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Project leader:	John Clarkson, University of Warwick
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Location of project:	University of Warwick, Wellesbourne Campus, Warwick.
Industry Representative:	Phil Langley, G's Growers
Date project commenced:	01/03/2018
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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

Experiments showed that commercial garlic products stimulated germination of *Sclerotium cepivorum* sclerotia *in vitro* while one also reduced white rot disease in salad onions in the field. The combined used of such germination stimulants with selected fungicides which also reduced disease shows promise as an integrated strategy for white rot disease.

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 *S. cepivorum* 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. Currently, 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 fungicides 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 the use of sclerotial germination stimulants (Coventry et al., 2006; Coley-Smith *et al*., 1986) 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. Two objectives were carried out in the current year (which was highly disrupted due to the Covid-19 pandemic and a member of staff leaving):

- Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia.
- Objective 4: Test combined treatments for their effect on white rot disease development

Summary

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

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. Garlic-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 with a particular focus on garlic oils and their constituent chemical compounds such as diallyl disulphide (DADS) or diallyl sulphide (DAS). Consequently, the current objective explored the development of *in vitro* assays to identify commercially available garlic products that could stimulate sclerotia germination.

Petri dish germination assays

A repeat experiment was carried out to determine the effects of different commercially available garlic products on germination of *S. cepivorum* sclerotia and results were generally consistent with those observed previously. Three NEMguard products (SC, DE, PCN) resulted in very high levels of germination (>86%) while use of the food grade garlic granules was less consistent. Germination of *S. cepivorum* sclerotia in response to DAS (used as a positive control treatment) was poor and has been less consistent throughout such experiments. This could be due to the more volatile nature of this compound resulting in

inconsistent levels over the experiments or perhaps a degradation of the compound while being stored over the duration of the project so far. Nonetheless, the results from these assays clearly indicate the potential of formulated garlic products to successfully stimulate germination of *S. cepivorum* sclerotia.

Soil based germination assays

A selection of the garlic products used in the Petri dish assays were examined for their effect on *S. cepivorum* sclerotia using a soil-based system under controlled temperature conditions to better replicate a field situation. Here, germination of sclerotia cannot be observed directly but is associated with a low recovery of intact sclerotia. Overall, results from two combined experiments were less clear than observed in the Petri-dish assays although both food grade garlic granules and NEMguard DE resulted in increased germination (non-recovery, >60%) of *S. cepivorum* sclerotia compared to the untreated control (<30% germination).

Field based germination assay

An extra small field experiment at Wellesbourne assessing selected garlic products for their effect on buried *S. cepivorum* sclerotia (in the absence of a crop) showed that NEMguard SC and NEMguard DE increased apparent germination (as measured by non-recovery) compared to an untreated control (germination 50-60%). Garlic granules had much lower levels of germination in this experiment compared to the soil box assay. As the viability of the sclerotia recovered was high for all treatments, this suggests that the products were not as effective in the field as they are in a controlled enviroment. This emphasises the need to perhaps increase doses of these products or attempt sealing of the soil surface by rolling.

Objective 4: Test combined treatments for their effect on white rot disease development in the field

Two field trials tested combinations of garlic products with fungicides and biological control agents in comparison with individual treatments for their effect on AWR disease in salad onions. Across both sites, NEMguard SC either alone or in combination with Signum or Trisoil significantly reduced white rot disease while the use of NEMguard DC either alone or in combination was less effective. The fungicides Perseus and Luna Privilege reduced white rot significantly at one site and decreased disease at the other. Their different modes of action and application timings means that they would be good candidates for inclusion in an integrated control strategy for AWR disease alongside NEMguard SC.

Conclusions

- *S. cepivorum* sclerotia were stimulated to germinate by commercial garlic products in *in vitro* Petri dish assays
- The germination stimulant effect was less clear when garlic products were tested in soil boxes under controlled conditions and in a small field trial
- NEMguard SC alone or in combination with other crop protection products reduced AWR disease in two field trials.
- The fungicides Perseus and Luna Privilege also reduced AWR disease and may therefore be additional useful components in an integrated control strategy.

Financial Benefits

None to report at this time.

Action Points

None to report at this time.

SCIENCE SECTION

Introduction

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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 the use of sclerotial germination stimulants (Coventry et al., 2006; Coley-Smith et al., 1986) 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.

Aims

To identify and test a range of treatments for the integrated control of AWR in bulb and salad onions.

Objectives for current year

Two objectives were carried out in the current year (which was highly disrupted due to the Covid-19 pandemic and a member of staff leaving).

- Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia
- Objective 4: Test combined treatments for their effect on white rot disease development in the field

Materials and methods

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

The work conducted in year 3 was interrupted by the COVID-19 outbreak, but comprised of experimental repeats to determine the effect of six garlic products on stimulating germination of *S. cepivorum* sclerotia using Petri dish and soil-based assays developed in year 2. In addition, a field experiment not originally included in the milestones was also included to test the effect of selected garlic products on germination of sclerotia under field conditions.

Production and conditioning of *S. cepivorum* sclerotia

As described previously (FV 449a annual report, 2020), *S. cepivorum* sclerotia were produced in conical flasks by inoculating a mixture of silica sand and cornmeal with mycelial agar plugs of *S. cepivorum*. Flasks were set up for two isolates (GS1 and WRAR13) and stored at room temperature in the dark for eight weeks. After this period, sclerotia from one flask for each isolate were harvested for conditioning by flotation with tap water. After a brief settling period, the very top part of the supernatant containing cornmeal residue and any empty/immature sclerotia was discarded after which the remaining sclerotia were captured on a 212 µm sieve. Sclerotia were then rinsed in water, dispensed into nylon bags (50 x 100 mm) and buried in 400 g of air-dried non-sterile field soil (silty clay loam, Dunnington Heath Series) in small plastic containers (100 x 120 x 65 mm; HotFormBoxes). Sterile distilled water (SDW) was then added to obtain a moisture content of 22% (w/w). The containers were stored 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 slight meniscus on the surface. 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. Fifty conditioned sclerotia from two isolates of *S. cepivorum* (GS1 and WRAR13) were placed on the nylon mesh in a 5 x 10 grid pattern, ensuring 5-10 mm between any two sclerotia (two Petri dishes per isolate per treatment). Garlic 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 weighed out and spread evenly across the vermiculite and nylon mesh at a rate of 13 mg (equivalent to 20 kg/ha) or 64 mg (equivalent to 100 kg/ha). 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. The six treatments comprised of four commercial and one experimental (PK02) garlic products (Ecospray, UK) and food grade garlic granules / powder (Barnes Williams, Cheltenham, UK). Diallyl disulphide (DAS; Sigma Aldrich, Poole, UK) dissolved in 2% Triton X was included as a positive control and the untreated control comprised 2% Triton X only; Table 1).

Table 1. Allium products tested for effect on germination of *S. cepivorum* sclerotia in Petri dish assay.

Treatment	**Application rate**
NEMguard SC	6% v/v
PK02	6% v/v
NEMguard PCN	20 kg/ha
NEMguard DE	20 kg/ha
Garlic Granule	100 kg/ha
Garlic Powder	100 kg/ha
Control untreated (2% Triton X)	-
Control untreated (unconditioned) (2% Triton X)	-
Control treated (DAS in 2% Triton X)	0.06% v/v
Control treated DAS (unconditioned) (DAS in 2% Triton X)	0.06% v/v

Sclerotia used in assays were conditioned except where indicated in control treatments

Germination of *S. cepivorum* sclerotia (mycelial or eruptive; Fig. 1) was assessed twice weekly, with any germinated / contaminated sclerotia then removed from the Petri dishes. The experiment was conducted over a period of 7 weeks after which any remaining (non-

germinated) sclerotia were assessed for viability by first squeezing with forceps to assess integrity (with those that collapsed being discarded). Those that remained intact were surface sterilised in 70% ethanol (v/v) for 1 min and washed twice in sterile distilled water for 30 s. 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 PDA agar cores arranged on in a Petri dish. Plates were sealed and incubated in the dark at 20°C and after 7 days, each sclerotium was examined for the production of mycelium and immature sclerotia (Fig. 2).

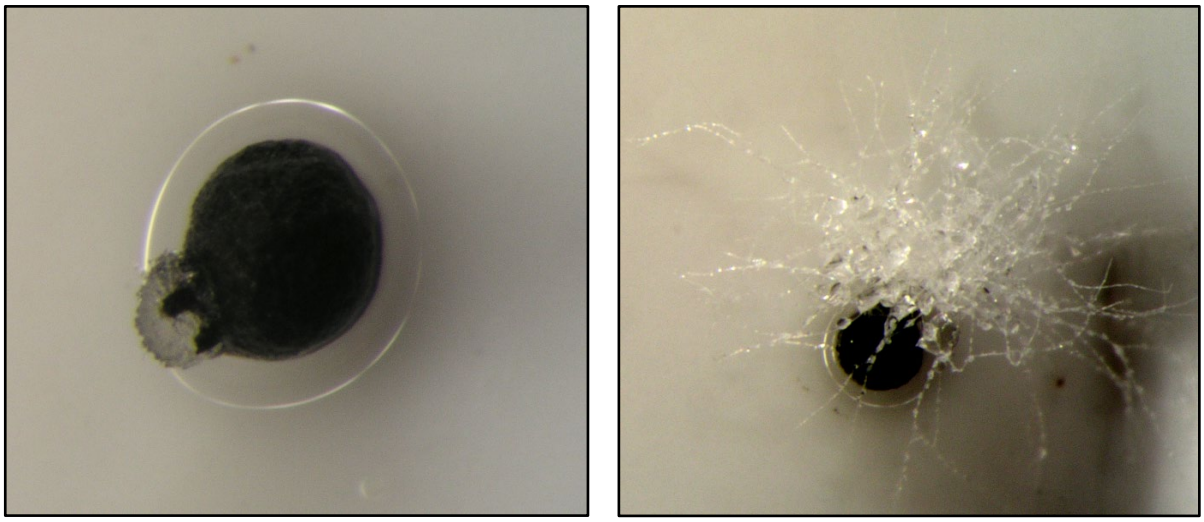

Figure 1. Germination of *S. cepivorum* sclerotium in response to DAS in vitro. Left, eruptive germination; right, mycelial growth.

Figure 2. 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 with *S. cepivorum* sclerotia

A soil-based assay was set up to build upon the results of the Petri dish system and provide a more realistic test for assessing the garlic product germination stimulants. This was a repeat of the same experiment carried out in 2019. Fifty conditioned *S. cepivorum* sclerotia were placed into a nylon bag (50 x 50 mm) which was then 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, Soakwaters Field, Wellesbourne), 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). The garlic product treatments were then incorporated into the soil at the specified rates (Table 2). Liquid products were added along with the SDW directly to both layers of soil, whilst the granular products were weighed before mixing into the soil in the individual containers. Individual containers were used for each of the two *S. cepivorum* isolates (GS1 and WRAR13), 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.

Table 2 Allium products tested for effects on germination of *S. cepivorum* sclerotia soil based assay

Treatment	**Application rate**
NEMguard SC	84 mL of 6% v/v
PK02	40 mL of 6% v/v
NEMguard DE	60 kg/ha (0.017 g)
Garlic Granule	100 kg/ha (0.084 g)
Control untreated (2% Triton X)	30 mL
Control treated (DAS in 2% Triton X)	84 mL of 0.06% v/v

Unlike the Petri dish system, no direct observation of *S. cepivorum* sclerotial germination is possible in this assay. However, if sclerotia germinate during the treatment period, only rind fragments should 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 and were gently rubbed on a sieve to break up any degraded sclerotia. Remaining sclerotia were then squeezed with forceps to make sure further degraded sclerotia were removed, with the remaining solid sclerotia being counted and placed in a 1.5 mL tube. These were then surface sterilised in 1 mL of 70% ethanol (v/v) for 1 min, then 1 mL of 1% sodium hypochlorite and finally washed three times in 1 mL of SDW. The sclerotia were resuspended in ~500 µL SDW and pipetted onto sterile filter paper in a sterile petri dish before being squashed as before and plated onto PDA cores as above. Viability was assessed as before (Fig. 2) after 7 days at 20˚C in the dark.

Field evaluation of germination stimulants of *S. cepivorum* sclerotia

In an additional experiment not originally included in the milestones for this objective, selected garlic products were tested for their effectiveness in inducing germination of *S. cepivorum* sclerotia buried in soil in the field. Thirty conditioned sclerotia (isolate GS1) were placed into a nylon bag (50 x 50 mm) which was then heat sealed. Plots (2 x 1.83 m) were marked out in the Quarantine Field at Warwick Crop Centre, Wellesbourne, in a 5 x 5 grid, with a 0.5 m space between each plot. Treatments consisted of four garlic products and an untreated control (Table 3) and these were applied by raking each into the soil down to a depth of 5 cm, with five replicate plots receiving each treatment. Immediately after product application, three bags of *S. cepivorum* sclerotia were buried per plot at an approximate soil depth of 5 cm (total of 15 bags per treatment). Bags remained in the soil for approximately 10 weeks between October to early December 2020, after which they were collected and washed to remove most of the excess soil. Bags were washed again to remove remaining soil and gently rubbed over a sieve to break apart germinated sclerotia. Intact sclerotia were individually squeezed with fine forceps as before and any further degraded sclerotia removed. The remaining sclerotia were added to a 1.5 mL tube before being surface sterilised in 1 mL of 1% Domestos bleach (4-5% available sodium hypochlorite) for 1 min. Sclerotia were washed four times and then resuspended in ~500 µL SDW. As before, they were placed on sterile filter paper and individually squashed before placing on cores of PDA, amended with chlortetracycline (20 mg L^{-1}). Viability was assessed as for the Petri dish and soil box assays.

Table 3. Garlic products tested for effect on germination of *S. cepivorum* sclerotia in the field

Product	**Dose (L or Kg* /ha)**	**Dose per Plot (2 x 1.8 m)**
NEMguard DE	20*	7.2 g
NEMguard SC	60	21.6 mL in 0.36 L Water
NEMguard PCN	60*	21.6 g
Garlic Granule	100*	36 g
Untreated	-	-

Objective 4: Test combined treatments for their effect on white rot disease development in the field

Field trial site selection

Two field trials were set up at field sites in Warwickshire (Stareton) and Cambridgeshire (Ely) to test the efficacy of different fungicides, the biological control agent Trisoil and NEMguard DE and SC alone or in combinations. The Ely site had developed white rot in the 2019 field trial while a commercial salad onion crop grown at the Stareton site had also shown disease symptoms in 2019.

Site and crop management

All cultivations, drilling and subsequent crop management were conducted by the host grower in accordance with commercial practice. 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. At Ely, untreated seed of *Allium fistulosum* cv. Totem was used in four double rows per bed, while at Stareton, *Allium fistulosum* cv. Yoda treated with Force (an insecticidal seed treatment), was used.

Treatments and application

Treatments were informed by results from previous field experiments in 2018 / 2019 and germination stimulant experiments. These consisted of the fungicides Signum (boscalid and pyraclostrobin; 1.5 kg ha^{-1}), Perseus (difenoconazole and fluxapyroxad; 2 L ha^{-1}), the biological control agent Trisoil (*Trichoderma atroviride* I-1237; 5 kg ha^{-1}) and the garlic products NEMguard DE (60 kg ha^{-1}; formulated as microgranules with 45% garlic extract) and NEMguard SE (6% v/v; suspension concentrate with 99.9% garlic extract) individually and in selected combinations, timings and application methods (Table 4). 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 1000 L ha^{-1} applied as either a concentrated banded spray 0.10 – 0.15 m width centred on each drill row 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]) across all sites for consistency. Treatments involving germination stimulants were applied 5 weeks prior to drilling as part of the initial bed forming [T-1] and were incorporated to 10 cm using the bed former. Selected treatments were also applied just prior to drilling [T0] and incorporated to 10 cm by hand raking. All application rates were based on manufacturer recommendations.

Table 4. Treatments and timings tested for AWR control in field trials at Ely and Stareton

Treat no.	T-1 bed form	T0 pre-drill	T1 emerge	T2 3-4 leaves	Application method
1			Signum	Signum	Banded
2			Signum	Signum	Whole Bed
3			Perseus	Perseus	Whole Bed
4		Luna Privilege			Whole Bed
5	NEMguard DE				Whole Bed
6	NEMguard DE		Signum	Signum	Whole Bed
7	NEMguard DE	Trisoil	Trisoil		Whole Bed + Banded
8	NEMguard SC				Whole Bed
9	NEMguard SC		Signum	Signum	Whole Bed
10	NEMguard SC	Trisoil	Trisoil		Whole Bed + Banded
11		Trisoil	Trisoil		Banded
12	Untreated				

Trial design

The trials comprised of a randomised block design of 5 blocks with 12 plots each. Treatment allocation to plots was conducted separately for each site. An additional blocking factor (side) was added to compensate for splitting the trial vertically either side of the central sprayer wheel markings, cutting through the blocks. Individual plot sizes varied between sites comprising of; 1.83 x 8 m at Ely and 1.83 x 10 m at Stareton. 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.

Plant establishment and 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. Scoring was carried out before application at T1, and 2-3 weeks post T1 and T2 application. Establishment was scored using an index of 0 to 5, where 5 was 80% to 100% establishment, and both vigour and leaf colour were assessed using a 0 to 3 index with 3 being normal.

AWR disease assessments

AWR disease incidence was assessed in the central two rows within 4 x 0.5 m pre-assigned lengths within each plot. 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. Markers were placed beside symptomatic plants and any new symptomatic plants added to the tally at the next assessment. 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 Stareton 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 plants showing foliar symptoms (chlorosis, necrosis and wilting). Consequently, the number of symptomatic plants was assessed monthly from the first treatment application (T1). Plant death was also recorded, and the plant removed in order to conform presence of white mycelium and/or sclerotia, after which the plant was recorded as white rot positive or negative. Plant population counts were conducted at the first assessment timing and subsequently used to calculate white rot incidence as a proportion of the established population.

Salad onion plants from the trial sites were harvested at a time beyond normal commercial practice to allow disease symptoms to develop further, being completed in early November 2020 at both sites. AWR disease 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 AWR disease, 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. Plant population counts were conducted at the first assessment timing and subsequently used to calculate white rot incidence as a proportion of the established population. At Stareton, to increase the sample size, the central 5 m of the middle two rows were harvested, rather than the 1 m row lengths.

Statistical analysis

The proportion of plants with AWR disease was calculated for each treatment and analysed by ANOVA as well as a generalised linear model (GLM) assuming a binomial distribution and logit link function (Genstat). The fitted terms were constant, block and treatment. Analysis was carried out on the AWR foliar symptom data collected and also the root symptom data at harvest (4 x 0.5 m row lengths at the Ely site and the two 5 m row lengths harvested as Stareton). All data were expressed as per metre of row to facilitate comparison between sites. In a further analysis “side” was included as a covariate at the Stareton site where treatments were not equally divided either side of the central wheelings. Duncan’s Multiple Range Tests were carried out to highlight significantly different treatments.

Results

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

Petri dish germination assays

As with previous experiments, all of the garlic product treatments with the exception of PK02 resulted in increased germination of the conditioned *S. cepivorum* sclerotia when compared to the untreated (conditioned and unconditioned, no DAS) controls (Fig. 3). NEMguard SC resulted in the most rapid response with 100% germination reached by 20 days post treatment for isolate WRAR13. NEMguard PCN and NEMguard DE resulted in similar final levels of germination for WRAR13, with 90% and 86% germination respectively, 50 days after application of treatment. Germination of GS1 sclerotia was slower for all treatments, usually resulting in lower levels than WRAR13, except for the NEMguard DE treatment. These data will be added to the data from two previous experiments and statistical analyses carried out for the final report. Viability of sclerotia at the end of the experiment was variable across the two Petri dishes for most treatments due to low and variable numbers of sclerotia remaining ungerminated at the end of the experiment (Fig. 4). Notably however, viability was low (<40%) for treatment PK02 where the majority of the sclerotia remained ungerminated as noted in previous experiments. There was also contamination of some of the control treatments resulting in low viability.

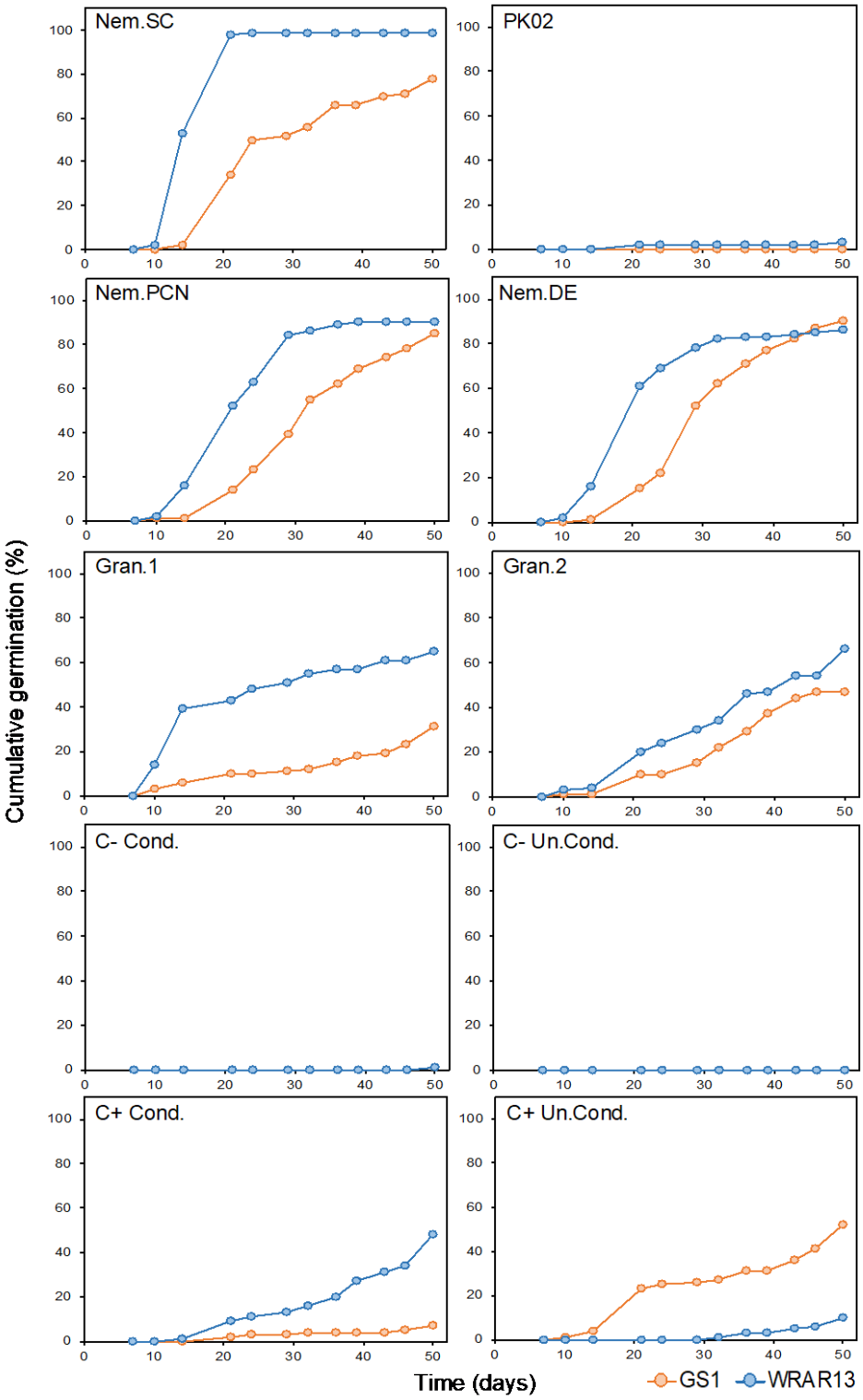

Figure 3. Effect of garlic products on the mean cumulative germination of conditioned *S. cepivorum* sclerotia (isolates GS1 and WRAR13). C-, treated with 2% Triton X only, C+, treated with diallyl sulphide (DAS; 0.06% v/v).

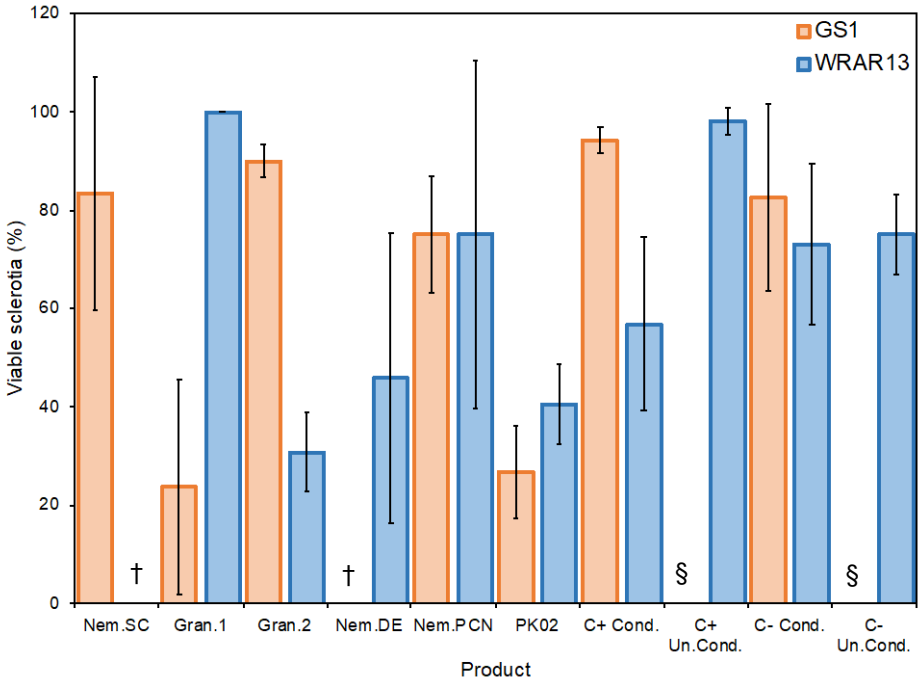

Figure 4. Viability of remaining ungerminated *S. cepivorum* sclerotia for isolates GS1 and WRAR13 following treatment with different garlic products. Error bars represent standard deviation across the two petri dishes per treatment. † No remaining sclerotia to test, § all remaining sclerotia contaminated therefore did not germinate.

Soil based germination assays for *S. cepivorum* sclerotia

The results from the two experiments carried out to determine the ability of different garlic products to stimulate germination of *S. cepivorum* sclerotia in the soil box assay under controlled conditions were combined. Although there was some variability across treatments and experiments, the garlic granules and NEMguard DE resulted in the highest levels of germinated sclerotia as measured by non-recovery (75-79% and 61-64% of sclerotia across the two *S. cepivorum* isolates respectively; Fig. 5). As for the Petri dish assay, PK02 resulted in a higher number of sclerotia recovered and the greatest number of non-viable sclerotia especially for isolate WRAR13. However, apparent germination for PK02 was still much higher (50% for isolate GS1 and 28% for WRAR13) compared with the results from the Petri-dish assays. The DAS control treatment did not appear to stimulate germination compared to the untreated control. Overall however, all treatments resulted in a reduction in viable *S. cepivorum* sclerotia compared to the untreated control.

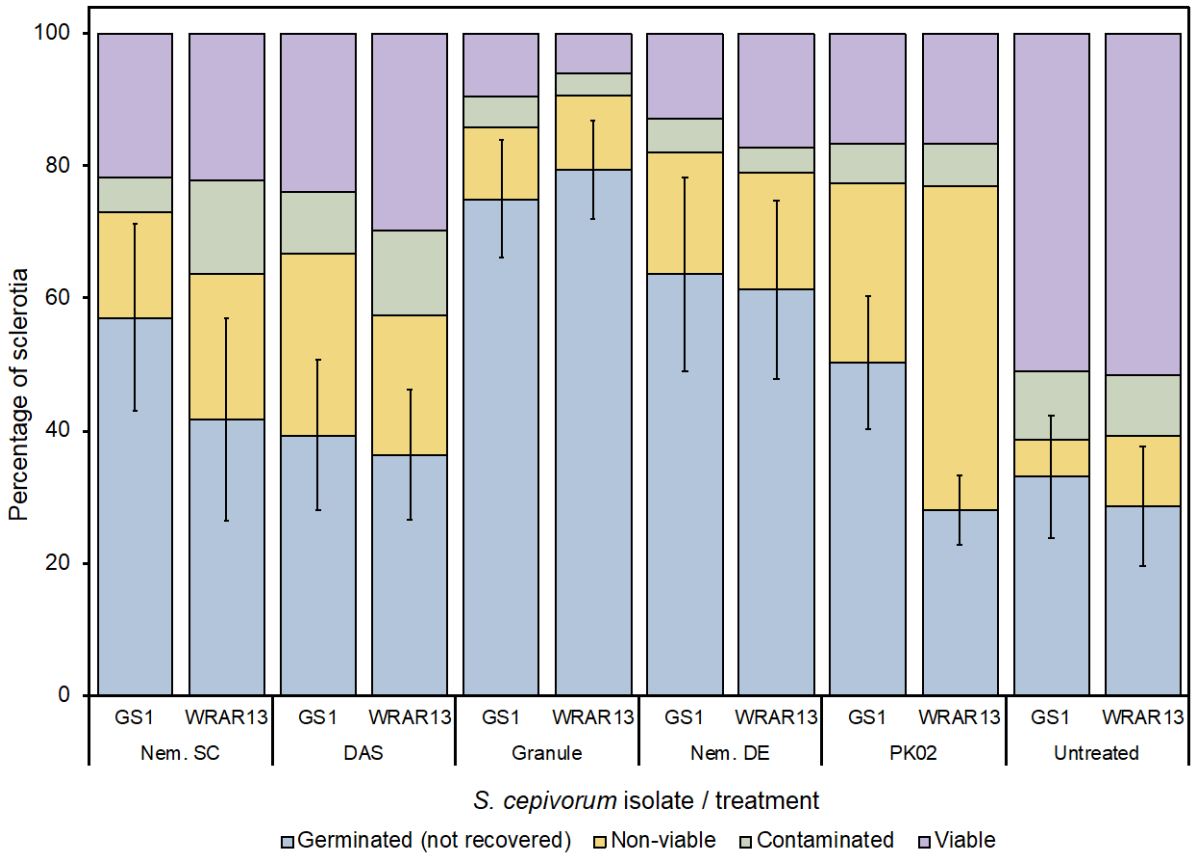

Figure 5. Effect of garlic products on the germination of *S. cepivorum* sclerotia (as measured by non-recovery, blue bars) in a soil box assay. Results of viability tests on remaining recovered sclerotia are indicated as non-viable (yellow), contaminated (green) or viable. Data from two combined experiments with error bars representing SEM of a total of six replicate soil boxes per treatment each containing 50 sclerotia.

Field evaluation of germination stimulants for *S. cepivorum* sclerotia

In the field experiment, apparent germination (as measured by non-recovery) of *S. cepivorum* sclerotia for the untreated control was unexpectedly high (42%) and only NEMguard SC and NEMguard PCN resulted in greater germination (57% and 51% respectively; Fig. 6) NEMguard SC resulted in the same level of germination in both the field and soil box assays (57% of sclerotia germinating). However, NEMguard DE performed considerably better in the soil box assay, resulting in 64% germination of sclerotia, compared to only 43% in the field (Figs. 5 and 6). The garlic granules resulted in 30% less germination in the field compared to when used in the soil box assay, a greater difference than observed for all other products. The viability of the intact (non-germinated) sclerotia remaining at the end of the experiment was high across all treatments.

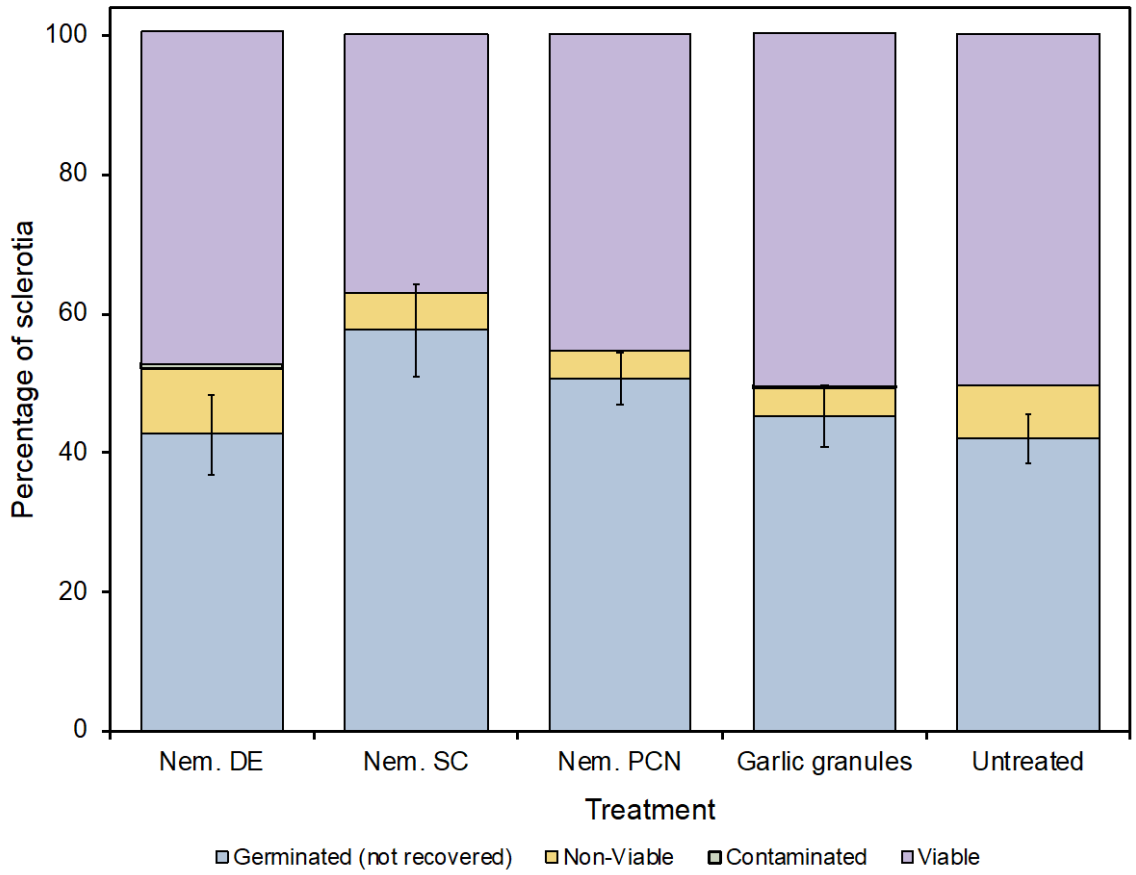

Figure 6. Effect of garlic products on the germination of *S. cepivorum* sclerotia (isolate GS1) in the field (as measured by non-recovery, blue bars). Results of viability tests on remaining recovered sclerotia are indicated as non-viable (yellow), contaminated (green) or viable. Error bars represent SEM of the five replicate plots per treatment each containing three bags of 30 sclerotia.

Objective 4: Test combined treatments for their effect on white rot disease development

Phytotoxicity and establishment

No phytotoxic effects were observed from any of the products tested at either of the field sites post T1 or T2 applications (data not shown).

Foliar assessments of AWR

There was little apparent AWR disease development as measured by leaf chlorosis or plant death at either of the two field trial sites (Table 5). At Ely, analysis was carried out for the last disease assessment on 07/10/20, while at Stareton, all treatments began to exhibit chlorosis as the crop developed beyond maturity (16/10/20, 29/10/20), and hence data from an assessment on 01/10/20 was selected. At these assessment times, the incidence of foliar symptoms consistent with AWR in untreated plants with was 1.9% at the Ely site (07/10/20) and 2.0% at Stareton (01/10/20). Following statistical analysis, none of the treatments at either site significantly reduced white rot symptoms compared to the untreated control, due to the apparent low disease levels. The additional covariate analysis for the Stareton data again resulted in no significant differences between treatments.

Harvest assessments of AWR

At both field trial sites, clear symptoms of AWR disease were observed on roots at harvest and incidence at Ely and Stareton was 11% and 28% plant infected in the untreated controls respectively (Fig. 7; Table 6). This suggested that the previous foliar assessments were not a good indication of AWR disease levels, perhaps because *A. fistulosum* salad onion varieties are very robust and do not exhibit above-ground symptoms when the plants are large, and infection occurs late in the year.

At the Ely site, 11% of plants in the untreated control had visible AWR symptoms on roots at harvest and all three treatments which included NEMguard SC either alone or in combination with Signum or Trisoil (Treatments 8, 9 and 10) resulted in a significant reduction in disease with less than 4% plants affected ($P<0.001$; Table 6). However, none of the other treatments resulted in significant reductions in AWR.

At Stareton, disease pressure was higher with 28% of plants in the untreated control having visible AWR symptoms on roots. Here, NEMguard SC in combination with Signum (Treatment 9) was the best effective treatment, significantly reducing disease to only 3.7% ($P<0.01$) in the analysis without the co-variate (Table 6). Disease levels for the other NEMguard SC treatments either alone or with Trisoil (Treatments 8 and 10) also reduced disease to approx.

12% plants affected but this was just outside the level of significance. However, when the covariate was introduced into the analysis, all NEMguard SC treatments significantly reduced AWR disease compared to the untreated control. Treatments with Perseus, Luna Privilege and NEMguard DC with Signum (Treatments 3,4 and 6) also significantly reduced AWR to 6.3, 5.9 and 10.5% respectively and this was also confirmed in the covariate analysis.

Overall therefore, treatments with NEMguard SC alone or in combination with other products significantly reduced AWR in both field trials with Perseus, Luna Privilege also showing promise.

Figure 7. Symptoms of AWR disease at harvest (Stareton site)

Table 5. Mean percentage of plants per m of row with foliar AWR disease symptoms at Ely on 07/10/20 and Stareton on 01/10/20. Data followed by different letters are significantly different following ANOVA. ANOVA analysis with and without and the co-variate is shown for the Stareton site.

	Treatment	**% of plants with AWR**		
		Ely	**Stareton (no covariate)**	**Stareton (co-variate)**
1	Signum T1 + T2 (banded)	0.39 a	1.20 a	1.15 a
2	Signum T1 + T2 (whole bed)	0.31 ab	0.69 a	0.84 a
3	Perseus T1 + T2 (whole bed)	0.95 ab	1.91 a	1.86 a
4	Luna Privilege T0	1.51 ab	0.59 a	0.64 a
5	NEMguard DE T-1	2.79 b	1.08 a	1.03 a
6	NEMguard DE T-1 with Signum T1 + T2 (whole bed)	0.80 ab	0.67 a	0.62 a
7	NEMguard DE T-1 with Trisoil T0 + T1 (banded)	0.30 a	0.95 a	1.00 a
8	NEMguard SC T-1	1.72 ab	2.04 a	2.09 a
9	NEMguard SC T-1 with Signum T1 + T2 (whole bed)	0.00 a	0.73 a	0.68 a
10	NEMguard SC T-1 with Trisoil T0 + T1 (banded)	0.70 a	1.27 a	1.22 a
11	Trisoil T0 + T1	0.74 a	1.76 a	1.71 a
12	Untreated control	1.88 ab	2.05 a	2.10 a
F-value		*0.097*	*0.623*	*0.618*
d.f.		*44*	*44*	*43*
S.e.d.		*0.874*	*0.874*	*0.869*
L.s.d.		*1.762*	*1.761*	*1.752*

Table 5. Mean percentage of plants with AWR per m of row at harvest for Ely on 02/10/20 and Stareton on 05/11/20. Data followed by different letters are significantly different following ANOVA. ANOVA analysis with and without and the co-variate is shown for the Stareton site. Cells highlighted in green indicate treatments resulting in a significant reduction in AWR compared to the untreated control.

	Treatment	**% plants with AWR at harvest**		
		Ely	**Stareton (no covariate)**	**Stareton (co-variate)**
1	Signum T1 + T2 (banded)	11.9 e	21.1 bcd	20.4 cde
2	Signum T1 + T2 (whole bed)	9.02 de	17.5 abcd	19.7 bcde
3	Perseus T1 + T2 (whole bed)	5.79 abcd	6.3 ab	5.6 ab
4	Luna Privilege T0	7.57 bcde	5.9 ab	6.6 abc
5	NEMguard DE T-1	9.56 de	22.0 cd	21.3 de
6	NEMguard DE T-1 with Signum T1 + T2 (whole bed)	8.15 cde	10.5 abc	9.8 abcd
7	NEMguard DE T-1 with Trisoil T0 + T1 (banded)	8.69 cde	22.8 cd	23.5 de
8	NEMguard SC T-1	2.47 ab	12.7 abcd	13.4 abcd
9	NEMguard SC T-1 with Signum T1 + T2 (whole bed)	1.81 a	3.7 a	3.0 a
10	NEMguard SC T-1 with Trisoil T0 + T1 (banded)	3.51 abc	12.1 abcd	11.4 abcd
11	Trisoil T0 + T1	11.2 de	21.4 bcd	20.6 cde
12	Untreated control	10.98 de	27.7 d	28.4 e
F-value		*<0.001*	*0.01*	*0.09*
d.f.		*44*	*44*	*43*
S.e.d.		*2.407*	*6.74*	*6.30*
L.s.d.		*4.852*	*13.58*	*12.71*

Discussion

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

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 (Entwistle et al., 1982; Coley-Smith et al., 1986; Somerville and Hall, 1987; 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 different assays to identify commercially available garlic products that could stimulate sclerotia germination.

Petri dish germination assays

A repeat Petri-dish experiment was carried out to determine the effects of different commercially available garlic products on germination of *S. cepivorum* sclerotia and results were generally consistent with those observed previously. All three NEMguard products (SC, DE, PCN) resulted in very high levels of germination (>86%) and these results are to a certain extent expected as although the products are formulated differently, they contain the same concentrations of polysulphides (45% w/w). However, use of the food grade garlic granules resulted in consistently less germination. 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 garlic products tested, PK02 resulted in little or 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 inhibitory to germination. Germination of *S. cepivorum* sclerotia in response to DAS was poor as has been less consistent throughout different experimental repeats. This could be due to the more volatile nature of this compound resulting in inconsistent levels over the experiments or perhaps a degradation of the compound while being stored over the duration

of the project so far. Nonetheless, the results from these assays clearly indicated the potential of formulated garlic products to successfully stimulate germination of *S. cepivorum* sclerotia.

Soil based germination assays

In this test, a selection of the garlic products used in the Petri dish assays 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. A second experiment of this assay was conducted, and the results combined with the assay carried out in 2019. Overall, results were less clear than observed in the Petri-dish assays although both garlic granules and NEMguard DE resulted in increased germination (non-recovery, >60%) of *S. cepivorum* sclerotia compared to the untreated control. The number of germinated sclerotia in the untreated controls was also somewhat higher than expected (33% and 29% for GS1 and WRAR13 respectively) while the number of germinated sclerotia from the DAS treatment was unexpectedly low. In addition, PK02 resulted in much higher germination of sclerotia (non-recovery) compared to the Petri dish assay although most of the recovered sclerotia were non-viable. These inconsistencies in the results observed in the soil assays compared to the Petri-dish assays could well be as a result of the indirect method of quantifying germinated sclerotia; for instance, non-recovery of sclerotia could also have been due to microbial degradation (as the soil used was not sterile) as well as due to germination. In addition, germinating *S. cepivorum* sclerotia may be more liable to microbial colonisation. Despite microbial degradation or contamination of sclerotia confounding the measurement of germination, what is clear is that most of the treatments tested reduced the final number of viable sclerotia compared to the untreated control (either directly or indirectly) and therefore still show potential for reducing the number of *S. cepivorum* sclerotia in soil.

Field based germination assay

The field experiment testing selected garlic products showed that NEMguard SC and NEMguard NEM had the greatest effect on germination of *S. cepivorum* (as measured by non-recovery) but were the only treatments that resulted in an increased germination compared to the untreated control. The garlic granules had much lower levels of germination in this experiment compared to the soil box assay, which could be due to the slow production of polysulphides as mentioned previously. As the viability of the sclerotia recovered was high for all treatments, this suggests that the products are not as effective in the field as they are in a controlled enviroment. One of the major differences here is that the products are not confined in a sealed system and so can escape through the air or leaching and prevailing

environmental conditions may exacerbate this. This emphasises the need to perhaps increase doses of these products or attempt sealing of the soil surface by rolling.

Objective 4: Test combined treatments for their effect on white rot disease development in the field

The two field trials carried out in 2020 tested combinations of sclerotial germination products with fungicides and biological control agents and comparison with individual treatments for the first time. Although the effect of treatments was not completely consistent across field sites, it was clear that NEMguard SC either alone or in combination with Signum or Trisoil significantly reduced white rot disease. In contrast, the use of NEMguard DC either alone or in combination was less effective, although combined with Signum, white rot disease was significantly reduced at the Stareton site. Based on the positive results from the *in vitro* tests, both NEMguard DC and NEMguard SC might have been expected to be effective, but it may be the case that the liquid formulation of the latter is more suitable in a field situation and perhaps allows better mobilisation of germination stimulant compounds within the soil profile. The benefits of combining NEMguard SC with the fungicide Signum is less clear, especially as Signum alone was ineffective, but previous published work combining the germination stimulant DADs and the fungicide procymidone resulted in total control of white rot in a field trial where the untreated control showed a disease incidence of ~35% (Villalta et al., 2004; Villalta et al., 2005). In contrast to DADs however, the formulation of garlic-derived compounds in products such as NEMguard, should in principal allow a more controlled and stable release of these germination stimulants over time with much less loss due to volatility. Finally, the fungicides Perseus and Luna Privilege both reduced white rot significantly at the Stareton site and also decreased disease at the Ely site. Their different modes of action and application timings means that they would be good candidates for inclusion in an integrated control strategy for AWR disease alongside NEMguard SC.

Conclusions

- *S. cepivorum* sclerotia were stimulated to germinate by commercial garlic products in *in vitro* Petri dish assays
- The germination stimulant effect was less clear when garlic products were tested in soil boxes under controlled conditions and in a small field trial
- NEMguard SC alone or in combination with other crop protection products reduced AWR disease in two field trials.

- The fungicides Perseus and Luna Privilege also reduced AWR disease and may therefore be additional useful components in an integrated control strategy.

Knowledge and Technology Transfer

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

- Presentation made at the Warwick Food GRP event, University of Warwick, 6th February 2020 (Alex McCormack).
- Presentation made at HIR Skane - Lökonferense (Onion Conference), Skane, Sweden, 11-12th February 2020 (Alex McCormack).

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