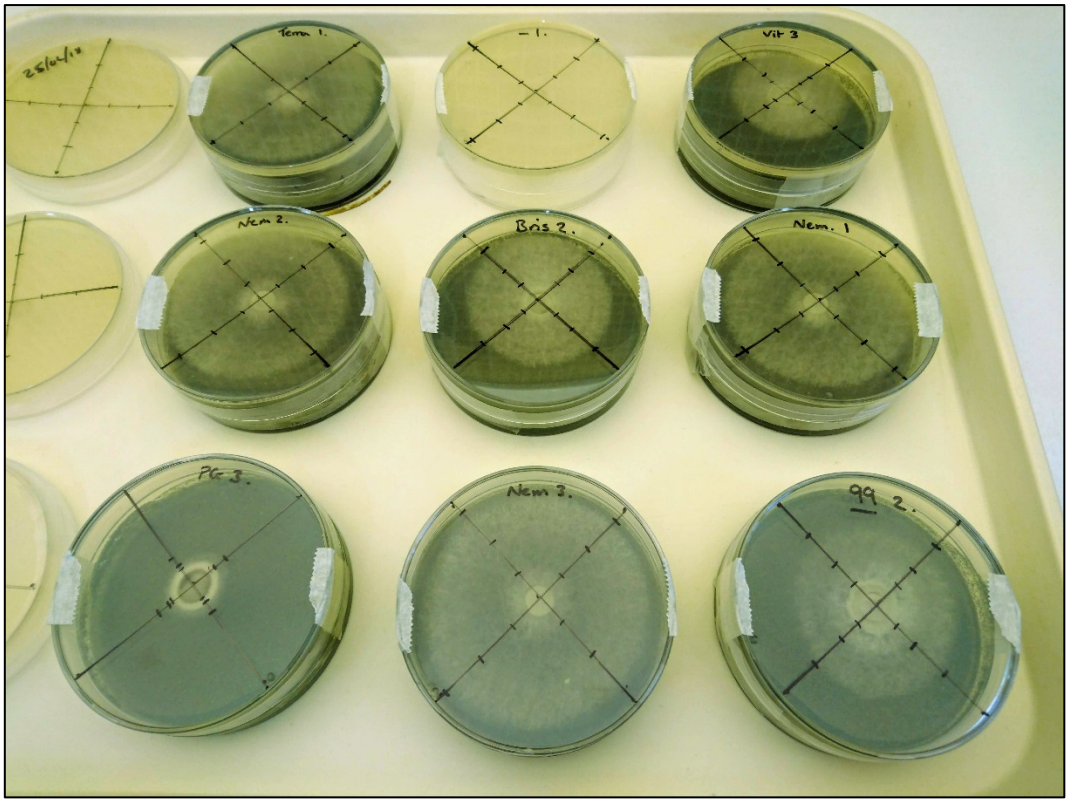

Figure 1.4. Radial growth of *S. cepivorum* on PDA in the presence of different biofumigant powders *in vitro*.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Statistical analysis

No statistical analysis was carried out as this experiment comprised of one replicate. Future repeats will be conducted after which suitable analysis will be undertaken.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Results

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease

Phytotoxicity

No phytotoxic effects of fungicide products tested were observed at any of the three field sites post T1 or T2 applications. Additionally, there was little variation in onion seedling establishment rate and vigour between plots (Table 2.0 and 2.2) at any of the three assessment timings. At site AWR002, only two assessments were possible (T0 and post T2, Table 2.1). Although, a decrease in seedling colour score was seen post T2 application at AWR001, with all plots exhibiting a score of 3 (yellowing) this was likely a consequence of herbicide damage and/or the extreme weather conditions. A decrease in vigour was also seen post T1 and T2 at AWR003, scoring 2 (medium vigour) at both timings; however as this effect was consistent between plots it was not deemed to be a treatment effect, but a consequence of difficult growing conditions. None of the phytotoxicity scores differed significantly from the untreated control at any site when examined using ANOVA (Appendix E, Table E1.0 – E1.2). Using a Fligner-Killeen test for homogeneity the datasets were found to be significantly different (p<0.001) and were therefore not combined for further analysis (Appendix E, Table E2.0)

Plant populations were below the target population rate at all of the sites post T1, but this effect was seen consistently across the treatments and therefore was not considered a treatment effect. At AWR001and AWR003 the target population was 20 plants per linear 0.5 m row, with the actual number established being 17.8 (range: 16.3 – 19.6) and 4.9 (range: 3.3 – 7.0) respectively. Similarly, at AWR002 the target population was 25 plants with 22.4 (range: 21.1 – 23.6) being established (Fig. 2.0). Some damage from capping at drilling and mechanical weeding were also seen at AWR003 (Fig. 2.1). Overall plant populations did not differ significantly from those of the untreated control at each site when examined using ANOVA (Appendix E, Table E1.0 – E1.2). Using a Fligner-Killeen test for homogeneity the datasets were found to be significantly different (p=0.046) and were therefore not combined for further analysis (Appendix E, Table E2.0).

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 2.0. Mean phytotoxicity scores for onion seedling establishment, vigour and colouration with respective error values (s.e.m) at Pre T1 and following T1 and T2 for site AWR001. Time intervals between product application and assessment was 20 days post T1 and 22 days post T2.

Trt	Pre T1 (GS 012)						Post T1 (GS 102/103)						Post T2 (GS 104)					
	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*
1	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
2	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
3	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
4	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
5	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
6	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
7	4.8	*0.2*	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	4.8	*0.2*	2.8	*0.2*	2.8	*0.2*
8	4.8	*0.2*	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
9	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
10	4.8	*0.2*	2.8	*0.2*	5.0	-	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	3.0	-
11	5.0	-	3.0	-	5.0	-	5.0	-	3.0	-	5.0	-	4.8	*0.2*	2.8	*0.2*	2.8	*0.2*

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 2.1. Mean phytotoxicity scores for onion seedling establishment, vigour and colouration with respective error values (s.e.m) at Pre T1 and Post T2 for site AWR002. Time intervals between product application and assessment was 7 days post T2.

Trt	Pre T1 (GS 101)						Post T2 (GS 104)					
	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*
1	3.8	*0.2*	1.8	*0.4*	4.0	*0.3*	4.2	*0.2*	2.0	*0.3*	5.0	-
2	3.6	*0.2*	2.6	*0.2*	4.6	*0.2*	4.6	*0.2*	2.2	*0.2*	5.0	-
3	4.2	*0.2*	2.6	*0.4*	4.8	*0.2*	4.8	*0.2*	2.4	*0.4*	5.0	-
4	4.0	*0.3*	2.4	*0.2*	4.4	*0.2*	4.6	*0.2*	2.4	*0.2*	5.0	-
5	3.8	*0.2*	2.0	*0.5*	3.8	*0.5*	3.8	*0.4*	2.0	*0.3*	5.0	-
6	3.8	*0.2*	2.6	*0.2*	4.4	*0.2*	4.8	*0.2*	2.4	*0.2*	5.0	-
7	3.6	*0.2*	2.2	*0.4*	4.0	*0.3*	4.0	*0.3*	2.0	*0.3*	5.0	-
8	3.6	*0.2*	2.2	*0.2*	4.2	*0.2*	4.4	*0.2*	2.2	*0.2*	5.0	-
9	4.0	-	2.2	*0.4*	4.4	*0.2*	4.8	*0.2*	2.6	*0.2*	5.0	-
10	3.8	*0.4*	2.0	*0.3*	4.0	*0.3*	4.2	*0.4*	1.8	*0.4*	5.0	-
11	3.6	*0.2*	2.6	*0.4*	4.8	*0.2*	4.6	*0.4*	2.2	*0.4*	5.0	-

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table 2.2. Mean phytotoxicity scores for onion seedling establishment, vigour and colouration with respective error values (s.e.m) at Pre T1 and following T1 and T2 for site AWR003. Time intervals between product application and assessment was 21 days post T1 and 20 days post T2.

Trt	Pre T1 (GS 011/012)						Post T1 (GS103/104)						Post T2 (GS 403)					
	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*	Est.	*S.e.m*	Vigour	*S.e.m*	Colour	*S.e.m*
1	2.4	*0.7*	3.0	-	5.0	-	3.0	0.8	2.0	-	5.0	-	2.2	*0.8*	2.0	-	5.0	-
2	2.8	*0.5*	3.0	-	5.0	-	2.8	*0.6*	2.0	-	5.0	-	2.2	*0.6*	2.0	-	5.0	-
3	3.0	*0.6*	3.0	-	5.0	-	3.0	*0.6*	2.0	-	5.0	-	2.8	*0.7*	2.0	-	5.0	-
4	2.6	*0.5*	3.0	-	5.0	-	2.2	*0.6*	2.0	-	5.0	-	2.8	*0.6*	2.0	-	5.0	-
5	2.6	*0.6*	3.0	-	5.0	-	2.8	*0.6*	2.0	-	5.0	-	2.8	*0.6*	2.0	-	5.0	-
6	1.8	*0.6*	3.0	-	5.0	-	2.2	*0.6*	2.0	-	5.0	-	2.2	*0.8*	2.0	-	5.0	-
7	2.2	*0.4*	3.0	-	5.0	-	2.0	*0.6*	2.0	-	5.0	-	2.2	*0.2*	2.0	-	5.0	-
8	3.0	*0.5*	3.0	-	5.0	-	3.2	*0.7*	2.0	-	5.0	-	2.4	*0.7*	2.0	-	5.0	-
9	2.4	*0.2*	3.0	-	5.0	-	2.6	*0.2*	2.0	-	5.0	-	2.0	*0.3*	2.0	-	5.0	-
10	2.6	*0.4*	3.0	-	5.0	-	2.2	*0.4*	2.0	-	5.0	-	1.8	*0.4*	2.0	-	5.0	-
11	2.4	*0.7*	3.0	-	5.0	-	2.4	*0.7*	2.0	-	5.0	-	2.6	*0.5*	2.0	-	5.0	-

© Agriculture and Horticulture Development Board 2019. All rights reserved

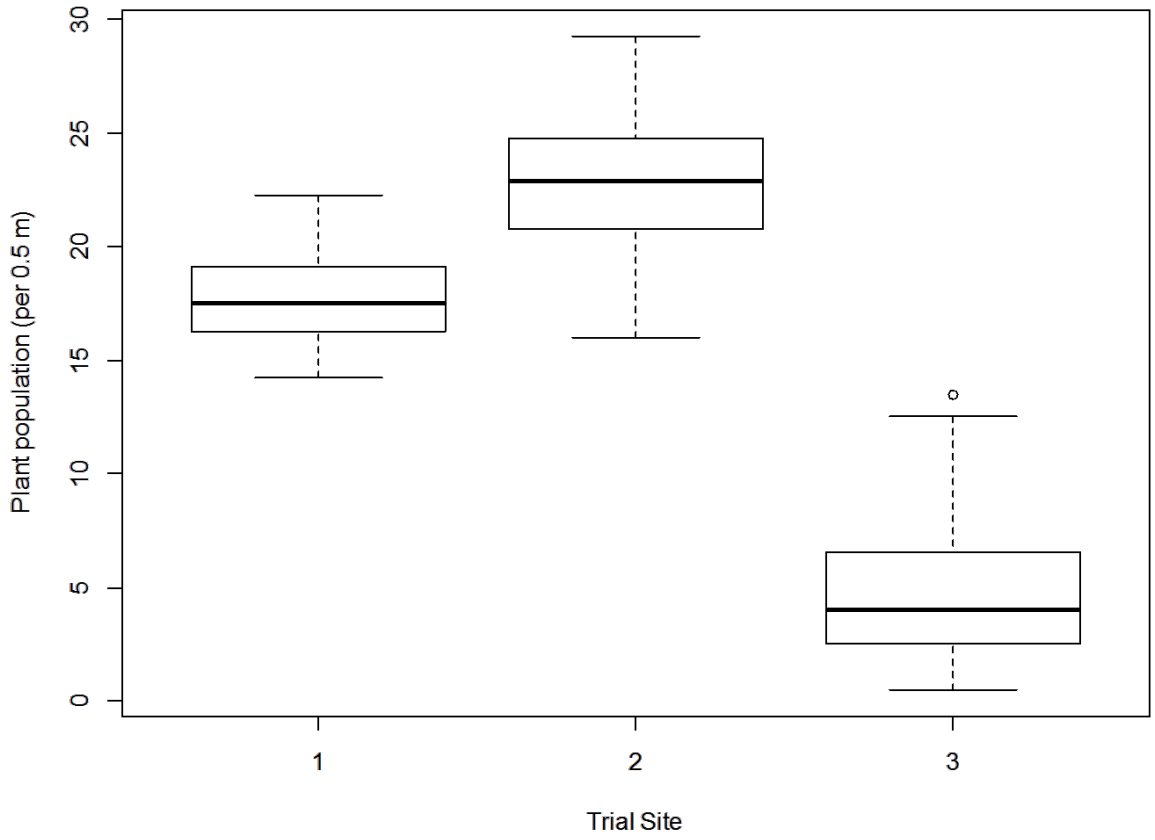

Figure 2.0. Boxplot showing the distribution of number of onion plants per linear 0.5 m plot length at the three field trial sites. 1, AWR001, 2, AWR002 and 3, AWR003.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 2.1. Plot at AWR003 showing low onion plant population. Image taken: 23/07/18. White markers denote the original assessment areas within the plot.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Allium white rot incidence

Foliar assessments of AWR

The incidence of AWR foliar symptoms was low at all of the test sites, as assessed by chlorosis and plant death during the growing season. No discolouration or chlorosis was seen at AWR001 until the second assessment timing (13/07/18), where potential symptoms were seen consistently across all of the plots (Fig. 2.2). As such, this was considered not to be a treatment effect, but as a result of herbicide damage. Consequently, no further foliar assessments were conducted at this site as these symptoms persisted throughout the season making assessment difficult. At AWR002 no symptoms were seen overall, with a single suspected plant found at the second assessment (18/07/18); although symptomatic plants were found in the guard plots at this timing (Fig. 2.3). A low incidence (<1% total) was recorded at AWR003 across all assessment timings.

Harvest assessments of AWR

At harvest the incidence of AWR infection, as determined by plants with sclerotia or white mycelium, was more prevalent than for the foliar assessments.

At AWR001, AWR incidence was high averaging 53.7% across the untreated plots (Fig. 2.5). Application of the conventional fungicides HDC F246, BAS 516 07F and HDC F247 resulted in a significant reduction in disease incidence (Fig. 2.5; Appendix E, Table E4.0) at both T1 and T1+T2 timings, compared to the untreated control. At T1, all three products were significantly different to the untreated but not to each other with mean AWR incidence of 8.3, 8.3 and 3.2% respectively (Fig. 2.5). However, at the second timing (T1+T2) HDC F247 was significantly different from HDC F246 and BAS 516 07F and resulted in 0.02% infection compared to 1.4 and 5.9%. The biological products Prestop and HDC F249 applied at both T1 and T2 timings, resulted in a mean AWR disease incidence of 49.7 and 46.5% respectively, and were not significantly different from the untreated control (53.7%). Use of the seed treatment product HDC F248 alone resulted in no significant disease reduction compared to the untreated control, with a mean AWR disease incidence of 49.7%. However, the addition of BAS 516 07F at T1 resulted in a significantly reduced disease incidence of 10.1%; likely as a consequence of the BAS 516 07F component, as this was comparable to the level achieved when this was used alone (8.3%).

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 2.2. Plot assessment area within the trial at AWR001, showing foliar discolouration, chlorosis, stress bulbing and soft necks caused by herbicide/weather damage in July/August 2018.

At AWR002, levels of AWR disease were much lower than at AWR001, with the untreated plots showing an average disease incidence of 9.3%. Consequently, no significant differences (p=0.0507; Appendix E, Table E4.0) were seen between any of the products at either of the timings tested (Fig. 2.6), although the close proximity to the threshold of p<0.05 would tend to suggest a general trend. Applications at T1 and T1+T2 of the products HDC F246, BAS 516 07F and HDC F247 showed mixed effects. In general, HDC F246 and HDC F247 led to a reduced incidence of AWR resulting in 4.4 and 4.6% respectively at T1, with repeat applications made at T1+T2 resulting in a further reduction to 1.7 and 1.4%. In comparison BAS 516 07F showed a slightly lower, but comparable, incidence to the control resulting in 7.5 and 7.4% at either timing. The biological products Prestop and HDC F249 were applied at both the T1 and T2 timings, and resulted in an average AWR disease incidence of 7.3 and 11.7 respectively; comparable to the untreated control at 9.3%. Use of the seed treatment product HDC F248 at T0 again showed comparable levels of AWR incidence (10.6%), to the untreated control. Whilst its use at T0 in combination with an application of BAS 516 07F at T1, led to a reduction in disease incidence to 4.5%.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 2.3. First confirmed sighting of AWR symptoms at the AWR002 site (18.07.18).

At AWR003, harvest assessments proved difficult as a consequence of low plant populations, and damage by herbicides and pests. In addition, whilst some plants showed white mycelium and sclerotia (Fig. 2.4) these were later confirmed to be a *Botrytis* sp. by isolation from several plant samples combined with DNA sequencing. None of the samples tested showed the typical disease symptoms of AWR nor could any of the target pathogen be isolated from these plants. Similarly, no symptomatic plants were seen within the growing season and consequently, no harvest data disease data for AWR is reported.

Using a Fligner-Killeen test for homogeneity the datasets for AWR001 and AWR002 were found to be significantly different (p<0.001) and were therefore not combined for further analysis (Appendix E, Table E2.0).

© Agriculture and Horticulture Development Board 2019. All rights reserved

Figure 2.4. Basal view of an infected onion plant from the AWR003 trial, showing large sclerotia and mycelial fragments typical of *Botrytis* spp.

© Agriculture and Horticulture Development Board 2019. All rights reserved

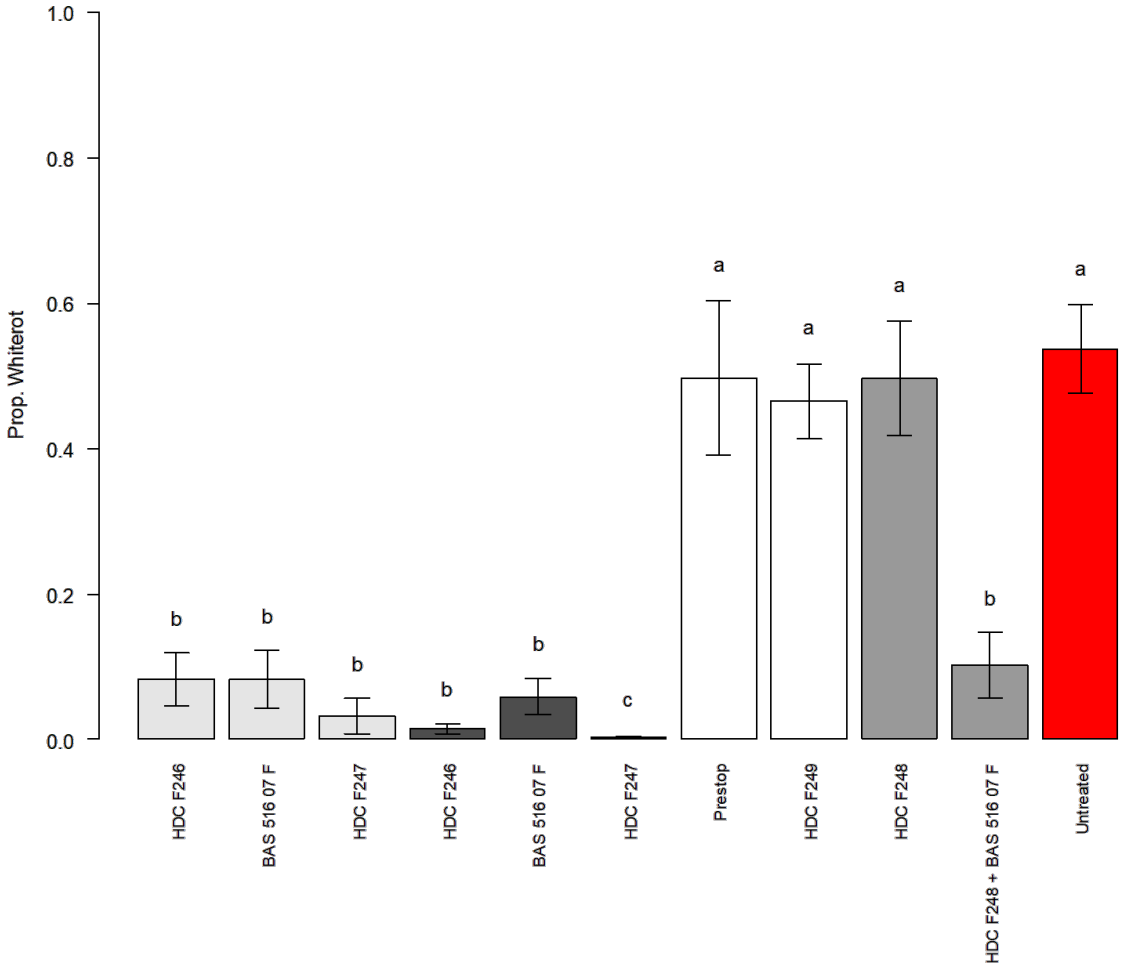

Figure. 2.5. Effect of different fungicide and biological treatments on the proportion of AWR infected plants at harvest for site AWR001. Key: light grey, application at T1; dark grey, application at T1+T2; white, biological products applied at T1+T2; mid-grey, seed treatment; red, untreated control. Error bars represent standard error of the mean (s.e.m). Bars with different letters are significantly different (GLM, p <0.05).

© Agriculture and Horticulture Development Board 2019. All rights reserved

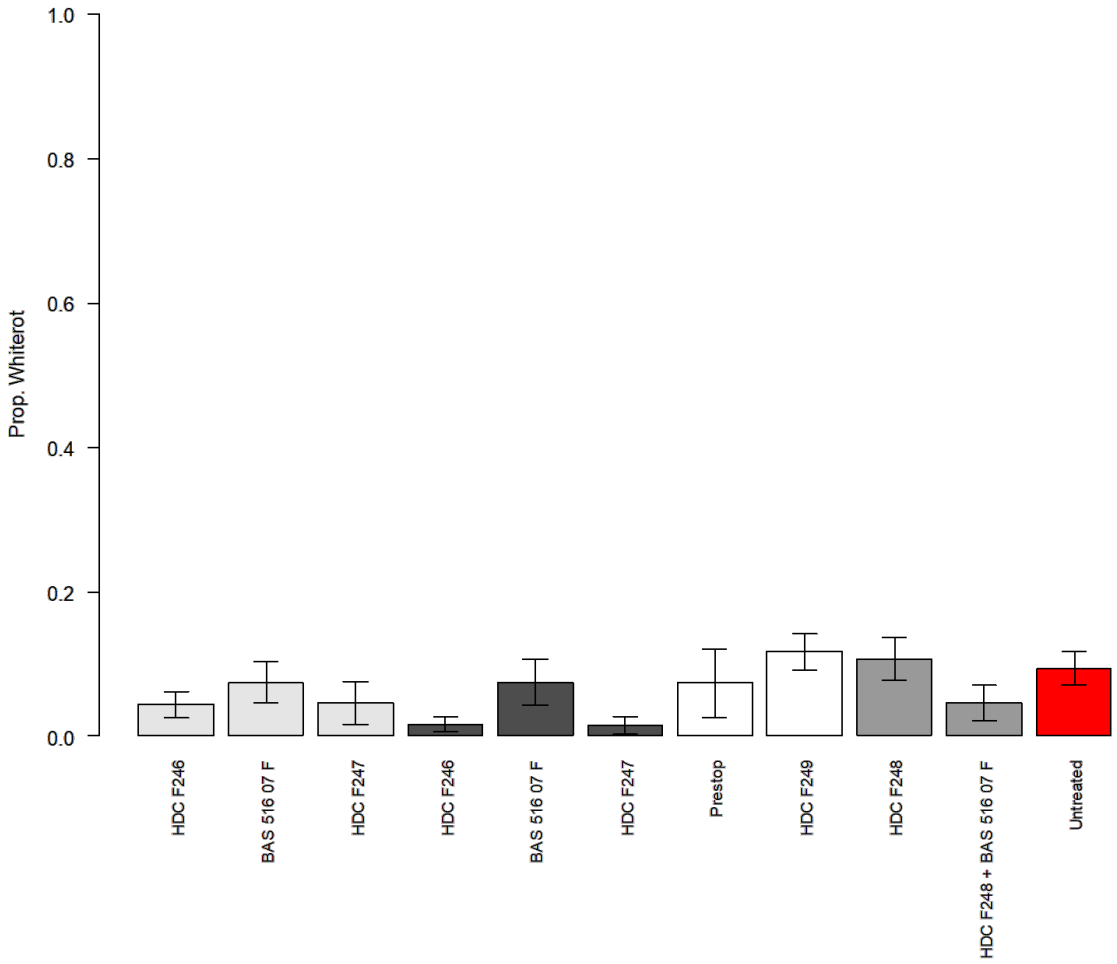

Figure. 2.6. Effect of different fungicide and biological treatments on the proportion of AWR infected plants at harvest for site AWR002. Key to treatments: light grey, application at T1; dark grey, application at T1+T2; white, biological products applied at T1+T2; mid-grey, seed treatment; red, untreated control. Error bars represent standard error of the mean (s.e.m). No significant differences due to low disease incidence (GLM, p = 0.0507).

© Agriculture and Horticulture Development Board 2019. All rights reserved

Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*

S. cepivorum sclerotia germination assay

Use of 'fresh' or dry stored *S. cepivorum* sclerotia

It has been suggested previously that sclerotial dormancy is broken by drying. Consequently, this effect was examined within two experiments using four *S. cepivorum* isolates to examine the additional factor of isolate variation.

In Experiment 1, fresh (un-dried) sclerotia were used to examine the relationship between conditioning sclerotia in combination with exposure to DAS, with this repeated for four UK *S. cepivorum* isolates. Using a GLM model, the main effect was found to be that of DAS (Table 2.3) resulting in a significant (p<0.01**) increase in sclerotial germination when examined alone. As a single explanatory variable, treatment with DAS resulted in an increased log-odds ratio (e^{β}) of 25.5 in germination response compared to the untreated (see Table 2.3, note a for explanation of log-odds ratio), which can be seen in Fig 2.7 where both conditioned and unconditioned sclerotia germinate in response to DAS. In comparison, conditioning of sclerotia had no significant effect (Table 2.3, p=0.15 NS) on germination, nor did Isolate choice (Table 2.3, p=0.46 NS, 0.86 NS and 0.90 NS) despite isolate WR3 responding at a much lower level overall compared to the other test isolates. When all of the terms were examined together using the model Prop._Germ.~DAS*Cond.+Isolate, DAS remained the main significant effect (Table 2.3, p<0.01**); although the log-odds ratio (e^{β}) decreased to 14.70, suggesting other factors were contributing to the response. This may likely be as a consequence of Isolate 2 (WR3) which was shown to be significantly different (Table 2.3, p<0.05*) to the other isolates, and would correlate with the lower levels of germination seen (c. 15% over all treatments) compared to others at >30%. Interestingly, no significant interaction was seen between treatment with DAS and conditioned sclerotia, although as p=0.052 it would suggest a general trend whereby conditioned sclerotia were more likely to germinate in response to DAS treatment as shown in Fig 2.7; with this similarly seen in the large log-odds ratio (e^{β}) of 11.17, which was greater than that of the other variables excepted DAS (14.70).

Once used in Experiment 1, the same batch of *S. cepivorum* sclerotia were air dried in a laminar flow hood overnight and stored for 7 weeks after which they were used in Experiment 2 to examine the effect of treatment with DAS and conditioning across the four isolates as before. As in Experiment 1, the main effect was as a consequence of treatment with DAS

© Agriculture and Horticulture Development Board 2019. All rights reserved

which significantly increased sclerotial germination (Table 1.2, p<0.01**) when examined both as an individual term and within the larger overall model (Prop._Germ.~DAS*Cond.+Isolate). None of the other explanatory variables (conditioning or isolate) had a significant effect as also observed in Experiment 1. In the overall model, Isolate 2 (WR3) was again found to be significantly different from the other isolates (Table 1.2, p<0.05*) suggesting that the sclerotial batch was contaminated or had not formed correctly during the culturing phase. As in Experiment 1, there was no significant interaction between DAS and conditioning, nor was the p-value nearing the significance threshold of p<0.05 as in Experiment 1, being p=0.96. Additionally, the lower log-odds ratio (e^{β}) of 1.09 compared to that of Experiment 1 (11.17) suggested it was less of an influence than previously, with this seen in the Conditioned + DAS treatments in Fig 2.8 (Experiment 2) compared to Fig 2.7 (Experiment 1).

Table 2.3. The effect of Diallyl sulphide (DAS) application, conditioning and isolate, on the germination of *S. cepivorum* sclerotia after 49 days. Experiment 1 utilised sclerotia 'fresh' undried sclerotia while Experiment 2 used the same batch of sclerotia which were dried and stored for 7 weeks. Figures are derived from separate (y~x1) and combined (y~x1*x2+x3) logistic regression models.

	Experiment 1				Experiment 2			
Effect	% Germ.	e^{β} [a]	s.e[b]	p-value[c]	% Germ.	e^{β} [a]	s.e[b]	p-value[c]
Intercept	5.33	0.06	*0.79*	-	7.25	0.08	*0.74*	-
+DAS	58.90	25.46	*0.86*	<0.01 **	49.70	12.62	*0.83*	<0.01 **
Intercept	18.80	0.23	*0.66*	-	13.50	0.16	*0.66*	-
+Cond.	45.40	3.58	*0.84*	0.15	43.40	4.19	*0.80*	0.06
Intercept	34.80	0.53	*0.48*	-	34.80	0.53	*0.78*	-
Isolate 2	15.20	0.34	*1.43*	0.46	11.40	0.24	*1.41*	0.33
Isolate 3	40.00	1.25	*1.20*	0.86	35.80	1.05	*1.10*	0.97
Isolate 4	38.40	1.17	*1.20*	0.90	31.80	0.88	*1.12*	0.91
Intercept	-	0.04	*0.81*	-	-	0.02	*1.43*	-
+DAS	-	14.70	*0.78*	<0.01 **	-	26.92	*1.44*	<0.05 *
+Cond.	-	1.84	*0.90*	0.52	-	11.24	*1.47*	0.13
Isolate 2	-	0.10	*0.76*	<0.05 *	-	0.10	*0.80*	<0.05 *
Isolate 3	-	1.64	*0.60*	0.43	-	1.09	*0.64*	0.90
Isolate 4	-	1.42	*0.60*	0.57	-	0.79	*0.65*	0.72
DAS*Cond	-	11.17	*1.10*	0.05	-	1.09	*1.59*	0.96

[a] e^{β} corresponds to the logodds-ratio for each explanatory variable. Figures greater that >0 indicate a positive response, whilst the inverse is true of figures which are <0. Similarly, the relative difference between figures within the same model indicate the magnitude of the effect relative to the control (intercept).
[b] standard error.
[c] Derived from a Wald's Test in comparison to the control (intercept).

© Agriculture and Horticulture Development Board 2019. All rights reserved

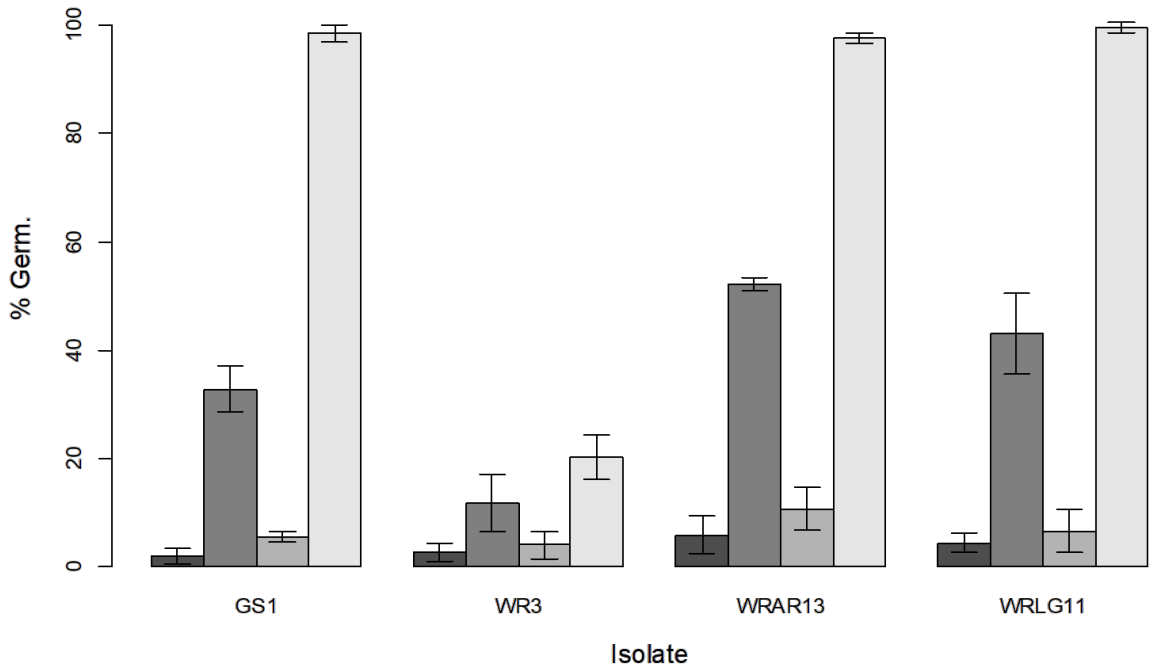

Figure 2.7. Mean germination of fresh conditioned and unconditioned *S. cepivorum* sclerotia with and without DAS for isolates GS1, WR3, WRAR13 and WRLG11 after 49 days. (Experiment 1). Key to treatments (L-R); dark grey, unconditioned -DAS; medium grey, unconditioned +DAS; grey, conditioned -DAS; light grey, conditioned +DAS. Error bars represent the standard error of the mean (s.e.m).

© Agriculture and Horticulture Development Board 2019. All rights reserved

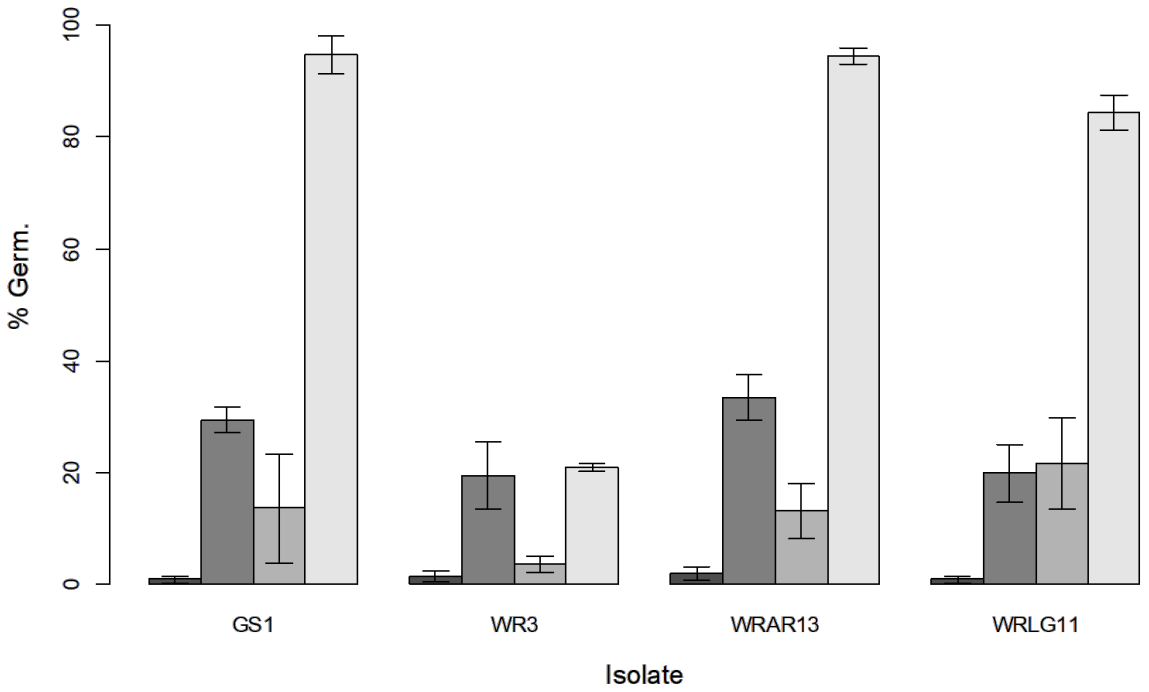

Figure 2.8. Mean germination of dry conditioned and unconditioned *S. cepivorum* sclerotia with and without DAS for isolates GS1, WR3, WRAR13 and WRLG11 after 49 days. (Experiment 2). Key to treatments (L-R); dark grey, unconditioned -DAS; medium grey, unconditioned +DAS; grey, conditioned -DAS; light grey, conditioned +DAS. Error bars represent the standard error of the mean (s.e.m).

When the two experiments are examined over the course of the 49 days a clear germination response can be seen for the conditioned *S. cepivorum* sclerotia treated with DAS within both Experiment 1 and 2 (Fig 2.9 and 2.10). In Experiment 1, all of the isolates reached >90% germination by 28 days, with the exception of WR3 which reached 40%. The same response was observed in Experiment 2, although both isolates GS1 and WRAR13 reached >90% germination earlier at 21 days, whilst WRLG11 reached a peak germination of *c.* 80% at 21 days with WR3 once again resulting in poor germination of <20%. For the other treatments, a much lower level of germination was observed as suggested by the GLM models in Table 1.2. In Experiment 1, some of the unconditioned sclerotia responded to treatment with DAS, reaching 10-40% germination by the end of the Experiment, but those not exposed to DAS remained largely dormant (<10%). Final germination was also reduced in Experiment 2 for unconditioned sclerotia treated with DAS resulting in 10-30% germination, although the unconditioned sclerotia without DAS also germinated to a similar level, reaching 10-20% by the end of the experiment.

© Agriculture and Horticulture Development Board 2019. All rights reserved

In summary, the results in Experiment 1 indicated that the use of 'fresh' conditioned *S. cepivorum* sclerotia in the assay resulted in a clear stimulation of germination in response to DAS (>90%) compared to an untreated control while germination of unconditioned sclerotia with or without DAS was low. Whilst drying of *S. cepivorum* sclerotia in Experiment 2 resulted in reduced levels of germination compared to Experiment 1, and also resulted in an increase in germination of unconditioned sclerotia (with or without DAS). Consequently, the use of fresh sclerotia is most appropriate for testing potential germination stimulants.

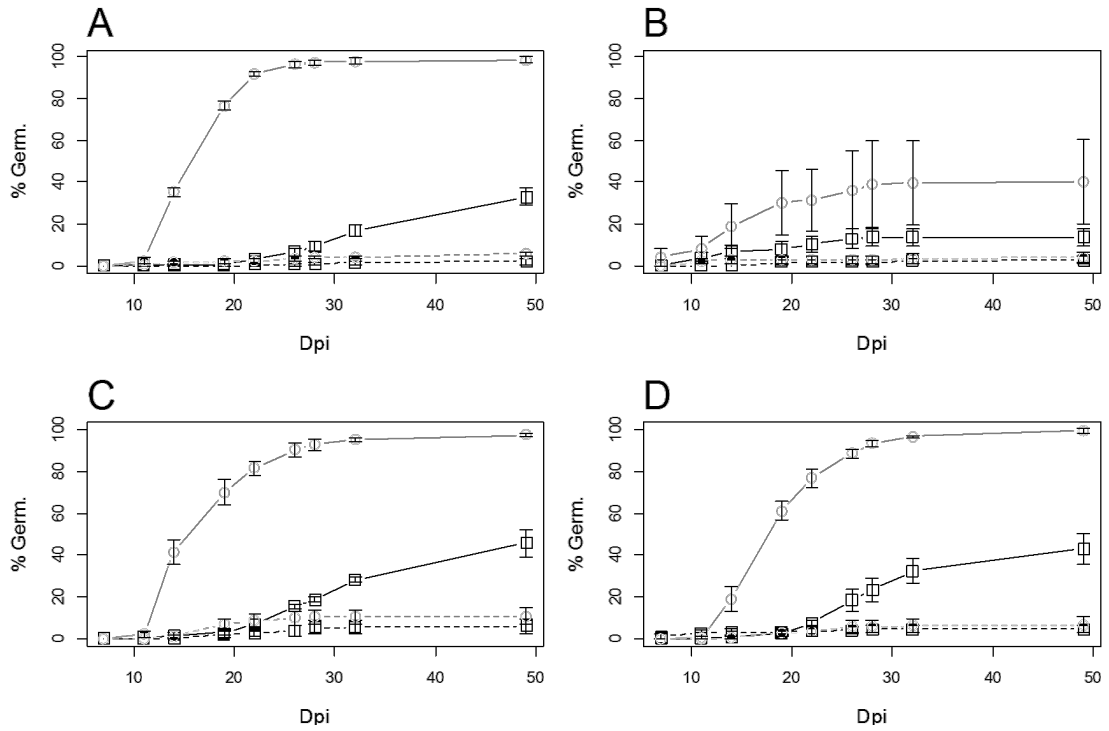

Figure 2.9. Mean cumulative germination of fresh conditioned and unconditioned *S. cepivorum* sclerotia with and without DAS for isolates A) GS1, B) WR3, C) WRAR13 and D) WRLG11 (Experiment 1). Key to treatments; solid lines, +DAS; broken lines, -DAS; open circles, conditioned sclerotia; closed circles, unconditioned sclerotia.

© Agriculture and Horticulture Development Board 2019. All rights reserved

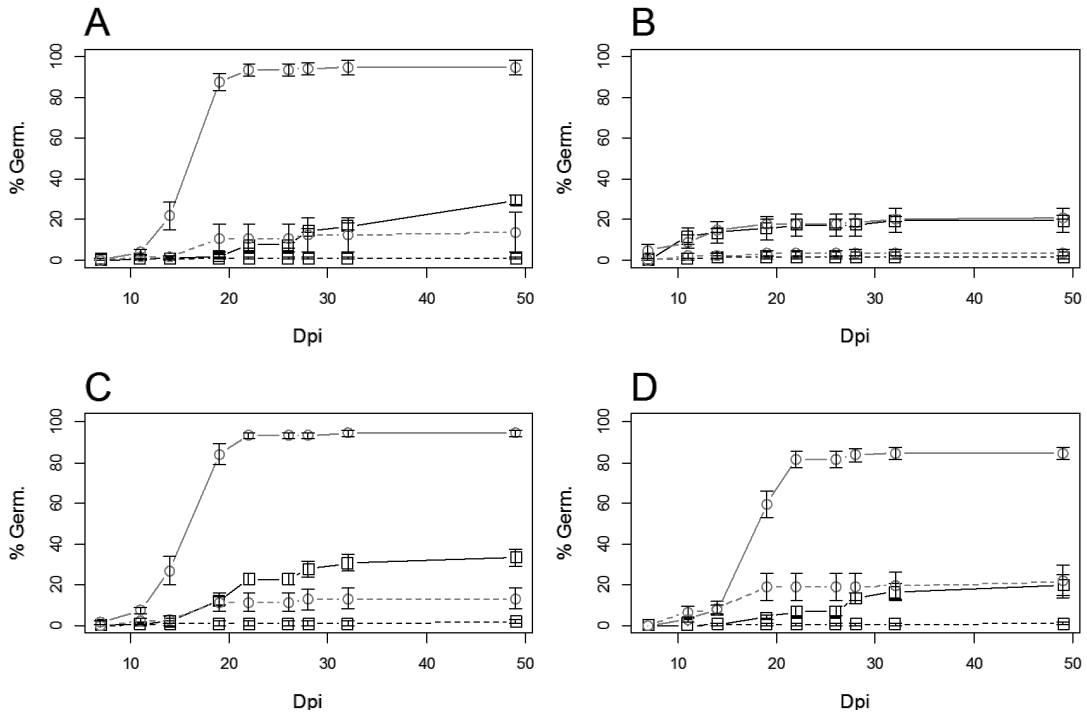

Figure 2.10. Mean cumulative germination of dry conditioned and unconditioned *S. cepivorum* sclerotia with and without DAS for isolates A) GS1, B) WR3, C) WRAR13 and D) WRLG11 (Experiment 2). Key to treatments; solid lines, +DAS; broken lines, -DAS; open circles, conditioned sclerotia; closed circles, unconditioned sclerotia.

Effect of *Allium* product treatments on germination of *S. cepivorum* sclerotia

Experiment 3, utilised the assay developed in the previous section to screen commercially formulated and un-formulated garlic derived products for their effect on sclerotial germination. Based on the previous results, fresh (undried) sclerotia of *S. cepivorum* isolates WRAR13 and GS1 were used.

Results are presented for one replicate (2 x plates of 50 sclerotia) only as replicates are being set up over time due to logistics of the experimental set up. Some differences were seen between the responses of the two isolates to the different treatments, but in general they behaved the same. Differences in the in germination response of *S. cepivorum* sclerotia was observed between the formulated products supplied by Ecospray Ltd. (HDC F261, F262, F263 and F264), with HDC F262 resulting in negligible germination, while HDC F264 resulted in the highest germination levels of 93.6 for WRAR13 and 89.1% for GS1 (Fig. 2.11). A lower germination response was seen with HDC F261 (36.6 and 55.4% germination for WRAR13 and GS1 respectively, whilst the modified version HDC F263 elicited a greater response at 60.5 and 68%. In comparison, the unformulated food garlic products (Granule 1 and 2)

© Agriculture and Horticulture Development Board 2019. All rights reserved

resulted in higher or comparable levels of germination to many of the formulated Ecospray products. Granule 1 resulted in 75.0% (WRAR13) and 53.3% (GS1) germination, somewhat lower than HDC F264 but comparable to HDC F261 and HDC F263. Granule 2 caused a slight increase in germination compared to granule 1 resulting in 88.0% (WRAR13) and 60.9% (GS1) germination. In this experiment the DAS positive control treatments did not respond as expected, with the conditioned sclerotia exposed to 0.5% v/v DAS (+DAS Cond.) resulting in low levels of germination (17.5% and 33.0% for WRAR13 and GS1 respectively), whilst germination for the unconditioned sclerotia (+DAS Un. Cond.) was greater (74.5% and 37.1% for WRAR13 and GS1 respectively). Conditioned and unconditioned sclerotia without DAS (-DAS Cond. and –DAS Un Cond.) generally did not germinate (1.0 to 3.2%) with the exception of GS1 –DAS Cond. where 29.3% responded. Hence, there was a higher background level of germination. The lack of a strong response for the +DAS positive control treatments, suggests that incorporating DAS at this concentration directly into the substrate was potentially fungitoxic or inhibitory and hence was adjusted in further work.

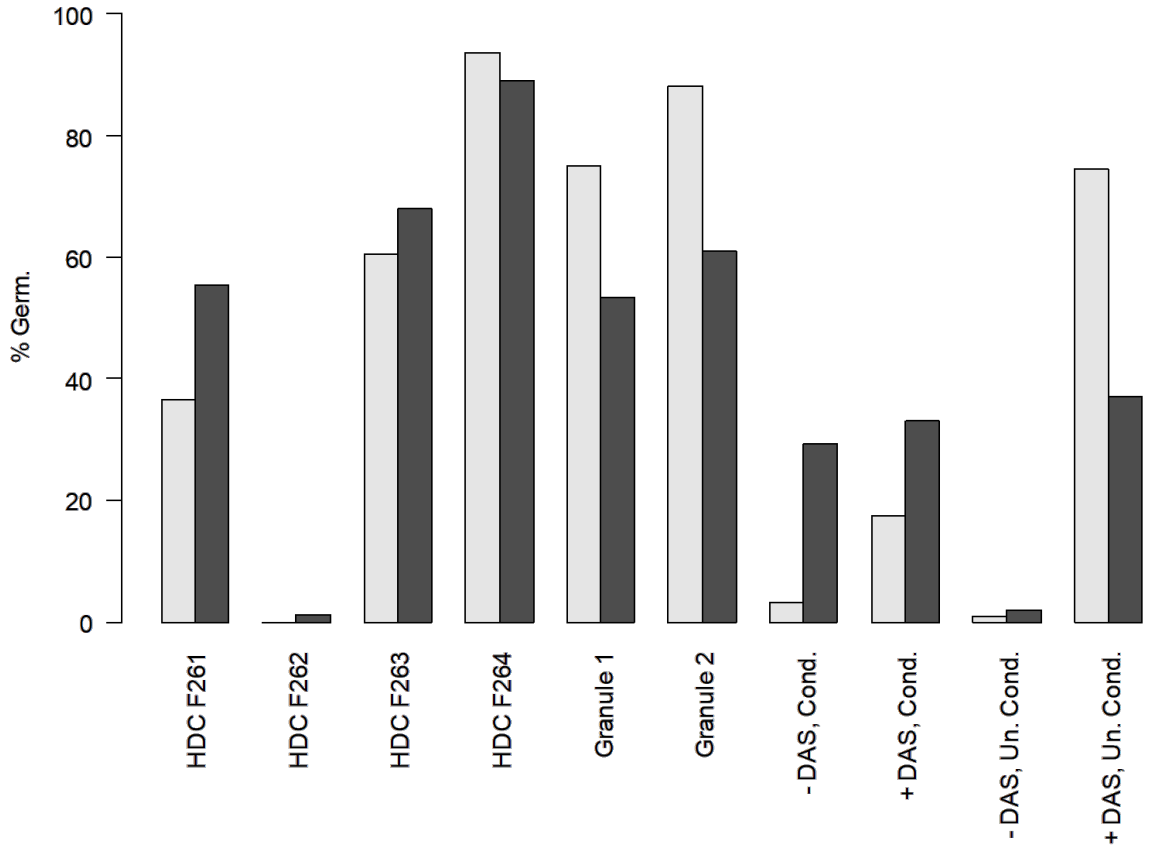

Figure 2.11. Mean percentage germination of *S. cepivorum* sclerotia when exposed to different *Allium* derived products after 50 days (Experiment 3). Light bars represent isolate WRAR13, whilst dark bars correspond to isolate GS1.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Differences in the rate of *S. cepivorum* sclerotial germination were also seen between the test products (Fig. 2.12). HDC F264 which resulted in the highest level of germination overall, attained 50% germination by 18-21 days and 90% between 45-50 days. Granule 1 and 2 caused the earliest response resulting in 21.1 and 17.9% germination by 10 days, but germination rate did not increase after this, resulting in 50% germination at 28 and 21-25 days for isolates WRAR13 and GS1 respectively. Both HDC F261 and HDC F263 resulted in a slow rate of germination until 18 days, when germination rapidly increased. However, HDC F261 did not reach 50% germination within the 50 day experiment time, whilst HDC F263 only reached this after 28-31 days. Based on previous experiments (Fig. 2.9 and 2.10.) conditioned *S. cepivorum* sclerotia in the presence of DAS should have responded the quickest, leading to 50% germination by 15-18 days and reaching 90% by 28 days. However, in this experiment little to no germination was seen before 17 days, with only 25.2% germination achieved after 50 days. Curiously, the unconditioned sclerotia responded to DAS better than those which were conditioned, reaching 50% germination by 38 days and 55.9% at 50 days. The unconditioned untreated control treatment did respond as expected resulting in 1.5% germination by 50 days.

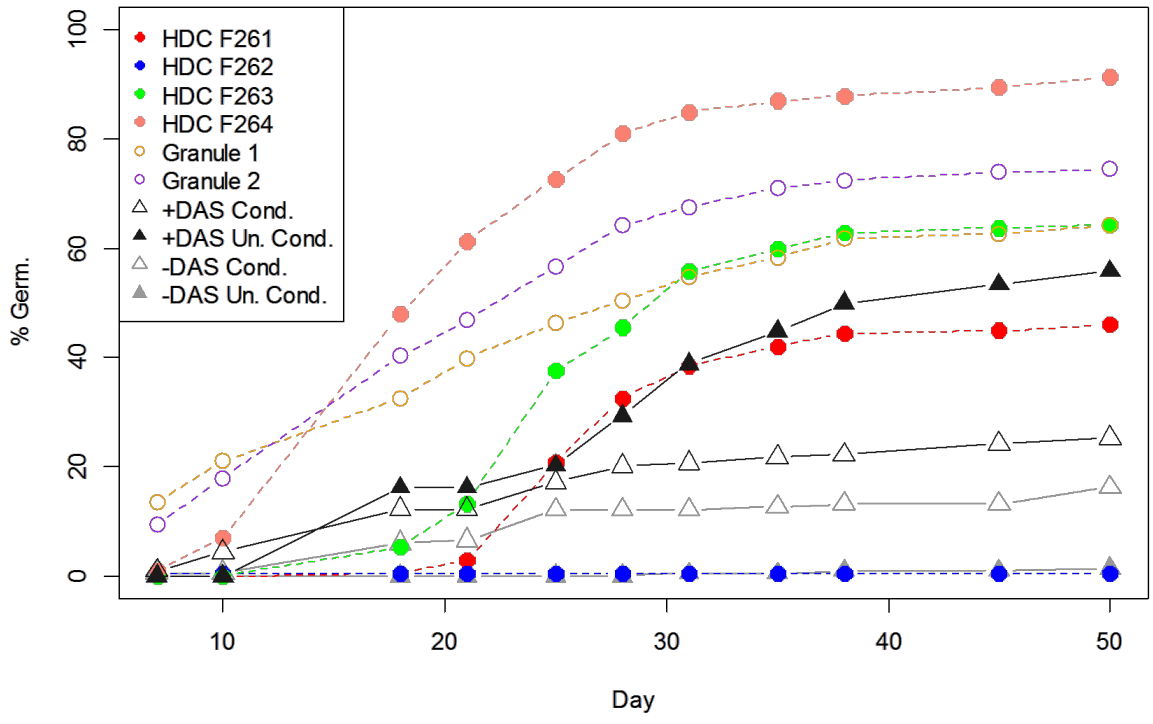

Figure 2.12. Mean cumulative germination of *S. cepivorum* sclerotia when exposed to different *Allium* derived products over 50 days (Experiment 3). Points represent the mean value for both isolates WRAR13 and GS1. Solid circles denote Ecospray products and open circles unformulated garlic products. Triangles depict positive and negative controls, with solid fill representing unconditioned sclerotia, and open symbols conditioned sclerotia.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Experiment 4, comprised of the same treatments as Experiment 3 with the exception that the concentration of DAS used in the positive control treatments was lowered to 0.1% v/v in order to counteract any inhibitory effects as suggested in Experiment 3. Additionally, product HDC F263 was replaced by HDC F265 which has the same polysulphide content as HDC F264 but with a different release profile. This experiment is ongoing and so the results to date (27/02/19) are reported. At 28 days the greatest response was from product HDC F261 where 75% of sclerotia from isolate GS1 germinated, although lower levels were seen with isolate WRAR13 where only 34% responding. In comparison, HDC F265 resulted in more consistent germination between isolates resulting in 35% germination for both WRAR13 and GS1; whilst HDC F264 resulted in 44% and 25% germination for WRAR13 and GS1 respectively. Isolate WRAR13 responded better to granule 1 and 2 resulting in 23% and 16% germination of WRAR13 and GS1 respectively, whilst GS1 only resulted in 9 and 3%. HDC F262 resulted in no germination response as observed previously in Experiment 3. The DAS positive control treatments responded as expected, but were still somewhat reduced compared to the same time point as Experiment 1 and 2. Conditioned sclerotia of each isolate exposed to DAS resulted in 26 and 25% germination for WRAR13 and GS1 respectively. Unconditioned sclerotia of WRAR13 similarly responded to a high level for WRAR13 (30%) but less so for GS1 (5%). Conditioned and unconditioned sclerotia of each isolate resulted in low levels of germination when not exposed to DAS (2-7%) suggesting that background levels of germination were low, and that the main effect was as a consequence of the treatments.

© Agriculture and Horticulture Development Board 2019. All rights reserved

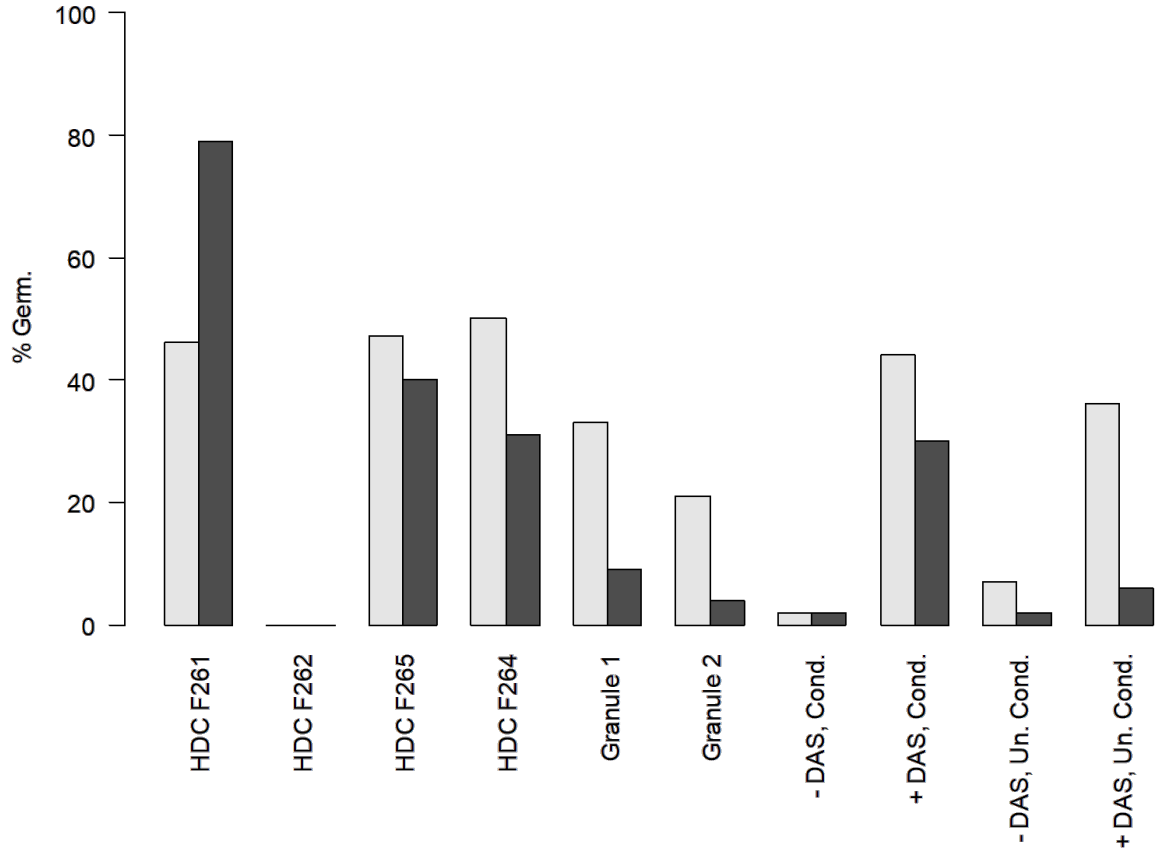

Figure 2.13. Mean percentage germination of *S. cepivorum* sclerotia when exposed to different *Allium* derived products over 28 days (Experiment 4). Light bars represent isolate WRAR13, whilst dark bars correspond to isolate GS1.

© Agriculture and Horticulture Development Board 2019. All rights reserved

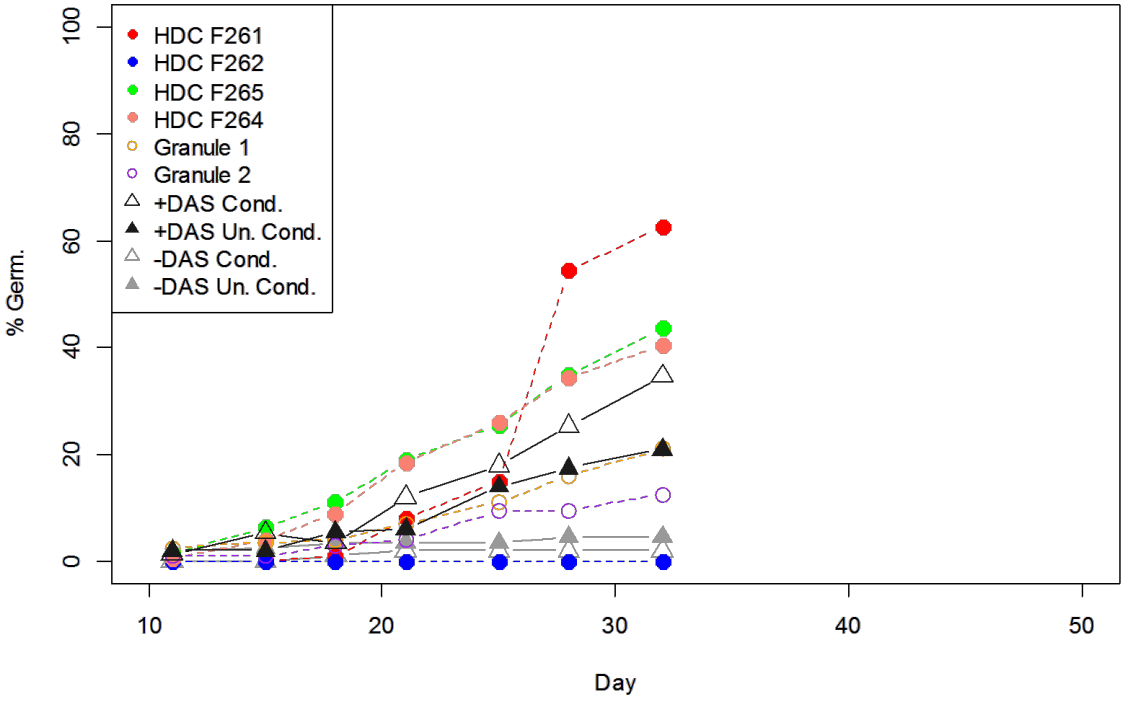

Figure 2.14. Mean cumulative germination of *S. cepivorum* sclerotia when exposed to different *Allium* derived products over 28 days (Experiment 4). Points represent the mean value for both isolates WRAR13 and GS1. Solid circles denote commercial products and open circles unformulated products. Triangles depict positive and negative controls, with solid fill representing unconditioned sclerotia, and open symbols conditioned sclerotia.

Based on the 28 day data (Fig. 2.14), the rate of germination of *S. cepivorum* sclerotia was different compared to that of Experiment 3. The greatest response was observed for HDC F261, whereby a mean cumulative germination of 54.5% achieved at 28 days over the two isolates. HDC F265 and HDC F264 resulted in a 35 and 34.5% germination by 28 days. This trend was similarly seen with both granules, but the overall level of germination was much lower at 16 and 9.5% for granule 1 and 2. No germination was seen with HDC F262. The DAS treated sclerotia also responded slowly, seeing a steady increase to 25.3% for conditioned sclerotia and 17.5% for unconditioned sclerotia, whilst those without DAS resulted in low levels (<5%) germination on average.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum

Using an *in vitro* method, a number of biofumigant plant powders were screened for their efficacy in reducing the mycelial growth of *S. cepivorum* (isolate WRAR13). Mycelial growth for the untreated controls was rapid, reaching the plate edge 3 days post inoculation (dpi). Little to no inhibition of mycelial growth was seen for Nemat and Terranova treatments (Fig 2.15), where growth was reduced by 9% and 4% respectively compared to the control. Whilst Brisant, Caliente 99 and Vitasso showed moderate reduction in growth of 32, 34 and 40% respectively while Pacific Gold resulted in the greatest level of inhibition with a reduction of 74% compared to the control after 3 days.

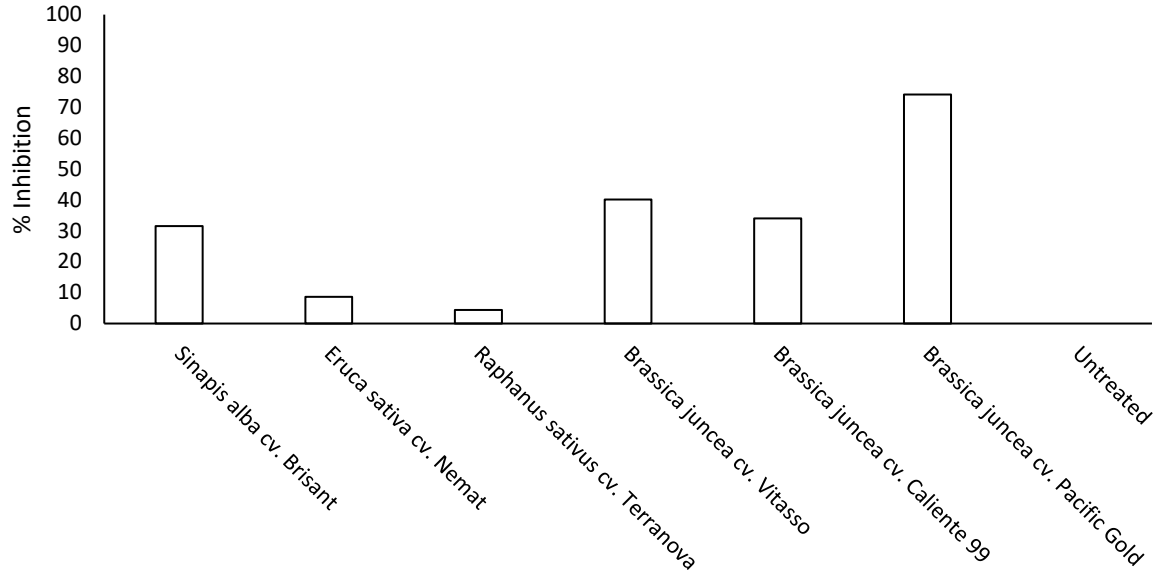

Figure 2.15. Percentage inhibition of *S. cepivorum* (WRAR13) mycelial growth in the presence of biofumigant powders derived from different plant species and varieties. Data derived from plate measurements taken across two perpendicular axes per plate (n=3) at 3 days post inoculation.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Discussion

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease.

Management of white rot in the UK is difficult due to the small number of approved active substances available through the HSE EAMU scheme. Whilst a range of crop protection products have shown promise elsewhere (Villalta *et al.*, 2004, 2010; Ferry-Abee, 2014), few have been recently examined under UK production systems and conditions. Consequently, the current work was undertaken to explore alternative active substances currently registered for other crops in the UK, and to examine their efficacy in field experiments.

Levels of AWR disease differed significantly between trial sites, with no confirmed incidence at the Lincolnshire site (AWR003), low levels at the Ely site (AWR002) and moderate incidence at the Wellesbourne site (AWR001). Although sites were selected with high risk of AWR, levels of inoculum were not known and this could explain the lack of disease at the Lincolnshire site. However, another major contributing factor to low disease levels at AWR002 and AWR003 was the warm and dry growing season where soil temperatures exceeding 20°C were recorded at emergence, which increased in the following weeks. Consequently, early infection by white rot would be unlikely as optimum conditions for sclerotial germination and infection occur at cooler temperatures of 14-18ºC (Crowe and Hall, 1980). Continuing high temperatures coupled with low moisture levels throughout July and August proved to be unconducive for disease development. Consequently, infection at the Ely and Wellsbourne sites was likely to have occurred 4-6 weeks before harvest when temperatures were within the optimum infection range (14-18 ºC; Appendix D). In addition to this, the difficult growing conditions also caused crop stress and bulbing, leading to a high incidence of *Botrytis* infection at AWR003 and subsequent rotting of the plants. As such no data on white rot incidence was reported from this site. Despite these conditions high AWR levels were recorded at the inoculated Wellesbourne site where irrigation was used to encourage disease development which was not possible for the Ely site where disease levels were much lower.

Differences in crop protection product efficacy was observed in trials AWR001 and AWR002. The three soil applied conventional fungicides resulted in a lower AWR disease incidence at AWR001 when compared to the untreated, with no significant difference between a single or double application of products HDC F246 or BAS 516 07F; however generally two applications resulted in better levels of control than one alone. The most consistent and best performing product in terms of efficacy was that of HDC F247, which led to a further 5.1% reduction in incidence compared to the other products after one application, whilst two

© Agriculture and Horticulture Development Board 2019. All rights reserved

applications resulted in almost no disease (0.02% incidence). Use of the fungicide HDC F248 as a seed treatment product proved ineffective against AWR disease in both AWR001 and AWR002, most likely as a consequence of late disease development.

This good level of AWR control is likely linked to the chemical basis for these treatments with all three being based on SDHI chemistry which has shown to be effective in other work (Villalta *et al.*, 2010; Ferry-Abee, 2014). For example, Villalta *et al.* (2010) found boscalid applied as a surface and later stem base spray reduced AWR disease incidence in multiple salad onion trials from 22.0, 28.8 and 15.1% (untreated) to 2.7, 2.7 and 0.5% respectively, resulting in a significant disease reduction and improved marketable yield. In the same study, fungicide applications as two stem base treatments alone were less effective in reducing disease levels (15.1% to 5.3%), but did provide an increase in marketable yield. In comparison, Ferry-Abee (2014) found boscalid to be ineffective in their field trials resulting in a disease scores of 3.6 and 4.2 compared to the untreated also at 3.6 and 4.2. However, both the total and marketable yields were significantly increased following applications. The findings of Villalta *et al.* (2010) are therefore similar to those found here, whereby disease incidence was reduced by the SDHI products following a single soil application, with a following repeat application three weeks later resulting in a greater reduction. Whilst the final incidence of AWR following fungicide treatments was higher in this project than observed by Villalta *et al.* (2010), the disease pressure within the current work was considerably higher; 53.7% in the untreated control at AWR001 compared to 15.1, 20 and 28.8% in their study. Additionally, the amount of a.s. applied was also different in the Villalta *et al.* (2010) work, where 500 g a.s ha^{-1} was applied at each application compared with 1200 g a.s ha^{-1} in this project, as a consequence of the concentrated band application. In contrast, Ferry-Abee (2014) applied 54.5 g a.s ha^{-1}, which may account for the lack of AWR control in their study. This is also supported by *in vitro* agar tests by Ferry and Davis (2010) where several SDHIs were examined for their ability to reduced myeclial growth of *S. cepivorum*. All of the SDHIs had comparativley higher EC_{50} values compared to triazole fungicides or other a.s. For example, 20 ppm of boscalid, 11 ppm of fluopyram or 2.5 ppm of penthiopyrad resulted in a 50% reduction, whilst only 0.4 ppm of fludioxonil or 0.8 ppm of tebuconazole was reqiured for the same effect. Together this suggests that greater concentrations of SHDIs are required compared to triazoles or other fungicide groups, particularly where disease pressure is high such as in the current study.

However, another consideration is that two of the SDHI products in the current project were also co-formulations with DMI and Qol fungicides, more specifically the triazole and strobilurin subgroups. As such this may potentially lead to enhanced disease control from the dual effects of each active substance. For example, tebuconazole is a commonly used DMI group

© Agriculture and Horticulture Development Board 2019. All rights reserved

fungicide which has been shown to be highly effective in the management of white rot (Clarkson *et al*., 2016) forming the main control option for UK growers under current EAMUs. Whilst Villalta *et al.* (2010) showed that azoxystrobin a Qol fungicide reduced AWR disease levels, although this was not as effective as triazoles or other SHDIs. Consequently, it is difficult to determine the efficacy of specific a.s's alone as two of the three were products were mixtures of fungicides that have shown efficacy individually. However, the improved disease reduction by BAS 516 07F may elude to synergistic effects of an SDHI and DMI mixture.

In comparison to the conventional fungicides, the biological control agents (BCAs) proved ineffective in controlling AWR with disease incidence being similar to the untreated control at both AWR001 and AWR002. This is potentially due to the pattern of disease development within the season and the biological nature of these products. For example, infection did not occur until later in the season (September/October), whilst applications of the BCAs occurred earlier at emergence and 3-4 leaf stage (May-July). As such, the BCAs may have been active at a time before infection occurred or were affected by the warm dry weather at application which also impacted on disease development. Similar variation has been seen in other studies with BCAs against AWR, with Noble (2013) reporting large variation between BCA products and test systems across multiple years of study. In this work, several BCAs (HDC F36, HDC F37, HDC F39, HDC F41, Prestop [previously known as HDC F42], HDC F43 and HDC F44) and a conventional fungicide were examined in pot and field experiments between 2010 and 2012. Large variation was seen in pot assays but HDC F39 and HDC F37 soil drenches were effective in reducing AWR disease by 50-60% compared to the controls in two of the three years of testing, whilst Prestop and HDC F41 were effective in one of three years. When used in the field, it was found that single or double drenches of some BCAs provided similar levels of control. Applications of Prestop and HDC F35 gave significantly improved control compared to the untreated control, with double applications of HDC F35 resulting in control comparable to the fungicide. However, this effect was only seen at one of the six trial sites and in one year only where disease pressure was low (7% infection), and as such made statistical analysis difficult. In comparison, the current study achieved high levels of AWR disease in the untreated plots, but BCA application timing and environmental conditions may have contributed to loss of product efficacy. Similar to Noble (2013) BCAs also showed high variation (as demonstrated by representative s.e.m.'s) whereby levels were lower than the untreated control in some replicates at AWR001. Consequently, multiple applications throughout the season will be attempted in year two.

© Agriculture and Horticulture Development Board 2019. All rights reserved

In summary, conventional fungicides applied once or twice early in crop development gave good levels of AWR control at AWR001, but these were applied at a much higher than recommended application rate due to the concentrated nature of band application as proof of concept. This approach was taken based on evidence in literature that high rates of a.s.'s are required to achieve good control under high disease pressure, with large application volumes to ensure penetration below the soil surface and into the root zone. As this type of use would not be supported commercially, now that effective actives have been identified, the approach in year 2 will be to determine the level of control achieved through whole plot applications at the recommended label rate.

Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*.

Sclerotium cepivorum persists between *Allium* crops as soil-borne sclerotia, which are robust survival structures that remain viable for up to 20 years (Coley-Smith, 1987) and which also constitute the primary inoculum for infection of onion crops. *Allium* based products can be used to reduce the levels of sclerotia by mimicking the natural root exudates of onion, causing them to germinate in the absence of a suitable host and exhaust nutrient reserves. The use of natural and synthetic *Allium* stimulants to control AWR has been reported previously (Esler and Coley-Smith, 1983; Coley-Smith and Parfitt, 1986; Somerville and Hall, 1987; Crowe *et al.*, 1994; Hovius and McDonald, 2002; Villalta *et al.*, 2004; Davis *et al.*, 2007), with a particular focus on garlic oils and their constituent chemical compounds such as diallyl disulphide (DADS) or diallyl sulphide (DAS). However, sometimes the effects have been inconsistent at the field scale, or inoculum load has not been reduced enough to prevent disease development and yield loss (Davis *et al.*, 2007). Consequently, the current objective explored the development of an *in vitro* assay to screen commercially available *Allium* products for their ability to cause sclerotia germination under controlled conditions.

Previously an *in vitro* assay to assess germination of *S. cepivorum* sclerotia was developed as part of Defra project HH3230SFV (Defra, 2009), but required further modifications and validation to be utilised with the commercial liquid and granular products tested here. Experiment 1 and 2, aimed to examine the germination response of 'fresh' and dried sclerotia from new isolates of *S. cepivorum* in both unconditioned (potentially dormant) and conditioned (dormancy potentially broken) states. In Experiment 1 using fresh sclerotia, DAS treatment resulted in a significant increase in the germination of both conditioned and unconditioned

© Agriculture and Horticulture Development Board 2019. All rights reserved

sclerotia. An interaction between DAS treatment and conditioning, was very close to being significant suggesting that conditioned sclerotia generally germinated at a higher level than unconditioned sclerotia as reported previously (Coley-smith *et al*., 1987; Gerbrandy, 1992). The same effect was observed in Experiment 2, where sclerotia were dried before use. In this case, germination was reduced compared with Experiment 1, although a greater level of germination was observed for conditioned sclerotia without DAS suggesting that drying sclerotia can lead to increased levels of constitutive germination. Consequently, the method used in Experiment 1 of producing 'fresh' sclerotia was chosen for screening *Allium* compounds for their ability to stimulate germination of *S. cepivorum* sclerotia.

In Experiment 3, a range of commercially formulated *Allium* products manufactuered by Ecospray Ltd. and unformulated food grade garlic granules where compared to DAS. In general the formulated products resulted in good levels of germination of *S. cepivorum* sclerotia from two isolates (30-90%) with the exception of the Experimental product HDC F262. The best response was elicited by HDC F264 which resulted in 90% germination, whilst 50% germination was reached after only 18-21 days. The food grade garlic granules and HDC F263 and HDC F261 also stimulated germination (46% - 74%). The differences between the products is most likely a consequence of formulation, polysulphide composition or stability all of which would affect both the magnitude and rate of the germination response. For example, the levels of stimulatory compounds in HDC F264 are high (45% w/w polysulphides) but are contained within the highly porous matrix of the granules leading to a controlled slow release of active compounds. The garlic granules contain lower levels of the polysulphide precursor Alliin (typically <1%) (Amagase *et al*., 2001), which also requires liberation through hydrolysis or microbial breakdown of the granules and this likely also leads to a slow production of polysulphides. As a consequence, both these products result in a long linear phase of germination over a period of 28 days, with the higher polysulphide concentration of HDC F264 resulting in a higher final germination level compared to the garlic granules. A similar effect was observed when DADS was compared with garlic juice for their ability to stimulate germination of *S. cepivorum* sclerotia (Somerville and Hall, 1987). Interestingly, both these products resulted in a plateau of germination at a similar time point (35-38 days) suggesting that the stimulatory compounds produced become exhausted or broken down at a similar rate. In contrast, the other products (HDC F261 and HDC F263) are a liquid formulation of polysulphides which result in a large release of active compounds soon after application. This action might be supported by the lack of germination before 21 days, after which a short steep linear phase of germination is seen before a final plateau at 38 days. Entwistle *et al.* (1982) showed how solutions of >20% DADS inhibited the germination of *S. cepivorum* sclerotia compared to lower concentrations (0.156 – 10%) where germination was

© Agriculture and Horticulture Development Board 2019. All rights reserved

increased, as also reported by Somerville and Hall (1987) in their experiments with allyl sulphide (AS). Whilst conversley DADs concentrations lower than 0.156% (Entwistle *et al.*, 1982) or 0.01% DADS (Coley-smith and Parfitt, 1986) result in little germination. These observations could therefore explain the behaviour of HDC F261 and HDC F263 here, with a large initial increase in germination stimulants causing an inhibition of germination initially until levels have decreased (through box venting for observation or natural decay within the headspace), after which they reach a suitable level to elicit a response. There is therefore likely an optimum concentration of active compounds for stimulating germination of *S. cepivorum* sclerotia with higher concentrations being inhibitory and lower concentrations having no effect.

Experiment 4, was set up to confirm the results obtained in Experiment 3 while reducing the concentration of the DAS positive control in order to reduce the previously observed inhibitory effect. DAS dose was reduced to 0.1% (v/v) while the product HDC F263 was replaced with HDC F265, which had the same polysulphide concentration as HDC F264 but with a different release profile. This experiment is currently ongoing, but in comparison with Experiment 3, germination of *S. cepivorum* sclerotia was lower for most of the products at the same time point with the exception of HDC F261. Conditioned and unconditioned *S. cepivorum* sclerotia exposed to the lower rate (0.1% v/v) of DAS exhibited higher germination levels of 35% and 21% respectively. Treatments without DAS resulted in <4.5% germination confirming that there is a low level of endogenous germination without exposure to *Allium* compounds.

Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum.

This preliminary experiment aimed to examine a selection of *Brassicaceous* biofumigant species for their effect on mycleial growth of *S. cepivorum in vitro*. In general, material from varities of *B. juncea* (Pacific gold, Caliente 99 and Vittasso) led to the greatest inhibition, although interestingly there was large variation between these. For example, Pacific Gold resulted in the highest level of inhibition (74% reduction in growth) compated to 40% for Vitasso led to 40% and 34% for Caliente 99. This is most likely due to differences in glucosinolate content, with HPLC analysis suggesting that the levels of the glucosinolate sinigrin were highest in Pacific Gold (approx. 7.0 μ mol/g DM) whilst Vitasso contained intermediate levels (5.0 μ mol/g DM) and Caliente 99 the lowest (2.5 μ mol/g DM). Of the other species, only *Sinapis alba* cv. Brisant caused an appreciable reduction in growth resulting in 32% inhibition.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Conclusions

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease.

- A good level of AWR control was achieved in the inoculated experiment at Wellesbourne (Warwickshire), but not at two commercial field sites in Cambridgeshire and Lincolnshire; with this linked to high disease pressure at Wellesbourne, and low levels at the two commercial sites.
- Fungicides based on SHDI and DMI chemistry gave the best levels of AWR control, with single or double applications proving to be similarly effective except for HDC 246 where two applications significantly improved control.
- Biological products were not effective in reducing AWR disease incidence at any of the sites, nor was a DMI fungicide seed treatment.
- Applications were made using a banded application and high water volume (1,000 L ha^{-1}) which likely contributed to their efficacy.

Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia *in vitro*.

- An *in vitro* (laboratory based) assay was developed and used to examine commercially developed and unformulated *Allium* extracts for their ability to stimulate germination of *S. cepivorum* sclerotia.
- Use of pure diallyl sulphide (DAS), previously identified as a germination stimulant, promoted germination of laboratory-produced *S. cepivorum* sclerotia which had been 'conditioned' in soil while little to no germination was observed in untreated sclerotia.
- Commercially formulated *Allium* products generally resulted in high levels of sclerotial germination, with product HDC F264 resulting in 90% germination.
- Unformulated products derived from food grade garlic granules resulted in 64-74% sclerotial germination.

Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum.

- A preliminary *in vitro* experiment showed that biofumigants were able to partially inhibit the mycelial growth of *S. cepivorum* on agar.
- Biofumigants which contained the glucosinolate sinigrin where generally more effective than those containing glucoraphanin or glucoerucin.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Knowledge and Technology Transfer

The results contained within the report have been presented at the below industry and grower events:

British Onion R&D Meeting, Spalding, 30th January 2019.

Hutchinson's Annual Vegetable Conference, Peterborough, 26th February 2019.

© Agriculture and Horticulture Development Board 2019. All rights reserved

References

Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., and Itakura, Y. (2001). Intake of garlic and its bioactive components. *The Journal of nutrition*, *131* (3), 955S-962S.

Clarkson, J.P., Payne, T., Mead, A., and Whipps, J.M. (2002). Selection of fungal biological control agents of Sclerotium cepivorum for control of white rot by sclerotial degradation in a UK soil. Plant Pathology 51, 735-745.

Clarkson, J.P., Mead, A., Payne, T., and Whipps, J.M. (2004). Effect of environmental factors and Sclerotium cepivorum isolate on sclerotial degradation and biological control of white rot by Trichoderma. Plant Pathology 53, 353-362.

Coley-Smith, J.R. and Parfitt, D. (1986). Some effects of diallyl disulphide on sclerotia of Sclerotium cepivorum: Possible novel control method for white rot disease of onions. Pesticide Science 17, 587-594.

Coley-Smith, J. R., Parfitt, D., Taylor, I. M., and Reese, R. A. (1987). Studies of dormancy in sclerotia of Sclerotium cepivorum. Plant Pathology, *36* (4), 594-599.

Coley-Smith, J.R., Mitchell, C.M., and Sansford, C.E. (1990). Long-term survival of sclerotia of Sclerotium cepivorum and Stromatinia gladioli. Plant Pathology 39, 58-69.

Coventry, E., Noble, R., Mead, A., Marin, F.R., Perez, J.A., and Whipps, J.M. (2006). Allium White Rot Suppression with Composts and Trichoderma viride in Relation to Sclerotia Viability. Phytopathology 96, 1009-1020.

Crowe, F.J., Hall, D.H., Greathead, A.S., and Baghott, K.G. (1980). Inoculum Density of Sclerotium cepivorum and the Incidence of White Rot of Onion and Garlic. Phytopathology 70, 64-69.

Davis, R.M., Hao, J.J., Romberg, M.K., Nunez, J.J., and Smith, R.F. (2007). Efficacy of Germination Stimulants of Sclerotia of Sclerotium cepivorum for Management of White Rot of Garlic. Plant Disease 91, 204-208.

DEFRA (2009). Factors Affecting the Inoculum potential of soilborne plant pathogens. DEFRA Final Report for Project HH3230SFV.

Defra Horticultural Statistics (2017). https://www.gov.uk/government/collections/horticultural-statistics.

Entwistle, A. R., Merriman, P. R., Munasinghe, H. L., & Mitchell, P. (1982). Diallyl-disulphide to reduce the numbers of sclerotia of Sclerotium cepivorum in soil. *Soil Biology and Biochemistry*, *14*(3), 229-232.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Entwistle, A. R. and Munasinghe, H. L. (1990). Evidence for damage in sclerotia of Sclerotium cepivorum following sublethal heat treatment. Proceedings of the Fourth International Workshop on Allium White Rot. Neustudt/Weinstrasse, Federal Republic of Germany. Biologische bundesanstalt fur Land- und Forst- wirtschaft, Messeweg 11/12, D-3300 Braunschweig, Federal Republic of Germany.

13-22.

Ferry-Abee, A.E. (2014). Control of Sclerotium cepivorum, causal agent of white rot of onion and garlic. Department of Plant Pathology. University of California.

Gerbrandy, S. J. (1992). Effects of different temperature treatments on dormancy of sclerotia of ten isolates of Sclerotium cepivorum. *Netherlands Journal of Plant Pathology*, *98*(5), 269-276.

McLean, K.L., Swaminathan, J., and Stewart, A. (2001). Increasing soil temperature to reduce sclerotial viability of Sclerotium cepivorum in New Zealand soils. Soil Biology and Biochemistry 33, 137-143.

Noble, R. (2013). Optimising field-scale control of Fusarium basal rot and white rot of onion using Trichoderma amended substrates and pellets, and onion residues. AHDB Final Report FV 219b.

Smolinska, U. (2000). Survival of Sclerotium cepivorum Sclerotia and Fusarium oxysporum Chlamydospores in Soil Amended with Cruciferous Residues. Journal of Phytopathology 148, 343-349.

Somerville, P. and Hall, D. (1987). Factors Affecting Sclerotial Germination of Sclerotium cepivorum, Secondary Sclerotia Formation, and Germination Stimulants to Reduce Inoculum Density. Plant Disease 71, 229-233.

Tsao, R., Yu, Q., Potter, J., Chiba, M. (2002). Direct and simultaneous analysis of Sinigrin and allyl isothiocyanate in mustards samples by High-Performance Liquid Chromatography. Journal of Agricultural and Food Chemistry 50, 4749-53

Villalta, O., Porter, I., Wite, D., Stewart, A., McLean, K., Hunt, J., and Craig, C. (2004). Integrated control strategy for onion white rot disease in spring onions and other bunching Allium crops. State Government of Victoria, Department of Primary Industries: Report on Project VG 01096.

Villalta, O., Porter, I., Wite, D., Stewart, A., and McLean, K. (2005). Stop the Rot - Managing Onion White Rot in Spring Onions. Horticulture Australia Limited Project VG01096.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Villalta, O., Wite, D., Porter, I.J., Ames, A., Imsic, M., Donald, C., Scoble, C., Riches, D., Mattner, S., Pung, H., Cross, S., Jones, S., Hay, F., Gent, D., Pilkington, S., Palmer, C., Pethybridge, S., and Duff, J. (2010). Project 2.1 Integrated management of soilborne pathogens(Sclerotinia beans, lettuce, carrots, celery and other). Victorian Department of Primary Industries (VICDPI) Project VG 07126.

Warmington, R. J. (2015). Pathogen diversity, epidemiology and control of Sclerotinia disease in vegetable crops. PhD Thesis, University of Warwick, UK.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Appendices

Appendix A. Detailed description of site and crop management

Table A1. Diary of key operations for each site.

Site	Operation	Date
AWR001	Drilling	23.04.18
	T1	17.05.18
	T2	21.06.18
	Harvest	22.10.18
AWR002	Drilling	23.04.18
	T1	13.06.18
	T2	11.07.18
	Harvest	26.09.18
AWR003	Drilling	05.05.18
	T1	15.06.18
	T2	13.07.18
	Harvest	21.09.18

© Agriculture and Horticulture Development Board 2019. All rights reserved

Appendix B. Detailed description of treatments, applications and conditions

Table B1. Treatment product details and other information.

Product Name/Code	Batch No.	Date of Manufacture
HDC F246	EFIC002495	-
BAS 516 07 F	12-P00528	01/01/16
HDC F247	FD-151136-001	27/11/15
Prestop	PCS 04267	-
HDC F249	17TP50	-

Table B2. Treatment application dates, and weather conditions at the time of application.

Site	Timing	Date	Conditions
AWR001	T1	17.05.18	Cool (10-15°C), light breeze, bright.
	T2	21.06.18	Warm (15-20°C), breezy, bright.
AWR002	T1	13.06.18	Warm (15-20°C), breezy, dull.
	T2	11.07.18	Warm (15-20°C), light breeze, bright.
AWR003	T1	15.06.18	Warm (15-20°C), moderate breeze, bright.
	T2	13.07.18	Warm (15-20°C), calm, dull.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Appendix C. Trial designs for the three individual sites.

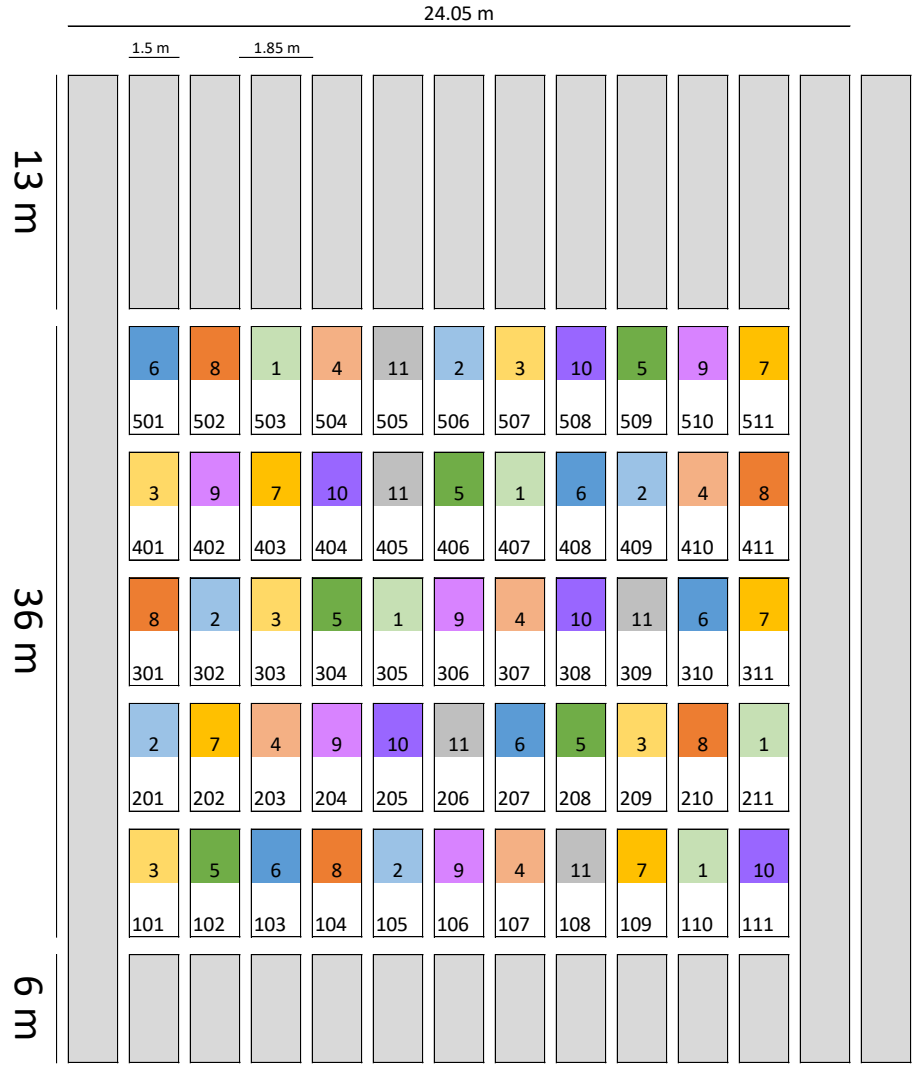

Figure C1.0. Wellesbourne (AWR001/18) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

© Agriculture and Horticulture Development Board 2019. All rights reserved

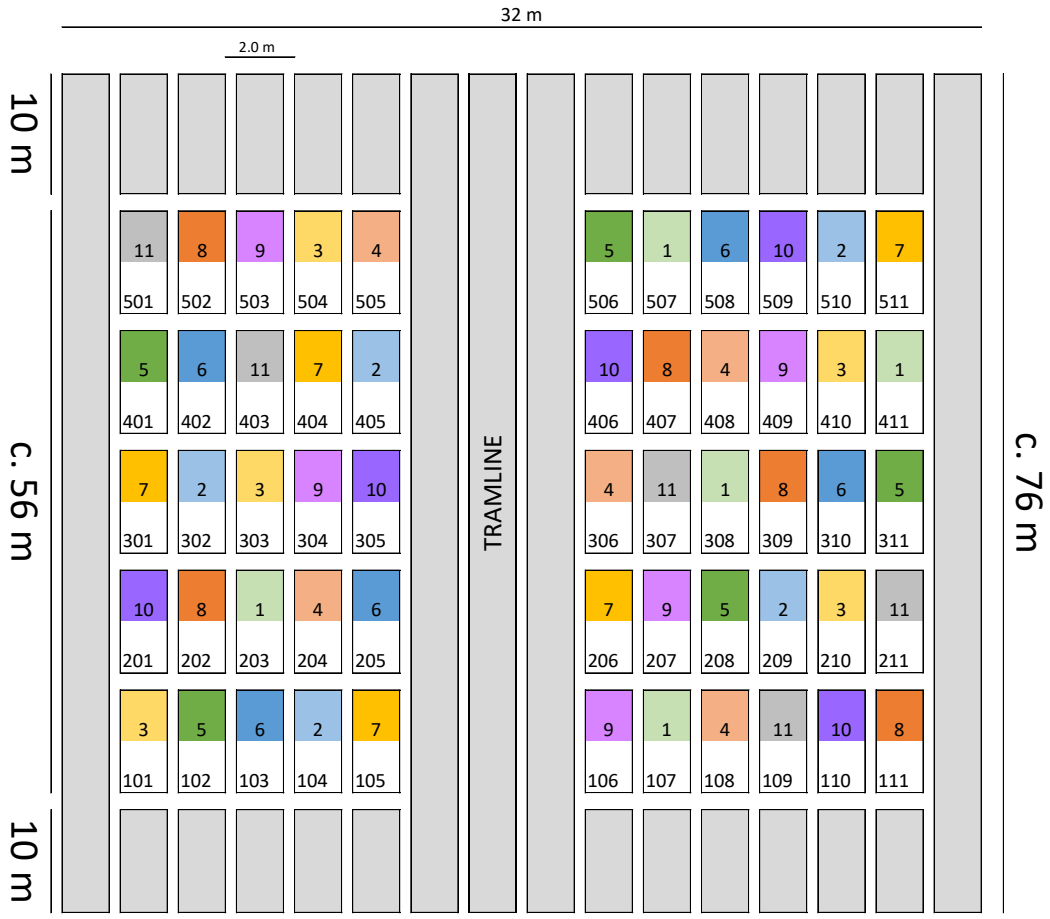

Figure C1.1. Cambridgeshire (AWR002/18) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

© Agriculture and Horticulture Development Board 2019. All rights reserved

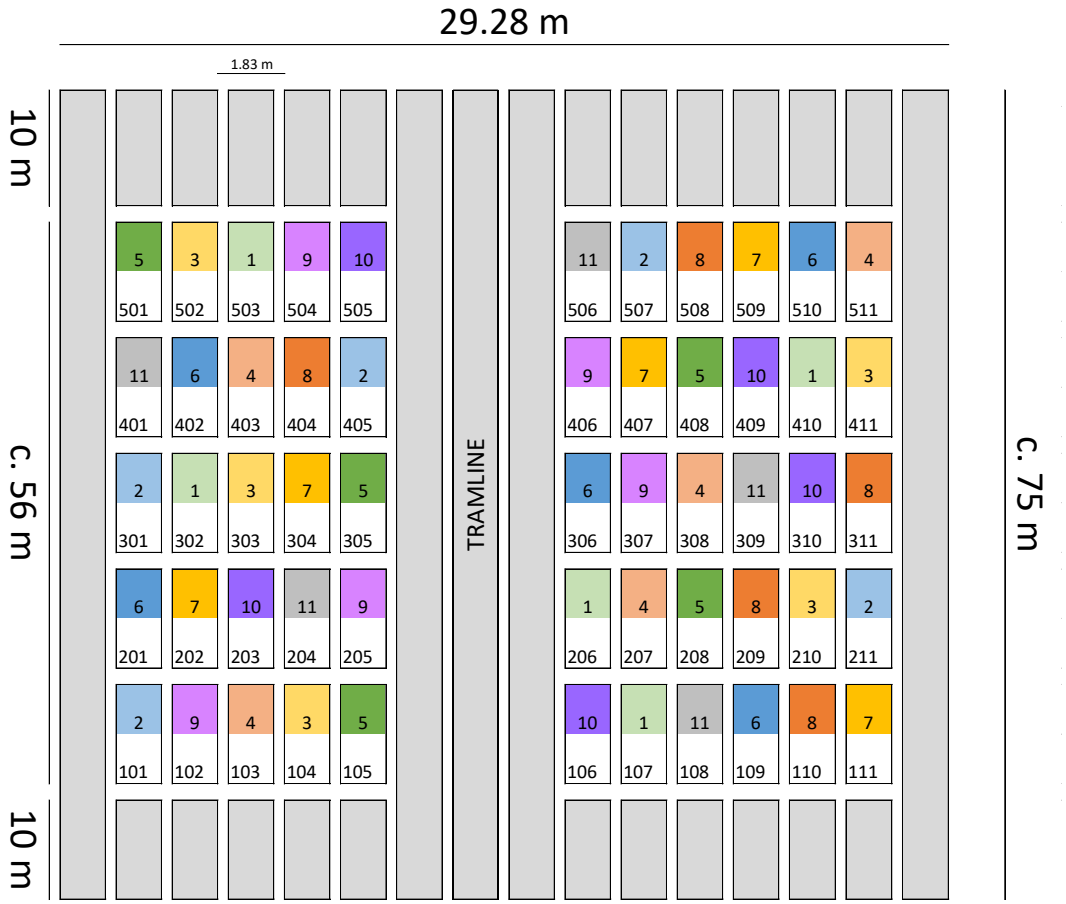

Figure C1.2. Lincolnshire (AWR003/18) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Appendix D. Weather Data

AWR001

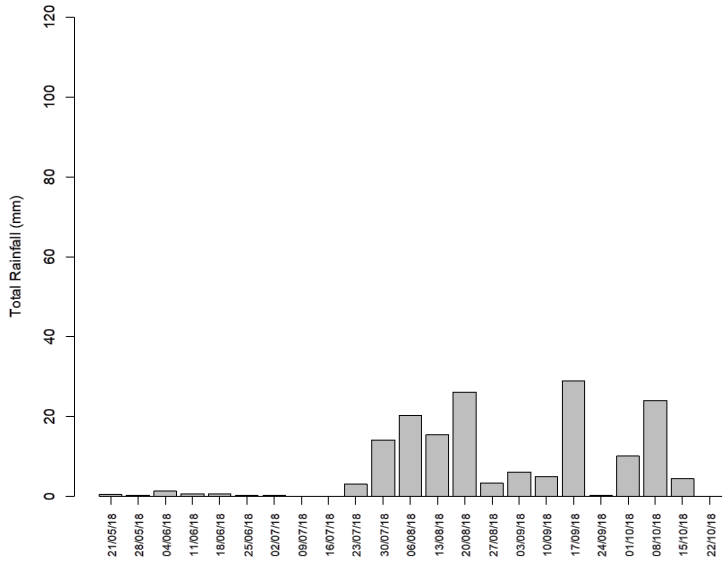

Figure D1.1. Total weekly rainfall (mm) at site AWR001. Date range: w/c 21/05/18 – w/c 22/10/18.

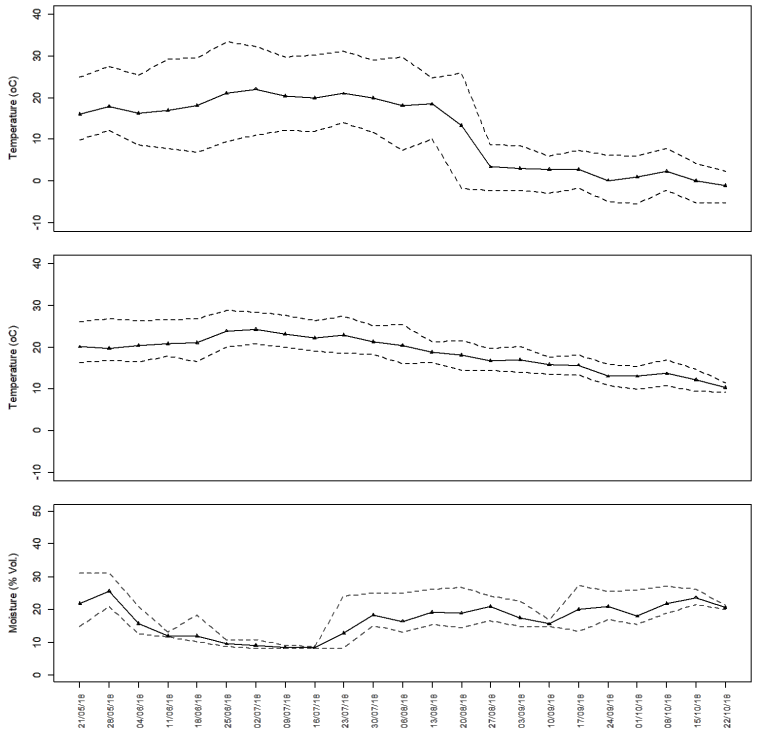

Figure D1.2. Weekly mean, max and minimum values for; Air temperature (Top), Soil temperature at 10 cm (Middle) and Soil moisture at 10 cm (Bottom) at site AWR001. Triangular points with solid line represents the mean temperature at each depth, with the flanking dotted lines showing the respective maximum and minimum values. Date range: w/c 21/05/18 – w/c 22/10/18.

© Agriculture and Horticulture Development Board 2019. All rights reserved

AWR002

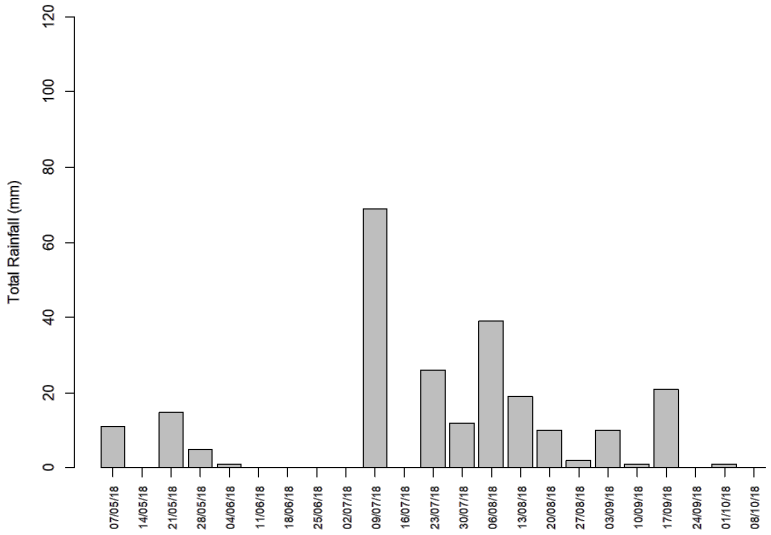

Figure D1.3. Total weekly rainfall (mm) at site AWR002. Date range: w/c 07/05/18 – w/c 08/10/18.

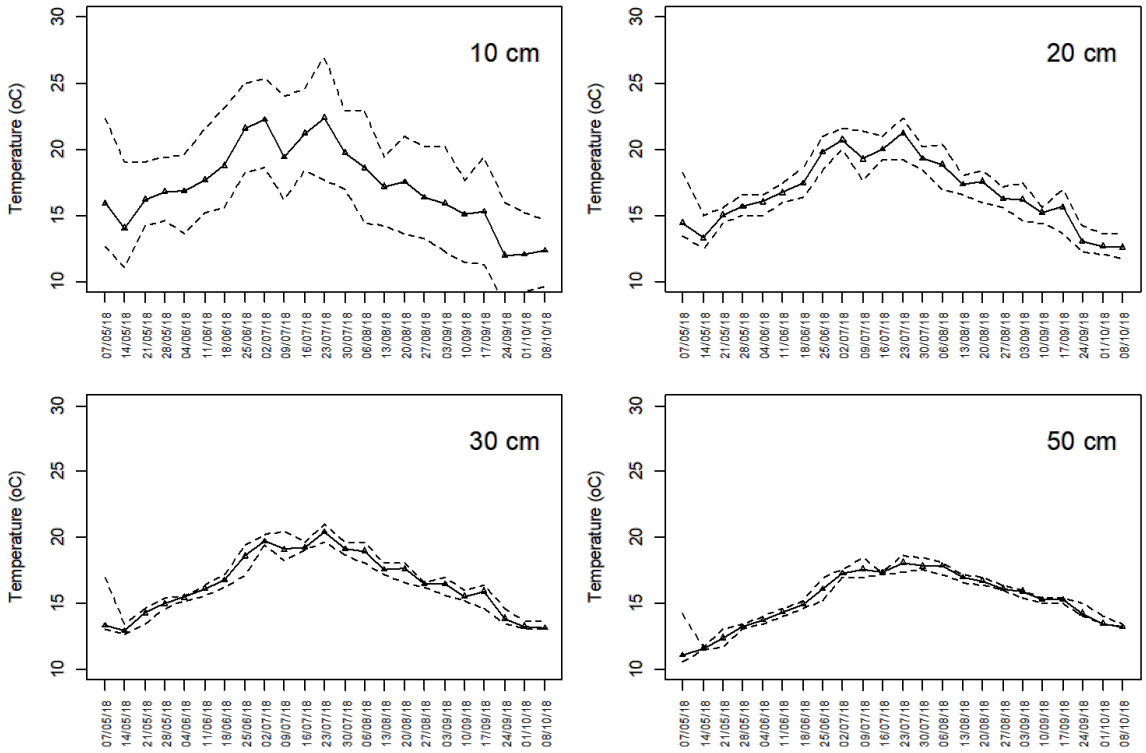

Figure D1.4. Weekly mean, max and minimum soil temperatures at site AWR002 taken at 10, 20, 30 or 50 cm depth. Date range: w/c 07/05/18 – w/c 08/10/18. Triangular points with solid line represents the mean temperature at each depth, with the flanking dotted lines showing the respective maximum and minimum temperatures recorded.

© Agriculture and Horticulture Development Board 2019. All rights reserved

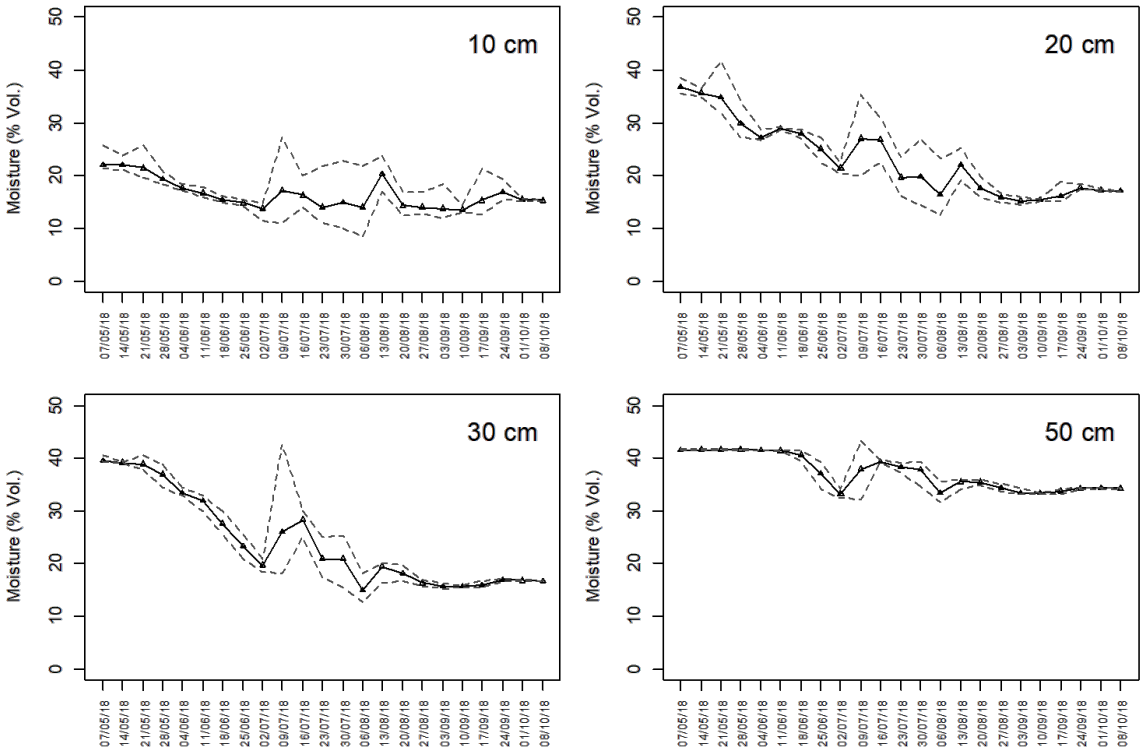

Figure D1.5. Weekly mean, max and minimum soil moisture at site AWR002 taken at 10, 20, 30 or 50 cm depth. Date range: w/c 07/05/18 – w/c 08/10/18. Triangular points with solid line represents the mean moisture at each depth, with the flanking dotted lines showing the respective maximum and minimum moisture recorded.

© Agriculture and Horticulture Development Board 2019. All rights reserved

Appendix E. Statistical analysis – Field Trials

Table E1.0. Summary of the Analysis of Variance (ANOVA) for establishment, vigour and colour scores conducted on field trial AWR001 in 2018.

	Df	SS	MS	*F*-value	*p*-value
Establishment					
Treatment	1	0.102	0.102	3.563	0.06
Timing	1	0.009	0.009	0.316	0.57
Block	1	0.109	0.109	3.795	0.05 NS
Residuals	161	4.628	0.029		
Vigour					
Treatment	1	0.061	0.061	3.407	0.07
Timing	1	0.009	0.009	0.511	0.48
Block	1	0.012	0.012	0.681	0.41
Residuals	161	2.864	0.018		
Colour					
Treatment	1	0.02	0.02	0.088	0.77
Timing	1	114.04	114.04	460.084	<0.001 ***
Block	1	0.01	0.01	0.049	0.83
Residuals	161	39.91	0.25		

Table E1.1. Summary of the Analysis of Variance (ANOVA) for establishment, vigour and colour scores conducted on field trial AWR002 in 2018.

	Df	SS	MS	*F*-value	*p*-value
Establishment					
Treatment	1	0.06	0.058	0.180	0.67
Timing	1	11.14	11.136	34.519	<0.001 ***
Block	1	0.02	0.018	0.056	0.81
Side	1	4.38	4.376	13.564	<0.001 ***
Residuals	105	33.88	0.323		
Vigour					
Treatment	1	0.00	0.001	0.002	0.97
Timing	1	0.23	0.227	0.468	0.50
Block	1	0.45	0.454	0.935	0.34
Side	1	0.67	0.670	1.379	0.24
Residuals	105	51.02			
Colour					
Treatment	1	0.00	0.001	0.004	0.95
Timing	1	13.13	13.127	59.333	<0.001 ***
Block	1	0.01	0.005	0.021	0.89
Side	1	0.51	0.509	2.300	0.13
Residuals	105	23.23	0.221		

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table E1.2. Summary of the Analysis of Variance (ANOVA) for establishment, vigour and colour scores conducted on field trial AWR003 in 2018.

	Df	SS	MS	*F*-value	*p*-value
Establishment					
Treatment	1	1.83	1.833	1.279	0.26
Timing	1	0.74	0.736	0.514	0.47
Block	1	7.88	7.882	5.499	<0.01 **
Residuals	161	230.78	1.433		
Vigour					
Treatment	1	0.00	0.00	0.000	1.00
Timing	1	27.50	27.50	483	<0.001 ***
Block	1	0.00	0.00	0.000	1.00
Residuals	161	9.17			
Colour					
Treatment	1	<0.001	<0.001	1.621	0.21
Timing	1	<0.001	<0.001	1.520	0.22
Block	1	<0.001	<0.001	2.026	0.16
Residuals	161	<0.001	<0.001		

Table E2.0. Comparison of variance between assessments at different field trials site using a Fligner-Killeen Test for homogeneity. Comparisons which are (p>0.05) not significantly different from each shown equal variance, and thus could be combined for comparison. *Key: Est., Establishemtn score; Vig., Vigour score; Col., Colour score; Exp., Experiment site; Prop., Proportion disease incidence at harvest.*

	Df	χ^2	*p*-value
Phytotoxicity Assessments			
Est.~ Exp.	2	140.95	<0.001 ***
Vig.~ Exp.	2	96.72	<0.001 ***
Col.~ Exp.	2	68.83	<0.001 ***
Plant populations			
Pop. ~ Exp.	2	6.17	<0.05 *
Disease incidence			
Prop. ~ Exp.	1	32.72	<0.001 ***

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table E3.0. Summary of the Analysis of Variance (ANOVA) for plant population counts conducted during phytotoxicity assessment at AWR001, AWR002 and AWR003.

	Df	SS	MS	*F*-value	*p*-value
AWR001					
Treatment	1	0.04	0.041	0.010	0.92
Block	1	0.10	0.096	0.024	0.88
Residuals	52	207.84	3.997		
AWR002					
Treatment	1	1.80	1.80	0.253	0.62
Time	1	1.40	1.36	0.192	0.66
Block	1	1.50	1.47	0.207	0.65
Side	1	97.47	97.47	13.725	<0.001 ***
Residuals	105	745.7	7.10		
AWR003					
Treatment	1	0.00	0.001	0.003	0.96
Time	1	1.70	1.698	8.368	<0.01 **
Block	1	2.78	2.776	13.680	<0.001 ***
Residuals	161	32.66	0.203		

© Agriculture and Horticulture Development Board 2019. All rights reserved

Table E4.0. Comparison of different treatments on the final percentage infection by *S. cepivorum* across two field trial sites (AWR001 and AWR002) in 2018. Percentage infection was derived from the mean value per treatment, which were then converted to proportional data (values of 0-1) before analysis. Analysis was conducted using a Generalised linear model (GLM), assuming a binomial distribution and a logit link function with the untreated control used reference factor (intercept).

	AWR001				
Effect	% Infection	e^{β} [a]	s.e[b]	*t- value*	*p*-value[d]
Untreated Treatment	53.7	1.11	*0.32*	3.45	-
1	8.3	-2.65	*0.32*	-5.53	<0.001 ***
2	8.3	-2.64	*0.48*	-5.53	<0.001 ***
3	3.2	-3.66	*0.48*	-5.30	<0.001 ***
4	1.4	-4.48	*0.69*	-4.55	<0.001 ***
5	5.9	-3.02	*0.99*	-5.58	<0.001 ***
6	2.2	-6.38	*0.54*	-2.61	<0.05 *
7	49.7	-0.17	*2.45*	-0.51	0.61
8	46.5	-0.30	*0.33*	-0.92	0.36
9	49.7	-0.17	*0.33*	-0.52	0.61
10	10.1	-2.42	*0.45*	-5.41	<0.001 ***
Block	-	*-0.32*	*0.07*	*-4.37*	*<0.001* ***

	AWR002				
Effect	% Infection	e^{β} [a]	s.e[b]	*t- value*	*p*-value[d]
Untreated Treatment	9.3	0.01	*0.48*	-4.20	-
1	4.4	0.16	*0.68*	-1.19	0.24
2	7.5	0.57	*0.58*	-0.42	0.68
3	4.6	0.18	*0.67*	-1.14	0.26
4	1.7	0.02	*0.97*	-1.87	0.07
5	7.4	0.56	*0.58*	-0.43	0.67
6	1.4	0.01	*1.03*	-1.90	0.06
7	7.3	0.54	*0.58*	-0.46	0.65
8	11.7	1.78	*0.53*	0.47	0.64
9	10.6	1.39	*0.54*	0.27	0.79
10	4.5	0.17	*0.67*	-1.15	0.25
Block	-	*0.83*	*0.10*	*-0.82*	*0.42*

[a] e^{β} corresponds to the logodds-ratio for each explanatory variable. Figures greater that >0 indicate a positive response, whilst the inverse is true of figures which are <0. Similarly, the relative difference between figures within the same model indicate the magnitude of the effect relative to the control (intercept).
[b] standard error.
[d] Derived from a Wald's Test in comparison to the control (intercept).

© Agriculture and Horticulture Development Board 2019. All rights reserved