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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

Commercial garlic products, which stimulate germination of *Sclerotium cepivorum* sclerotia in the absence of an onion host and biofumigant plants were shown to have potential as components of an integrated disease strategy for *Allium* white rot disease in lab and controlled environment experiments. A combination of garlic products and fungicides reduced the incidence of white rot in field experiments.

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 soil-borne 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 FV499.

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 practised commercially. *S. cepivorum* sclerotia constitute the primary inoculum for infection of onion crops and garlic-based products have the potential 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, one of the main focuses of the project was to develop assays to identify and test commercially available garlic products that could stimulate sclerotial germination. Another potential approach to reduce the levels of sclerotia is the use of biofumigant crops. These are specific brassica plants such as mustards which contain glucosinolates (GLS), and when these plants are crushed and incorporated into soil in the presence of adequate moisture, the GLS are hydrolysed to release toxic isothiocyanates (ITCs). Various studies have previously demonstrated that ITCs have activity against plant pathogens and can also reduce the viability or weaken sclerotia.

The main aim of this project was to identify and test a range of treatments for the integrated control of AWR in bulb and salad onions. The objectives were:

- Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease and generate preliminary data on the effect of selected products on Fusarium basal rot.
- Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia.
- Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum.
- Objective 4: Test combined treatments for their effect on *Allium* white rot disease development.

Summary

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease and generate preliminary data on effect of selected products on *Fusarium* basal rot

This objective was completed in Year 2 and full results are described in full in the project annual reports published in 2019 and 2020. In summary, field trials were conducted with salad onions at three sites over two years (2018, 2019) at an inoculated site at Wellesbourne (Warwickshire) and two commercial field sites in Cambridgeshire and Lincolnshire. A range of chemical fungicides and biological treatments (coded products) were tested at recommended rates and were applied as a concentrated band along the row in 2018 or as whole plot applications in 2019. Application timings were performed on a growth stage basis at emergence and at three to four true leaves across all sites. Some treatments comprised of seed-applied products. Good levels of AWR disease in untreated control plots were only evident at the inoculated site at Wellesbourne in 2018 (54% disease incidence at harvest) and at the Cambridgeshire site in 2019 (39% disease incidence at harvest) and these were therefore the only trials where data could be analysed. At Wellesbourne in 2018 where products were applied as a concentrated band, several fungicides based on SDHI and DMI chemistry significantly reduced disease incidence (Luna Privilege (HDC F246), Signum (BAS 516 07F) and Perseus (HDC F247)) and hence there were good levels of AWR control, with single or double applications proving to be similarly effective except for Luna Privilege where two applications significantly improved control. Biological products tested were not effective in reducing AWR disease incidence and nor was a DMI fungicide seed treatment. At Cambridgeshire in 2019 where applications were made over entire plots, no significant control of AWR disease was observed for any of the fungicide or biological treatments.

In 2019, an additional field trial was conducted at an inoculated site at Wellesbourne to assess the effect of a range of chemical fungicides and biological treatments for control of *Fusarium* basal rot. Products were applied at recommended rates, either as a concentrated band along the row or as whole plot applications at emergence and at three to four true leaves. High disease pressure resulted in a good level of *Fusarium* symptoms with 59% of plants dead or with basal rot at harvest in the untreated control. Although none of the fungicides applied as whole bed applications resulted in a significant decrease in disease, treatment with Rudis (HDC F273) decreased disease incidence slightly (44% dead / diseased plants at harvest). Small decreases in disease were also apparent for some treatments where the fungicides were applied as banded applications but again this was not

significant. The efficacy of Rudis was also improved using the banded application approach but was just outside the level of significance despite attaining a final disease incidence of 31.4% dead/diseased plants at harvest compared with 59% in the untreated control. No decrease in disease was observed for any of the biopesticide treatments tested. Finally, the number of remaining healthy onion bulbs per plot was significantly greater ($P<0.001$) in plots which had received Rudis.

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

Petri dish germination assays

A repeat experiment was carried out to determine the effects of different commercially available garlic products (Ecospray, UK) on germination of *S. cepivorum* sclerotia (from two isolates) in a Petri-dish assay where *S. cepivorum* sclerotia are placed on vermiculite and directly observed for germination over time following treatment at 15ºC. This experiment built on the assay development and the first sets of results that are fully described in the annual project reports for 2019, 2020 and 2021. Treatments tested were NEMguard SC (HDC F261), NEMguard DE (HDC F264), NEMguard PCN (HDC F265), an experimental product PK02 (HDC F261; not to be released commercially) and garlic granules. Results in the current test were generally consistent with those carried out previously with all products resulting in significant stimulation of *S. cepivorum* sclerotial germination compared with an untreated control with the exception of PK02. NEMguard SC, NEMguard DE and garlic granules resulted in very high levels of sclerotial germination (>80%) for *S. cepivorum* isolate (WRAR13), whilst the other isolate (GS1) was more responsive to NEMguard SC (88% germination) but less so to garlic granules and NEMguard DE (26 & 35% germination, respectively). In contrast, PK02 did not stimulate germination of sclerotia from either isolate and resulted in a reduction in viability of sclerotia (proportions of non-viable sclerotia ≥49%) as measured by plating onto agar. This initial test indicated that all the garlic products have the potential to stimulate germination of *S. cepivorum* sclerotia with the exception of PK02 which appeared to have a direct toxic effect on sclerotia.

Soil based germination assays

A repeat experiment was carried out using the same selection of garlic products used in the Petri dish assays for their ability to stimulate germination of *S. cepivorum* sclerotia (from two isolates) using a soil-based system under controlled temperature and moisture conditions to better replicate a field situation. This experiment added additional data to the results from two previous experiments which were fully described in the annual project reports for 2020 and 2021. Here, sclerotia are buried in mesh bags in soil (standard moisture content) contained in plastic boxes, treatments applied and

boxes incubated for 8 weeks at 15ºC. In this system, germination of sclerotia cannot be observed directly and hence is assumed to be associated with a low recovery of intact sclerotia after 8 weeks (sclerotia decay after germination). All garlic product treatments including PK02 resulted in a high level of germination of sclerotia of *S. cepivorum* isolate WRAR13 (≥81% non-recovery) and a moderate level in isolate GS1 (38-64% non-recovery) with NEMguard SC and NEMguard DE generally resulting in the most germination. Results for each garlic product varied across the three soil box experiments carried out over the course of the project (possibly due to different batches of *S. cepivorum* being used which can vary in their ability to germinate) but NEMguard SC and NEMguard DE generally resulted in good levels of sclerotial germination. In contrast, PK02 did not stimulate germination in the first two soil box tests in 2019 and 2020 but did in the test in 2021. This could be due to a slight adjustment of dose in the test in 2021. Nonetheless overall the results clearly indicate the potential of garlic products to stimulate germination of *S. cepivorum* sclerotia in soil.

Field based germination assay

An experiment was carried out from May to July 2021 to test the effect of NEMguard DE, NEMguard SC, NEMguard PCN and garlic granules on germination of *S. cepivorum* sclerotia buried in soil in small field plots at Wellesbourne in the absence of a crop. This was a repeat of an experiment conducted October - December 2020 fully described in the annual project report 2021. As for the lab-based soil germination assay, sclerotia are buried in mesh bags, and treatments applied with sclerotia recovered after 10-12 weeks. Again, as germination of sclerotia cannot be observed directly, this is assumed to be associated with a low recovery of intact sclerotia. The results showed that NEMguard SC and NEMguard PCN slightly increased germination (31 and 36%, respectively, as measured by non-recovery) compared to an untreated control (germination 20%). However, garlic granules, NEMguard DE and NEMguard SC resulted in lower levels of germination in this experiment compared to the soil box assay. By comparison, in the field experiment October - December 2020, *S. cepivorum* sclerotial germination across all treatments and also the untreated control was in the range of 44-52%, and there was less variation observed amongst the garlic treatments, as sclerotial germination for the garlic granules, NEMguard DE and NEMguard PCN treatments was in the range of 46-47%. NEMguard SC was the only treatment which resulted in a slightly elevated level of germination (52%) compared with the untreated control, but this effect was not significant. These data suggest that the garlic products were not as effective in stimulating germination of *S. cepivorum* sclerotia in the field as they are in a controlled enviroment and indicates the need to perhaps increase doses of these products or attempt sealing of the soil surface by rolling.

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

Preliminary experiments were conducted to explore the potential of using biofumigant crop plants to reduce the viability of *S. cepivorum* as another approach for controlling AWR and also for reducing inoculum and resulting onion basal rot caused by *Fusarium oxysporum* f.sp. *cepae* (FOC).

Soil-based assays with *S. cepivorum* sclerotia

Four biofumigant plants cv. Caliente 199, cv. Rojo (both brown mustard, *Brassica juncea*), cv Brisant (white mustard, *Sinapis alba*) and Bento (radish, *Raphanus sativus*) were evaluated for their ability to reduce the viability of *S. cepivorum* sclerotia in soil using a similar approach comparable to that used in the garlic product soil-based box assay. *S. cepivorum* sclerotia (isolate WRAR13) were buried in mesh bags in soil amended with dried biofumigant material within sealed plastic boxes and water added to achieve a standard soil moisture content. Sclerotia were then recovered after 8 weeks incubation at 15ºC, and viability assessed by plating onto agar. Unexpectedly, non-recovery of sclerotia from three of the biofumigants, Bento, Brisant and Caliente 199 (containing the respective GLS compounds glucoraphanin, sinalbin and sinigrin) was high (30-61%) suggesting that germination had been stimulated. In contrast, Rojo (GLS sinigrin) elicited a low level of sclerotial germination (non-recovery 2%) but reduced viability (45% non-viable sclerotia). These results suggested that some biofumigants may stimulate germination of sclerotia while others might directly reduce viability. Both these modes of action are potentially useful in reducing the numbers of *S. cepivorum* in soil, hence contributing to disease control.

FOC seedling assay

A bulb onion seedling assay with FOC-infested compost was developed as fully described in the annual project report 2020. This approach induced high levels of seedling mortality and provided a suitable system to examine the efficacy of selected biofumigants in decreasing FOC inoculum and reducing disease symptoms. Biofumigant material was initially incubated for 4 weeks at 20°C with FOC inoculum (5 x 10^5 CFUs g^{-1}) to allow time for the treatments to kill FOC spores. Onion seeds were then sown into the FOC/biofumigant treated compost in modules which were incubated in a growth room at 25ºC (16 h daylength) and seedling survival assessed over 4-5 weeks. After 34 days, survival of onion seedlings for the untreated inoculated control was 76% while no onion seedlings survived for the inoculated control (FOC only) and the Brisant and Rojo biofumigant treatments. In contrast, seedling survival for Caliente 199 and Bento treatments was significantly greater with 7.5% and 14% seedling survival respectively. Although this is still low compared to the untreated control, this experiment used high levels of FOC inoculum but nonetheless provided some preliminary

evidence that some biofumigants might be useful as a potential control approach for *Fusarium* basal rot disease of onion by reducing inoculum.

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

This objective was completed in Year 3 and full results are described in full in the annual project report published in 2021. In summary, two field trials located at Stareton (Warwickshire) and Ely (Cambridgeshire) tested combinations of garlic products with fungicides and biological control agents in comparison with individual treatments (Table 21) for their effect on AWR disease in salad onions. Applications (depending on treatment) were made as banded or whole bed sprays at T-1 (bed-forming), T0 (pre-drilling), T1 emergence or T2 (3-4 leaves). 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 resulted in a significant reduction in disease with less than 4% plants affected ($P<0.001$; Table 21). 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 was the most effective treatment, significantly reducing disease to only 3.0% ($P<0.05$). All NEMguard SC treatments also significantly reduced AWR disease compared to the untreated control. Treatments with Perseus, Luna Privilege and NEMguard DC with Signum also significantly reduced AWR to 5.6, 6.6 and 9.8%, respectively. Across both sites therefore, 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

- Commercial garlic products, in particular NEMguard DC and NEMguard SC, effectively stimulated germination of *S. cepivorum* sclerotia in lab-based controlled environment experiments but were less effective in a small field trial. Increased rates and/or optimised application may improve efficacy.

- Biofumigants cv. Bento, Brisant and Caliente 199 stimulated germination of *S. cepivorum* sclerotia in lab-based controlled environment experiments.
- Biofumigant plants cv. Caliente 199 and Bento reduced disease due to *Fusarium* in a seedling assay.
- NEMguard SC alone or in combination with the fungicide Signum reduced AWR disease in two field trials while the fungicides Perseus and Luna Privilege also demonstrated activity in one field trial.
- Overall selected garlic products, biofumigants and fungicides demonstrated activity against *S. cepivorum* either by stimulating germination of sclerotia, reducing viability or preventing infection. An integrated control programme incorporating all these elements would enhance management of AWR disease.
- Further work should i) further investigate the potential of biofumigant crops to stimulate germination and / or reduce viability of *S. cepivorum* sclerotia, ii) optimise use of Nemguard garlic products in the field through adjusting rates and / or improving application to maximise polysulphide retention in the soil iii) confirm the value of combining garlic products with effective fungicides.

Financial Benefits

Financial benefits of the different control approaches investigated in this project are difficult to quantify. It is clear however that Nemguard products currently approved for use in carrot, parsnip, sugar beet and potatoes for nematode control would also have added value for *Allium* white rot control in a rotation where they are used as they potentially stimulate germination of *S. cepivorum* sclerotia in the absence of an *Allium* host. Use of these garlic products potentially combined with biofumigation crops may also have the potential to clean up land that is heavily contaminated with *S. cepivorum* sclerotia, but this would represent a considerable financial investment. Combining Nemguard products with effective fungicides would seem a cost-effective way of tackling *Allium* white rot in the short-medium term with biofumigant crops providing an additional break crop in rotations which would increase soil fertility, promote beneficial microbes and again add to the reduction in inoculum.

Action Points

- Avoid spreading sclerotia of *S. cepivorum* between fields through contamination of machinery or equipment.
- Consider the use of garlic-based products in the rotation to potentially reduce the number of *S. cepivorum* sclerotia in the soil in the absence of an *Allium* host.
- Where possible, combine biofumigants, garlic products and fungicides a more long-term and integrated control approach for *Allium* white rot.

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 *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 FV499.

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 practised commercially. *S. cepivorum* sclerotia constitute the primary inoculum for infection of onion crops and garlic-based products have the potential 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, one of the main focuses of the project was to develop assays to identify and test commercially available garlic products that could stimulate sclerotial germination. Another potential approach to reduce the levels of sclerotia is the use of biofumigant crops. These are specific brassica plants such as mustards which contain glucosinolates (GLS), and when these plants are crushed and incorporated into soil in the presence of adequate moisture, the GLS are hydrolysed to release toxic isothiocyanates (ITCs). Various studies have previously demonstrated that ITCs have activity against plant pathogens and can also reduce the viability or weaken sclerotia Smolinska (2000).

The main aim of this project was to identify and test a range of treatments for the integrated control of AWR in bulb and salad onions. The objectives were:

- Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease and generate preliminary data on effect of selected products on *Fusarium* basal rot
- Objective 2: Test *Allium* products for their effect on the germination of *S. cepivorum* sclerotia.
- Objective 3: Test biofumigants for their ability to reduce viability of *S. cepivorum* sclerotia and reduce *Fusarium* inoculum.
- Objective 4: Test combined treatments for their effect on *Allium* white rot disease development.

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease and generate preliminary data on effect of selected products on *Fusarium* basal rot

Materials and methods and results

This objective was completed in Year 2 and full results are described in full in the project annual reports published in 2019 and 2020. In summary, field trials were conducted with salad onions at three sites over two years (2018, 2019) at an inoculated site at Wellesbourne (Warwickshire) and two commercial field sites in Cambridgeshire and Lincolnshire. A range of chemical fungicides and biological treatments (coded products) were tested at recommended rates and were applied as a concentrated band along the row in 2018 or as whole plot applications in 2019. Application timings were performed on a growth stage basis at emergence and at three to four true leaves across all sites. Some treatments comprised of seed applied products. Good levels of AWR disease in untreated control plots were only evident at the inoculated site at Wellesbourne in 2018 (54% disease incidence at harvest) and at the Cambridgeshire site in 2019 (39% disease incidence at harvest) and these were therefore the only trials where data could be analysed. At Wellesbourne in 2018 where products were applied as a concentrated band, several fungicides based on SDHI and DMI chemistry significantly reduced disease incidence (Luna Sensation (HDC F246), Signum (BAS 516 07F) and Perseus (HDC F247)) and hence there were good levels of AWR control, with single or double applications proving to be similarly effective except for Luna Sensation where two applications significantly improved control. Biological products tested were not effective in reducing AWR disease incidence and nor was a DMI fungicide seed treatment. At Cambridgeshire in 2019 where applications were made over entire plots, no significant control of AWR disease was observed for any of the fungicide or biological treatments.

In 2019, an additional field trial was conducted at an inoculated site at Wellesbourne to assess the effect a range of chemical fungicides and biological treatments for control of *Fusarium* basal rot. Products were applied at recommended rates, either as a concentrated band along the row or as whole plot applications at emergence and at three to four true leaves. High disease pressure resulted in a good level of *Fusarium* symptoms with 59% of plants dead or with basal rot at harvest in the untreated control. Although none of the fungicides applied as whole bed applications resulted in a significant decrease in disease, treatment with Rudis (HDC F273) decreased disease incidence slightly (44% dead / diseased plants at harvest). Small decreases in disease were also apparent for some treatments where the fungicides were applied as banded applications but again this was not significant. The efficacy of Rudis was also improved using the banded application approach but was

just outside the level of significance despite attaining a final disease incidence of 31.4% dead/diseased plants at harvest compared with 59% in the untreated control. No decrease in disease was observed for any of the biopesticide treatments tested. Finally, the number of remaining healthy onion bulbs per plot was significantly greater ($P<0.001$) in plots which had received Rudis.

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

Materials and methods

Production and conditioning of *S. cepivorum* sclerotia

As described previously (FV449a annual report 2020), *S. cepivorum* sclerotia for isolates GS1 and WRAR13 were produced in conical flasks by inoculating a mixture of silica sand and cornmeal with mycelial agar plugs of *S. cepivorum*. Flasks were stored at room temperature in the dark for eight weeks and after this period, sclerotia from one flask for each isolate were harvested, dispensed into nylon bags (50 x 100 mm) and buried in air-dried non-sterile field soil (silty clay loam, Dunnington Heath Series) in sealable plastic containers. Sterile distilled water (SDW) was then added to obtain a moisture content of 22% (w/w) and the containers were stored at 15°C for a minimum of 8 weeks in order to condition the sclerotia for germination.

Petri dish germination assays

In 2019 and 2020, experiments were carried out to determine the effects of different commercially available garlic products (Ecospray, UK) on germination of *S. cepivorum* sclerotia (from two isolates) in a Petri-dish assay. Individual sterile Petri dishes (90 mm) were filled with 4.8 g of autoclaved fine vermiculite (1.0 to 3.0 mm) ensuring a level surface. The vermiculite was saturated using 28 mL of SDW and a single 50 x 50 mm square of autoclaved nylon mesh (160 µm) was placed into the centre of the dish ensuring good contact with the underlying vermiculite. 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. Garlic product treatments were then applied to individual Petri dishes according to the application rates shown in Table 1. The six treatments comprised of four garlic products (Ecospray, UK) three of which are commercial and one experimental (PK02), and food grade garlic granules / powder (Just Ingredients, Wotton under Edge, 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).

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). Once placed into the containers, Petri dish lids were removed, and the individual containers sealed, placed inside individual gripper bags and incubated at 15°C for 8 weeks in the dark.

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

Treatment	Application rate (per plate)
NEMguard SC	2 ml of 90% v/v (6% v/v)
PK02	2 ml of 90% v/v (6% v/v)
NEMguard PCN	13 mg (20 kg/ha)
NEMguard DE	13 mg (20 kg/ha)
Garlic granules	64 mg (100 kg/ha)
Garlic powder	64 mg (100 kg/ha)
Control treated (DAS in 2% Triton X)	0.06% v/v
Control treated DAS (unconditioned) (DAS in 2% Triton X)	0.06% v/v

N.B. Sclerotia used in assays were conditioned except where indicated in control treatments.

Germination of *S. cepivorum* sclerotia (myceliogenic or eruptive; Fig. 1) was assessed twice weekly, with any germinated / contaminated sclerotia removed from the Petri dishes. The experiment was conducted over a period of seven weeks after which any remaining (non-germinated) sclerotia were assessed for viability by first squeezing with forceps to assess integrity. Collapsed sclerotia were discarded and intact (non-germinated) sclerotia were counted, surface sterilised in 70% ethanol (v/v) for 1 min and washed twice in SDW for 30 s. This was followed by a modified version of the agar drop viability test of Clarkson *et al*. (2002), whereby a proportion of the remaining *S. cepivorum* sclerotia were squeezed using sterile forceps to burst the rind and individually placed on 10 mm cores of potato dextrose agar (20 mg L^{-1}) amended with chlortetracycline arranged 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 typical mycelium and immature sclerotia. The experimental setup was repeated three times over 2019 and 2020 with one replicate box of 100 sclerotia for each of the above treatments.

Modified Petri dish and viability assays

A final Petri dish assay was completed as part of a supervised undergraduate student project in May 2021 to supplement the data from 2019 and 2020 with the following amendments to the above method. Thirty conditioned sclerotia from the same two isolates of *S. cepivorum* (GS1 and WRAR13) were placed on the nylon mesh in a 5 x 6 grid pattern, ensuring 5-10 mm between any two sclerotia. Two replicate plates each with 30 sclerotia were prepared for each sealable plastic container. Only

four products were included from Table 1: NEMguard SC, PK02, NEMguard DE and garlic granules. The rates of application remained the same and three replicate boxes of 60 sclerotia were prepared for each treatment/isolate combination (total of 180 sclerotia per treatment). Germination was assessed as above over seven weeks and remaining intact sclerotia were surface sterilised in 1 ml 1% sodium hypochlorite (20% v/v Domestos bleach) for 1 min followed by four washes in 1 ml SDW. Viability testing was carried out by squashing sclerotia with forceps to break the rind and plating into individual wells of 96 well plates filled with PDA amended with chlortetracycline (20 mg L^{-1}). Plates were incubated in the dark at 15°C for 7 days, after which sclerotia were assessed for growth of typical *S. cepivorum* mycelium (Fig. 2) or signs of contamination.

Calculations and statistical analyses for Petri dish assays

S. cepivorum sclerotia can be lost at two different stages in the Petri-dish assays: firstly, due to contamination by other microorganisms in the Petri dish during the assay, and secondly during surface sterilisation due to their small size (when some can go missing). The following calculations were made to take account of these losses (example for modified Petri dish assay where total number of sclerotia per replicate box = 60 but the same approach was applied for data from the previous experiment where there were 100 sclerotia per replicate box). Mean values in the results were calculated across replicate boxes.

1) % Germinated = no. sclerotia germinated in Petri dish by final assessment / (60 – no. sclerotia contaminated during the assay) x 100
2) % Viable = no. sclerotia (remaining in Petri dish, not germinated) resulting in mycelial growth in viability assay / (60 – no. contaminated and lost during sterilisation process) x 100
3) % Non-viable = no. sclerotia without mycelial growth in viability assay / (60 – no. contaminated and lost during sterilisation process) x 100
4) % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)

Percentage data from the Petri dish assay above was logit-transformed before being subjected to ANOVA analyses to determine levels of significance. Least significant differences (LSD) were calculated to enable comparison of treatments with the untreated control.

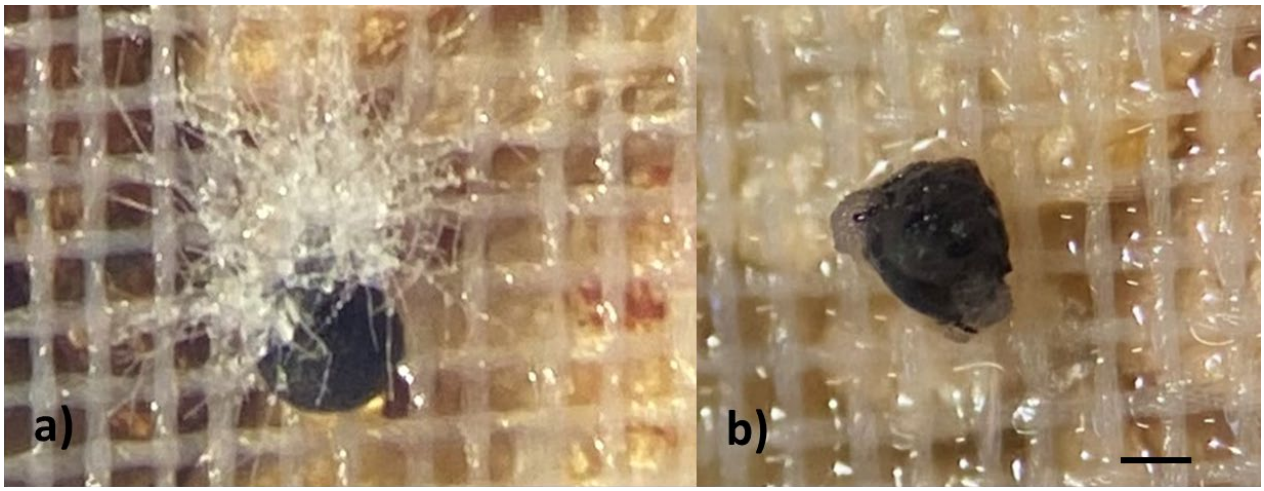

Figure 1. Myceliogenic (a) and eruptive (b) germination of *S. cepivorum* sclerotia *in vitro* in the Petri dish assay. Bar = approx. 200 µm.

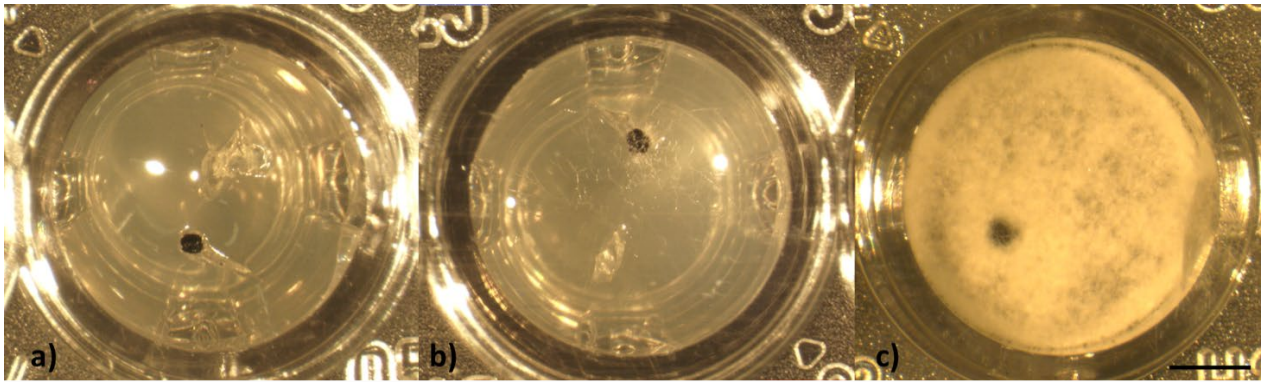

Figure 2. Amended viability assay in 96 well plates showing a) non-viable *S. cepivorum* sclerotium (no mycelial growth) and viable sclerotia showing characteristic mycelium developing after b) 4 and c) 7 days. Bar = 2 mm.

Soil-based box assays with *S. cepivorum* sclerotia

A repeat experiment was carried out using the same selection of garlic products used in the Petri dish assays for their effect on *S. cepivorum* sclerotia (from two isolates) using a soil-based box system under controlled temperature and moisture conditions to better replicate a field situation. This experiment added additional data to the results from two previous experiments carried out in 2019 and 2020 which were fully described in the annual project reports published 2020 and 2021. Batches of 50 conditioned *S. cepivorum* sclerotia (isolates GS1 and WRAR13) were placed into nylon mesh bags (160 µm mesh aperture, 50 x 50 mm), sealed and kept moist on damp tissue. Untreated controls were first prepared by filling small plastic containers (100 x 120 x 65 mm) with 200 g of air-dried unsterile field soil (silty clay loam, Soakwaters Field, Wellesbourne). SDW was then added to obtain a moisture content of 17% (w/w) and a single nylon bag of sclerotia placed on the top of the soil layer. A further 200 g of soil was added to cover the bag and wetted to obtain the same moisture

content. Treated boxes were then similarly prepared with the 400 g soil containing garlic products at the rates specified in Table 2. Liquid products were diluted appropriately with SDW and applied as a drench to the top layer of soil, whilst the granular products were mixed into the soil in both layers before addition to the individual containers. Soil boxes were prepared for each of the two *S. cepivorum* isolates (GS1 and WRAR13), and there were three replicate boxes of 50 sclerotia for each isolate/treatment combination. The containers were sealed, placed inside individual polythene gripper bags and incubated at 15°C for 8 weeks in the dark. As indicated previously, 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, brittle and liable to collapse. An effective treatment would therefore result in a low recovery of sclerotia in this assay. At the end of the incubation period, nylon bags of sclerotia were recovered from each soil box, rinsed in tap water to remove soil and then gently rubbed on a sieve to break up and remove any degraded or hollow sclerotia. Sclerotia that remained were then gently squeezed with forceps to remove any more that were degraded. The final remaining number of firm sclerotia were then counted and placed in a 1.5 ml tube. These intact sclerotia were subjected to surface sterilisation and viability testing in 96 well plates as described for the Petri dish assays above.

Table 2. Garlic products tested for effects on germination of *S. cepivorum* sclerotia in the soil-based box assay.

Treatment	Dose per box	Field rate (L or kg/ha)
NEMguard SC	38 ml of 0.133% (v/v)	60 L/ha
PK02	38 ml of 0.133% (v/v)	60 L/ha
NEMguard DE	17 mg	20 kg/ha
Garlic granules	84 mg	100 kg/ha

Calculations and statistical analyses for soil-based box assays

During the surface sterilisation process, sclerotia can be lost due to their small size. The following calculations were made to take account of this loss:

1) % Germinated = no. sclerotia germinated (as measured by non-recovery) / 50 x 100
2) % Viable = no. sclerotia showing mycelial growth in viability assay / (50 – no. lost during sterilisation process) x 100
3) % Non-viable = no. sclerotia without mycelial growth in viability assay / (50 – no. lost during sterilisation process) x 100
4) % Eliminated = % sclerotia eliminated through germination or through direct effect on viability

Percentage data from the soil box assay above was logit-transformed before being subjected to ANOVA analyses to determine levels of significance. Least significant differences (LSD) were calculated to enable comparison of treatments with the untreated control.

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

In additional experiments 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. An experiment was carried out from May to July 2021 to test the effect of NEMguard DE, NEMguard SC, NEMguard PCN and garlic granules on germination of *S. cepivorum* sclerotia buried in soil in small field plots at Wellesbourne in the absence of a crop. This was a repeat of an experiment conducted October - December 2020 fully described in the annual project report 2021. Nylon mesh bags (160 µm aperture, 50 x 50 mm) containing 30 conditioned sclerotia (isolate GS1) were prepared, sealed and kept moist on damp tissue. Plots (2 x 1.83 m) were marked out in a 5 x 5 grid, with a 0.5 m space between each plot in the Quarantine Field. Treatments consisted of four garlic products (Table 3) applied by raking each into the soil down to a depth of 5 cm and an untreated control was also included. There were five replicate plots for each treatment. Immediately after product application, three bags of 30 *S. cepivorum* sclerotia were buried in each plot at an approximate soil depth of 5 cm (total of 15 bags per treatment). Bags remained in the soil for approximately 10 weeks after which they were retrieved and washed to remove soil. As for the soil box assays, an effective treatment which stimulates germination would result in a low recovery of sclerotia. Hence, the retrieved sclerotia were rubbed over a sieve and gently squeezed with fine forceps to remove any that were hollow / degraded and intact sclerotia counted and subjected to surface sterilisation and viability testing in 96 well plates as described as described for the soil box assays above.

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

Treatment	Dose (L or Kg/ha)	Dose per Plot (2 x 1.8 m)
NEMguard DE	20 kg/ha	7.2 g
NEMguard SC	60 L/ha	21.6 mL in 0.36 L Water
NEMguard PCN	60 kg/ha	21.6 g
Garlic Granules	100 kg/ha	36 g

Results

Petri dish germination assays

Petri dish assay experiments assessing effect of garlic products on germination of *S. cepivorum* sclerotia were carried out in 2019, 2020 and 2021. The results from tests in 2019 and 2020 were presented in annual project reports for 2020 and 2021 but are summarised and analysed here for comparison with the final experiment carried out in 2021.

All the Petri dish assays conducted 2019-2021 consistently demonstrated that the NEMguard products (DE, SC and PCN), garlic granules and garlic powder all resulted in significantly increased germination of *S. cepivorum* sclerotia for both isolates GS1 and WRAR13 ($P<0.001$) compared to the untreated controls (Figs. 3,4 Tables 4-8). However, the response of the sclerotia to these products was variable between experiments; garlic granules/powder typically stimulated a lower level of germination (41-67% and 27-81% in 2019-2020 and 2021, respectively) compared to 77-93% and 34-100% for the NEMguard product treatments (Tables 5 and 7). Germination of conditioned sclerotia (isolates GS1 and WRAR13) in response to DAS in 2019-2020 experiments was also in the lower range (34-44%) than that observed with the NEMguard products, although this was still significantly higher ($P<0.001$) than the untreated control sclerotia (0.3-1%; Tables 7 and 8). In contrast, there was negligible sclerotial germination for both *S. cepivorum* isolates following treatment with PK02 (≤1%).

In the 2021 Petri dish assay, treatment with NEMguard DE resulted in the most rapid response in the Petri dish assay with 93% germination reached by 15 days post-treatment for sclerotia of *S. cepivorum* isolate WRAR13 (Fig. 3, Table 4). However, on this occasion, isolate GS1 was far less responsive to NEMguard DE, as only 35% of sclerotia had germinated for this treatment 43 days after application. Treatment with NEMguard SC resulted in high final levels of sclerotial germination for both *S. cepivorum* isolates GS1 and WRAR13 (89 and 93% germination, respectively). Although the rate of germination for sclerotia of isolate WRAR13 treated with garlic granules was slightly slower, the final proportion that germinated was still high (80%). In contrast, garlic granules elicited a far lower level of germination in sclerotia of isolate GS1 as only 27% germinated by the end point.

Viability of remaining *S. cepivorum* sclerotia of isolates GS1 and WRAR13 at the end of assessing germination in the 2021 experiment across all treatments was significantly lower ($P<0.001$) across all treatments relative to untreated control sclerotia (Fig. 4, Table 6). The proportion of viable sclerotia can be considered as a measure of the ‘live’ propagules that remain after a garlic compound

treatment has eliminated sclerotia either via inducing germination or by directly exerting toxic effects. In contrast to any other treatment, the experimental garlic compound PK02 was in the latter category; this product did not induce germination of sclerotia of either *S. cepivorum* isolate in 2019-2020 or 2021 experiments (Tables 6 and 8), but significantly reduced sclerotial viability (2021; P<0.001; Fig. 4, Table 6).

The efficacy of the garlic treatments in reducing the sclerotial inoculum 'load' was also determined by calculating the percentage of sclerotia eliminated (% germinated + % non-viable sclerotia). The percentage of sclerotia eliminated in the Petri dish assay in 2021 ranged from 30-79% and 49-100% in GS1 and WRAR13 isolates, respectively, across the four garlic treatments, compared to only 6% in the untreated controls (Table 5).

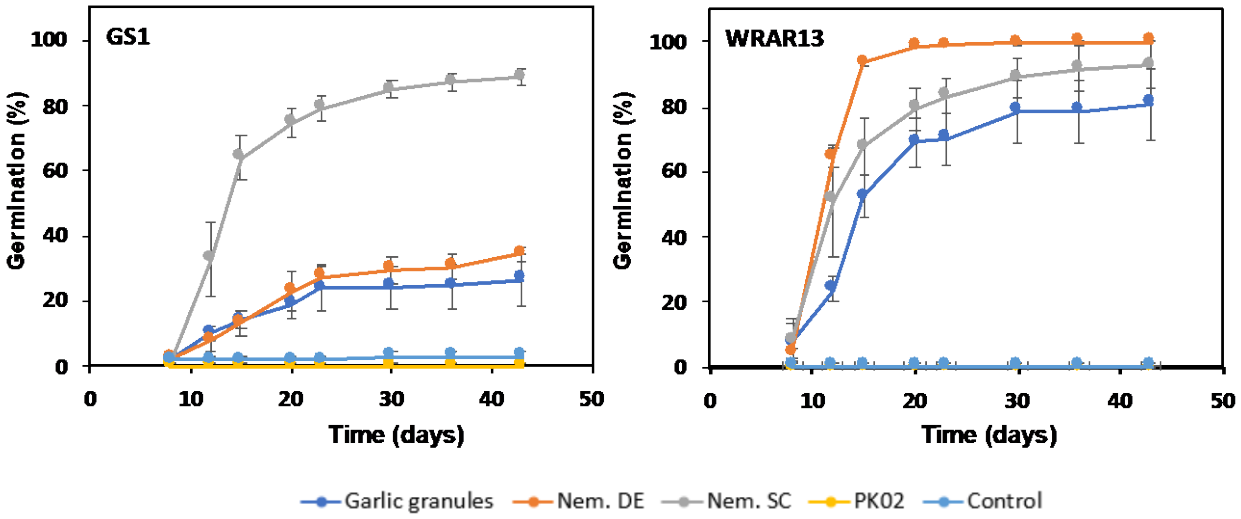

Figure 3. Effect of garlic products on the mean cumulative germination of conditioned *S. cepivorum* sclerotia for isolates GS1 and WRAR13 in the 2021 Petri dish assay.

Table 4. Mean cumulative percentage germination of *S. cepivorum* sclerotia isolates GS1 and WRAR13 following treatment with garlic products in the 2021 Petri dish assay. Data represent mean values for three replicates of 60 sclerotia.

Days	***S. cepivorum* isolate GS1**					***S. cepivorum* WRAR13**				
	Garlic granules	Nem. DE	Nem. SC	PK02	Control	Garlic granules	Nem. DE	Nem. SC	PK02	Control
8	2.22	2.26	0.56	0.00	1.70	7.78	4.44	8.33	0.00	0.56
12	10.00	7.95	33.08	0.00	1.70	24.00	64.44	51.44	0.00	0.56
15	13.95	13.10	63.93	0.00	1.70	52.47	93.33	67.62	0.00	0.56
20	19.00	22.72	74.60	0.00	1.70	69.06	98.33	79.37	0.00	0.56
23	23.72	27.24	78.95	0.00	1.70	70.19	98.89	83.30	0.00	0.56
30	24.06	29.50	84.64	0.00	2.82	78.66	99.44	88.83	0.00	0.56
36	24.62	30.44	86.97	0.00	2.82	78.66	100.00	91.63	0.00	0.56
43	26.88	34.14	89.17	0.00	2.89	80.90	100.00	92.78	0.00	0.56

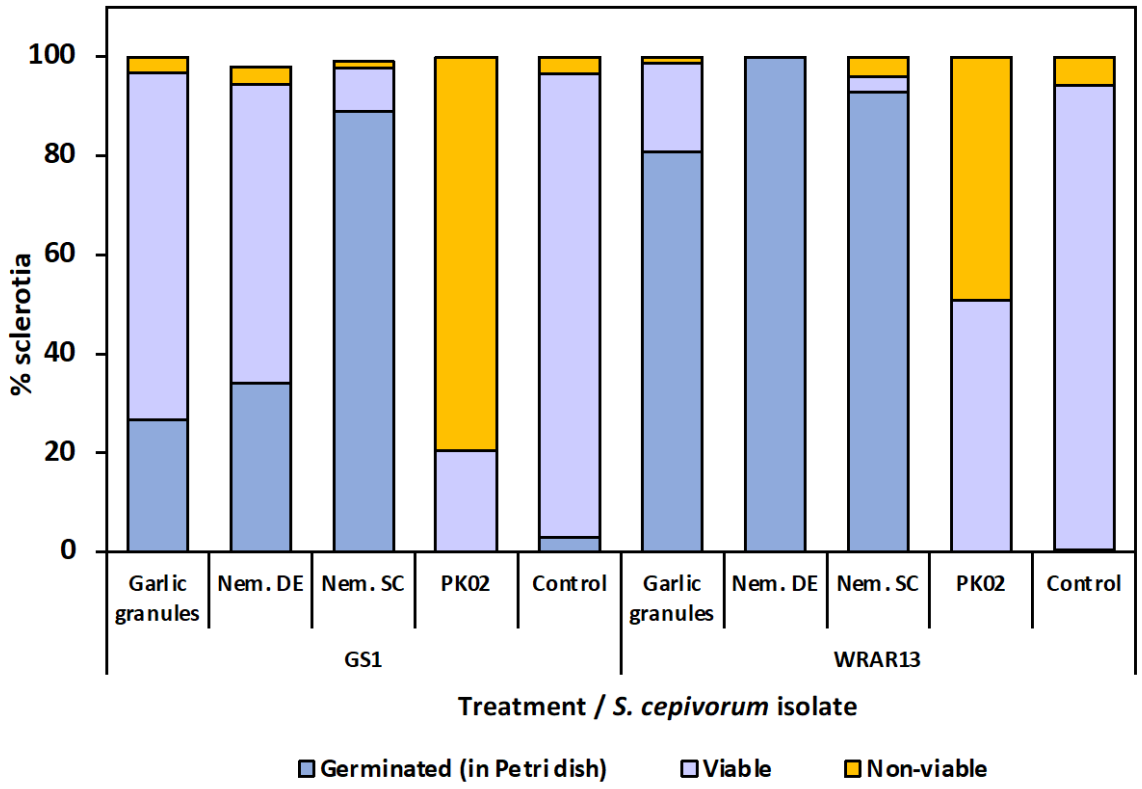

Figure 4. Effect of garlic products on germination of *S. cepivorum* sclerotia (blue bars) and on viability of remaining ungerminated sclerotia; viable (purple bars) and non-viable (yellow bars) for two isolates GS1 and WRAR13 in the 2021 Petri dish assay after 43 days.

Table 5. Final percentage of germinated *S. cepivorum* sclerotia and percentage viable and non-viable sclerotia for isolates GS1 and WRAR13 43 days after treatment with garlic products in the 2021 Petri dish assay. Percentages of viable and non-viable sclerotia as determined in subsequent viability testing are also presented as well as the percentage eliminated (either by germination or non-viability). Data represent mean values for three replicates of 60 sclerotia.

Isolate	Treatment	% Germinated[1]	% Viable[2]	% Non-viable[3]	% Eliminated[4]
GS1	Garlic granules	26.88	70.05	2.85	29.72
	NEMguard DE	34.14	60.43	3.56	37.70
	NEMguard SC	89.17	8.65	1.15	90.32
	PK02	0.00	20.59	79.41	79.41
	Control	2.89	93.65	3.26	6.15
WRAR13	Garlic granules	80.90	17.95	1.15	82.05
	NEMguard DE	100.00	0.00	0.00	100.00
	NEMguard SC	92.78	3.33	3.89	96.67
	PK02	0.00	50.88	49.12	49.12
	Control	0.56	93.57	5.85	6.40

[1] % Germinated = no. sclerotia germinated in Petri dish by final assessment / (60 – no. sclerotia contaminated during the assay) x 100
[2] % Viable = no. sclerotia (remaining in Petri dish, not germinated) resulting in mycelial growth in viability assay / (60 – no. contaminated and lost during sterilisation process) x 100
[3] % Non-viable = no. sclerotia without mycelial growth in viability assay / (60 – no. contaminated and lost during sterilisation process) x 100
[4] % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)
N.B. The percentage of sclerotia lost in the assay due to contamination or during the viability plating process was in the range of 0-15%

Table 6. Logit transformed values and indication of significant differences from untreated control following ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolates GS1 and WRAR13 for the 2021 Petri dish assay.

Treatment	*S. cepivorum* isolate			
	% Germinated[1]		% Viability[2]	
	GS1	WRAR13	GS1	WRAR13
Garlic granules	-1.05	1.99	0.88	-2.06
NEMguard DE	-0.64	4.11	0.41	-4.11
NEMguard SC	2.02	3.15	-2.22	-3.42
PK02	-4.11	-4.11	-1.34	0.03
Untreated control	-3.22	-3.86	2.49	2.87
LSD	1.56		1.45	
Treatments that are significantly different from untreated control (P<0.001)				
Treatments that are not significantly different from untreated control				

[1] % Germinated = no. sclerotia germinated in Petri dish by final assessment / (60 – no. sclerotia contaminated in Petri dish) x 100 (logit transformed values)
[2] % Viable = no. sclerotia (remaining in Petri dish, not germinated) resulting in mycelial growth in viability assay / (60 – no. contaminated and lost during sterilisation process) x 100 (logit transformed values).

Table 7. Final percentage of germinated *S. cepivorum* sclerotia for isolates GS1 and WRAR13 50 days after treatment with garlic products for the 2019-2020 Petri dish assays. Data represent mean values for three replicates of 100 sclerotia.

Treatment	Conditioned Sclerotia[1]	*S. cepivorum* isolate % Germinated[2]	
		GS1	WRAR13
Garlic granules	+	40.48	66.98
Garlic powder	+	61.00	62.33
NEMguard DE	+	76.84	86.26
NEMguard PCN	+	92.50	88.67
NEMguard SC	+	93.01	89.60
PK02	+	0.00	1.02
DAS	-	50.66	40.67
DAS	+	44.33	33.85
Untreated control	-	0.67	0.00
Untreated control	+	1.01	0.33

[1] In this experiment, sclerotia used to test treatments were conditioned in unsterile soil for at least 8 weeks at 15°C (+) while those used in DAS and untreated control treatments were either conditioned or unconditioned (used directly from the sterile sand/cornmeal culture media) (-).
[2] % Germinated = no. sclerotia germinated in Petri dish / (100 - no. sclerotia contaminated in Petri dish) x 100. N.B. The percentage of sclerotia that became contaminated in the Petri dish was in the range of 0-5%

Table 8. Logit transformed values and indication of significant differences from untreated control for garlic products based on ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolates GS1 and WRAR13 for the 2019-2021 Petri dish assays.

Treatment	Conditioned sclerotia	*S. cepivorum* isolate % Germinated[2]	
		GS1	WRAR13
Garlic granules	+	-0.337	0.697
Garlic powder	+	0.468	0.502
NEMguard DE	+	1.415	1.881
NEMguard PCN	+	2.938	2.065
NEMguard SC	+	3.478	2.488
PK02	+	-4.612	-4.136
DAS	-	0.016	0.193
DAS	+	-0.472	-0.749
Untreated control	-	-4.242	-4.615
Untreated control	+	-4.001	-4.381
LSD	2.413		
Treatments that are significantly different from untreated control (P<0.001)			
Treatments that are not significantly different from untreated control			

[1] In this experiment, sclerotia used to test treatments were conditioned in unsterile soil for at least 8 weeks at 15°C (+) while those used in DAS and untreated control treatments were either conditioned or unconditioned (used directly from the sterile sand/cornmeal culture media) (-).
[2] % Germinated = no. sclerotia germinated in Petri dish / (100 - no. sclerotia contaminated in Petri dish) x 100 (logit transformed values).

Soil-based box assays with *S. cepivorum* sclerotia

Soil-based box assays were conducted in 2019, 2020 and 2021 to evaluate the ability of garlic products to stimulate germination of *S. cepivorum* sclerotia in more natural conditions. The results from tests in 2019 and 2020 were presented in annual project reports for 2020 and 2021 but are summarised and analysed here for comparison with the final experiment carried out in 2021.

In the soil-based assay conducted in 2021, the NEMguard products (DE and SC), garlic granules and PK02 all significantly stimulated germination (as measured by non-recovery; see materials and methods) of *S. cepivorum* sclerotia from isolates GS1 and WRAR13 relative to the untreated control ($P<0.001$; Table 10). However, it was noted that sclerotia of isolate WRAR13 were more responsive to the garlic products than those of isolate GS1, as high levels of germination were achieved with all four treatments (83-93% for WRAR13; blue bars Fig. 5, Table 9). The results for isolate GS1 were more variable, as the granular treatments of NEMguard DE and garlic granules resulted in 37 and 39% germination, respectively, compared to 52 and 64% for the liquid treatments of NEMguard SC and PK02. This differential response between the two isolates was also evident in the Petri dish assay with garlic granule and NEMguard DE treatments (2021; Fig. 4, Tables and 5). The percentage of sclerotial germination in the untreated controls in the soil-based assay, similarly to the Petri dish assay, was negligible (1 and 7% for *S. cepivorum* isolates WRAR13 and GS1, respectively).

The proportion of *S. cepivorum* sclerotia (isolates GS1 and WRAR13) that remained at the end of the 2021 soil-box experiment and were also viable was significantly lower ($P<0.001$) across all treatments relative to the untreated controls (Fig. 5, Table 10). In contrast to the Petri dish system, the reduction in the viable portion of sclerotia remaining in the PK02-treated soil this time was attributed to the stimulation of germination, and not to direct toxic effects on the sclerotia.

The efficacy of the garlic treatments in reducing the sclerotial inoculum 'load' was also determined by calculating the percentage of sclerotia eliminated (% germinated + % non-viable sclerotia). Overall, the garlic treatments resulted in high levels of elimination of sclerotia in the soil, mainly due to inducing germination (38-64% and 83-93% elimination in GS1 and WRAR13 isolates, respectively; Table 9).

In the soil-based assay conducted in 2019, moderate to high levels of sclerotial germination were observed following treatment with the same four garlic products and DAS (60-96% and 37-95% across *S. cepivorum* isolates GS1 and WRAR13, respectively; Table 11). However, owing to the unusually high level of sclerotial germination for the untreated control soil in this particular assay (41 and 47% for isolates WRAR13 and GS1 respectively), a significant increase in sclerotial germination

compared with the untreated control was only observed with the NEMguard DE and garlic granule treatments for both isolates, and also the NEMguard SC for isolate GS1 ($P<0.001$; Table 12).

In the soil-based assay conducted in 2020, the level of sclerotial germination following treatment with garlic products was lower than in the 2019 / 2021 assays (19-55% and 19-63% across the *S. cepivorum* isolates GS1 and WRAR13, respectively; Table 11). Only the garlic granule treatment significantly increased sclerotial germination relative to the untreated control for both isolates in the 2020 assay while NEMguard DE significantly increased sclerotial germination for isolate WRAR13 (Table 12).

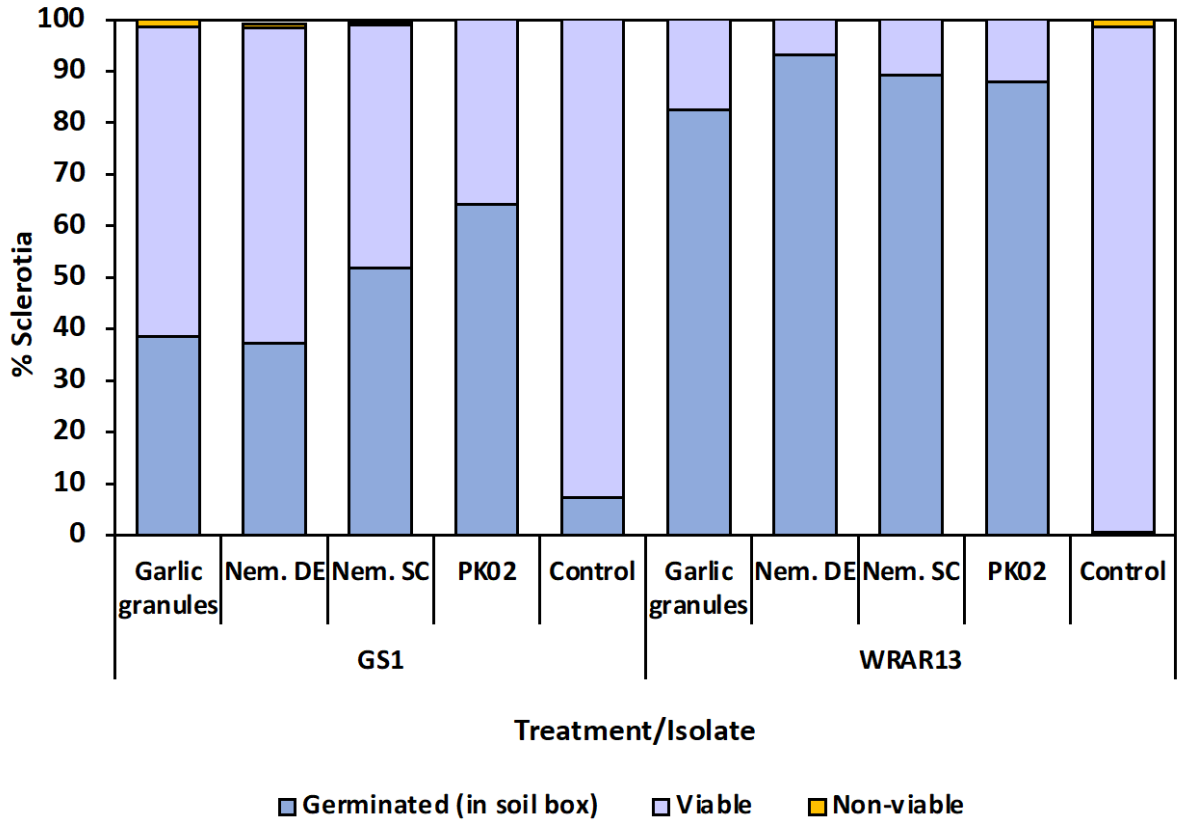

Figure 5. Effect of garlic products on germination of *S. cepivorum* sclerotia (as measured by non-recovery, blue bars) and on viability of remaining ungerminated sclerotia; viable (purple bars) and non-viable (yellow bars) for two isolates GS1 and WRAR13 in a soil box test. Data represent mean values for three replicates of 50 sclerotia.

Table 9. Final percentage of germinated *S. cepivorum* sclerotia (as measured by non-recovery) for isolates GS1 and WRAR13 after treatment with garlic products for the 2021 soil-based box assay. Percentages of viable and non-viable sclerotia as determined in subsequent viability testing are also presented as well as the percentage eliminated (either by germination or non-viability). Data represent mean values for three replicates of 50 sclerotia.

Isolate	Treatment	% Germinated[1]	% Viable[2]	% Non-viable[3]	% Eliminated[4]
GS1	Garlic granules	38.67	60.00	1.33	40.00
	NEMguard DE	37.33	61.14	0.67	38.00
	NEMguard SC	52.00	46.90	0.67	52.67
	PK02	64.00	36.00	0.00	64.00
	Control	7.33	92.67	0.00	7.33
WRAR13	Garlic granules	82.67	17.33	0.00	82.67
	NEMguard DE	93.33	6.67	0.00	93.33
	NEMguard SC	89.33	10.67	0.00	89.33
	PK02	88.00	12.00	0.00	88.00
	Control	0.67	97.94	1.36	2.03

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (50) x 100
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[3] % Non-viable = no. sclerotia without mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[4] % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)
N.B. The percentage of sclerotia lost in the assay due to contamination or during the viability plating process was in the range of 0-6%

Table 10. Logit transformed values and indication of significant differences from untreated control for garlic products based on ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolates GS1 and WRAR13 for the 2021 soil-based box assay.

Treatment	*S. cepivorum* isolate			
	% Germinated		% Viable	
	GS1	WRAR13	GS1	WRAR13
Garlic granule	-0.44	1.52	0.39	-1.52
NEMguard DE	-0.52	2.48	0.46	-2.48
NEMguard SC	0.08	2.19	-0.13	-2.19
PK02	0.57	1.88	-0.57	-1.88
Untreated control	-2.38	-3.69	2.38	3.53
LSD	0.94		0.99	
Treatments that are significantly different from untreated control (P<0.001)				

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (50) x 100 (logit transformed values)
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100 (logit transformed values).

Table 11. Final percentage of germinated *S. cepivorum* sclerotia (as measured by non-recovery) for isolates GS1 and WRAR13 after treatment with garlic products for the 2019 and 2021 soil-based box assays. Percentages of viable and non-viable sclerotia as determined in subsequent viability testing are also presented as well as the percentage eliminated (either by germination or non-viability). Data represent mean values for three replicates of 50 sclerotia.

Isolate	Treatment	% Germinated[1]	% Viable[2]	% Non-viable[3]	% Eliminated[4]
2019 soil box assay					
GS1	Garlic granules	94.67	2.00	3.33	98.00
	NEMguard DE	96.00	0.00	4.00	100.00
	NEMguard SC	88.00	6.00	6.00	94.00
	PK02	72.67	2.67	24.67	97.33
	DAS	60.00	8.00	32.00	92.00
	Untreated control	46.67	46.00	7.33	54.00
WRAR13	Garlic granules	95.33	0.67	4.00	99.33
	NEMguard DE	90.00	0.67	9.33	99.33
	NEMguard SC	63.33	16.00	20.67	84.00
	PK02	37.33	2.67	60.00	97.33
	DAS	46.67	27.33	26.00	72.67
	Untreated control	41.33	45.33	13.33	54.67
2020 soil box assay					
GS1	Garlic granules	55.33	17.33	27.33	82.67
	NEMguard DE	31.33	26.00	42.67	74.00
	NEMguard SC	26.00	37.33	36.67	62.67
	PK02	28.00	30.67	41.33	69.33
	DAS	18.67	40.00	41.33	60.00
	Untreated control	19.33	56.00	24.67	44.00
WRAR13	Garlic granules	63.33	11.33	25.33	88.67
	NEMguard DE	32.67	34.00	33.33	66.00
	NEMguard SC	20.00	28.67	51.33	71.33
	PK02	18.67	30.67	50.67	69.33
	DAS	26.00	32.00	42.00	68.00
	Untreated control	16.00	58.00	26.00	42.00

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (50) x 100
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[3] % Non-viable = no. sclerotia without mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[4] % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)
N.B. No sclerotia were recorded as lost during the viability assays.

Table 12. Logit transformed values and indication of significant differences from untreated control for garlic products following ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolates GS1 and WRAR13 for the 2019 and 2020 soil-based box assays.

Treatment	*S. cepivorum* isolate			
	% Germinated[1]		% Viable[2]	
	GS1	WRAR13	GS1	WRAR13
2019 soil box assay				
Garlic granules	2.86	3.19	-3.45	-3.69
NEMguard DE	3.24	2.51	-3.93	-3.69
NEMguard SC	1.88	0.73	-2.58	-2.32
PK02	0.94	-0.51	-3.37	-3.21
DAS	0.52	-0.25	-2.56	-1.46
Untreated control	-0.17	-0.44	-0.20	-0.17
LSD	1.76		1.97	
Treatments that are significantly different from untreated control (P<0.001)				
Treatments that are not significantly different from untreated control				
2020 soil box assay				
Garlic granules	0.21	0.53	-1.78	-2.13
NEMguard DE	-0.76	-0.72	-1.05	-0.70
NEMguard SC	-1.03	-1.41	-0.50	-0.96
PK02	-0.91	-1.42	-0.79	-0.89
DAS	-1.42	-1.10	-0.79	-1.03
Untreated control	-1.45	-1.70	0.28	0.31
LSD	0.84		1.56	
Treatments that are significantly different from untreated control (P<0.05)				
Treatments that are not significantly different from untreated control				

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (50) x 100 (logit transformed values)
[2] % Viable = no. sclerotia (remaining in Petri dish, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100 (logit transformed values).

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

Two field experiments were set up to evaluate the effect of NEMguard DE, NEMguard SC, NEMguard PCN and garlic granules on germination of *S. cepivorum* sclerotia (isolate GS1) buried in small plots at Wellesbourne in the absence of a crop. The first experiment was carried out October - December 2020 and is fully described in the annual project report 2021. The second was carried out May to July 2021. Data and analyses for both experiments are presented here for comparison.

In the field experiment in May-July 2021, germination of *S. cepivorum* sclerotia (as measured by non-recovery) for the untreated control was unexpectedly high (20%; Fig. 6, Table 13). No significant difference in the level of sclerotial germination was found between sclerotia from the untreated control plots and those treated with the garlic granules, NEMguard DE and SC treatments (22, 25 and 31% germination, respectively, Fig. 6, Tables 13 and 14). However, in plots treated with NEMguard PCN, 36% of sclerotia germinated, which was significantly higher than in control plots ($P<0.05$). Overall, the response of *S. cepivorum* sclerotia to the garlic treatments in the 2021 field trial was lower compared with results from the Petri-dish or soil box assays.

By comparison, in the field experiment October-December 2020, *S. cepivorum* sclerotial germination across all treatments and also the untreated control was in the range of 44-52%, which was generally greater than the levels measured in the field experiment in 2021 which ranged from 20-36% (Fig. 6, Table 13). In 2020, there was less variation observed amongst the garlic treatments, as sclerotial germination for the garlic granules, NEMguard DE and NEMguard PCN treatments was 46-47%. NEMguard SC was the only treatment which resulted in a slightly elevated level of germination (52%) compared with the untreated control, but this effect was not significantly different from the control (Tables 13 and 14).

When the remaining intact sclerotia were examined from the field, a high proportion were found to be viable across all treatments (54-71% and 41-49% viable in 2021 and 2020, respectively), and there was no significant effect compared to sclerotia from untreated plots (Tables 13 and 14). Only 9-12% of sclerotia were non-viable from the untreated control plots, and across the garlic treatments the level of non-viable sclerotia did not exceed 10% in both years (Fig. 6, Table 13).

These data suggest that the garlic products were not as effective in stimulating germination of *S. cepivorum* sclerotia in the field as they are in a controlled enviroment and indicates the need to perhaps increase doses of these products or attempt sealing of the soil surface by rolling.

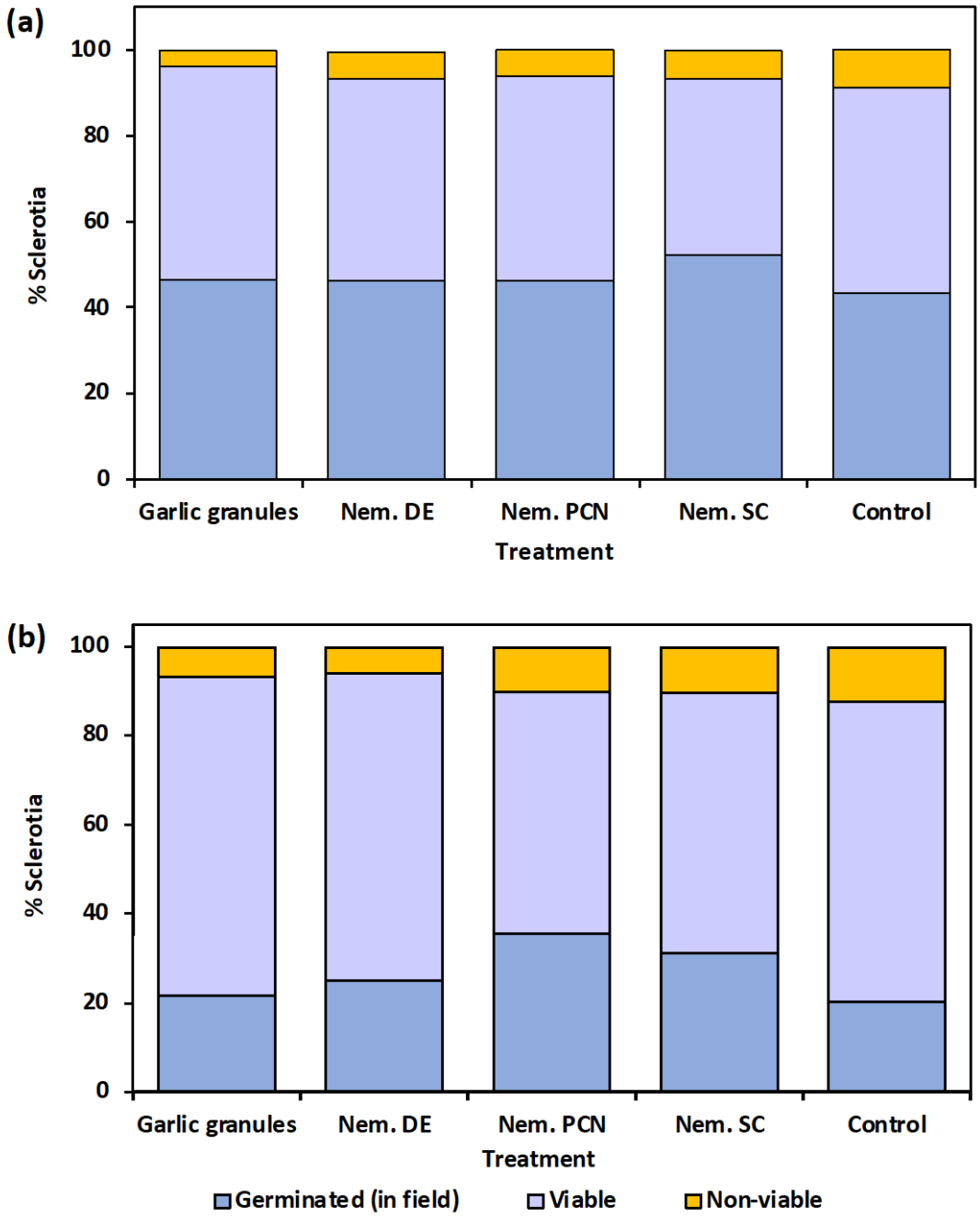

Figure 6. Effect of garlic products on the germination of *S. cepivorum* sclerotia (isolate GS1) in field experiments carried out a) Oct-Dec 2020 and b) May-July 2021 as measured by non-recovery (blue bars) and on viability of remaining ungerminated sclerotia; viable (purple bars) and non-viable (yellow bars). Values represent means of five replicate plots per treatment each containing three bags of 30 sclerotia.

Table 13. Final percentages of germinated *S. cepivorum* sclerotia (isolate GS1), measured by non-recovery, following treatment with garlic products in field experiments in Oct-Dec 2020 and May-July 2021. Percentages of viable and non-viable sclerotia as determined in subsequent viability testing are also presented. Values represent means of five replicate plots per treatment each containing three bags of 30 sclerotia.

Treatment	% Germinated[1]	% Viable[2]	% Non-viable[3]	% Eliminated[4]
Field experiment 2020				
Garlic granules	46.67	49.36	3.80	50.47
NEMguard DE	46.22	46.91	6.24	52.46
NEMguard PCN	46.22	47.52	6.22	52.44
NEMguard SC	52.22	40.86	6.71	58.94
Untreated control	43.56	47.56	8.89	52.44
Field experiment 2021				
Garlic granules	21.78	71.47	6.69	28.47
NEMguard DE	25.11	69.04	5.78	30.89
NEMguard PCN	35.56	54.35	10.03	45.59
NEMguard SC	31.11	58.49	10.24	41.35
Untreated control	20.22	67.53	12.14	32.36

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (30) x 100
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated)resulting in mycelial growth in viability assay / (30 – no. contaminated and lost during sterilisation process) x 100
[3] % Non-viable = no. sclerotia without mycelial growth in viability assay / (30 – no. contaminated and lost during sterilisation process) x 100
[4] % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)
N.B. The percentage of sclerotia that were lost from any one plot during the viability sterilisation process was in the range of 0-3% (2020 and 2021)

Table 14. Logit transformed values and indication of significant differences from untreated control for garlic products following ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolate GS1 for the 2020 and 2021 field experiments.

Treatment	2020		2021	
	% Germinated[1]	% Viable[2]	% Germinated[1]	% Viable[2]
Garlic granules	-0.12	-0.04	-1.39	1.00
NEMguard DE	-0.14	-0.12	-1.13	0.88
NEMguard PCN	-0.16	-0.09	-0.64	0.13
NEMguard SC	0.11	-0.41	-0.84	0.35
Untreated	-0.29	-0.10	-1.57	0.99
LSD	0.72	0.64	0.78	0.94
Treatments that are significantly different from untreated control (P<0.05)				
Treatments that are not significantly different from untreated control				

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (30) x 100 (logit transformed values)
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (30 – no. contaminated and lost during sterilisation process) x 100 (logit transformed values).

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

Materials and methods

Soil-based box assays with *S. cepivorum* sclerotia

The soil box assay described in Objective 2 was adapted to test the effects of selected biofumigant plants on the viability of *S. cepivorum* sclerotia. A range of *Brassica* spp. were grown in the glasshouse, harvested at the start of flowering and the foliage dried and milled as described fully in the annual project report 2020. Levels of the main GLS produced in each crop were quantified by HPLC as detailed in the annual project report 2020. Nylon mesh bags containing 50 conditioned *S. cepivorum* sclerotia of isolate WRAR13 were prepared as described in Objective 2. Four selected biofumigants with high GLS content were selected and appropriate amounts combined with soil (Soakwaters, Wellesbourne) to achieve a standardised GLS concentration of 100 µmol per soil box (Table 15). Each soil box was prepared by first dispensing 200 g of the soil/biofumigant mix into the box and then adding an appropriate volume of SDW to adjust the moisture content of the soil component to 17% and that of the biofumigant component to 75%. A nylon mesh bag containing the *S. cepivorum* sclerotia was then immediately placed onto the damp soil/biofumigant layer and then the remaining 200 g of soil/biofumigant added and wetted to achieve the same moisture content. Untreated control boxes were set up with unamended soil. Boxes were sealed immediately, placed inside individual plastic gripper bags and incubated at 15°C for 8 weeks in the dark. Retrieval of intact sclerotia and subsequent viability testing was carried out as described in Objective 2. There were three replicate boxes of 50 *S. cepivorum* sclerotia for each of the four biofumigant treatments and two separate experiments were carried out.

Calculations and statistical analyses for soil-based box assays

As outlined in Objective 2, sclerotia can be lost due to their small size in the surface sterilisation process. The following calculations were made to take account of this loss:

1) % Germinated = no. sclerotia germinated (as measured by non-recovery) / 50 x 100
2) % Viable = no. sclerotia showing mycelial growth in viability assay / (50 – no. lost during sterilisation process) x 100
3) % Non-viable = no. sclerotia without mycelial growth in viability assay / (50 – no. lost during sterilisation process) x 100
4) % Eliminated = % sclerotia eliminated through germination or through direct effect on viability

Percentage data from the soil box assays above was combined and logit-transformed before being subjected to ANOVA analyses to determine levels of significance. Least significant differences (LSD) calculated to enable comparison of treatments with the untreated control.

Table 15. Biofumigants tested for effects on germination of *S. cepivorum* sclerotia in the modified soil-based assay.

Biofumigant species	Cultivar	Glucosinolate	Glucosinolate conc. (µmol g^{-1} dry wt.)	Amount (g) for 100 µmol per box
Raphanus sativus	Bento	Glucoraphanin	10.0	10.0
Sinapis alba	Brisant	Sinalbin	5.4	18.5
Brassica juncea	Caliente 199	Sinigrin	8.3	12.0
Brassica juncea	Rojo	Sinigrin	4.1	24.4

Fusarium oxysporum f.sp. *cepae* (FOC) seedling assay

Initial work was conducted in 2019 to identify a level of FOC inoculum that consistently induced damping-off disease symptoms in an onion seedling bioassay (see annual project report 2020). This assay was then used to examine the efficacy of four selected biofumigants (as used for the *S. cepivorum* assay above) in reducing the level of FOC inoculum in artificially infested compost. FOC inoculum was prepared as described by Taylor *et al*. (2013) by adding five 5 mm agar plugs taken from an actively growing culture of FOC (isolate FUS2) to a sterile wheat bran / Levington M2 compost mix in 1 L flasks and incubating in the dark at 25°C for 4-8 weeks. Colony forming units (CFUs) were then quantified as a measure of spore concentration by series dilution onto PDA amended with chlortetracycline (20 mg L^{-1}). Mixtures consisting of FOC inoculum, milled biofumigant and Levington FS2 compost were prepared in large polythene gripper bags (30 x 30 cm) to a total weight of 1 kg (Table 16). The FOC inoculum was added to achieve a final concentration of $5x10^5$ CFUs g^{-1} while the amount of biofumigant incorporated was adjusted such that the same concentration of GLS compound was achieved as for the *S. cepivorum* (100 µmol per soil box) in the equivalent volume of F2S compost (volume of 400 g Soakwaters soil = 146 g F2S compost). Finally, SDW was added to adjust the moisture content of the compost component to 70% and that of the biofumigant to 75%. After adding the water, bags were quickly sealed to trap any ITCs released from the immediate hydrolysis of the GLS compounds and incubated for 4 weeks at 20°C in the dark. Bags of compost for control treatments consisting of either FOC inoculum and compost only or compost only were also prepared.

Table 16. Biofumigants tested for effects on FOC inoculum level in infected compost.

Biofumigant species	Cultivar	GLS	Glucosinolate conc. (µmol g^{-1} dry wt.)	Amount (g) for 1 kg FOC compost
Raphanus sativus	Bento	Glucoraphanin	10.03	68.5
Sinapis alba	Brisant	Sinalbin	5.4	126.7
Brassica juncea	Caliente 199	Sinigrin	8.3	82.2
Brassica juncea	Rojo	Sinigrin	4.1	167.1

Following the incubation period, a small amount of each biofumigant/compost mixture was retained in order to determine if any treatments had reduced FOC CFU counts through serial dilution onto a *Fusarium*-selective medium consisting of PDA amended with malachite green (2.5 mg L^{-1}). The bulk of each biofumigant/compost mixture was then used to fill four 30-cell module trays such that each individual cell contained approximately 11 g of mixture (equating to ~5 µmol GLS/cell). Onion seed (cv. Hytech) was then sown into modules which were then placed in trays lined with capillary matting and arranged in a randomised block design in a controlled environment room at 20°C (16 h day length). The compost mix was kept moist by watering from below via the matting. Germination (recorded as emergence to reach loop stage) and (post-emergence) damping-off due to FOC was assessed twice weekly for 34 days. In order to capture both effects of pre- and post-emergence damping off due to FOC, the percentage of healthy seedlings surviving at each time point was calculated for each module tray using the equation below:

(Cumulative no. of germinated seeds – cumulative no. seedlings damped off) / 30 x 100.

Calculations and statistical analyses for FOC seedling assay

The mean percentage of surviving onion seedlings was calculated for the 16- and 34-day timepoints for each treatment and ANOVA carried out following logit transformation of the data to determine levels of significance. Least significant differences (LSD) were calculated to enable comparison of treatments with the untreated control.

Results

Soil-based box assays with *S. cepivorum*

The results from the two soil-based box experiments carried out to evaluate the effects of different biofumigants on *S. cepivorum* sclerotia from isolate WRAR13 were combined. The expectation was that biofumigants would have a direct toxic effect on the sclerotia by reducing their viability as assessed by growth on agar. However, it was clear when *S. cepivorum* sclerotia were recovered from the boxes that many were not intact, suggesting that they had germinated. Germination of sclerotia (as measured by non-recovery) was significantly increased ($P<0.001$) for Brisant, Bento and Caliente 199 biofumigant treatments compared to the untreated control with levels of 31, 53 and 61%, respectively (Fig. 7, blue bars, Tables 17 and 18). In contrast, germination of sclerotia from soil treated with Rojo was only 2%. Although Rojo had no significant effect on sclerotial germination, there was evidence of a direct toxic effect, as 45% were unviable and 41% remained viable which was significantly less ($P<0.001$) than the 98% viability observed for the untreated control (Fig. 7, Tables 17 and 18). In addition, it was also noted that mycelial growth from viable sclerotia treated with Rojo was impeded, as hyphae were far slower to emerge than those growing from healthy untreated sclerotia. The percentage of viable sclerotia remaining in soil amended with Brisant, Bento and Caliente 199 biofumigants was also significantly reduced compared with the untreated control (23-41% viable; $P<0.001$) but for Bento and Caliente 199 treatments, this was due to the fact that there were few remaining sclerotia as very high levels of sclerotial germination was observed for these two treatments. However, as well as inducing germination, Brisant appeared to have a direct toxic effect on *S. cepivorum* sclerotia as 20% of sclerotia were non-viable (Fig. 7, Table 17). Overall, the proportion of sclerotia eliminated by the biofumigant treatments either via stimulating germination or by toxic effects was in the range of 47-64% (Table 17).

Incorporation of the biofumigants in some cases also resulted in extensive growth of other colonising fungi as mycelial growth was clearly observed on the surface of the soil following the incubation period, particularly in Brisant and Rojo treatments (Fig. 8). This may have contributed to the fact that a proportion of all sclerotia in the biofumigant treatments were contaminated in the viability assay (6 to 12%), despite the surface sterilisation steps employed in the protocol (Fig. 7, green bars, Table 17).

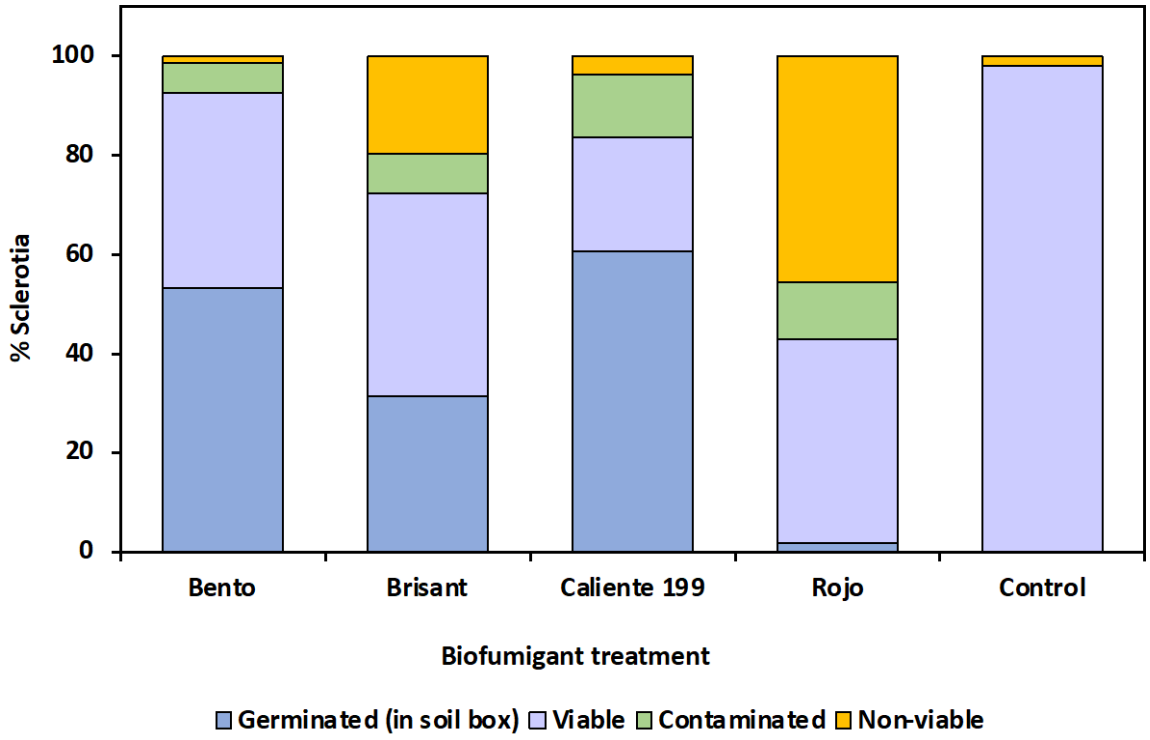

Figure 7. Effect of biofumigant treatments on the germination of *S. cepivorum* sclerotia (isolate WRAR13) in a soil-based assay as measured by non-recovery (blue bars) and on viability of remaining ungerminated sclerotia; viable (purple bars) and non-viable (yellow bars) in a soil box test. The proportions of contaminated sclerotia in the viability assay are also shown (green bars). Data from two combined experiments with a total of six replicate soil boxes per treatment each containing 50 sclerotia.

Table 17. Final percentage of sclerotia (isolate WRAR13) that showed germination, measured by non-recovery, following treatment with biofumigants in a soil-based assay. Percentages of viable and non-viable sclerotia as determined in subsequent viability testing are also presented as well as the percentages eliminated (either by germination or non-viability) and contaminated in the viability assay. Data represent mean values calculated from two combined experiments with a total of six replicate soil boxes per treatment each containing 50 sclerotia.

Treatment	Glucosinolate	% Germinated[1]	% Viable[2]	% Contaminated[3]	% Non-viable[4]	% Eliminated[5]
Bento	Glucoraphanin	53.38	39.27	6.00	1.35	54.73
Brisant	Sinalbin	31.33	41.00	8.00	19.67	51.00
Caliente 199	Sinigrin	60.67	23.00	12.67	3.67	64.33
Rojo	Sinigrin	1.69	41.22	11.56	45.52	47.22
Control	None	0.00	98.00	0.00	2.00	2.00

[1] % Germinated = no. sclerotia germinated (measured by non-recovery) / (50) x 100
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[3] % Non-viable = no. sclerotia without mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100
[4] % Contaminated = no. sclerotia with fungal contamination in viability assay / (50 – no sclerotia lost during sterilisation process)
[5] % Eliminated = % germinated sclerotia + % non-viable (sclerotia eliminated through germination or through a direct effect on viability)
N.B. The percentage of sclerotia that were lost during the viability sterilisation process was in the range of 0-2%

Table 18. Logit transformed values and indication of significant differences from untreated control for biofumigants following ANOVA for percentage germination and viability of *S. cepivorum* sclerotia isolate WRAR13.

Treatment	Glucosinolate	% Germinated[1]	% Viable[2]
Bento	Glucoraphanin	0.27	-0.71
Brisant	Sinalbin	-1.49	-0.57
Caliente 199	Sinigrin	0.48	-1.28
Rojo	Sinigrin	-3.43	-0.46
Untreated control	None	-3.93	3.41
LSD		2.20	1.60
Treatments that are significantly different from untreated control (P<0.001)			
Treatments that are not significantly different from untreated control			

[1] % Germinated = no. sclerotia germinated (as measured by non-recovery) / (50) x 100 (logit transformed values)
[2] % Viable = no. sclerotia (remaining in mesh bag, not germinated) resulting in mycelial growth in viability assay / (50 – no. contaminated and lost during sterilisation process) x 100 (logit transformed values).

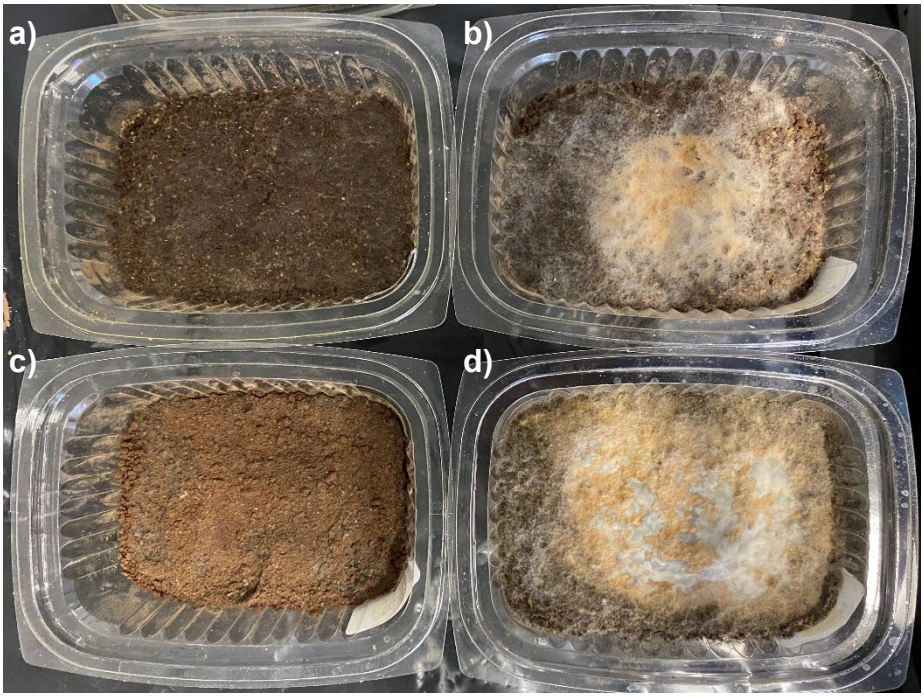

Figure 8. Soil box assay to test the effects of biofumigant treatments on the germination and viability of *S. cepivorum* sclerotia. Soil treated with biofumigants led to mycelial growth of other colonising fungi during the 8-week incubation period. Low fungal colonisation was observed in soil treated with a) Caliente 199 and c) Bento biofumigants but extensive colonisation was apparent for b) Brisant and d) Rojo biofumigants.

Fusarium oxysporum f.sp. *cepae* (FOC) seedling assay

Upon addition of water to the compost/biofumigant mixtures to adjust moisture content at the start of the experiment, the level of FOC inoculum was slightly diluted from $5x10^5$ to $3x10^5$ CFUs g^{-1}. Following incubation for four weeks at 20ºC, the number of CFU g^{-1} was reduced slightly in the FOC only control to $1x10^4$ CFU g^{-1} while the levels for Caliente 199, Rojo and Brisant were slightly less, varying between $3x10^3$ and $7x10^3$ CFU g^{-1} (Fig. 9). The remaining treatment Bento was the only biofumigant to substantially reduce FOC inoculum to a $4x10^2$ CFU g^{-1}.

Artificial inoculation of compost with FOC at a concentration of $5x10^5$ CFU g^{-1} was successful in causing high levels of pre- and post-emergence seedling damping off in the onion seedling bioassay. In the inoculated control treatment, there was considerable pre-emergence damping-off with a maximum seedling emergence of only 18% at 10 days, after which seedling survival declined such that 30 days post-inoculation, no healthy onion seedlings remained (Fig. 10, Table 19). In contrast in the uninoculated control, 75% of seedlings were healthy at the end of the experiment. Interestingly, onion seedlings emerged quickly in FOC-inoculated compost treated with Caliente 199 and Brisant

biofumigants, as respective levels of 74 & 77% healthy seedlings were recorded at 10 days post-inoculation compared to 21% in the uninoculated control (Fig. 10, Table 19). After 16 days, there was a significantly greater proportion of heathy onion seedlings (P>0.001) in all treatments relative to the FOC-inoculated control (Fig. 9, Tables 19 and 20). However, the proportion of healthy seedlings then rapidly declined in the Brisant treatment due to post-emergence damping off, and complete seedling mortality was observed by 24 days (Figs.10 and 11,Table 19). The percentage of healthy seedlings only reached 38% in FOC-infected compost amended with Rojo after 13 days, and post-emergence damping off also rapidly occurred such that there were no surviving seedlings after 24 days.

In contrast to the other two biofumigants, both Bento (which substantially reduced FOC inoculum as measured by CFUs) and Caliente 199 treatments resulted in a significantly greater percentage of healthy seedlings remaining at the end of the experiment after 34 days compared with the FOC-inoculated control (P<0.001, Figs. 10 and 11, Tables 19 and 20) although this was still very low (14 and 7.5% respectively) compared with the uninoculated control (76%). In contrast to Caliente 199, onion seedlings were slow to emerge in FOC-infested compost treated with Bento and the proportion of healthy seedlings only reached a maximum of 35% at 16 days compared to 60% in the uninoculated control and 40% for Caliente 199 at this time point.

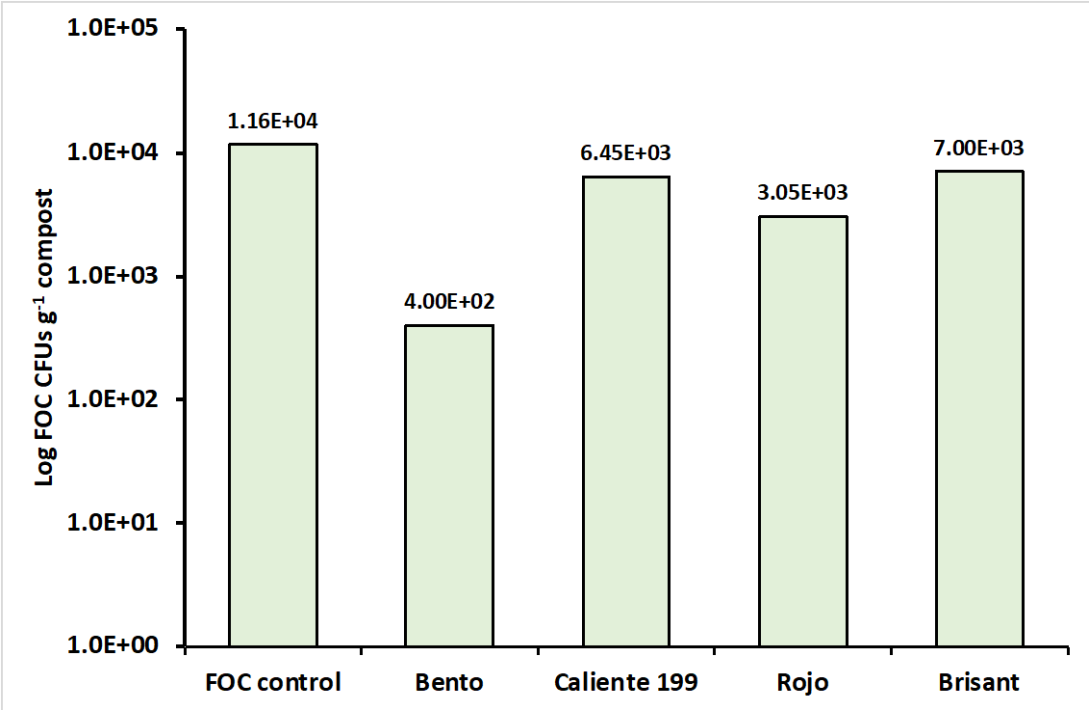

Figure 9. Effect of four biofumigant treatments on FOC inoculum level after incubation for 4 weeks at 20ºC.

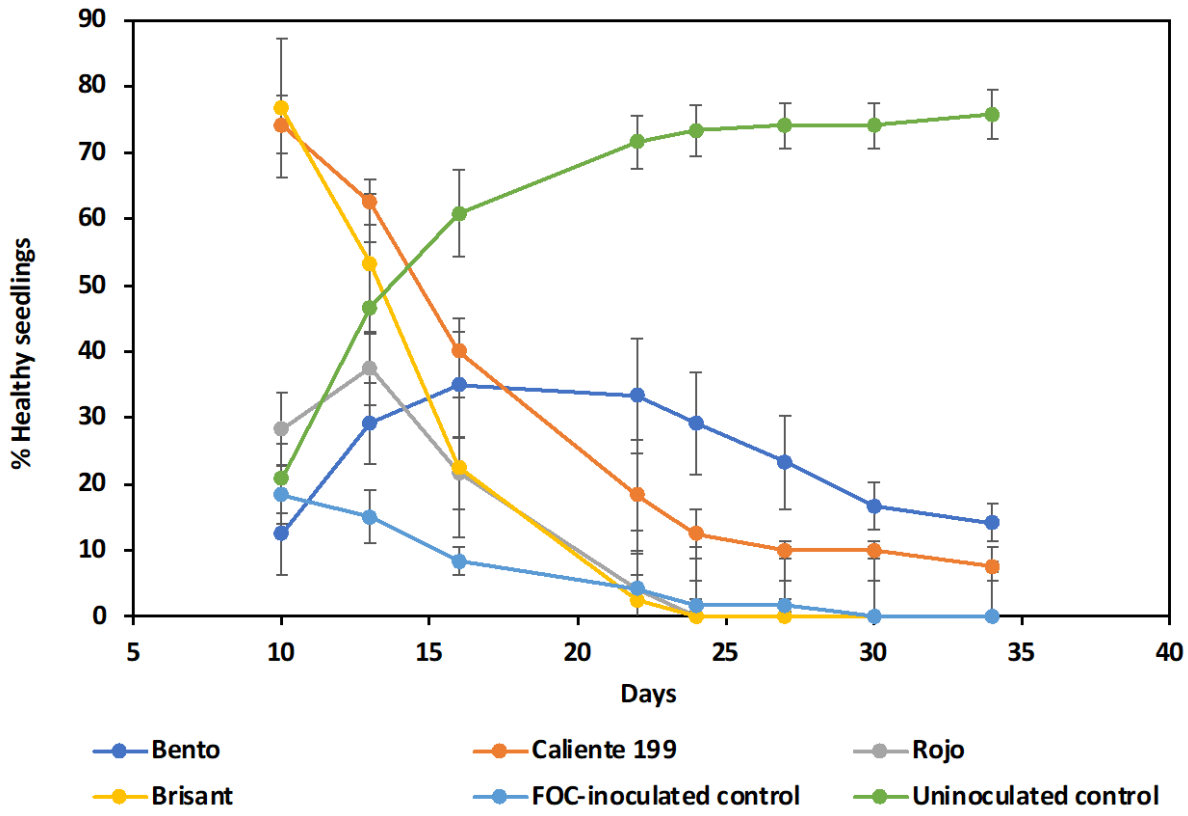

Figure. 10. Effect of four biofumigant treatments on onion seedling survival in compost infested with FOC. Data are means of four replicate seedling trays, each containing 30 seedlings. Error bars are standard error of the mean.

Table 19. Effect of four biofumigant treatments on percentage onion seedling survival in compost infested with FOC. Data are mean percentage values calculated from four replicate seedling trays, each containing 30 seedlings.

Days	Bento	Caliente 199	Rojo	Brisant	FOC control	Uninoculated control
10	12.50	74.17	28.33	76.67	18.33	20.83
13	29.17	62.50	37.50	53.33	15.00	46.67
16	35.00	40.00	21.67	22.50	8.33	60.83
22	33.33	18.33	4.17	2.50	4.17	71.67
24	29.17	12.50	0.00	0.00	1.67	73.33
27	23.33	10.00	0.00	0.00	1.67	74.17
30	16.67	10.00	0.00	0.00	0.00	74.17
34	14.17	7.50	0.00	0.00	0.00	75.83

Table 20. Logit transformed values and indication of significant differences from untreated control for biofumigants for percentage of healthy seedlings remaining in FOC-inoculated compost after 16 and 34 days.

Treatment	% Healthy seedlings remaining	
	16 Days	34 Days
Bento	-0.632	-1.674
Caliente 199	-0.392	-2.188
Caliente Rojo	-1.239	-3.434
Brisant	-1.165	-3.434
FOC-inoculated control	-2.152	-3.434
Uninoculated control	0.436	1.081
LSD (Treatment vs FOC-inoculated control)	0.6828	0.3819
Treatments that are significantly different from the FOC-inoculated control (P<0.001)		
Treatments that are not significantly different from the FOC-inoculated control		

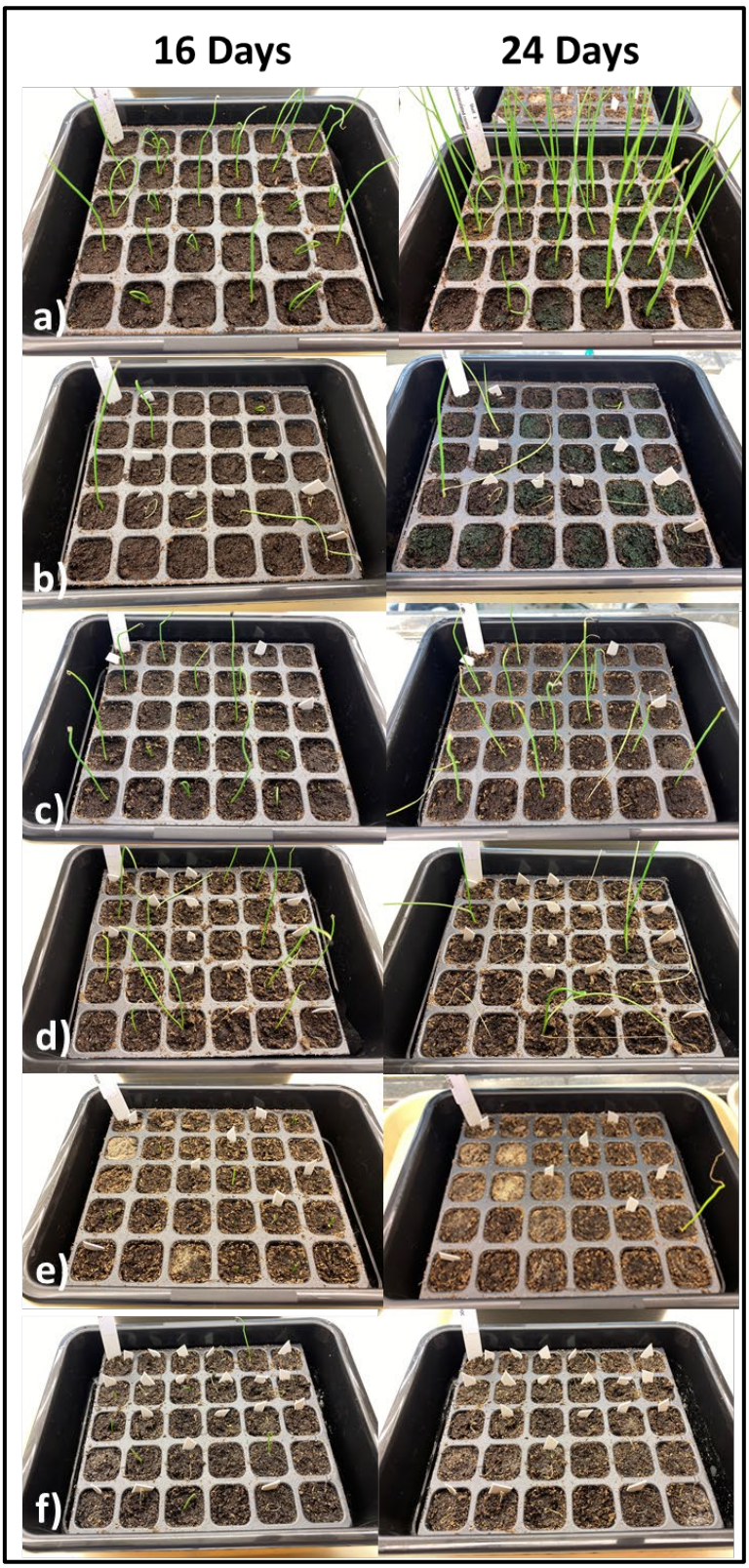

Figure 11. Effect of four biofumigant treatments on onion seedling germination and survival in compost inoculated with FOC at 16 and 24 days post-inoculation for a) uninoculated control, b) FOC-inoculated compost, c) Bento, d) Caliente 199, e) Rojo and f) Brisant

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

Materials and methods and results

This objective was completed in Year 3 and full results are described in full in the project annual report published in 2021. In summary, two field trials located at Stareton (Warwickshire) and Ely (Cambridgeshire) tested combinations of garlic products with fungicides and biological control agents in comparison with individual treatments (Table 21) for their effect on AWR disease in salad onions. Applications (depending on treatment) were made at label rates as banded or whole bed sprays at T-1 (bed-forming), T0 (pre-drilling), T1 emergence or T2 (3-4 leaves). 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 resulted in a significant reduction in disease with less than 4% plants affected ($P<0.001$; Table 21). 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 was the most effective treatment, significantly reducing disease to only 3.0% ($P<0.05$). All NEMguard SC treatments also significantly reduced AWR disease compared to the untreated control. Treatments with Perseus, Luna Privilege and NEMguard DE with Signum also significantly reduced AWR to 5.6, 6.6 and 9.8%, respectively. Across both sites therefore, NEMguard SC either alone or in combination with Signum or Trisoil significantly reduced white rot disease while the use of NEMguard DE 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.

Table 21. 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. Cells highlighted in green indicate treatments resulting in a significant reduction in AWR compared to the untreated control.

	Treatment	**Ely**	**Stareton**
1	Signum T1 + T2 (banded)	11.9 e	20.4 cde
2	Signum T1 + T2 (whole bed)	9.02 de	19.7 bcde
3	Perseus T1 + T2 (whole bed)	5.79 abcd	5.6 ab
4	Luna Privilege T0	7.57 bcde	6.6 abc
5	NEMguard DE T-1	9.56 de	21.3 de
6	NEMguard DE T-1 with Signum T1 + T2 (whole bed)	8.15 cde	9.8 abcd
7	NEMguard DE T-1 with Trisoil T0 + T1 (banded)	8.69 cde	23.5 de
8	NEMguard SC T-1	2.47 ab	13.4 abcd
9	NEMguard SC T-1 with Signum T1 + T2 (whole bed)	1.81 a	3.0 a
10	NEMguard SC T-1 with Trisoil T0 + T1 (banded)	3.51 abc	11.4 abcd
11	Trisoil T0 + T1	11.2 de	20.6 cde
12	Untreated control	10.98 de	28.4 e
F-value		*<0.001*	*0.09*
d.f.		*44*	*43*
S.e.d.		*2.407*	*6.30*
L.s.d.		*4.852*	*12.71*

Discussion

Objective 1: Test fungicides and biological control agents for their effect on *Allium* white rot disease and generate preliminary data on effect of selected products on *Fusarium* basal rot

Management of white rot in the UK with fungicides is difficult due to the small number of approved active substances available through the HSE EAMU scheme. Whilst a range of crop protection products have shown promise elsewhere (Villalta *et al.*, 2004, 2010; Ferry-Abee, 2014), few have been examined previously under UK production systems and conditions.

At the field trial at Wellesbourne in 2018 where products were applied as a concentrated band, several fungicides based on SDHI and DMI chemistry significantly reduced disease incidence (Luna Privilege (HDC F246), Signum (BAS 516 07F) and Perseus (HDC F247)) and hence there were good levels of AWR control, with single or double applications proving to be similarly effective except for Luna Privilege where two applications significantly improved control. SDHI chemistry has shown to be effective against AWR disease in other work (Villalta *et al.*, 2010; Ferry-Abee, 2014) while tebuconazole, a commonly used DMI group fungicide has also been shown to be highly effective in the management of white rot (Clarkson *et al*., 2016) and was presvoiusly a major control option for UK growers. In comparison to the conventional fungicides, the biological control agents proved ineffective in controlling AWR disease. Variation in performance of BCAs is common as reported by Noble (2013) and this work reported Prestop as being effective, which was not observed in this study. In summary, fungicides applied once or twice early in crop development gave good levels of AWR control but these were applied at a much higher than recommended application rate due to the concentrated nature of band application as proof of concept. This approach was taken based on evidence in literature that high rates of are required to achieve good control under high disease pressure, with large application volumes to ensure penetration below the soil surface and into the root zone. As this type of use would not be supported commercially, the approach in the year 2 field trials was through whole plot applications at the recommended label rate. Unfortunately, this did not result in any significant reductions in disease.

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

Sclerotium cepivorum sclerotia are constitutively dormant (i.e. will not germinate even in the presence of an appropriate stimulus) for weeks to months after forming but following a 'conditioning' period in they will germinate in response to specific compounds in *Allium* root exudates, principally di-allyl sulphide (DAS) and di-allyl disulphide (DADS) (Coley-Smith and King, 1969). Soil temperature and moisture are key factors affecting both sclerotial germination and subsequent infection with temperatures of 14-18ºC and moist soil at -30 kPa being optimum (little activity < 9ºC or > 24ºC; <-100 kPa or in very wet soils; Crowe and Hall, 1980). Soil inoculum can therefore be reduced if *S. cepivorum* sclerotia can be stimulated to germinate by *Allium* (principally garlic) based products in the absence of host plants as they exhaust their nutrient reserves in the absence of a host. Natural *Allium* products and synthetic germination stimulants such as diallyl disulphide (DADS) have been extensively researched for AWR disease control and have provided near eradication of sclerotia in infested soil when applied during periods of conducive temperatures (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) found that 0.5ml/m^2 DADS reduced sclerotial inoculum density in field trials by ~90% while the same study also showed that garlic powder applied at rates of 112 kg/ha and 224 kg/ha were able to achieve similar reductions in inoculum density. Ferry-Abee (2014) also found DADS to be effective at controlling white rot disease in a naturally infested field, reducing the number of sclerotia by up to 80% and increasing marketable yield by 40% (Table 3) while garlic oil reduced numbers of sclerotia by 50%. Other *Allium* products such as garlic granules and 'garlic juice' may also have potential as stimulants of sclerotial germination as highlighted by Villalta *et al*. (2005). A commercial product Alli-Up was risk assessed by the USA Environmental Protection Agency (EPA) in 2003 for use in AWR disease management by direct soil injection. However, this product increased in price and subsequently was withdrawn from the market (Ferry-Abee, 2014).

The development of formulated garlic-based products by Ecospray in the UK, principally for control of nematodes therefore presented an opportunity for control of AWR disease control and hence in this project experiments were carried out to assess the effects of different commercially available garlic products on germination of *S. cepivorum* sclerotia in Petri-dish assays, soil-based box tests and in small field plots. In Petri-dish assays, all garlic products resulted in very high levels of sclerotial germination with NEMguard SC and NEMguard DE particularly effective while an experimental product PK02 did not stimulate germination and resulted in a reduction in viability of sclerotia. The soil-based box tests confirmed that the same garlic products had a similar effect in soil (although results were more variable) and again NEMguard SC and NEMguard DE generally resulted in good

levels of sclerotial germination. Although PK02 did not stimulate germination in the first two soil box tests this did occur in the third which was most likely due to a slight adjustment of dose in this last test. When experiments were carried out in small field plots with selected garlic products over two years (in the absence of a host), the products NEMguard SC and NEMguard PCN only slightly increased germination compared to an untreated control.

The different NEMguard products were all shown to stimulate germination of *S. cepivorum* sclerotia fairly consistently *in vitro* and this is to a certain extent expected as although the products are formulated differently, they contain the same concentrations of polysulphides (45% w/w). The use of food grade garlic granules resulted in less consistent germination and this is likely as a 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 often resulted in little or no germination of *S. cepivorum* sclerotia and this is attributed to the very high concentrations of polysulphides formulated in this experimental product which are inhibitory to germination.

There was some variation in results within and between the different Petri-dish and soil box assays and this can be attributed to a number of factors including the use of different isolates and batches of *S. cepivorum* sclerotia (which may vary in their ability to germinate depending on their 'conditioning' status) or slight variation in experimental conditions. The soil box tests also used an indirect method of quantifying germinated sclerotia where non-recovery was assumed to be directly associated with germination. However, non-recovery of sclerotia could also have been due to microbial degradation (as the soil used was not sterile) and in addition, germinating *S. cepivorum* sclerotia may be more liable to microbial colonisation. Despite microbial degradation or contamination of sclerotia potentially confounding the measurement of germination in these tests, 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.

The lack of good efficacy of NEMguard SC and NEMguard PCN in stimulating germination of *S. cepivorum* sclerotia in small field plots is disappointing and suggests that the products may not be as effective in the field as they are in a controlled enviroment and indicates the need to perhaps increase application rates of these products or attempt sealing of the soil surface by rolling to prevent loss of volatile *Allium* compounds. In addition, soil-type has been shown to affect efficacy of garlic-based products both on *S. cepivorum* and nematodes (Hovius and McDonald, 2002; Eder *et al*., 2021)

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

Certain crops, particularly *Brassica* spp., have biofumigation effects due to the conversion of glucosinolates to isothiocyanates (ITCs) following maceration and incorporation into the soil. There are very few studies which have examined biofumigation for control of AWR disease although Smolinska (2000) demonstrated that *B. juncea* and *B. napus* residues reduced the number of *S. cepivorum* sclerotia recovered from soil as well their viability. However, the nutrient content of soil being amended with the plant residues appeared to affect their efficacy, with a reduced effect in a more nutrient rich soil. Incorporation of cruciferous residues also led to a 'sharp increase' in the level of soil microorganisms present in the system within one month of application and this was suggested as another mechanism by which sclerotia were reduced in number. Villalta *et al*. (2010) also found that treatment with a mustard oil product (Voom®) killed mycelium and sclerotia of *S. cepivorum* but when this product was applied in the field via shank-injection, only ~25% reduction in disease incidence was observed.

Therefore, preliminary work in this project assessed the potential of biofumigation as a useful component of an integrated control strategy for AWR disease. Four biofumigant plants cv. Caliente 199, cv. Rojo (both brown mustard, *Brassica juncea*), cv Brisant (white mustard, *Sinapis alba*) and Bento (radish, *Raphanus sativus*) were evaluated for their ability to reduce the viability of *S. cepivorum* sclerotia in soil box tests using a similar approach comparable to that used in the garlic product soil-based box assay. Unexpectedly, non-recovery of sclerotia from three of the biofumigants, Bento, Brisant and Caliente 199 was high suggesting that germination had been stimulated while in contrast Rojo elicited a low level of sclerotial germination but reduced viability. These results suggest that some biofumigants may stimulate germination of sclerotia while others might directly reduce viability although both these modes of action are potentially useful in reducing the numbers of *S. cepivorum* in soil, hence contributing to disease control. The potential different modes of action of biofumigants merits further investigation especially in relation to stimulation of sclerotial germination which has not been previously reported; it could be that additional compounds as well as ITCs are released from biofumigants which may stimulate this response. For instance, dimethyl disulphide (DMDS) can also be generated through the breakdown of *Allium* tissues (Arnault *et al*., 2013) which could have a stimulatory or toxic effect on *S. cepivorum* depending on concentration. Other modes of action such as promoting an increased in microbial activity as mentioned above could also be a factor. Finally, Caliente 199 and Bento treatments has some effect on FOC, suggesting that biofumigants may be useful in reducing inoculum and combating both AWR disease and basal rot in onion.

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. 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 one field 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

- Commercial garlic products, in particular NEMguard DC and NEMguard SC, effectively stimulated germination of *S. cepivorum* sclerotia in lab-based controlled environment experiments but were less effective in a small field trial. Increased rates and/or optimised application may improve efficacy.
- Biofumigants cv. Bento, Brisant and Caliente 199 stimulated germination of *S. cepivorum* sclerotia in lab-based controlled environment experiments.
- Biofumigant plants cv. Caliente 199 and Bento reduced disease due to *Fusarium* in a seedling assay.
- NEMguard SC alone or in combination with the fungicide Signum reduced AWR disease in two field trials while the fungicides Perseus and Luna Privilege also demonstrated activity in one field trial.

- Overall selected garlic products, biofumigants and fungicides demonstrated activity against *S. cepivorum* either by stimulating germination of sclerotia, reducing viability or preventing infection. An integrated control programme incorporating all these elements would enhance management of AWR disease.
- Further work should i) further investigate the potential of biofumigant crops to stimulate germination and / or reduce viability of *S. cepivorum* sclerotia, ii) optimise use of Nemguard garlic products in the field through adjusting rates and / or improving application to maximise polysulphide retention in the soil iii) confirm the value of combining garlic products with effective fungicides.

Knowledge and Technology Transfer

The results contained within the report have been presented annually to agronomists at VCS and *Allium* and *Brassica* Centre and also at the following 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).
- Presentation made at Onion and Carrot Conference 20th November 2019 (John Clarkson)

References

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

Arnault, I., Fleurance, C., Vey, F., Fretay, G.D., and Auger, J. (2013). Use of Alliaceae residues to control soil-borne pathogens. *Industrial Crops and Products*, **49**, 265-272.Clarkson, J.P., Payne, T., Mead, A., and Whipps, J.M. (2002). Selection of fungal biological control agents of *Sclerotium cepivorum* for control of white rot by sclerotial degradation in a UK soil. *Plant Pathology*, **51**, 735-745.

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

Clarkson, J.P., Wedgwood, E., and Hamilton, M. (2016). Onions: Investigation into the control of White Rot in bulb and salad onion crops. AHDB Project Report FV499.

Coley-Smith, J.R. and King, J.E. (1969). The production by species of *Allium* of alkyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum* Berk. *Annals of Applied Biology*, **64**, 289-301.

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

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

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

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

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

Crowe, F., Debons, J., Darnell, T., Thornton, M., McGrath, D., Koepsell, P., Laborde, J., and Redondo, E. (1994). Control of *Allium* White Rot with DADS and Related Products. Proceedings of the 5th International Workshop on *Allium* White Rot. Cordoba, Spain.

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

Eder, R., Consoli, E., Krauss, J., and Dahlin, P. (2021). Polysulfides Applied as Formulated Garlic Extract to Protect Tomato Plants against the Root-Knot Nematode *Meloidogyne incognita*. *Plants*, **10**, 394.

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

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

Esler, G. and Coley-Smith, J.R. (1983). Flavor and odour characteristics of species of *Allium* in relation to their capacity to stimulate germination of sclerotia of *Sclerotium cepivorum*. *Plant Pathology*, **32**, 13-22.

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

Hovius, M.H.Y. and McDonald, M.R. (2002). Management of *Allium* white rot [*Sclerotium cepivorum*] in onions on organic soil with soil-applied diallyl disulfide and di-N-propyl disulfide. *Canadian Journal of Plant Pathology*, **24**, 281-286.

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

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

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

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

Taylor, A., Vagany, V., Barbara, D. J., Thomas, B., Pink, D. A. C., Jones, J. E., and Clarkson, J. P. (2013). Identification of differential resistance to six *Fusarium oxysporum* f. sp. *cepae* isolates in commercial onion cultivars through the development of a rapid seedling assay. *Plant Pathology*, **62**, 103-111.

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

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

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