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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.

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.

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GROWER SUMMARY

Headline

Three field trials in 2019 did not identify effective treatments against *Allium* white rot disease due to low disease levels at two sites, while a third trial showed good disease pressure but no reduction in disease for any of the treatments. Lab experiments showed that *Allium* products stimulated 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 soilborne fungal pathogen causes estimated losses of 2-15% in UK onion equating to approximately £7M per annum. 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 FV 449.

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*. Three objectives were carried out in the current year;

1) Test fungicides and biological control agents for their effect on *Allium* white rot disease.
   1b) Generate preliminary data on effect of selected products on Fusarium basal rot.
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* sclerotia and reduce *Fusarium* inoculum.

Summary

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

- No significant control of AWR disease was observed for fungicide or biological treatments in a field trial carried out at an inoculated site at Wellesbourne (Warwickshire) and two commercial field sites in Cambridgeshire and Lincolnshire. This was due to low disease levels at the first two sites (<1%), and whilst the third site attained moderate disease, there were no differences between treatments. This could be due to the high organic matter content of the soil at this site which may have compromised fungicide efficacy. These results are in contrast to those from 2018 where some fungicide products resulted in significant reduction of AWR disease at the Wellesbourne site.

Objective 1b: Test fungicides and biological control agents for their effect on *Fusarium* basal rot

- No significant level of FOC disease control was observed for fungicide or biological treatments in an inoculated field trial carried out at Wellesbourne (Warwickshire).

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.
- Commercially formulated *Allium* products generally resulted in high levels of sclerotial germination for products HDC F264, HDC F265 and HDC F261.
- Unformulated food grade garlic granule products resulted in moderate sclerotial germination.
- When examined in a soil-based system, HDC F264 resulted in the lowest rate of sclerotia survival (<1%) due to stimulation of sclerotial germination, along with an unformulated garlic granules (<2%). Other products were also stimulatory but were more variable in their efficacy.

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

- Two batches of plant biofumigant material was produced in the glasshouse for experimental work. However, analysis suggested that the levels of key glucosinolates were low. This material will be used in future experiments, but further biofumigant plants are being produced.
- A seedling based assay for FOC established that 1 x10^6 cfu/g of compost would result in consistent high levels of onion seedling mortality, providing a suitable system to examine the effect of plant biofumigants on the pathogen in future experiments.

Financial Benefits

None to report at this time.

Action Points

None to report at this time.

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 soilborne fungal pathogen causes estimated losses of 2-15% in UK onion equating to approximately £7M per annum. 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.

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

Objectives were taken from the project proposal document, although only those which were applicable to year 2 are shown.

1) Test fungicides and biological control agents for their effect on *Allium* white rot disease / generate preliminary data on effect of selected products on Fusarium basal rot.
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* sclerotia and reduce *Fusarium* inoculum.

Materials and methods

Objective 1a: 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 (AWR006) 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 and in the first year's trial (2018). The three field sites allowed for the examination of other factors such as soil-type and local environmental conditions.

Table 1.0. Individual locations of the field experiments in 2019 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 attained from Metoffice.gov.uk.

AWR006/19	AWR004/19	AWR005/19
University of Warwick Wellesbourne Warwick Warwickshire CV35 9EF Grid Ref.: SP 274569 Soil texture: Loam pH: 7.3 OM%: 3.0 CEC: 12.0 Ave. rainfall: 614.8 mm Ave. temp.: 5.9-14.5 ºC	G's Growers Ltd. Redmere Fen Ely Cambridgeshire CB7 4SS Grid Ref.: TL 640864 Soil texture: Peat pH: 7.6 OM%: 38.7 CEC: 583.0 Ave. rainfall: 573.9 mm Ave. temp.: 5.7-14.5 ºC	Oldershaws of Moulton Ltd. Roman Bank, Spalding Lincolnshire PE12 8BA Grid Ref.: TF 327267 Soil texture: Loamy clay pH: - OM%: - CEC: - Ave. rainfall: 610.1mm Ave. temp.: 6.3-13.8 ºC

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 AWR006 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 in late April / early May across the three sites at a seed rate of 48 seeds per linear meter, with an aim of establishing 40 plants per meter. There were four drill rows per bed at all of the sites, with single drill lines used at Wellesbourne and double lines at the other sites.

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 449) and results from year 1 trials (Table 1.1). Applications were made using hand held applicators (Vermorel 2000 HP, Berthoud 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 either a concentrated banded spray 0.10 – 0.15 m width centred on each drill row (Fig. 1.0) or a whole bed application across the plot width; both applications used a medium spray quality. Applications were applied based on growth stage (GS) at emergence (BBCH GS 011/012 [T1]) and at three to four true leaves (BBCH GS 103/104 [T2]) (Table 1.1) across all sites for consistency. Selected treatments were also applied prior to drilling [T0] and were incorporated to 10 cm using the bed former based on manufacturer recommendations. For details on treatment products see Appendix A.

Trial design

The three field trials comprised of a randomised block design of 5 blocks with 12 plots each. Treatment allocation to plots was conducted separately for each site. At sites AWR004 and AWR005 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 AWR006, 2 x 8 m at AWR004 and 1.83 x 8 m at AWR005. 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 B.

Figure 1.0. Top left, product application at T1, showing 0.10-0.15 m band centred on drill line. Top right, whole plot view after treatment showing application bands to each of the four drill lines. Bottom, whole plot application at T0 prior to incorporation with bed former.

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

No.	Name/Code	FRAC Group	App. Method	Timing	Dose[1]
1	HDC F246	7	Band	T1 + T2	0.625
2	BAS 516 07F	7 + 11	Band	T1 + T2	1.5*
3	HDC F247	7 + 3	Band	T1 + T2	2.0
4	HDC F246	7	Whole Bed	T1 + T2	0.625
5	BAS 516 07F	7 + 11	Whole Bed	T1 + T2	1.5*
6	HDC F247	7 + 3	Whole Bed	T1 + T2	2.0
7	Prestop	-	Band	T1 + T2	5.0*
8	HDC F249	-	Band	T1 + T2	2.5*
9	HDC F275	-	Band	T1 + T2	2.5*
10	HDC F272	7 + 3	Whole Bed	T0	1.5
11	HDC F246	7	Whole Bed	T0	0.625
12	Untreated	-		-	-

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

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

AWR disease incidence was assessed in the central two rows of salad onions for 4 x 0.5 m pre-assigned lengths within each plot at sites AWR004 and AWR006. 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 approach aimed to capture the patchy nature of the disease and allowed for the same plants to be assessed over time. Due to low plant establishment at AWR005 these lengths were increased to 1 m each.

AWR disease incidence is difficult to assess non-destructively, but is commonly done so by counting the number of salad onion 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 pathogen (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 AWR004 and AWR006, and subsequently used to calculate white rot incidence and plant death as a proportion of the established population. Populations at AWR005 were assessed when the disease was first seen within individual plots.

Figure 1.1. Experimental plot at site AWR001 in 2018, 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

Salad onion plants from the trial sites were harvested in September and October 2019. Harvest assessments comprised of carefully lifting the plant and root system from each assessment area 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 white mycelium and/or an aggregated mass of small (<2 mm) 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 (v3.6.2 [2019.12.12]) 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 were 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 observed 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 plot assessment areas, and the means calculated for individual treatments at 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.

Objective 1b: Test fungicides and biological control agents for their effect on *Fusarium* basal rot

Field trial site selection

The field trial was situated in the quarantine field area at Wellesbourne, utilising a field which has previously been inoculated with both FOC infested crop residues and with lab-produced FOC inoculum in 2016. Since this time bulb onions have been grown at this site each year with high levels of disease incidence.

Site and crop management

All cultivations, drilling and subsequent crop management were conducted by University of Warwick staff in accordance with local practice with agronomic advice provided by Andrew Richardson from the Allium and Brassica Centre.

Bulb onions (cv. Hytech) were chosen as the test crop due to good susceptibility to FOC (AHDB POBOF 452). Seed was supplied by Elsoms seeds Ltd. (Spalding, UK), and sown at a rate of 30 seeds per linear meter, with an aim to achieve 20-25 plants established in each of four single drill rows per bed.

Treatments and application

Active ingredients (a.i) were selected based on previous reports of efficacy against FOC in the literature and AHDB/manufacturer suggestions (Table 1.3). Applications were made as for AWR in the previous section.

Trial design

The trial trials comprised of a randomised block design of 4 blocks with 15 plots each. Treatment allocation to plots was conducted in GenStat (version 17). Individual plot sizes were 1.83 x 6 m 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 B.

Crop assessments

FOC incidence in the developing crop

Disease incidence was assessed in the central two rows of bulb onions over a length of 4 m with this marked using static markers inserted into the soil throughout the season. Similar to AWR, FOC 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 weekly from the 1st treatment application (T1). Plant death was also recorded, with this comprising of removing the whole plant and observing for basal rot or root decay after which the plant was recorded as positive or negative and removed from the plot. When positive for FOC, a single wooden pea stick (c. 20 cm length) was inserted to mark its place and this was used to assess cumulative plant death throughout the trial. Plant population counts were conducted at the first assessment timing (GS 101/102) and subsequently used to calculate FOC incidence and plant death as a proportion of the established population.

FOC incidence at harvest

Once the majority of the bulb onion crop had formed bulbs and reached >50% leaf fall, the trial was harvested. This comprised of removing the bulbs from the soil and placing on the plot surface to cure for one-week during a period of dry weather. Bulbs were then assessed for rotting by gently squeezing the basal plate; if soft then the bulb was recorded as infected with FOC and discarded in the field, whilst those which were firm were recorded as healthy and placed into net bags. The number of soft bulbs was then added to the cumulative count of dead plants during the growing season to form the final assessment of dead/diseased plants.

FOC storage assessment

The healthy bulbs from the field then stored in nets under well ventilated conditions in an unheated glasshouse for one week to cure, before being moved to an unheated but dry store for 4 weeks. Following storage, bulbs were then graded by size into <40, 40-60, 60-80 and >80mm diameter before recording the weight and number of each grade.

Statistical analysis

Statistical analysis was conducted in Rstudio (v3.6.2 [2019.12.12]) using the base functions along with additional packages where required.

FOC incidence and plant death were averaged across the individual plot assessment areas, and the means calculated for individual treatments at each time point. The mean cumulative incidence and initial plant count for each plot was then used to calculate the relative proportion of plants with *Fusarium* symptoms and 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.

Table 1.3. List of treatments tested for FOC control including product identifier, active ingredient(s), time of application, active concentration and product dose.

No.	Name/Code	FRAC Group	App. Method	Timing	Dose[1]
1	HDC F246	7	Band	T1 + T2	0.625
2	BAS 516 07F	7 + 11	Band	T1 + T2	1.5*
3	HDC F247	7 + 3	Band	T1 + T2	2.0
4	HDC F273	3	Band	T1 + T2	0.4
5	HDC F246	7	Whole Bed	T1 + T2	0.625
6	BAS 516 07F	7 + 11	Whole Bed	T1 + T2	1.5*
7	HDC F247	7 + 3	Whole Bed	T1 + T2	2.0
8	HDC F273	3	Whole Bed	T1 + T2	0.4
9	HDC F274	-	Band	T1 + T2	0.5*
10	Prestop	-	Band	T1 + T2	5.0*
11	HDC F249	-	Band	T1 + T2	2.5*
12	HDC F246	7	Whole Bed	T0	0.625
13	HDC F273	3	Whole Bed	T0	0.4
14	HDC F272	7 + 3	Band	T1+T2	1.5
12	Untreated	-		-	-

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

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

The work conducted in year 2 followed on from the initial *in vitro* Petri dish assays developed in year 1. This utilised freshly harvested *S. cepivorum* sclerotia (not dried) produced in maizemeal sand and conditioned in soil with products incorporated into vermiculite on which the sclerotia were placed.

In addition to these Petri dish assays, a second soil-based system was developed to further evaluate products. This comprised of the sclerotia being buried in field soil treated with each

product in a sealed plastic container for 8 weeks. Following treatment, sclerotia were then recovered and assessed for viability.

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*. Triplicate flasks were setup for each of the *S. cepivorum* isolates used (WRAR13 and GS1), 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 others 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 200 g of air-dried unsterile field soil (silty clay loam, Dunnington Heath Series), and a single nylon bag placed on top. An additional 200 g of soil was then added and SDW added to obtain a 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 placed in an incubator (MLR-352-PE, Sanyo Panasonic Biomedical, Loughborough, UK) at 15°C for 8 weeks in order to condition the sclerotia for germination.

Petri dish germination assays

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 28 ml of SDW resulting in a fine film 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 5 x 10 grid pattern, ensuring 5-10 mm between any two sclerotia (Fig. 1.2). *Allium* product treatments were then applied to individual Petri dishes, with liquid solutions added in a total volume of 2 ml pipetted evenly across the

vermiculite. Granular products were weighted out and spread evenly across the vermiculite and nylon mesh. Treatments were applied to two Petri dishes per treatment, with both of these being contained in a single sealable plastic container (1.75 L; 290 x 150 x 70 mm; Sistema, New Zealand). Once placed into the containers, Petri dish lids were removed, and the individual containers sealed and placed in an incubator at 15°C in the dark. Germination of sclerotia was then assessed over time as described below.

Treatments

In year 1, two experiments (Experiments 1-2, 2018) were conducted to develop an *in vitro* method to screen sclerotial stimulants. Using this system several products supplied by Ecospray Ltd. (Thetford, UK) were examined with most of these proving to be effective in stimulating sclerotial germination *in vitro* (Experiment 3, 2018). Consequently, these products were further examined in year 2 of the project, with several experimental repeats required due to the change in experimental system and variability in the test products.

In Experiment 4, conditioned *S. cepivorum* sclerotia of isolates WRAR13 and GS1 were exposed to six treatments comprising of four commercial *Allium* products (HDC F261, HDC F262, HDC F264, HDC F265; EcoSpray Ltd, Thetford, UK), two sizes of food grade garlic granules (Barnes Williams, Cheltenham, UK), DAS (Sigma Aldrich, Poole, UK) and an untreated control (2% Triton X)(Table 1.4.). The concentration of the DAS positive control was changed to 0.06% v/v compared to the 0.5% v/v used in the first year, following initial experiments to optimise rates (data not shown). The experiment was repeated twice in total, and analysed together.

Figure 1.2. Top, Petri dish containing vermiculite and nylon mesh with *S. cepivorum* sclerotia. Bottom, New plastic container with two separate Petri dishes.

Germination and viability assessments 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 Petri dishes. Experiments were conducted over a period of 7 weeks, after which the majority of sclerotia in the positive (DAS) control had germinated. Any remaining (non-germinated) sclerotia were assessed for viability by first squeezing with forceps to assess integrity, with those that collapsed being discarded. For those that remained intact, 20 sclerotia (or fewer depending on germination levels) were then surface sterilised in 70% ethanol (v/v) for 30 sec and 50% sodium hypochlorite (11% available chlorine) for 30 sec, followed by two rinses in sterile distilled water and allowed to air dry. This was followed by a modified version of the agar drop viability test of Clarkson *et al*. (2002), whereby the *S. cepivorum* sclerotia were squeezed

using sterile forceps to burst the rind and individually placed on 10 mm agar cores taken from a 90 mm Petri dish containing PDA amended with chlortetracycline (20 mg L^{-1}). Twenty agar cores with sclerotia were then placed into a clean sterile 90 mm Petri dish sealed with parafilm (Bemis, USA) and incubated in the dark at 20°C. After 7 days, each sclerotium was examined for the production of mycelium and immature sclerotia (Fig 1.4).

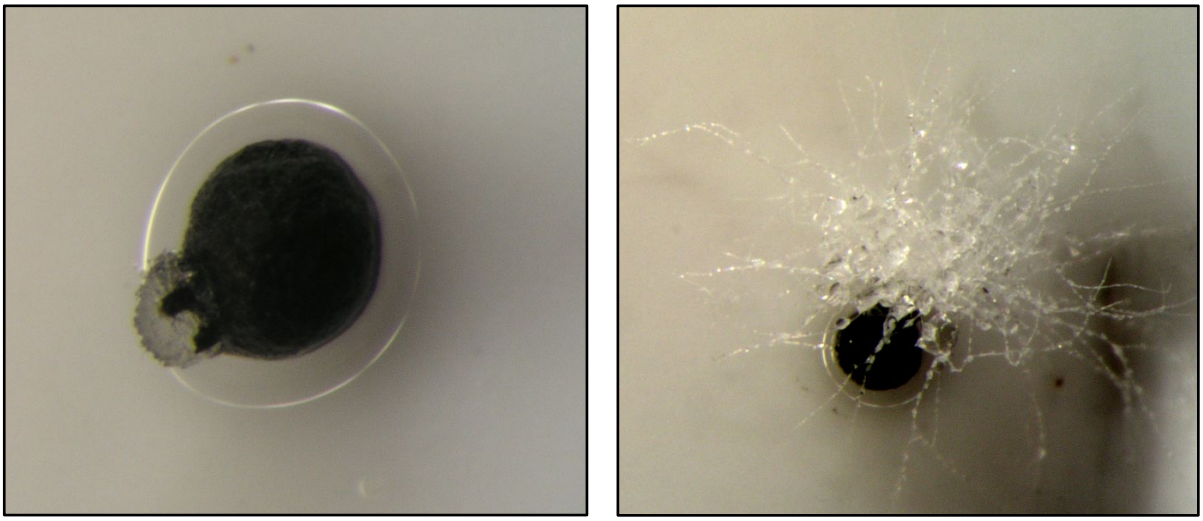

Figure 1.3. Germination of *S. cepivorum* sclerotium in response to DAS *in vitro*. Left, eruptive germination; right, mycelial growth.

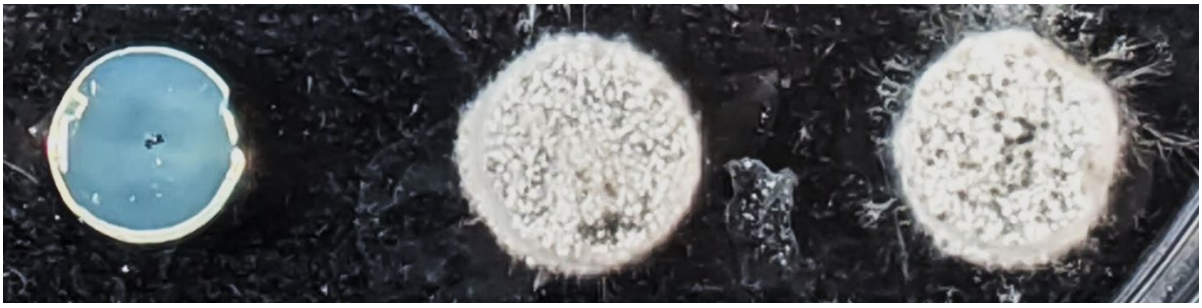

Figure 1.4. Left, unviable *S. cepivorum* sclerotium with no mycelium produced. Middle and right, viable sclerotia showing mycelial growth and immature sclerotia formation after 7 days.

Soil based germination assays for *S. cepivorum* sclerotia

A soil-based assay was used to build upon the results of the Petri dish system and provide a more realistic test for assessing the *Allium* product germination stimulants. Fifty conditioned *S. cepivorum* sclerotia were placed into a nylon bag (50 x 50 mm) before heat sealing. Small plastic containers (100 x 120 x 65 mm; HotFormBoxes) were filled with 200 g of air-dried unsterile field soil (silty clay loam, Dunnington Heath Series), and a single nylon bag placed on top. An additional 200 g of soil was then added and SDW added to obtain a total moisture content of 22% (w/w). The *Allium* product treatments were then incorporated into the soil at the same rate as used in the Petri dish assay. Liquid products were added along with the

SDW directly to both layers of soil, whilst the granular products were mixed with 3 kg of soil before weighing into the individual containers.

Individual containers were used for each of the two *S. cepivorum* isolates, with each isolate/treatment combination being replicated three times, and the experiment repeated twice. The containers were sealed placed in an incubator (MLR-352-PE, Sanyo Panasonic Biomedical, Loughborough, UK) at 15°C for 8 weeks.

Treatments

Experiment 5, utilised the soil-based assay to examine some of the same *Allium* products used in year 1 and in Experiment 4. This comprised of treating conditioned *S. cepivorum* sclerotia of isolates WRAR13 and GS1 with four commercial *Allium* products (HDC F261, HDC F262, HDC F264, HDC F265; supplied by EcoSpray Ltd, Thetford, UK), a food grade garlic granule (Barnes Williams, Cheltenham, UK), 0.06% v/v DAS (Sigma Aldrich, Poole, UK) and an untreated control (2% Triton X) (Table 1.5.).

Germination and viability assessments

Unlike the Petri dish system, no direct observation of *S. cepivorum* germination was possible. However, if *S. cepivorum* sclerotia germinated during the treatment period, only rind fragments would remain or they would be hollow and brittle. Hence, an effective treatment would result in a low recovery of sclerotia in the assay. Therefore following treatment, the nylon bags containing sclerotia were recovered from the containers and rinsed in tap water to remove any soil. The sclerotia were then surface sterilised in 70% ethanol (v/v) for 30 sec, then 50% sodium and allowed to air dry. A sterile metal spatula was then gently rubbed against the sclerotia to shatter any that were hollow or degraded, before plating out any remaining intact sclerotia to assess viability as described before.

Statistical analysis

For the Petri dish-based assays, the number of germinated *S. cepivorum* sclerotia was averaged (mean) across treatment replicates at each assessment time, followed by transformation to proportional data by dividing by the total number of sclerotia. 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.

For the soil-based assays, the number of recovered and viable sclerotia was averaged (mean) separately across treatment replicates followed by transformation to proportional data by dividing by the total number of sclerotia. The proportional data was then analysed using a

generalised linear model (GLM) assuming a binomial distribution and logit link function, with comparison to the negative control (2% Triton).

Table 1.4. *Allium* products tested for effects on germination of *S. cepivorum* sclerotia in the Petri dish assay (Experiment 4).

No.	Product(s)	Active ingredient(s)	Application rate[1]	Isolate[2]	Conditioned[3]
1	HDC F261	Unknown	6% v/v	1	+
2	HDC F261	Unknown	6% v/v	2	+
3	HDC F262	Unknown	6% v/v	1	+
4	HDC F262	Unknown	6% v/v	2	+
5	HDC F265	Unknown	20 kg/ha	1	+
6	HDC F265	Unknown	20 kg/ha	2	+
7	HDC F264	Unknown	20 kg/ha	1	+
8	HDC F264	Unknown	20 kg/ha	2	+
9	Garlic Granule	-	100 kg/ha	1	+
10	Garlic Granule	-	100 kg/ha	2	+
11	Garlic Powder	-	100 kg/ha	1	+
12	Garlic Powder	-	100 kg/ha	2	+
13	Control (-)	-	-	1	+
14	Control (-)	-	-	1	-
15	Control (-)	-	-	2	+
16	Control (-)	-	-	2	-
17	Control (+)	Diallyl sulphide	0.06% v/v	1	+
18	Control (+)	Diallyl sulphide	0.06% v/v	1	-
19	Control (+)	Diallyl sulphide	0.06% v/v	2	+
20	Control (+)	Diallyl sulphide	0.06% v/v	2	-

[1]Application rates were derived from on-label rates or through manufacturer guidance, before being converted to a rate per Petri dish based on surface area; [2]Isolates: 1, GS1 and 2, WRAR13; [3]+, Sclerotia were conditioned in unsterile soil for 8 weeks; -, Sclerotia remained in the sterile sand/cornmeal culture media and were consequently not conditioned.

Table 1.5. *Allium* products tested for effects on germination of *S. cepivorum* sclerotia in the soil based assay (Experiment 5).

No.	Isolate	Product(s)	Active ingredient(s)	Label Rate[1]	Rate per Unit[2]
1	1	DAS	Diallyl sulphide	-	84 ml of 0.06% v/v
2	2	DAS	Diallyl sulphide	-	84 ml of 0.06% v/v
3	1	HDC F261	Unknown	6% v/v	84 ml of 6% v/v
4	2	HDC F261	Unknown	6% v/v	84 ml of 6% v/v
5	1	HDC F264	Unknown	60 kg ha^{-1}	0.017 g
6	2	HDC F264	Unknown	60 kg ha^{-1}	0.017 g
7	1	HDC F262	Unknown	6% v/v	40 ml of 6% v/v
8	2	HDC F262	Unknown	6% v/v	40 ml of 6% v/v
9	1	Garlic Granule	Unknown	100 kg ha^{-1}	0.084 g
10	2	Garlic Granule	Unknown	100 kg ha^{-1}	0.084 g
11	1	Control (-)	-	-	84 ml
12	2	Control (-)	-	-	84 ml

[1]Application rates were derived from on-label rates or through manufacturer guidance. [2]On-label rates were converted to a rate per unit (400 g of unsterile soil) based on unit surface area.

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

Production of biofumigant plant material and dried powders

Eight *Brassica* spp. with known biofumigant properties (Table 1.6), and untreated seed sourced from the respective seed breeding company. Each variety/species was sown into seven 7.5 L pots containing compost (Levington's M2) with the addition of a nitrogen and sulphur containing fertiliser to an equivalent rate to 100 kg ha^{-1} of and 20 kg ha^{-1} respectively; as this has been suggested to improve glucosinolate production (M. Back 2018, personal communication). Each variety/species was sown at a rate of 7-14 seeds per pot, depending upon germination rate, before being thinned to 5 seedling per pot at the first true leaf stage. Plants were maintained in a glasshouse compartment at 18-20°C with no additional lighting. Two batches of each variety were produced, the first being grown between April – July 2019, and the second July – December 2019.

Once the majority of each variety had reached mid-flowering (BBCH GS 65) the material was harvested for drying and milling (Fig 1.5). This comprised of cutting the stem at soil level with secateurs, chopping the material into 10-20 cm sections, before placing into labelled paper bags. For those species which formed a large tap root (*Raphanus sativus*) the shoot portion was cut at the point of the first leaf, while the root was removed from the pot and also harvested into a separate bag. Fresh weights were taken of the material from each pot, before drying at 80°C for 24-48 hrs after which the dry weight was recorded and the material milled to a fine powder (<2 mm). The powder was stored in air tight zip lock bags at -20°C to preserve the GLS content, before using HPLC to assess the quantity of key GLSs (Tsao *et al*., 2002).

Experiments aiming to utilise these powders are currently underway and as such no data is available for reporting at the time of writing. However, these experiment involve the use of biofumigant powders in a soil and compost based *in vitro* system to examine their effects on *S. cepivorum* sclerotia survival and FOC inoculum reduction.

Table 1.6. List of biofumigants species and varieties.

Treatment	Species	Variety	Supplier
1	*Brassica carinata*	Cappuchino	RAGT
2	*Sinapis alba*	Brisant	RAGT
3	*Brassica juncea*	Caliente 199	Tozer Seeds
4	*Brassica juncea*	Caliente Rojo	Tozer Seeds
5	*Eruca sativa*	Nemat	Tozer Seeds
6	*Eruca sativa*	Trio	RAGT
7	*Raphanus sativus*	Terranova	Tozer seeds
8	*Raphanus sativus*	Contra	Elsoms

Figure 1.5. Biofumigant plants grown under glass at Wellesbourne 2019. Top left, plants during vegetative growth. Top right, *B. juncea* at mid-flowering prior to harvest. Bottom left, dried plant material.

FOC seedling assay development

Previous work (Clarkson *et al*., 2018) had shown that between artificial inoculation using $1x10^3$ – $1x10^6$ CFUs g^{-1} FOC resulted in high rates of basal rot in mature onion plants, when onion seedlings were transplanted into inoculated compost. These concentrations of FOC inoculum were evaluated for use in a seedling assay, whereby onion seed was planted into infested compost and pre- and post-emergence damping-off due to the pathogen recorded. The aim of this initial work was to identify a level of FOC inoculum that resulted in consistent disease for use in future experiments to assess the ability of the biofumigants to kill FOC spores.

Inoculum of *Fusarium oxysporum* f. sp. *cepae* (FOC) was prepared as described by Clarkson et al. (2018; AHDB POBOF 452). Individual 500 ml conical flasks were filled with a mixture of wheat bran and M2 compost before autoclaving to sterilise. To each flask three 5 mm agar cores, taken from an actively growing culture of FOC (isolate FUS2), were added before resealing and incubating in the dark at ambient temperature. Flasks were agitated weekly to evenly disperse the inoculum for 4-8 weeks. Colony forming units (CFUs) were then calculated by taking 1 g from the flask and performing serial plating onto antibiotic media.

The inoculated bran/compost mix was diluted using unsterile compost (FS2; Levington, UK) to achieve ten-fold concentrations of $1x10^2$ – $1x10^6$ CFUs g^{-1} in a total volume of 600 g. This was then split across three 35 cell module trays, and each cell sown with one untreated onion seed (cv. Hytech). Trays were then arranged in a randomised complete block design in a controlled environment room set to 16/8 hrs Day/Night at 23-25°C. The experiment was repeated twice on separate days. Trays were assessed daily for seedling emergence and post-emergence damping-off for 28 days. Seedling survival was calculated by subtracting the cumulative number of damping-off individuals per day from the cumulative number of germinated seedlings per day.

Statistical analysis

Data from the last day of assessment (28 dpi) was used to examine differences between treatments and the control treatments in both experiments. This comprised of calculating the mean percentage onion seedling survival for each treatment across replicates for each experimental repeat, before comparing for equal variance using an F-test or other appropriate test to examine variation between repeats. If no significant differences occurred between repeats then the data was combined for additional statistical power.

Results

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

Phytotoxicity

As found in year 1, no phytotoxic effects were seen for any of the fungicide products at any of the three field trial sites post T1 or T2 applications. There was also little variation in onion seedling establishment rate and vigour between plots at any of the three assessment timings. The target number of plants (50 per linear row m) was established at site AWR004 (Cambs) resulting in 53.5 per linear row m (range: 32 - 68) and also at AWR005 (Lincs) where 56.4 per linear row m were established (range: 38 – 72.5). At AWR006 (Wells), plant populations were below the target rate averaging 28.6 per linear row m (range: 0 – 40); this was due to difficult establishment conditions and pest damage caused by moles.

Allium white rot incidence

Foliar assessments of AWR

Foliar symptoms of AWR, such as chlorosis and wilting, were low or not present across all three field trials. First symptoms were evident in trial AWR004 at the first assessment date in June 2019. However, the incidence was low ranging between 0.7 and 1.6 % on average across all treatments, and remained low at between 1.3 and 3.6 % in July. No further assessments were then conducted until harvest in September. No foliar symptoms of AWR were observed for any of the assessments in AWR005. AWR incidence at AWR006 was similar to that of AWR004, remaining low at no more than 0.3% overall.

Harvest assessments of AWR

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

At AWR004, AWR incidence was moderate, averaging 39.2% across the untreated plots (Fig. 2.0, A). However, no significant differences were observed between treatments. Applications of fungicides used in year 1 (HDC F246, BAS 516 07F and HDC F247) resulted in 39.4, 39.6 and 28.4% respectively when applied twice as banded applications. In comparison, two applications across the whole bed width proved to be less effective resulting in 43.8, 50.4 and 44.3% infection respectively. Product HDC F246 was also applied as a single treatment at drilling, following advice on proposed label usage, but this also proved ineffective, resulting

in 46.6% disease incidence. In addition to the fungicides from year 1 an additional product HDC F272 was also included as a novel fungicide new to EU markets. However this was also ineffective resulting in 41.4% infection when applied twice across the whole bed. Three biological products were also tested, including Prestop and HDC F249 as used in year 1. In addition to these, HDC F275 was also included in this year's trial, with all three products applied three times as banded applications. However, none of these biological products showed a reduction in AWR with Prestop, HDC F247, and HDC F275 resulting in 43.0%, 59.4% and 34.2% disease incidence respectively.

At AWR005, none of the plots showed any typical disease symptoms of AWR, nor was any symptomatic plants observed within the growing season. Consequently, no harvest data disease data for AWR is reported.

At AWR006 which had previously been inoculated with *S. cepivorum* sclerotia, disease incidence was much lower than observed in year 1 (Fig. 2.0, B), with the untreated control plots averaging <1%. It is not clear why this was, given high disease pressure observed in year 1 and in previous salad onion crops, but as a consequence, no significant differences could be detected between any of the treatments. In general, applications of the fungicides from year 1 (HDC F246, BAS 516 07F and HDC F247) as two banded applications resulted in much lower levels of disease than when applied across the whole bed. The application of HDC F272 twice as a whole bed application or HDC F246 applied at drilling seemed to be just as effective as the other fungicides applied in this way averaging 3.4 and 5.8% disease incidence. Similarly, three banded applications of the biologicals (Prestop, HDC F249 and BIO1) resulted in low levels of disease but these were not significantly different from the untreated.

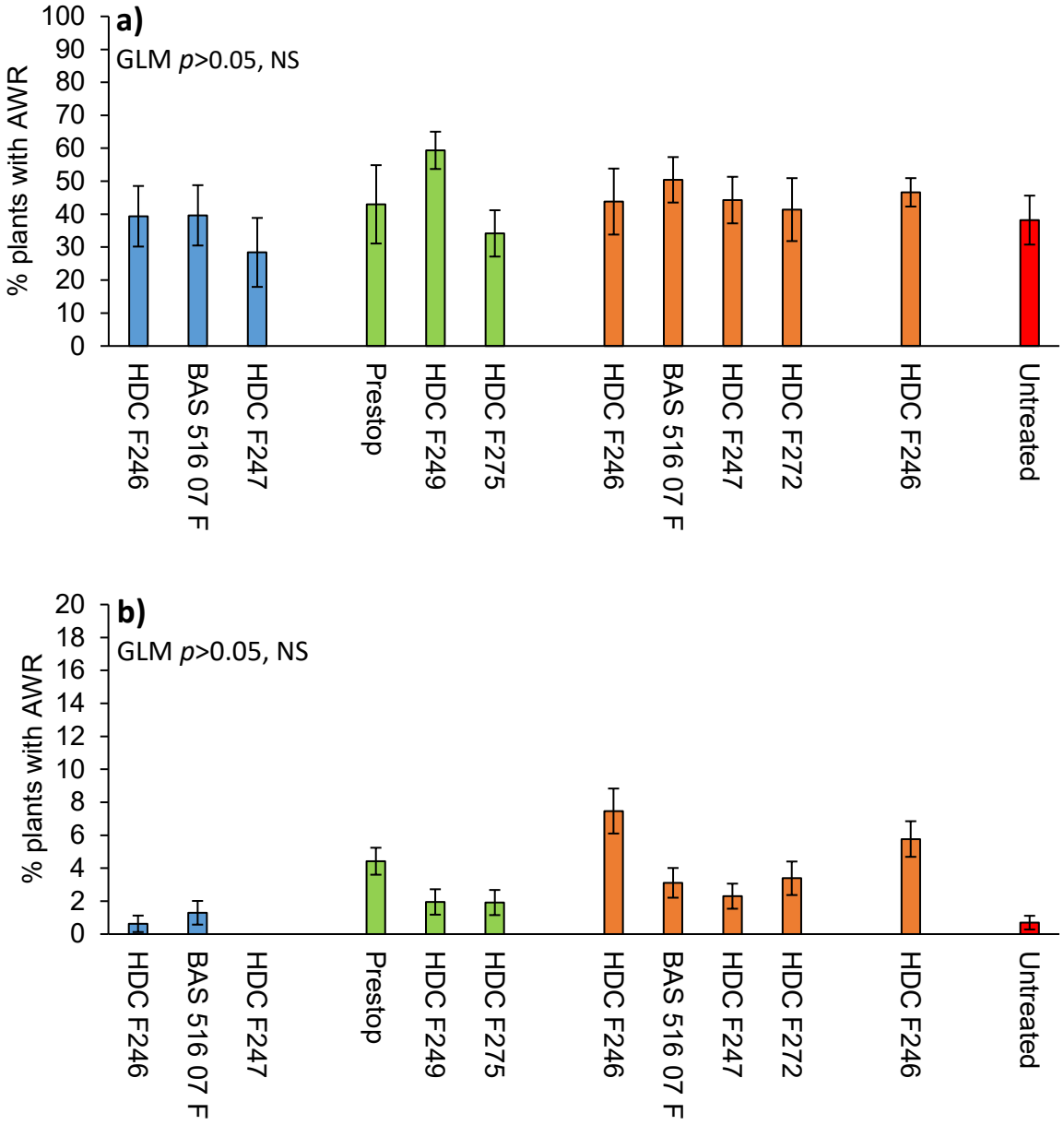

Figure. 2.0. Effect of different fungicide and biological treatments on the proportion of AWR infected plants at harvest for a) field site AWR006 and b) field site AWR004. Key; blue, band applied fungicides (x2 applications); green, band applied biologicals (x3 applications); orange, whole plot applied fungicides (x2 applications); yellow, whole plot applied fungicides applied at sowing (x1 application); red, untreated control. Error bars represent standard error of the mean (s.e.m).

Objective 1b: Test fungicides and biological control agents for their effect on *Fusarium* basal rot

Phytotoxicity

No phytotoxic effects were observed in the bulb onions post application, with all treatments resulting in an establishment score of between 3 and 4.25, with a vigour rating of 3 and a colour score of 5. Some variation in plant populations was seen with plots ranging from 5.5 to 6.8 plants per linear row meter, somewhat below the target of 15. However, this could not be attributed to any treatment effects and was likely as a consequence of the high level of FOC inoculum present at the site which led to seedling damping-off.

Fusarium basal rot incidence

Foliar assessments of FOC

Disease incidence due to FOC was recorded weekly, by recording the cumulative number of dead onion plants during the season and the number of diseased bulbs at harvest. Incidence of foliar wilting symptoms was also recorded at the same time, but cumulative data were variable. Therefore, the cumulative proportion of dead plants in relation to the total number of plants per plot and the number of wilted plants observed each week are presented.

Basal rot disease progression was initially slow, with few plants showing either wilting or plant death at the first few assessments in late June and early July (Fig 2.1 and Fig 2.2). However, by the end of July, a combination of high rainfall and warmer temperatures resulted in greater disease incidence and development. By the time of harvest in early September the number of dead plants averaged 59.2% in the untreated plots, with many of the other treatment plots being similarly affected. None of the fungicides applied as whole bed applications showed a significant decrease in diseased/dead plants at harvest, although HDC F273 resulted in a slight decrease in both disease incidence and plant death; resulting in 44.0% dead plants at harvest. This was also the case for treatments where the fungicides were applied as band applications, although this generally resulted in lower (bit not significant) disease levels. The efficacy of HDC F273 was also improved using this application approach, but was just outside the level of significance (GLM p=0.054); despite attaining a final disease incidence of 31.4% at harvest, or a reduction of 47.0% compared to the untreated. No decrease in disease was seen with any of the biopesticides. Applications of HDC F246 or HDC F273 at sowing across the whole bed led to a reduction in disease development early on, but this was not sustained throughout the season resulting in disease levels similar to the untreated at harvest (Fig. 2.1).

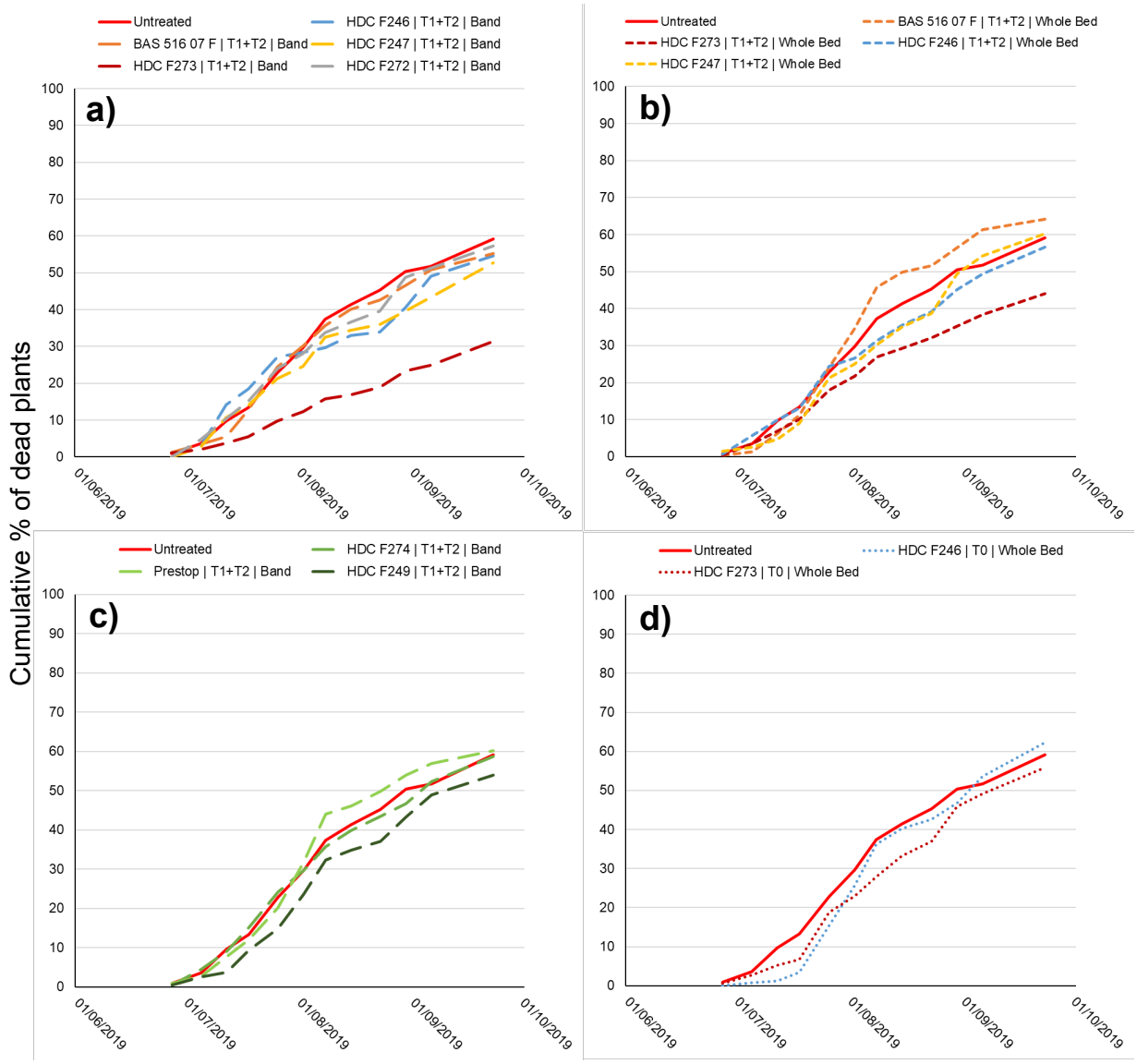

Figure 2.1. Effect of different fungicide and biological treatments on cumulative mean percentage of dead plants due to FOC infection in the 2019 field trial. a) Fungicides applied as 2x banded applications; b) Biologicals applied as 2x banded applications; c) Fungicides applied as 2x whole bed applications; d) Fungicides applied as 1x whole bed applications at sowing.

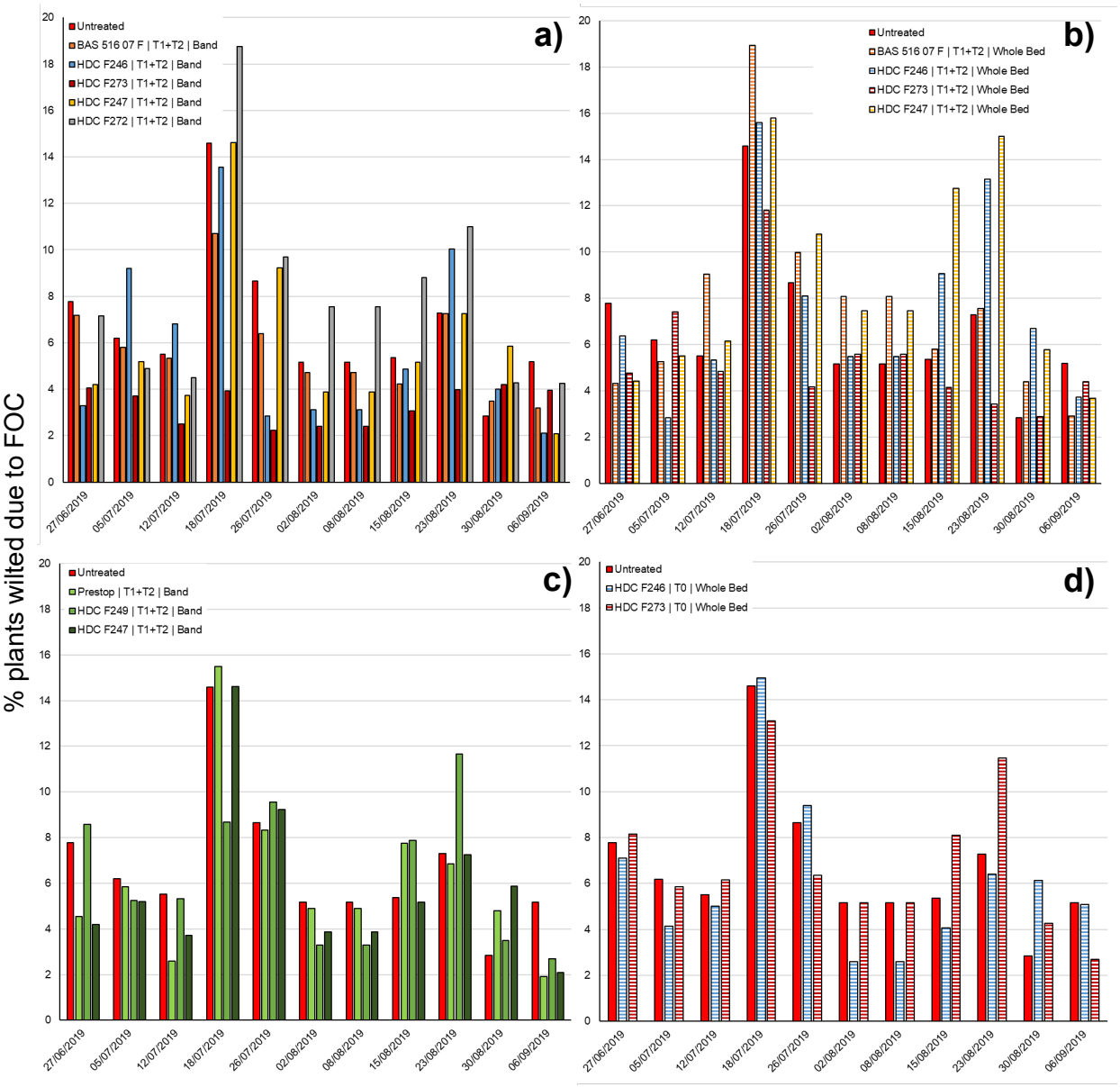

Figure 2.2. Effect of different fungicide and biological treatments on average (mean) percentage of plants showing wilt symptoms caused by FOC in the 2019 field trial. a) Fungicides applied as 2x banded applications; b) Biologicals applied as 2x banded applications; c) Fungicides applied as 2x whole bed applications; d) Fungicides applied as 1x whole bed applications at sowing.

Harvest/Storage assessments of FOC

Bulb number

At harvest, the number of onion bulbs per plot was significantly greater (p<0.001) in plots which had received treatment HDC F273 at T1+T2 both as a band or whole bed application compared to the untreated; however use at T0 did not have any effect (Table 2.0). In general, the banded application of HDC F273 resulted in a mean of 31.8 bulbs per plot compared with 28.3 bulbs per plot for the whole bed application and 21.25 bulbs per plot for the untreated.

When examined by grading size, this larger number of bulbs was attributed to a significantly (p<0.01) greater number of smaller (40-60 mm) bulbs for these treatments (10.8 and 10.0 bulbs per plot respectively) compared with the untreated (5.5 bulbs per plot). This effect was also observed for the biological HDC F249 (T1+T2, banded) application where both grades 40-60 mm and >80mm contained significantly (p<0.01) more bulbs compared with the untreated. However, overall bulb number remained similar to the untreated.

Bulb yield

No significant differences in overall onion bulb yield or yield by grade was observed across treatments, with all treatments averaging between 2.28 and 4.56 kg; however yield for HDC F273 at T1+T2 banded and whole plot applications were generally higher than the untreated (Table 2.1). This could be related to the low plant populations across the trial as a consequence of damping-off, resulting in little competition between plants and thus lower competition for resources.

Table. 2.0. Effect of different fungicide and biological treatments on mean number of onion bulbs per plot in by size grade. Numbers in italics represent the standard error of the mean (s.e.m) for each category. Significance values by grade are derived from a GLM assuming a Poisson distribution, with individual t-tests used to examine within grade differences compared to the untreated. Asterisks denote significance at *p<0.05 and **p<0.01.

	Bulb Size (mm)								
Product	<40		40-60		60-80		>80		*Total*
T1+T2 Banded									
HDC F246	3.0	*1.22*	7.3	*3.07*	10.0	*4.60*	0.5	*0.29*	*20.75*
BAS 516 07 F	2.8	*0.63*	7.5	*0.65*	8.3	*2.46*	0.3*	*0.25*	*18.75*
HDC F247	2.5	*0.87*	6.8	*1.70*	9.8	*2.75*	1.5	*0.96*	*20.50*
HDC F273	3.5	*1.04*	10.8**	*2.78*	15.5	*2.78*	2.0	*1.15*	*31.8***
HDC F272	2.0	*1.08*	4.8	*1.11*	11.3	*1.80*	2.8	*1.55*	*20.75*
HDC F274	3.5	*1.19*	5.3	*2.36*	11.3	*3.30*	1.3	*0.25*	*21.25*
Prestop	1.8	*1.18*	9.3	*2.87*	9.0	*2.97*	1.3	*0.95*	*21.25*
HDC F249	3.0	*1.35*	10.0*	*3.94*	11.0	*3.08*	0.3*	*0.25*	*24.25*
T1+T2 Bed Width									
HDC F246	2.0	*0.71*	3.8	*0.85*	8.5	*2.33*	2.3	*0.85*	*16.50*
BAS 516 07 F	1.8	*0.48*	4.8	*2.56*	9.5	*2.33*	0.8	*0.48*	*16.75*
HDC F247	1.5	*0.96*	5.0	*3.08*	11.3	*2.59*	1.5	*1.19*	*19.25*
HDC F273	4.5	*1.19*	10.0*	*2.89*	11.0	*3.29*	2.8	*1.31*	*28.3**
T0 Bed Width									
HDC F273	3.3	*1.44*	7.0	*2.42*	11.3	*3.20*	0.0	*0.00*	*21.50*
HDC F246	2.0	*0.71*	7.5	*2.25*	10.0	*3.29*	1.8	*1.18*	*21.25*
Untreated	2.8	*0.48*	5.5	*1.26*	11.0	*3.24*	2.0	*0.71*	*21.25*
p value	0.419	-	<0.01	-	0.391	-	<0.001	-	<0.001

Table. 2.1. Effect of different fungicide and biological treatments on mean yield (kg) of onion bulbs per plot by size grade. Numbers in italics represent the standard error of the mean (s.e.m) for each category. No significant differences could were seen between treatments, as assessed by a GLM assuming a Poisson distribution, with individual t-tests used to examine within grade differences compared to the untreated.

	Bulb Size (mm)								
Product	<40		40-60		60-80		>80		*Total*
T1+T2 Banded									
HDC F246	0.1	*0.03*	0.6	*0.23*	2.0	*0.88*	0.2	*0.09*	*2.74*
BAS 516 07 F	0.1	*0.01*	0.6	*0.05*	1.5	*0.47*	0.1	*0.08*	*2.28*
HDC F247	0.0	*0.02*	0.5	*0.09*	1.7	*0.51*	0.4	*0.26*	*2.63*
HDC F273	0.3	*0.03*	0.9	*0.22*	2.8	*0.59*	0.6	*0.45*	*4.56*
HDC F272	0.1	*0.02*	0.4	*0.08*	2.0	*0.34*	0.8	*0.49*	*3.25*
HDC F274	0.1	*0.03*	0.4	*0.19*	1.9	*0.61*	0.4	*0.10*	*2.83*
Prestop	0.0	*0.02*	0.8	*0.21*	1.6	*0.56*	0.4	*0.30*	*2.83*
HDC F249	0.1	*0.01*	0.8	*0.34*	2.1	*0.54*	0.1	*0.07*	*3.04*
T1+T2 Bed Width									
HDC F246	0.1	*0.18*	0.3	*0.23*	1.7	*0.53*	0.7	*0.37*	*2.79*
BAS 516 07 F	0.0	*0.01*	0.4	*0.23*	1.7	*0.46*	0.2	*0.15*	*2.35*
HDC F247	0.0	*0.02*	0.4	*0.22*	2.0	*0.42*	0.5	*0.37*	*2.91*
HDC F273	0.1	*0.03*	0.8	*0.22*	2.1	*0.59*	0.9	*0.45*	*3.86*
T0 Bed Width									
HDC F273	0.1	*0.04*	0.6	*0.20*	1.8	*0.52*	0.0	*0.00*	*2.47*
HDC F246	0.1	*0.03*	0.5	*0.14*	1.9	*0.64*	0.5	*0.34*	*2.99*
Untreated	0.1	*0.01*	0.4	*0.10*	1.9	*0.54*	0.6	*0.21*	*2.99*
p value	1.000	-	1.000	-	1.000	-	0.540	-	0.970

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

Petri dish germination assays

In Experiment 4, different *Allium* products were tested for their ability to stimulate germination of freshly harvested conditioned sclerotia of *S. cepivorum* isolates GS1 and WRAR13 using the Petri dish system. All of the treatments resulted in increased germination off the conditioned sclerotia with the exception of HDC F262 (Fig. 2.3) compared to the untreated (conditioned and unconditioned) controls. Product HDC F261 resulted in the most rapid response with 100% germination reached by 28 dpi for isolate GS1, whilst HDC F265 also achieved 100% germination for isolate GS1 by 49 dpi. In comparison, HDC 264, the two garlic granules and the DAS control (C+ Cond.) resulted in lower germination levels of 40-80% germination by 49 dpi. This level of germination was also achieved by the unconditioned DAS control (C+ Un.Cond.) but the effect was highly variable between replicates. Following statistical analysis of the mean proportion of sclerotia germinated at the end of the experiment (49 dpi), HDC F261 significantly ($p<0.05$) increased the likelihood (log-odds ratio or e^{β}) of a sclerotia germinating, whilst the negative control (C- Cond.) significantly decreased this (Table 2.2). Other products which decreased the likelihood of germination included the second negative control (C- Un.Cond.) and HDC F262; however these were not significant. Applications of DAS to both conditioned and unconditioned sclerotia resulted in an smaller increased chance of germination, along with Garlic Granule 1; but none of these led to a significant increase (p=0.06 - 0.09). Whilst applications of Garlic Granule 2, HDC F264 and HDC F265 significantly increased the likelihood of germination but to a lesser degree than HDC F261.

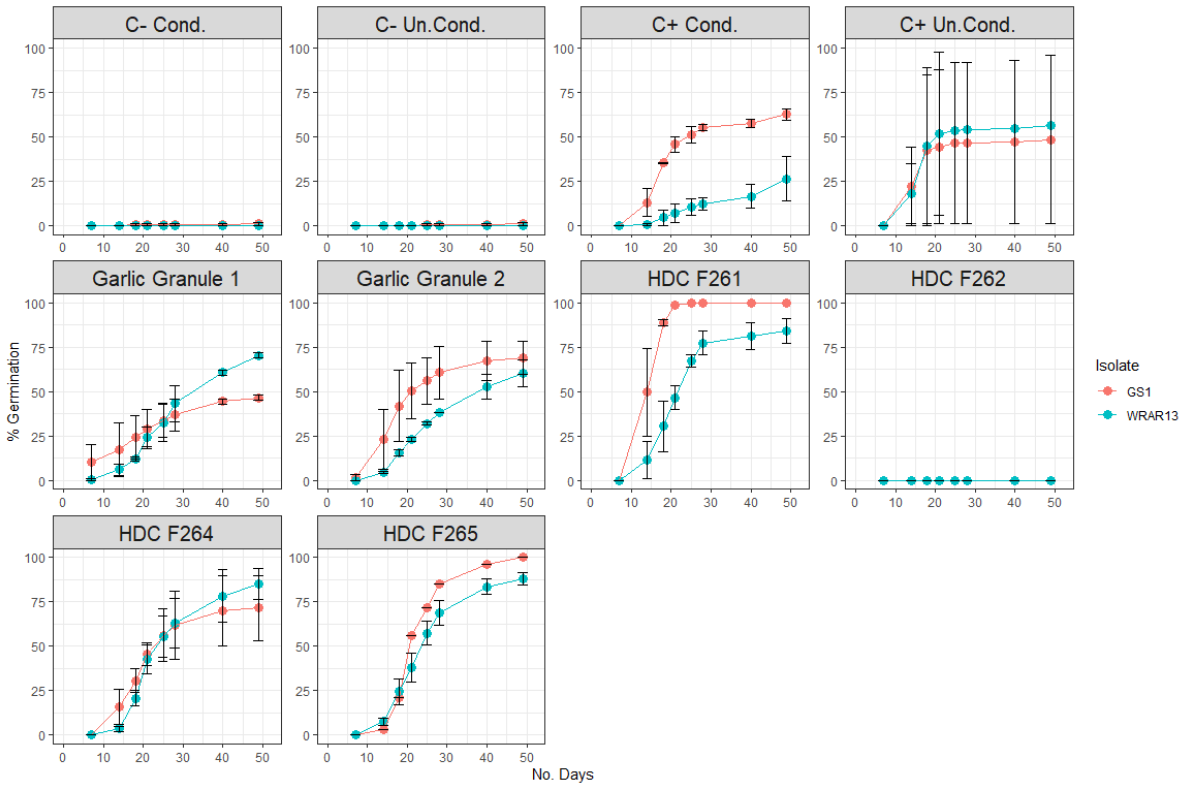

Figure 2.3. Effect of *Allium* products on the mean cumulative germination of conditioned *S. cepivorum* sclerotia (isolates GS1 and WRAR13). Key: C-, sterile distilled water only, C+, positive control treated with Diallyl sulphide (0.06% v/v). Error bars represent standard error of the mean (s.e.m). Note: Only one experimental repeat is available for product HDC F265, due to experimental error.

Table 2.2. Effect of *Allium* products on the final percentage germination of *S. cepivorum* sclerotia (isolates GS1 and WRAR13) at 49 dpi. Percentage germination was derived from the mean value per treatment for each isolate (GS and WRAR13), which were then converted to proportional data (values of 0-1) before analysis. Analysis was conducted on the data for both isolates combined using a Generalised linear model (GLM), assuming a binomial distribution and a logit link function with the untreated control used as a reference factor (intercept).

	% Germination					
Effect	GS1	WRAR13	e^{β} [a]	s.e[b]	*t- value*	*p*-value[d]
Control (-) Cond. *Intercept*	1.0	0.0	-7.18	*2.98*	-2.41	<0.05 *
Control (-) Un. Cond.	1.0	0.0	-1.07×10^{-14}	*4.09*	0.00	1.00
Control (+) Cond.	5.0	26.4	5.23	*2.93*	1.79	0.09
Control (+) Un. Cond.	48.5	55.9	5.57	*2.93*	1.90	0.07
HDC F261	100.0	84.0	8.07	*3.00*	2.69	<0.05 *
Granule 1	46.5	70.4	5.84	*2.93*	2.00	0.06
Granule 2	69.2	60.5	6.14	*2.93*	2.10	<0.05 *
HDC F264	71.6	85.2	6.86	*2.94*	2.33	<0.05 *
HDC F265	100.0	88.0	7.91	*3.03*	2.61	<0.05 *
HDC F262	0.0	0.0	-14.21	*212.8*	-0.01	0.99
Isolate	-	-	*-0.19*	*0.38*	*-0.52*	*0.61*
Block	-	-	*1.21*	*0.38*	*3.15*	*<0.05 **

[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).

Soil based germination assays for *S. cepivorum* sclerotia

In Experiment 5, a selection of the *Allium* products used in the Petri dish system (Experiment 1-3, 2018 and Experiment 4, 2019) were examined for their ability to stimulate germination of *S. cepivorum* sclerotia using a soil-based system. As indicated previously a low percentage recovery of sclerotia would indicate that germination had occurred.

At the end of the experiment, the number of recovered sclerotia was somewhat lower than expected in the untreated controls (-Control) with 53.3 and 58.7% for isolates GS1 and WRAR13 respectively (Fig. 2.4. The positive control (DAS) resulted in a higher than expected recovery of 40.0 and 53.3% for isolates GS1 and WAR13, while recovery for HDC F262 was 27.3 and 62.7% and for HDC F261 was 12.0 and 36.7%. The lowest rate of recovery was for the Garlic Granule treatment at 5.3 and 4.7% for isolates GS1 and WAR13, with HDC F264 also exhibiting very low recovery at 4.0 and 10.0%.

S. cepivorum sclerotia that were recovered were then assessed for viability as described previously. Levels of viability were high in those recovered from the untreated control (-Control) with 86.3 and 77.2% of the sclerotia remaining viable after treatment (Fig 2.4). In contrast, the large number of recovered sclerotia from treatment HDC F262 were largely unviable with only 9.9 and 4.2% germinating on agar. Few sclerotia were recovered from the Garlic Granule treatment and HDC F264, but those that were showed variable levels of viability at 37.7 and 14.9% for the granules. Whilst those from HDC F264 remained almost unviable at 0.0 and 0.07%. The DAS and HDC F261 treatments resulted in more variable viability levels of 20.0 and 51.2%, and 50.0 and 43.6% for isolates GS1 and WAR13 respectively.

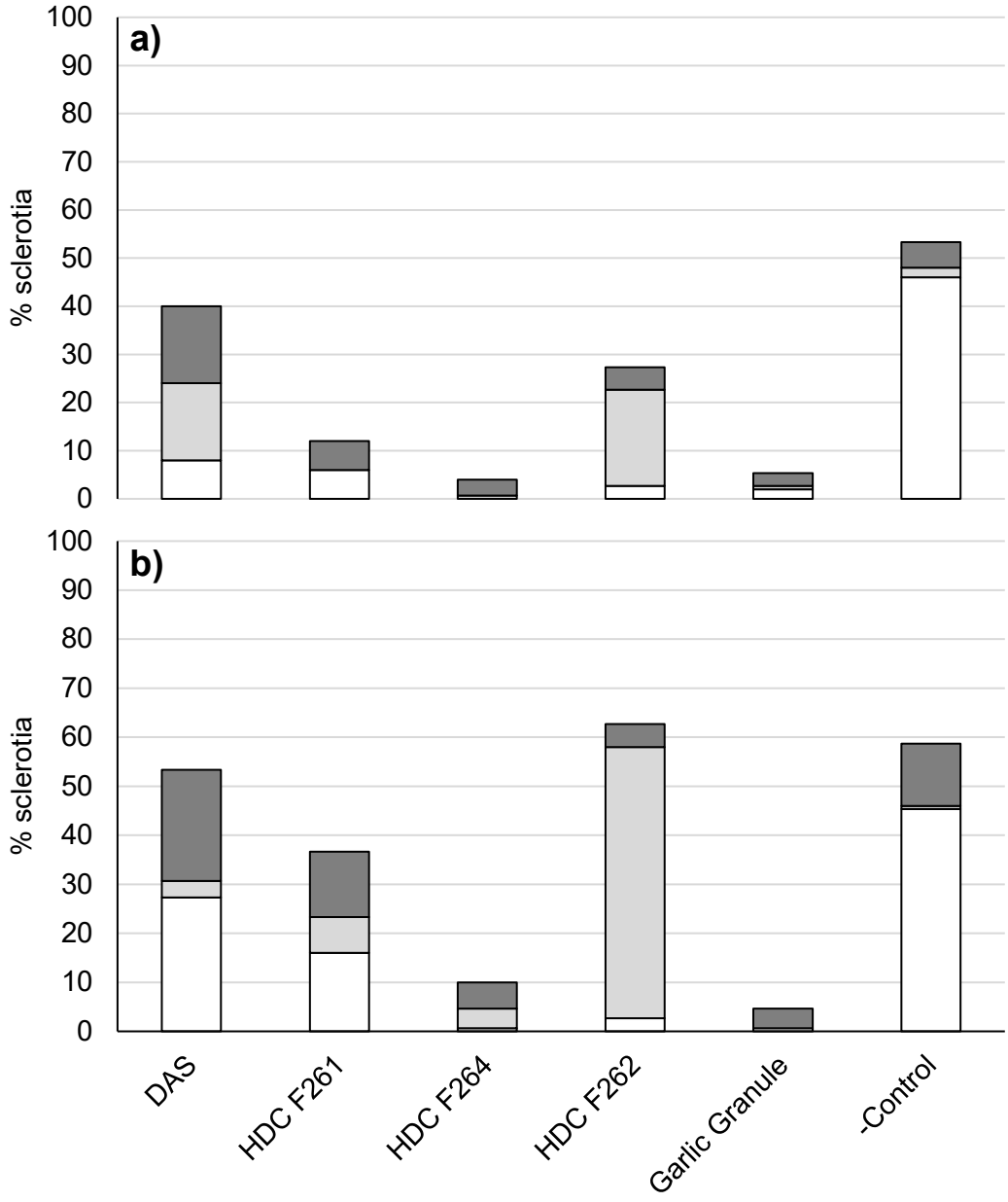

Figure 2.4. Effect of *Allium* products on the percentage of recovered *S. cepivorum* sclerotia and the proportion that were viable (white bar), unviable (light grey) and contaminated (dark grey) for a) isolate GS1 and b) isolate WRAR13 in unsterile field soil.

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

Production of biofumigant plant material and dried powders

Good levels of seed germination were achieved across all of the varieties enabling five seedlings to be established per 7.5 L pot, although some seedlings were lost during the growth of batch 2 due to external factors resulting in only 2-3 seedlings. However, this appeared to have little impact on the amount of biomass produced by each species/variety between batches. The variety Cappuchino produced the highest amount of biomass overall, yielding 123 g of dry material (batch 2), whilst other such as Caliente Rojo only achieved 31 g dry weight (batch 2) (Fig 2.5).

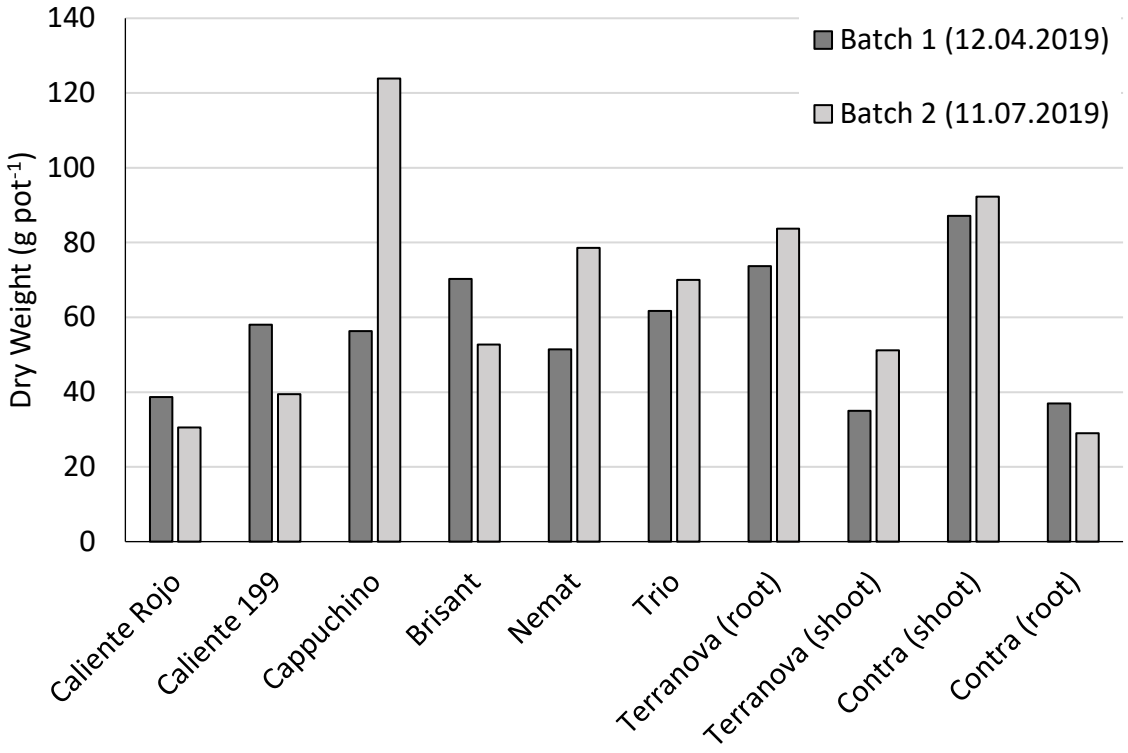

Figure 2.5. Biomass production of different biofumigant plant varieties grown under glass in 2019.

Glucosinolate analysis was conducted for the main GSL for each variety using HPLC. Concentration of GSL was highest for Terranova (shoot) (batch 2) at a concentration of 28 µmol g^{-1} of dry weight whilst other varieties such as Nemat and Trio only reached 1.06 – 1.9 µmol g^{-1} of dry weight (Fig 2.6).

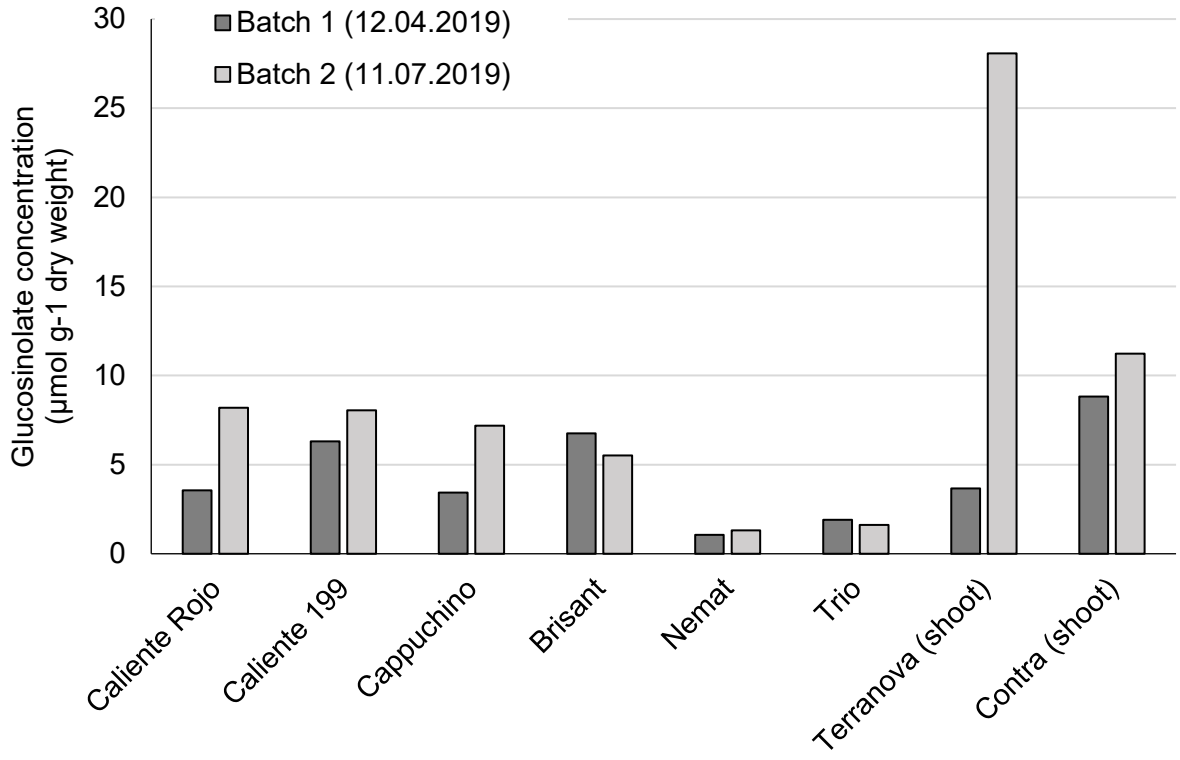

Figure 2.6. Glucosinolate content for different biofumigant plant varieties grown under glass in 2019.

FOC seedling assay development

Artificial inoculation of compost with different concentrations of FOC inoculum proved successful in reducing the emergence of onion seedlings and causing post-emergence damping-off (Fig 2.7, Fig. 2.8). The greatest reduction in emergence was for the highest FOC concentration of 1.0 x10^6 CFU g^{-1} compost, resulting in a reduction of 19.5% seedling emergence compared to the control treatment. The greatest effect of inoculation with FOC was post-emergence damping-off, where concentrations of 1.0 x10^6 CFU g^{-1} compost resulted in 51.4% of plants affected by 28 dpi. Overall both pre and post-damping off for this treatment reduced the number of surviving onion seedlings to only 10.9% by 28 dpi (Fig. 2.8). Similar effects were observed at the lower concentration of 1 x 10^5 CFU g^{-1} compost which resulted in 28.6% of seedling survival. FOC concentrations of 1 x 10^2 – 1 x10^4 CFU g^{-1} compost resulted in 68.1-81.9% seedling survival.

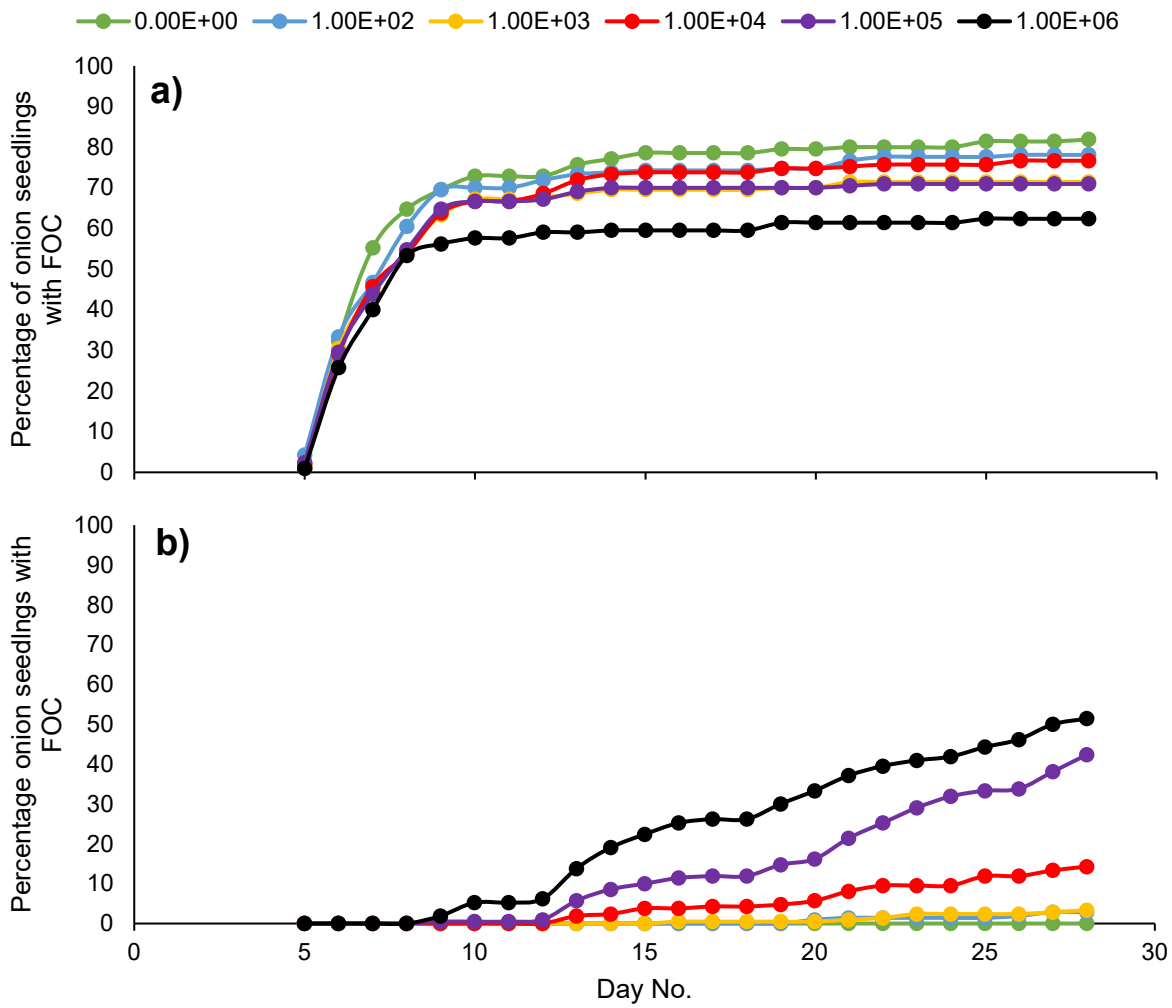

Figure 2.7. Effect of different FOC inoculum concentrations (CFU g^{-1} compost) on the percentage of onion seedlings a) emerged and b) with post-emergence damping-off over 28 days.

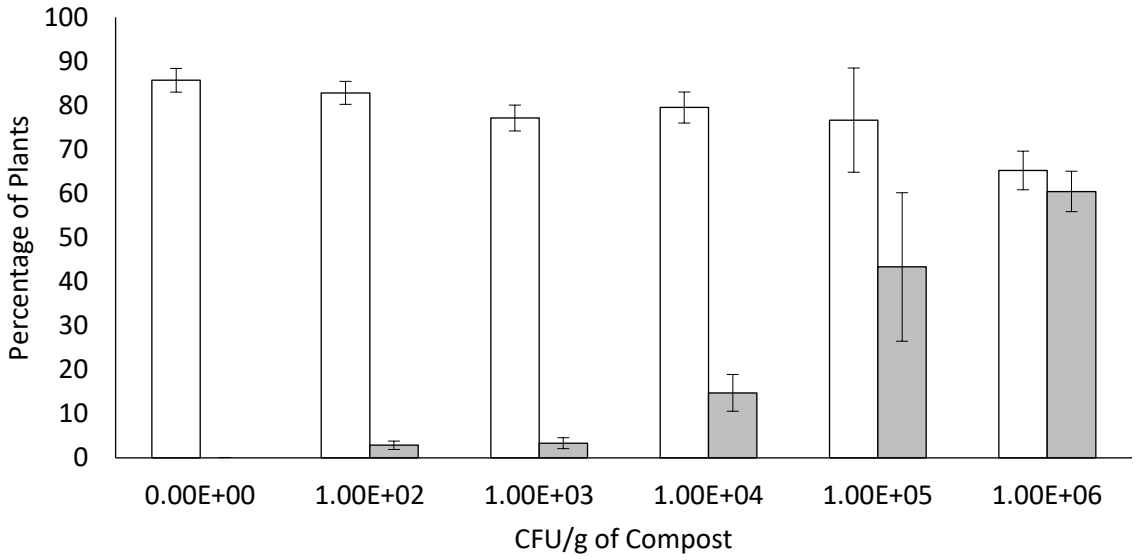

Figure 2.8. Effect of different FOC inoculum concentrations (CFU g^{-1} compost) on the percentage emergence (white bars) and damping-off (grey bars) of onion seedlings at 28 dpi. Error bars represent the standard error of the mean (s.e.m).

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 ingredients 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 examined under UK production systems and conditions. Consequently, the current work was undertaken to explore alternative active ingredients 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 (AWR005), moderate levels at the Ely site (AWR004) and low incidence at the Wellesbourne site (AWR006). 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 in particular. In comparison to year 1, disease levels were significantly lower at Wellesbourne (AWR006) compared with 2018 (AWR001), with only 0.69% infection in the untreated control compared with 53.7% in the previous year. This is perhaps linked to the high level of germination/infection seen within year 1, leading to depleted inoculum levels, or due to ploughing which may have buried previously built up inoculum. Consequently, the site will be re-inoculated in the spring of 2020 before trials are established, to ensure adequate inoculum is present.

No significant differences were observed between any of the products and the untreated controls in any of the trials. Whilst both trials AWR005 and AWR006 had very low AWR levels which would prevent identification of effective products, this was not the case for AWR004 where 38.2% of plants affected by AWR in the untreated plots. In this trial, no treatments were found to be effective, with banded applications of HDC F246, BAS 516 07 F and HDC F247 resulting in 39.3, 39.6 and 28.4% disease incidence respectively. This is surprising given that these same three treatments resulted in significantly reduced disease levels at Wellesbourne in 2018 (AWR001), and showed a similar trend at Ely 2018 (AWR002) where disease pressure was low. This lack of disease control may be due to the high levels of organic matter in this soil, a factor which is known to reduce efficacy of some pesticides. Similarly, when these products were applied to the whole bed a decrease in efficacy was seen compared to the banded applications. This is perhaps not unsurprising as the amount of active ingredient applied per given treatment area is less in the whole bed applications. The newer products HDC F272 (T1+T2) and HDC F246 applied pre-planting (T0) across the whole bed similarly also resulted in poor disease control. As observed in year 1, none of the biologicals were

effective in reducing disease which may have been due to application methods and timings, along with differences in optimal environmental conditions for individual products.

Objective 1b: Test fungicides and biological control agents for their effect on *Fusarium* basal rot

Good levels of FOC infection in bulb onions were attained in the inoculated area at Wellesbourne, although disease progression was slow at the start of the season, before increasing during warmer weather. At harvest, basal rot disease incidence had reached 59.2% in the untreated plots. Fungicides applied as banded applications were largely ineffective with the application of HDC F246 resulting in 54.6%, BAS 516 07F, 55.2% and HDC F247, 52.8% disease respectively. When applied as whole bed applications efficacy was reduced further. In comparison, HDC F273 showed a good level of disease control when used as a banded applications with just 31.4% disease incidence, but this was just outside the level of significance. This product was less effective as a whole bed application (44.0% disease) or when applied pre-planting (55.9%). None of the biologicals were effective in reducing disease incidence.

Few studies have examined the effects of soil applied fungicides or biological control agents against FOC in onion; however some products have been examined as seed/set treatments in other *Allium* spp. For example, Sintayehu *et al.* (2011) demonstrated that prochloraz (DMI, FRAC 3), tebuconazole (DMI, FRAC 3) and, a mixture of carbendazim (MBC, FRAC 1) and metalaxyl (PA, FRAC 4), applied as a set dip or dressing to shallot bulbs, provided up to 87% control in two years of field trials. Similarly, Naik and Burden (1981) found dipping onion sets in benomyl (MBC, FRAC 1) + mancozeb (FRAC M03) to be effective in reducing basal rot of onion by up to 77% in two years of field trials, whilst captan (FRAC M04) and thiram (FRAC M03) proved to be ineffective. Whilst providing a limited comparison, these studies support the performance of HDC F273 which like prochloraz and tebuconazole, is a demethylation inhibitor (DMI, FRAC3). Consequently, this product tended towards significant control when applied as a banded applications, and showed some effect as a whole bed application at seedling stages but not at pre-planting. Similarly, HDC F273 has also performed well in manufacturer trials when used in a similar manner as a banded applications. However, as this is not the intended on-label usage it is not clear if this product will be useful for growers where the on-label approval is for pre-planting applications across the whole bed. However, efficacy may be improved for this application method in a commercial situation where disease pressure is much lower than in our inoculated field area.

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

Petri dish germination assays

S. 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.

In year 1 a modification of an *in vitro* system developed as part of Defra project HH3230SFV (Defra, 2009), was validated and utilised to examine the effect of commercial liquid and granular products on germination of *S. cepivorum* sclerotia. Using this system, it was found that freshly-harvested (undried) sclerotia gave the highest and most consistent responsive to stimuli such as DAS or commercial products, as suggested in the literature (Coley-smith *et al*., 1987; Gerbrandy, 1992).

Previously in year 1, we reported the preliminary results from Experiment 4 whereby the concentration of the positive DAS control was reduced to elicit a better germination response of sclerotia and an additional product HDC F265 was included. Since then two experimental repeats have been achieved, and the results combined. As expected, the control treatments comprising conditioned and unconditioned sclerotia without DAS, did not respond within the experimental period (49 days). Reducing the concentration of DAS to 0.06% v/v, improved germination of the positive controls to 26.4 and 62.7% for isolates WRAR13 and GS1 respectively compared to 17.5% and 33.0% in Experiment 3 (2018) where the higher rate of 0.5% v/v was used. However, these were still below the expected >90% as seen in Experiment 1 and 2 (2018), suggesting that this concentration may be inhibitory. For example, Entwistle *et al.* (1982) showed that solutions of >20% DADS inhibited the germination of *S.*

cepivorum sclerotia compared to lower concentrations (0.156 – 10%) where germination was increased, as also reported by Somerville and Hall (1987) in their experiments with allyl sulphide (AS). Conversely DADs concentrations lower than 0.156% (Entwistle *et al.*, 1982) or 0.01% DADS (Coley-smith and Parfitt, 1986) resulted in little germination. Together these studies, and our results here, demonstrate the difficulty in optimising DAS concentration to elicit a high and consistent germination response.

Unusually, a higher than expected level of germination (55.9 and 48.5%) was seen in the unconditioned sclerotia exposed to DAS over the two experiments with 100 and 96.0% germination for the first and 11.9 and 1.0% for the second. It is not fully understood why this was, but it could be as a consequence of different ages of sclerotia when used. As observed in Experiment 3, the commercially formulated *Allium* products resulted in a strong and consistent germination response, but this varied between products. Product HDC F261 resulted in the strongest germination response with isolate GS1 reaching 100% germination by 21 dpi and isolate WRAR13 84.0% by 49 dpi. This was an improvement over Experiment 3, where <50% germination was achieved between the two isolates; and perhaps due to the use of a smaller container (1.75 L) compared to Experiment 3 (2.7 L). HDC F265 was also effective in stimulating germination but achieved this over a longer period, reaching 88.0 and 100.0% germination by 49 dpi. A similar formulation HDC F264 also showed the same trend, resulting in 85.2 and 71.6% germination by 49 dpi. This difference between HDC F264 and F265 is interesting given that they are both formulated to contain the same concentrations of polysulphides (45% w/w) and were applied at the same rate. However, the rate of polysulphide release from the carrier material is different, which may account for the improved efficacy of HDC F265. Similarly, HDC F264 resulted in >90% germination in Experiment 3, so again attributable to the change in system. Use of the food grade garlic granules resulted in inconsistent germination between isolates, with granule 1 resulting in 70.4 and 46.4% and granule 2 60.5 and 69.2% by 49 dpi. This is likely as consequence of the 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 likely also leads to a slow production of polysulphides. In contrast to all the other products tested, PK02 resulted in no germination of sclerotia from either *S. cepivorum* isolate and this is attributed to the high concentrations of polysulphides formulated in this experimental product which are inhibit germination.

Soil based germination assays

In Experiment 5, a selection of the *Allium* products used in the Petri dish system (Experiment 1-3, 2018 and Experiment 4, 2019) were examined using a soil-based system to better

replicate a field situation. Here, germination of *S. cepivorum* sclerotia cannot be observed directly but is associated with a low recovery of intact sclerotia. The number of recovered sclerotia in the untreated controls was somewhat lower than expected (53.3 and 58.7% for each isolate respectively) and this could possibly be due to microbial degradation or as a consequence of poor sclerotia formation in culture. However, the majority of these recovered sclerotia were viable. Similarly, a high number of sclerotia (40.0 and 53.3%) were recovered from the DAS treatment although fewer were viable. Product HDC F261, which had performed well in the Petri dish assays, resulted in a moderate level of recovery of sclerotia (12.0 and 36.7%) while both the Garlic Granule (5.3 and 4.7%) and product HDC F264 (4.0 and 10.0%) resulted in lower levels of recovery. This confirms the results of the Petri dish assays whereby HDC F264 proved to be effective in stimulating moderate to high levels of germination. Several other studies have shown that garlic granules applied at high rates (>100 kg ha^{-1}) can be as effective as treatment with DADS (Davis *et al*., 2007). The experimental product HDC F262 which has very high levels of polysuplhides resulted in a large proportion of sclerotia recovered (27.3 and 62.7%), few of these (2.7 and 2.7%) were viable. This confirms previous results in the Petri dish system where HDC F262 resulted in no germination and a reduction in viability.

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

Two batches of biofumigants were grown under glass within 2019, before being dried and milled to a fine powder. Glucosinolate concentration was then measured using HPLC, for single key glucosinolates known to be assiated with each plant species. For example, Sinigrin (2-propenyl glucosinolate) was measured for the varieties of *Brassica juncea* and *Brassica carinata*, Sinalbin (4-hydroxybenzyl glucosinolate) for those belonging to *Sinapsis alba*, Glucoerucin (4-methylthiobutyl glucosinolate) for *Eruca sativa*, and Glucoraphenin (4-methylsufinyl-3-butenyl glucosinolate) for *Raphanus sativus*.

The concentration of key GSLs varied considerably both between and within biofumigants species, but was comparatively low compared to other studies (Kirkegaard and Sarwar, 1998). For example, in their study the concentration of sinigrin in the shoots of *B. juncea* ranged from 0.1 – 18.7 µmol g^{-1}, and 10.0 – 20.02 µmol g^{-1} for *B. carinata*. Consequently, our results are at the lower end of this range for *B. juncea* (3.55-8.19 µmol g^{-1}), but considerably lower in *B. carinata* var. Cappuchino (3.43 and 7.18 µmol g^{-1}). This trend was also similarly seen with sinalbin being present at 6.75 and 5.51 µmol g^{-1} compared to 9.0-14.4 µmol g^{-1} in their study. Levels of glucoerucin in *E. sativa* and glucoraphanin in *R. sativus* were not measured in their study. Further biofumigant plants will be gorwn for the project in 2020.

Finally, the initial work to determine the effect of soil-applied FOC inoculum on onion seedling survival when applied at different concentrations has successfully identified levels (1 x 10^5 and 1 x 10^6 CFU g^{-1} that results in consistent damping-off disease symptoms. This bioassay can therefore be used a rapid means of determining the effect of different biofumigants on FOC inoculum in the final year of the project.

Conclusions

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

- No significant control of AWR disease was observed for fungicide or biological treatments in a field trial carried out at an inoculated site at Wellesbourne (Warwickshire) and two commercial field sites in Cambridgeshire and Lincolnshire. This was due to low disease levels at the first two sites (<1%), and whilst the third site attained moderate disease, there were no differences between treatments. This could be due to the high organic matter content of the soil at this site which may have compromised fungicide efficacy. These results are in contrast to those from 2018 where some fungicide products resulted in significant reduction of AWR disease at the Wellesbourne site.

Objective 1b: Test fungicides and biological control agents for their effect on *Fusarium* basal rot

- No significant level of FOC disease control was observed for fungicide or biological treatments in an inoculated field trial carried out at Wellesbourne (Warwickshire).

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.
- Commercially formulated *Allium* products generally resulted in high levels of sclerotial germination for products HDC F264, HDC F265 and HDC 261.
- Unformulated food grade garlic granule products resulted in moderate sclerotial germination.

- When examined in a soil-based system, HDC F264 resulted in the lowest rate of sclerotia survival (<1%) due to stimulation of sclerotial germination, along with an unformulated garlic granules (<2%). Other products were also stimulatory but were more variable in their efficacy

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

- Two batches of plant biofumigant material was produced in the glasshouse for experimental work. However, analysis suggested that the levels of key glucosinolates were low. This material will be used in future experiments, but further biofumigant plants are being produced.
- A seedling based assay for FOC established that 1 x10^6 cfu/g of compost would result in consistent high levels of onion seedling mortality, providing a suitable system to examine the effect of plant biofumigants on the pathogen in future experiments.

Knowledge and Technology Transfer

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

Warwick Food GRP event, University of Warwick, 6th February 2020.

HIR Skane - Lökonferense (Onion Conference), Skane, Sweden, 11-12th February 2020.

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Appendices

Appendix A. Detailed description of treatments, applications and conditions

Table A1. 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	-

Appendix B. Trial designs for the three individual sites.

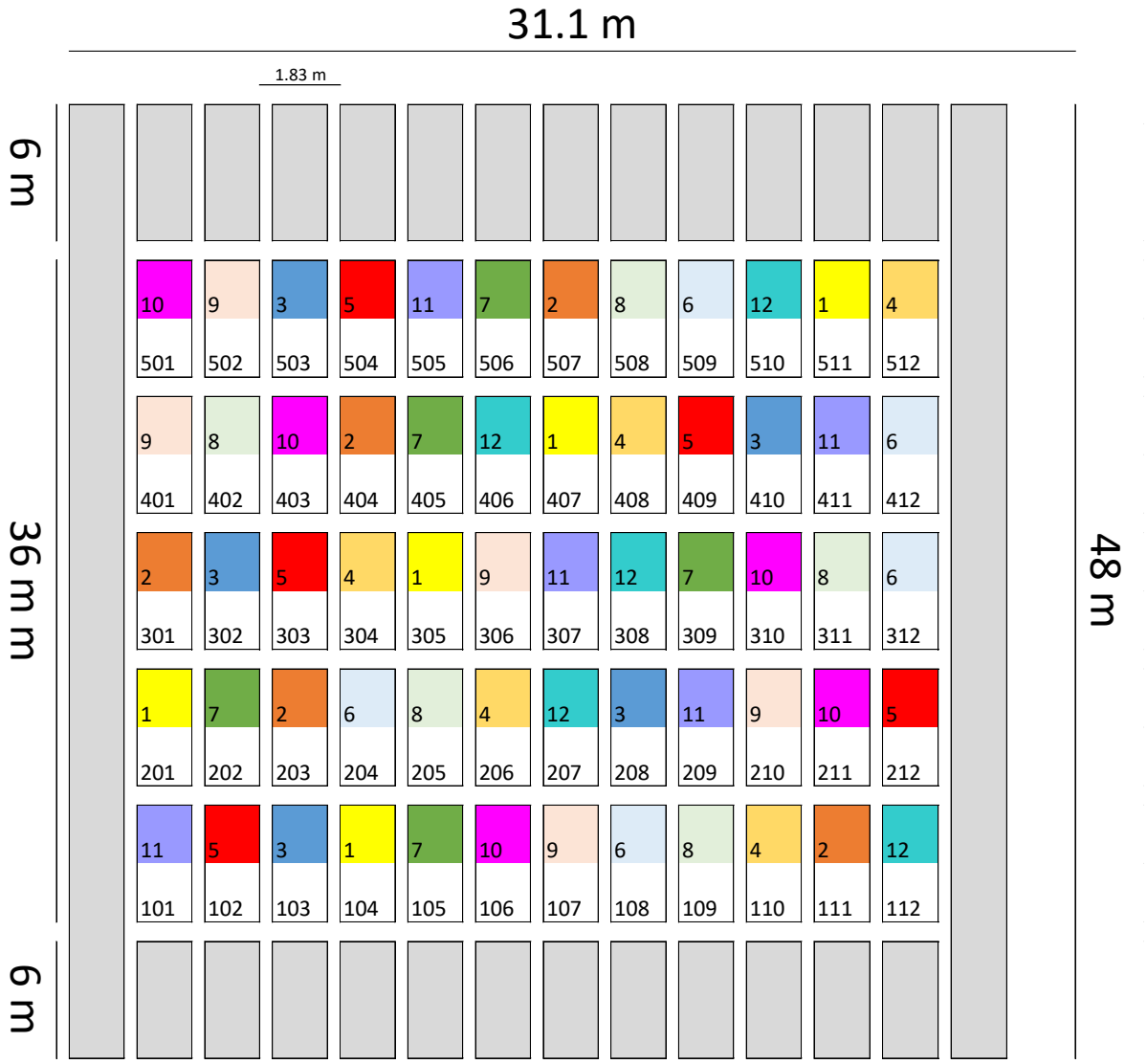

Figure B1.0. Wellesbourne (AWR006/19) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

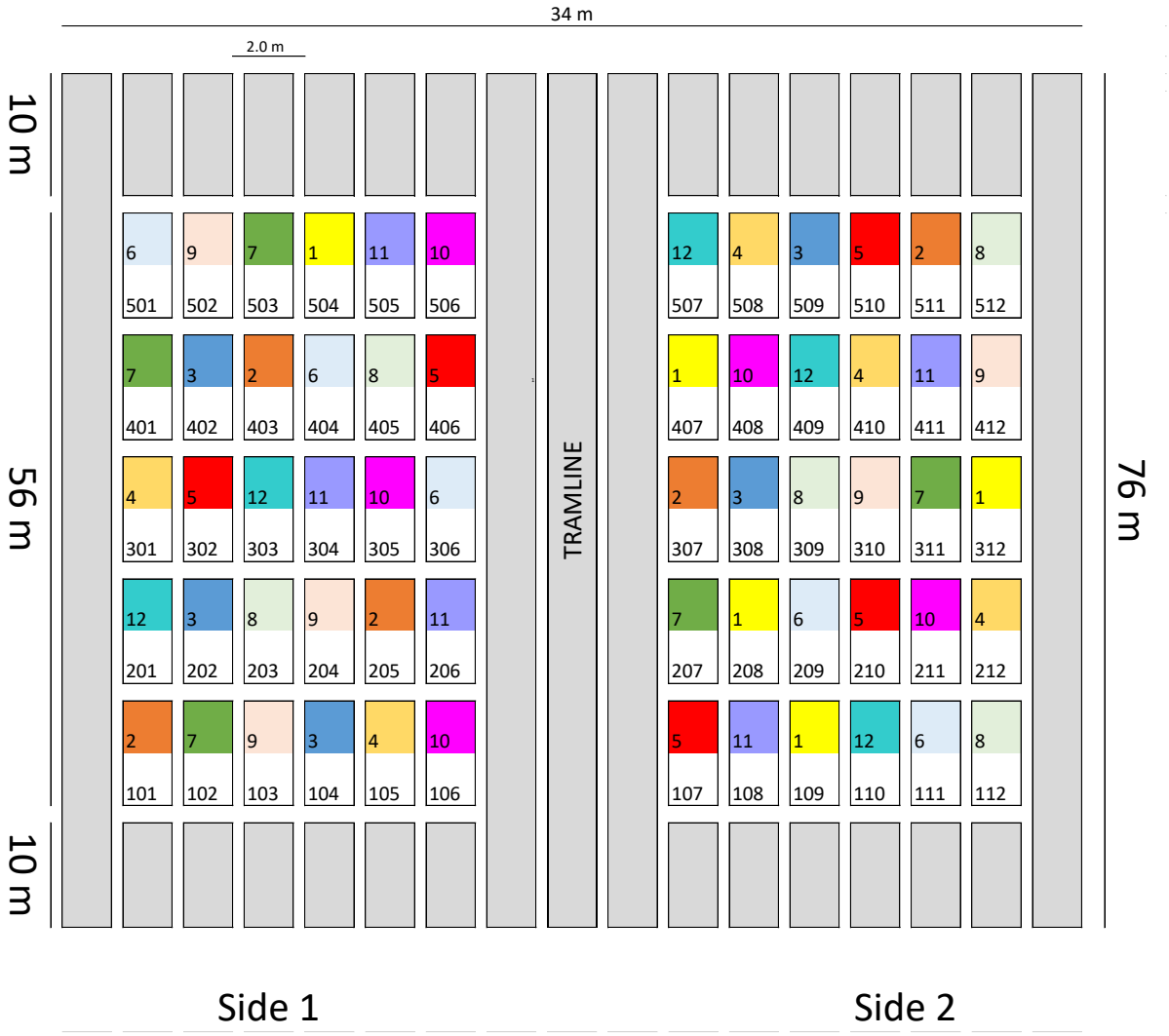

Figure B1.1. Cambridgeshire (AWR004/19) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

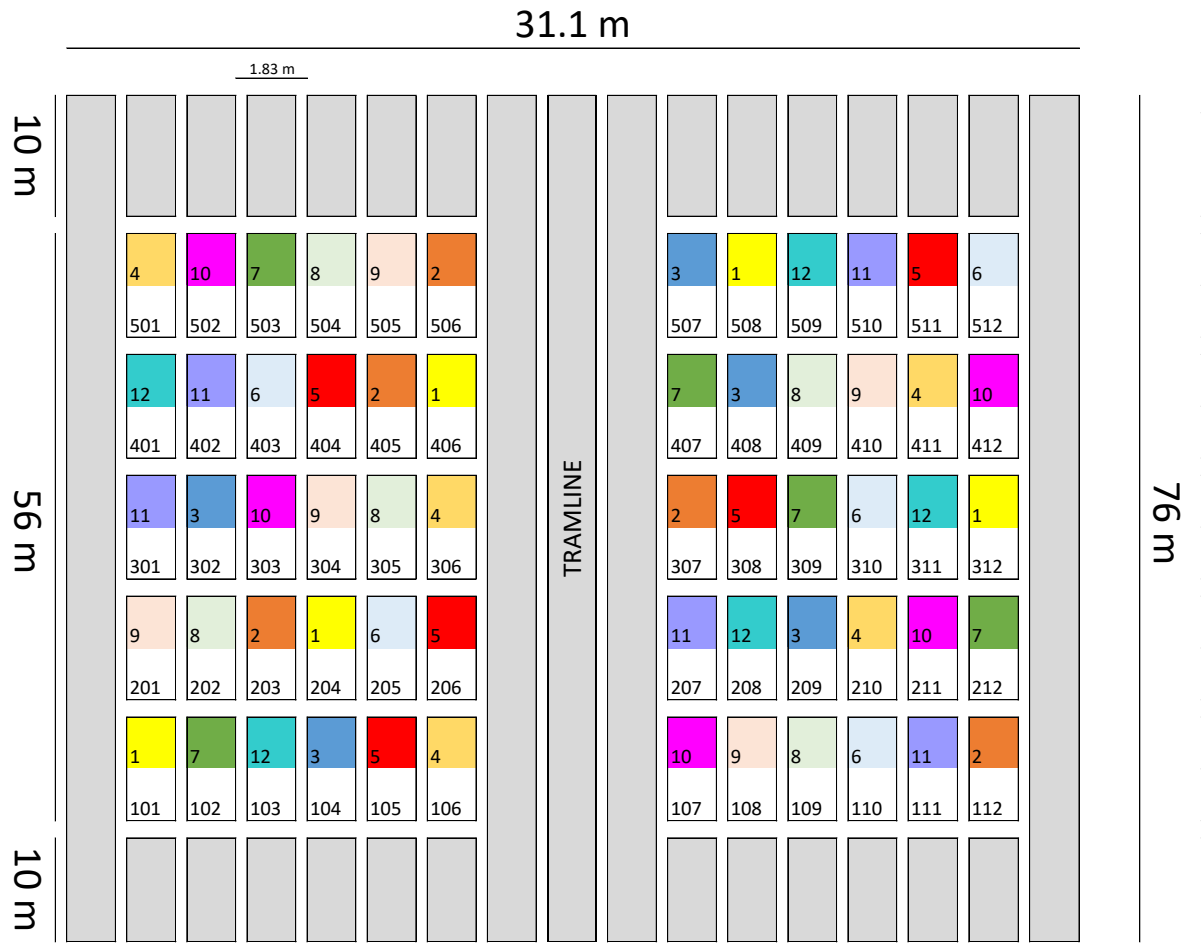

Figure B1.2. Lincolnshire (AWR005/19) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot.

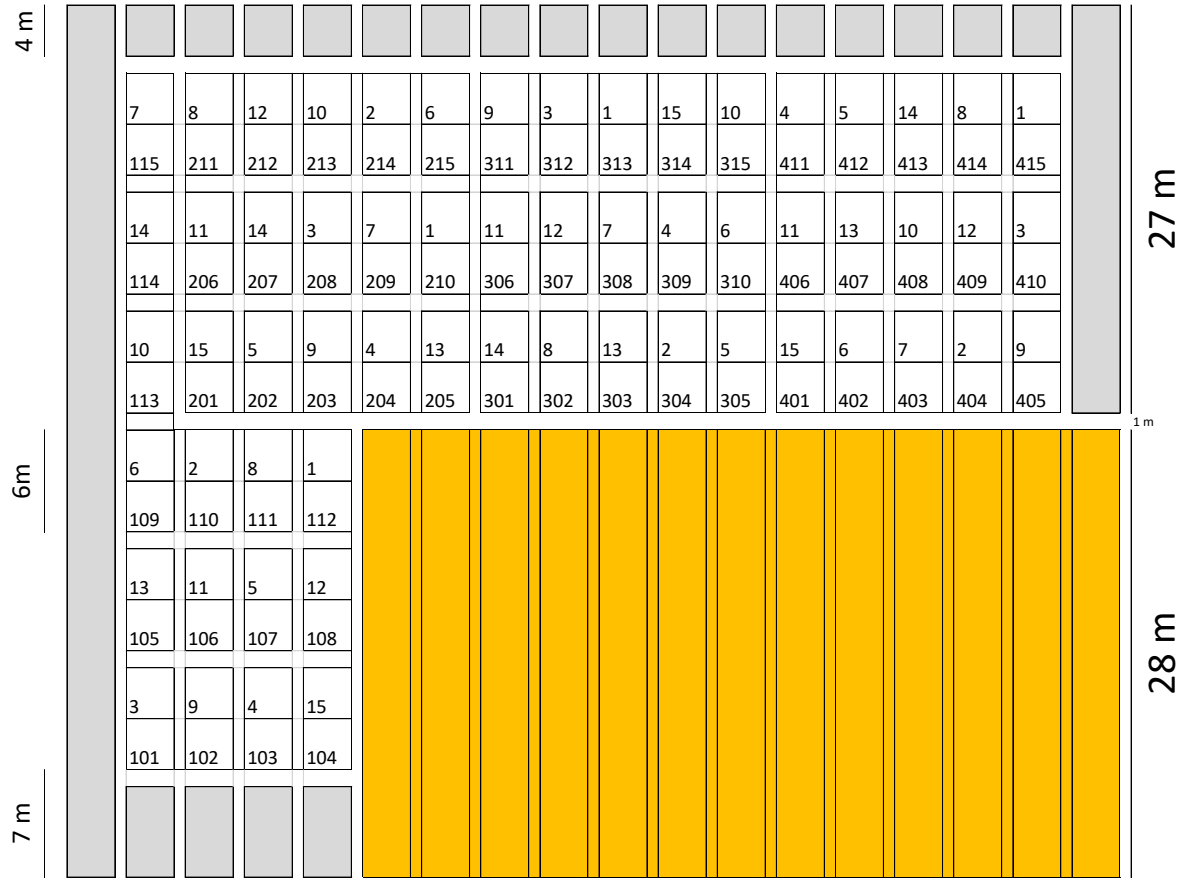

Figure B1.3. Wellesbourne Fusarium (FOC) trial layout with randomisation plan. Plot numbers are indicated on the bottom of each plot, whilst treatment numbers are at the top of each plot

