Grower Summary

Integrated control of Allium white rot

FV 449a

Final report, February 2022

Project title:	Integrated control of Allium white rot
Project number:	FV 449a
Project leader:	John Clarkson, University of Warwick
Report:	Final report, February 2022
Previous report:	February 2021
Key staff:	Nicole Pereira, University of Warwick Sascha Jenkins, University of Warwick James Howell, VCS Erika Wedgewood, ADAS
Location of project:	University of Warwick, Wellesbourne Campus, Warwick.
Industry Representative:	Phil Langley, G's Growers
Date project commenced:	01/03/2018
Date project completed (or expected completion date):	28/02/2022

DISCLAIMER

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

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.

Nicole Pereira
Researcher
Warwick Crop Centre, School of Life Sciences, University of Warwick
Signature: *Nicole Pereira* Date: 17/03/22

John Clarkson
Professor
Warwick Crop Centre, School of Life Sciences, University of Warwick
Signature: *John Clarkson* Date: 17/03/22

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.