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| **Project leader:** | Dr John Clarkson, Warwick Crop Centre, University of Warwick |
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# 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

Potential new treatments and approaches for the control of *Allium* white rot have been identified in a literature review. These include new chemistry, biological control, sclerotial germination stimulants and different methods of soil disinfestation. These approaches should be integrated in subsequent experimental work.

## Background

*Allium* white rot, caused by *Sclerotium cepivorum*, can result in severe crop losses in both bulb and salad onion crops in the UK. Intensive cropping over many years has led to build-up of sclerotial inoculum of the fungus (long-lived resting bodies) in many of the fen and silt soils most suitable for *Allium* crops as well as in other areas. Once a field becomes infested, it is very difficult and costly to continue growing the crop as the sclerotia can survive for at least 20 years. There is therefore an urgent need to identify and develop integrated strategies for effective and long-term management of the disease through reducing the inoculum potential of infested soil, hence facilitating the sustainable production of onion crops.

The main aim of this first phase of the project, was to review the recent research on *Allium* white rot, focussing primarily on physical, chemical and biological approaches to disease control and to assess where possible the efficacy, practicality and cost of different disease management options. Results of this review will be discussed with the British Onion Producers Association (BOPA) and the AHDB in order to identify the most promising, practical and economic treatments to test in laboratory, glasshouse and field experiments as appropriate in a second phase of the project.

## Summary

### *Review method and scope*

A literature search was undertaken to review relevant scientific publications, conference reports, project reports (AHDB and from overseas) and technical information concerning *S. cepivorum* and its control. Published information on related sclerotial pathogens (e.g. *Sclerotium rolfsii* and *Sclerotinia* species) and other soilborne pathogens such as species of *Verticillium* and *Fusarium* was also included where relevant. ADAS carried out the majority of the review with support and editing provided by Warwick. The key topics covered were: biology and epidemiology of *S. cepivorum*, germination of *S. cepivorum* sclerotia, detection and quantification of *S. cepivorum* in soil, chemical control, biological control, sclerotial germination stimulants, soil disinfestation, improving soil health and integrated control.

### Main outcomes of the review

The main approaches for management of *Allium* white rot were identified and associated treatments with potential for further investigation are as follows:

*1. Chemical control*

*Review summary*: tebuconazole has been widely used for white rot control in the UK and in many other parts of the world, but it is clear that alternative chemistry including azoxystrobin, boscalid, penthiopyrad and procymidone has good potential, given recent studies in Australia and USA (77-95% disease reduction). It is also evident that effective control with fungicides depends on the application method, with the most benefit from targeted seedbed sprays just after sowing and follow-up foliar sprays.

*Next steps*: test alternative chemistry using targeted application methods.

*2. Biological control*

*Review summary:* despite extensive research in many different countries, only one microbial product (Tenet) is registered specifically for use against white rot in New Zealand and Australia. However, a recent AHDB project FV 219b demonstrated that some UK registered biocontrol agents such as Serenade ASO (*Bacillus subtilis*) and Prestop (*Gliocladium catenulatum*) may reduce disease (70-77% disease reduction) although results were not always consistent due to variation in inoculum pressure. Since then other products have come onto the market that may also have potential. Again, application method is of key importance with in-furrow treatments being the most effective option for delivering these products to the root zone.

*Next steps:* Test the most effective biological control products available using targeted applications to the root zone.

### 3. Sclerotial germination stimulants

*Review summary: S. cepivorum* sclerotia only germinate in response to sulphur-related chemical compounds released from the roots of *Allium* plants. One of these with a high stimulatory effect is diallyl disulphide (DADS) which has been used to artificially initiate germination in the absence of a host, resulting in up to 80-90% reduction in viable sclerotia. Similarly, composted onion waste has also been used to achieve the same result but application rates and supplies are limited. Currently, there are no longer any commercial producers of DADS but other products based on garlic that are being developed for other crop protection applications, or for the food industry, may be a viable alternative. These include Ecospray products that are available in the UK and which contain actives which may stimulate germination of sclerotia.

*Next steps:* identify commercial sources of *Allium*-based products and test feasibility of use and efficacy.

### *4. Soil disinfestation*

*Review summary:* Sclerotia of *S. cepivorum* can also be killed by other methods including chemical and biofumigation, soil solarisation, steaming, flooding and anaerobic disinfestation. The use of chemical fumigants has now been severely restricted but recently a new product based on dimethyl disulphide (DMDS) aimed at nematode control has also been shown to have activity against *S. rolfsii* and registration is now being sought in the UK. DMDS is present both in composted onion waste and brassica residues and is recognised as a fungitoxic compound and hence may have potential against white rot. Biofumigation through the chopping and incorporation of certain brassica crops (usually mustards or radish) into soil has also received attention for control of soilborne diseases, but remains largely untested against *S. cepivorum*. The main problem of using this approach as a control measure is the cost and logistics of fitting a biofumigant crop into a rotation and most crops will not produce adequate biomass over the winter period. However, a number of biofumigant-derived products such as mustard meals and oils are coming onto the market and may have potential for more immediate application. Of the other soil treatments, anaerobic disinfestation (ASD) also holds some promise. This process involves incorporating an organic feedstock such as a green manure, irrigation and sealing with plastic to initiate anaerobic conditions. ASD has been shown to reduce disease caused by *S. rolfsii* and, while untested for control of white rot, other studies have shown that flooding and high soil moisture conditions reduce viability of *S.*

*cepivorum* sclerotia. Finally, steaming and soil solarisation although potentially effective for white rot control, are probably impractical due to cost and the UK climate, respectively.

*Next steps:* test most promising approaches; biofumigant crop products, DMDS fumigation and ASD.

*5. Improving soil health*

Application of organic amendments such as green manures, manures and composts may have a general beneficial effect on soil health through increased microbial activity and lead to general suppression of soilborne diseases including sclerotial pathogens. However, there is little evidence that this approach is particularly effective for control of *S. cepivorum*.

*Next steps:* although soil health / microbial activity must be maintained to maximise potential disease suppression, this approach is difficult to test in the short-term.

*6. Integrated control*

No single management practice has resulted in complete control of *Allium* white rot, and for that reason, some studies have sought to combine a number of different approaches. Generally, this has resulted in an enhanced level of disease control and notable examples include combinations of biological control agents and fungicides, DADS and fungicides, and biological control agents with green waste.

*Next steps:* develop and test integrated control approach based on individual treatments identified above.

## Financial Benefits

The bulb onion and salad onion sectors at 8,945 ha and 1601 ha respectively, were worth £97M and £24M in 2013 (Defra horticultural statistics, 2014). White rot can greatly reduce marketable yields and cause significant financial losses and it is estimated that a minimum of 2-3% of UK bulb onions are affected annually equating to around £2.9M of lost crop. In salad onions the level of damage is higher at around 10-15% equating to £3.6M. Reduction of these losses by just 50% through development of an integrated control approach would increase the value of bulb and salad onions by £3.3M annually.

*Allium* white rot control needs to be sustainable and remain effective over the medium to long term, which is challenging due to the diminishing availability of effective fungicides/chemical soil sterilants and the absence of other fully effective disease management options. Integrated disease management programmes that combine a wide range of management options, considering soil, seed/set and crop, offer the greatest potential for long-term sustainable control of white rot. Integrated white rot control programmes that restrict the use of chemical fungicides through the adoption of alternatives will help to manage/prevent resistance in the remaining, or new, chemical fungicides.

Integrated control could for example include the use of anaerobic digestion solids in ASD (£125/ha) or germination stimulant (£180/ha) to reduce sclerotial inoculum followed by fungicide (£18/ha) or biological (£288/ha) treatments. The possible overall outlay of around £500 would be financially worthwhile given crop values of over £10,000 per hectare.

Areas of silt and fen soils with high yield potential have been abandoned due to high *Allium* white rot levels and other growing areas are also infested. It is now possible for growers to send soil samples for molecular quantification of *S. cepivorum* inoculum although the value of this in predicting disease levels has yet to be assessed. Adoption of integrated strategies that take account of density of sclerotia and integrated approaches to reduce inoculum and disease may allow some areas to be brought into production and current areas to remain in production.

## Action Points

- Map areas of white rot infested land using crop records and/or the results from soil samples, and ensure farm staff are aware of them.
- Take care that your own, or hired, farm equipment does not bring contaminated soil onto “clean” land.
- Consider preventative use of fungicides (including seed treatment) if there is a white rot risk in the field.
- Be aware that a combination of methods including cultural, biological and chemical controls will be required for the reduction of white rot sclerotia in soil and the prevention of crop infection.

# SCIENCE SECTION

## Introduction

*Allium* white rot, caused by *Sclerotium cepivorum*, can result in severe crop losses in both bulb and salad onion crops in the UK.  Intensive cropping over many years has led to build-up of sclerotial inoculum of the fungus (long-lived resting bodies), in many of the fen and silt soils most suitable for *Allium* crops as well as in other areas. Once a field is infested, it is very difficult and costly to continue growing the crop as the sclerotia can survive for at least 20 years.  There is therefore an urgent need to identify and develop integrated strategies for effective and long-term management of the disease through reducing the inoculum potential of infested soil, hence facilitating the sustainable production of onion crops.

Considerable research effort has been expended worldwide to increase understanding of *Allium* white rot disease and its control. Therefore, the aim of this first phase of the project was to review this research, focussing primarily on physical, chemical and biological approaches to disease control and to assess where possible the efficacy, practicality and cost of different disease management options. Results of this review will be discussed with BOPA and AHDB in order to identify the most promising, practical and economic treatments that are appropriate to UK onion growers and costs then provided by Warwick / ADAS to test these in laboratory, glasshouse and field experiments as appropriate in a second phase of the project. Specific objectives in the first phase were:

1) To critically review recent worldwide research on *Allium* white rot and its control;
2) To identify from the review, the most feasible and promising control treatments for management of *Allium* white rot in the UK and cost of application.

## Materials and methods

A literature search was undertaken principally using Google Scholar and Web of Science to review relevant scientific publications, conference reports, project reports (AHDB and from overseas) and technical information concerning *S. cepivorum* and its control. Effort was focused primarily on publications from 2000 onwards to build on comprehensive reviews published by Coley-Smith (1990) and Entwistle (1990).  Although work was mainly directed toward *S. cepivorum*, published information on related sclerotial pathogens (e.g. *Sclerotium rolfsii* and *Sclerotinia* species) and other soilborne diseases was also included where judged to be relevant to control of *Allium* white rot. The review team aimed to interpret the relevance of information in publications and also to report the degree of efficacy of treatments and *S. cepivorum* inoculum level (disease control/inoculum reduction) and the variability / repeatability of results where available. ADAS carried out the majority of the review with support and editing provided by Warwick. The key topics covered were:

- Biology and epidemiology of *S. cepivorum*
- Germination of *S. cepivorum* sclerotia
- Detection and quantification of *S. cepivorum* in soil
- Chemical control
- Biological control
- Sclerotial germination stimulants
- Soil disinfestation
- Integrated control

## Results: a review of research on *Allium* white rot

### *Introduction*

White rot caused by the soil-borne fungus *Sclerotium cepivorum* is one of the most important and destructive diseases of *Allium* species, with onion and garlic particularly affected. It is commonly a major limiting factor for commercial production particularly where *Allium* crops are grown in cool conditions that are conducive to pathogen growth and reproduction. Losses vary year to year but it is estimated that a minimum of 2-3% of UK bulb onions are affected annually, equating to approximately £2.9M of lost crop. In salad onions, the level of damage is higher at around 10-15% equating to £3.6M (AHDB, project tender document). The development of improved, sustainable methods for management of *Allium* white rot caused by *S. cepivorum* is a priority for the British Onion Producers Association (BOPA).  Many of the fen and silt soils in the eastern and south eastern areas of the UK where bulb and salad onions were traditionally grown, are heavily infested with *S. cepivorum* resting bodies (sclerotia) and because these can survive for at least 20 years (Coley-Smith *et al.*, 1990), it is no longer economic to grow *Allium* crops in these areas. Inoculum densities as low as 0.1 sclerotia/L of soil can cause economic losses with 1 sclerotia/L soil typically causing losses between 30-60% and 10 sclerotia/L soil causing near total crop loss (Davis *et al.*, 2007). Partly as a consequence of this, onion growers have moved to sandier soils which often have lower yield potential and some of these areas are now also infested with *S. cepivorum*. Given this situation, and the trend of reducing industry returns, there is now an increasing need to develop sustainable methods of onion production through effective mitigation of *Allium* white rot.

### *Biology and epidemiology of S. cepivorum*

*Sclerotium cepivorum* can affect plants at any stage of development, causing root collapse and death with foliar symptoms usually only first observed after root infection is well established. Foliage of infected plants turns yellow and then white followed by plant wilting and death (Fig 1). With high levels of soil infestation, plants may die suddenly across large areas of a field; in less infested areas, plants may be affected in smaller patches, with those in the centre dying first (Koike *et al.*, 2007; Crowe, 2008). White rot may continue to cause decay of infected bulbs in storage, and may spread between bulbs if temperature and moisture are adequate. In addition to the plant symptoms, white fluffy mycelium may also be seen on roots and around the base of bulbs, eventually moving upwards and inwards and numerous small (poppy seed-sized) black sclerotia (resting bodies) eventually form in the decaying tissues (Fig. 1). Often, in advanced stages of the disease, the roots and bulbs become soft and rotten due to secondary decay organisms. The fungus can spread in soil along the crop rows to infect neighbouring plants, and further spread across the field or between fields can occur through movement of infested soil and infected plant material, water/irrigation, and high winds.

**Figure 1.** Foliar and stem base symptoms of *Allium* white rot showing yellowing leaves, fluffy white mycelium and formation of sclerotia.

*Sclerotium cepivorum* has no known sexual stage and hence reproduces asexually through production and germination of sclerotia with no dispersal spores (conidia) produced. Sclerotia germinate specifically in response to certain chemical compounds exuded from *Allium* roots resulting in the production of hyphae which grow through the soil and cause direct infection. This is in contrast to the related species *Sclerotium rolfsii* which has a wide host range (including onion), but which is not established in the UK. Isolates of *S. cepivorum* may vary in their ability to infect roots and in the ability of sclerotia to germinate in response to temperature and root exudates. However, genetic variability in *S. cepivorum* has not been well studied although several genetically distinct clones were identified through DNA fingerprinting and mycelial compatibility tests (Couch and Kohn, 2000; Earnshaw *et al.*, 2000). Several contemporary UK isolates of *S. cepivorum* have been collected and are now in the process of being characterised molecularly through sequencing of housekeeping genes (AHDB Horticulture Project CP 113; Clarkson, 2015). Increased knowledge of the genetic diversity of the fungus may help to improve understanding of the biological variation of different isolates.

### ***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 on decayed host plants, although a clear relationship between dormancy and environmental factors has yet to be clearly established. However, dormancy was broken following ‘conditioning’ of sclerotia in soil at 5-15°C after 2-18 weeks (DEFRA, 2009). Following this conditioning period, sclerotia can potentially survive for at least 20 years until *Allium* host plants are grown nearby (Coley-Smith *et al.*, 1990; Crowe *et al.*, 2005).

As indicated previously, *S. cepivorum* sclerotia germinate in response to specific compounds in root exudates such as n-propyl, methyl and allyl cysteine sulphides uniquely produced by the *Allium* genus. Principal amongst these is di-allyl disulphide (DADS) which has a strong stimulatory effect on non-dormant sclerotia resulting in high levels of germination (Coley-Smith and King, 1969). These compounds have been shown to stimulate germination as far as 10 cm away (Coley-Smith, 1960; Entwistle, 1990). The fungal mycelium resulting from sclerotia germination then grows at least 1-2 cm through soil before directly infecting onion roots or the basal plate. 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). Bulb onions that have been established from transplants are thought to be more severely affected than direct seeded crops because of more vigorous root systems that develop earlier in the season and hence encounter more soilborne sclerotia. *S. cepivorum* sclerotia will eventually die under conditions of prolonged soil moisture saturation, the rate of mortality increasing as temperature increases (Crowe and Hall, 1980).

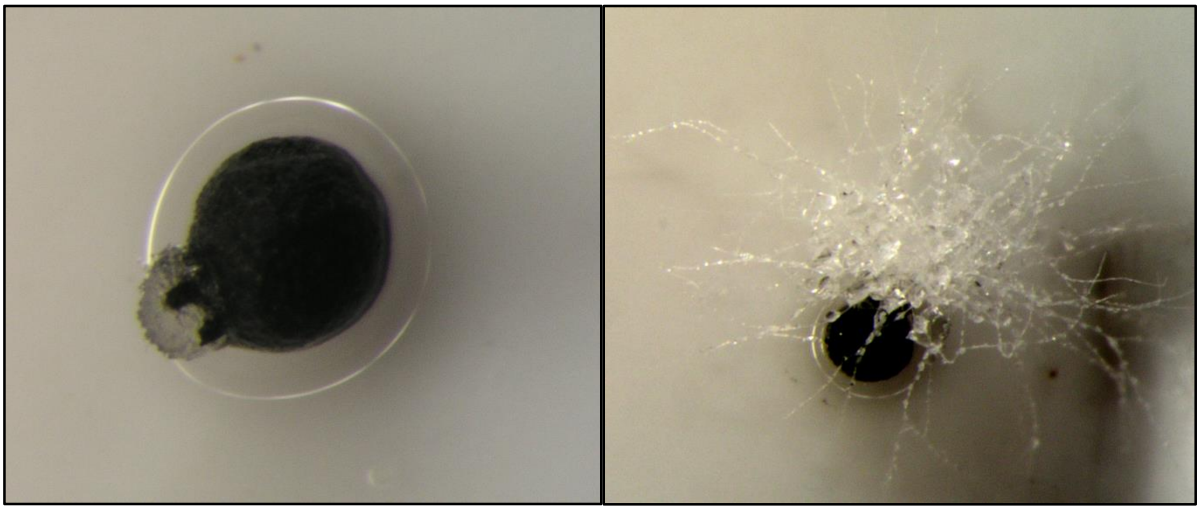

**Figure 2.** Germination of *S. cepivorum* sclerotium in response to DADS *in vitro*. Left, eruptive germination; right, mycelial growth.

### *Detection and quantification in soil*

Many studies have attempted to detect and quantify *S. cepivorum* sclerotia by soil sieving and also relate the number of sclerotia in soil to disease levels (e.g. Crowe *et al.* (1980); Adams (1981)). Although useful as a broad indication of risk, sieving is time-consuming, difficult to do in some soils, and the relationship between sclerotial density and infection is not always clear as it is confounded by dormancy and environmental factors. More recently, a bulk soil DNA extraction method and a PCR detection test for *S. cepivorum* has been developed at Fera (Woodhall *et al.*, 2012) which is now available as a commercial service utilising a soil sample of up to 1 kg. Improved knowledge of sclerotial density, distribution and dormancy status would inform cropping decisions and subsequent disease management treatments.

### *Chemical control*

In general, fungicide control of white rot in bulb onions has not been very effective. Drench applications with dicarboximide fungicides (e.g. iprodione (Rovral); vinclozolin (Ronilan)) was initially useful but enhanced microbial degradation of these compounds in some soils led to a decline in their performance (Entwistle, 1985; Entwistle, 1992; Koike *et al.*, 2007). More recent studies investigating a range of fungicides (See Table 1 for details and levels of efficacy) have highlighted the efficacy of triazole fungicides, particularly tebuconazole which is now used for white rot control in several countries (Koike *et al.*, 2007). In the UK, tebuconazole seed treatment followed by two foliar sprays starting 8-10 weeks after sowing gave significant control of white rot in salad onions in HDC project FV 004e (Paterson and Wood, 2001), although there was some reduction in seedling emergence. Tebuconazole is still approved in the UK as a foliar and seed treatment in onions. Very recently, Ferry-Abee (2014) demonstrated that tebuconazole, as well as penthiopyrad and fluopyram, were effective for control of white rot on onions in the USA. However, unlike Villalta *et al.* (2004), Ferry-Abee (2014) did not find boscalid to be an effective chemical control for white rot based on increases in marketable yield.

Research on white rot control in spring onions in New Zealand indicated that some strobilurin (e.g. azoxystrobin) and SDHI fungicides (e.g. boscalid) can also reduce disease levels (Villalta *et al.*, 2004) when applied as a seed treatment or as stem base sprays from shortly after emergence. Villalta *et al.* (2004) also showed procymidone to be effective at reducing incidence of white rot in onions but also noted it had a negative effect on seedling emergence. However, fungicides for soilborne disease control can often vary in their efficacy and generally control is more consistent under low disease pressure situations when there are low numbers of sclerotia in the soil and when environmental conditions are less conducive to disease development (e.g. in spring-sown salad onions). Fungicide control in full-season bulb onions is more challenging as conditions conducive to infection are more prolonged and seed treatments therefore only provide initial protection.

Effective fungicide application methods, with appropriate volumes of water and timing of sprays are essential for good control of white rot with in-furrow application (treated seed) and soil surface sprays or drenches providing protection around the root zone (Gladders *et al.*, 1987). Standard foliar spray applications are generally not suitable for delivering fungicide treatments for control of *Allium* white rot unless targeted at the seedbed. New technologies in spray application that target fungicide placement more effectively and novel fungicides with better activity against *S. cepivorum* may provide opportunities to improve control of the disease.

**Table 1.** Summary of recent studies resulting in control of *Allium* white rot with fungicides

| **Active ingredient** | **Efficacy** | **Application method and rate** | **Experimental details** | **Reference** |
|---|---|---|---|---|
| **Tebuconazole**<br>(approved for use on onions in UK) | 85% reduction in disease incidence (untreated control, 79.6% incidence) | Seed treatment 0.4g a.i./ 100,000 seeds then a foliar spray 0.25L a.i./ha | Artificially inoculated field site (0.4g sclerotia/m$^2$ (added 3 weeks prior to drilling) | Paterson and Wood, 2001 |
| **Tebuconazole**<br>(approved for use on onions in UK) | Up to 63% reduction in disease incidence (untreated control, 71% incidence) | Seed treatment | Inoculated glasshouse seedling bioassay | Clarkson *et al.*, 2006 |
| **Tebuconazole**<br>(approved for use on onions in UK) | 81% reduction in disease incidence (untreated control, 47% incidence) | Seed treatment | Artificially inoculated field site (50,000 sclerotia/m$^2$) | Clarkson *et al.*, 2006 |
| **Boscalid**<br>(approved for use on onions in UK) | ~90% reduction in disease incidence (untreated control, ~22% incidence) | Soil surface spray after sowing. Second spray to stem base 3 weeks after sowing (No rates given) | Australian field trial at 'high pressure site' | Villalta *et al.*, 2004 |
| **Procymidone**<br>(not approved for use in UK) | 77% reduction in disease incidence (untreated control, ~43% incidence) | 2x foliar sprays (2L a.i./ha) | Australian field trial with sprays at week 3 and week 7 after planting | Villalta *et al.*, 2004 |
| **Procymidone** (not approved for use in UK) + **Azoxystrobin**<br>(approved for use on onions in UK) | ~78% reduction in disease incidence (untreated control, ~43% incidence) | 3x foliar sprays (2x procymidone at 2L a.i./ha and 2000L/ha water; 1x azoxystrobin, a.i. rate not given, but applied in 500L water/ha) | Australian field trial:<br>Procymidone sprays at week 3 and week 7 after planting, azoxystrobin at week 10 after sowing | Villalta *et al.*, 2004 |
| **Procymidone** (not approved for use in UK) + **Boscalid** (approved for use on onions in UK) | ~95% reduction in disease incidence under low disease pressure (untreated control, 6% incidence)<br>~76% reduction in disease incidence under higher disease pressure (untreated control, ~26% incidence) | Two sprays of procymidone, first to the soil surface after sowing, second to stem base 5 weeks after sowing. Supplemented with one boscalid spray to the stem base 8 weeks after sowing | Under high disease pressure 2 applications of boscalid give better control. | Villalta *et al.*, 2004 |

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| **Penthiopyrad**<br>(registered in UK for use on cereals and top fruit, NOT onions) | Increased marketable yield by ~2 metric tonnes/ha compared to control (from 2.9t in untreated control to 4.6t) | Spray applied at 56.1g a.i./ha<br>in a 10-15cm width over seed at planting | Californian field trial. Disease pressure 28 sclerotia/kg soil. | Ferry-Abee, 2014 |
| **Fluopyram** (approved in UK on cereal crops) | Increased marketable yield by 1.5 metric tonnes/ha compared to control (from 1.9t in untreated control to 3.4t) | Spray applied at 46.7g a.i./ha in a 10-15cm width over seed at planting | Californian field trial. Disease pressure 34 sclerotia/kg soil. | Ferry-Abee, 2014 |
| **Tebuconazole** (approved on onions in UK) + **Penthiopyrad** (registered in UK for cereals and top fruit, NOT onions) + **Fludioxonil** (approved for use of onions in UK) | Increased marketable yield by ~6 metric tonnes/ha (from 1.7t in untreated control to 7.8t) | Sprays applied in a 10-15cm width over seed furrow at planting (tebuconazole 90.9 g a.i./ha; penthiopyrad 46.7 g a.i/.ha, fludioxonil 40.1g a.i./ha | Californian field trial. Disease pressure 45 sclerotia/kg soil. | Ferry-Abee, 2014 |
| **Tebuconazole** (approved for use of onions in UK) + **Penthiopyrad** (registered in UK for use on cereals and top fruit, NOT onions) | Increased marketable yield by 5.5 metric tonnes/ha (from 1.7t in untreated control to 7.2t) | Sprays applied in a 10-15cm width over seed furrow at planting<br>(tebuconazole 90.9 g a.i./ha; penthiopyrad 46.7 g a.i./ha) | Californian field trial. Disease pressure 45 sclerotia/kg soil. | Ferry-Abee, 2014 |

### *Biological control*

Due to problems in the past with fungicide control, many studies have examined the potential of microbial biological control agents on *Allium* white rot. A range of organisms have been studied (See Table 2 for details and levels of efficacy) which likely employ different control mechanisms such as antibiosis, parasitism, defence induction, and competition for nutrients (Utkhede and Rahe, 1983; Entwistle, 1990; DEFRA, 1999; Clarkson and Whipps, 2002; Clarkson *et al.*, 2002; DEFRA, 2002; Clarkson *et al.*, 2004; DEFRA, 2005; McLean *et al.*, 2005; Clarkson *et al.*, 2006; Whipps *et al.*, 2009; Heydari and Pessarakli, 2010; Bélanger *et al.*, 2012; McLean *et al.*, 2012b; Junaid *et al.*, 2013; Shalaby *et al.*, 2013).

In the UK, *Trichoderma viride* strain S17A was first recognised as being an efficient mycoparasite of *S. cepivorum* in Defra funded research at Warwick (Clarkson *et al.*, 2002; Clarkson *et al.*, 2004). Work followed on understanding how environmental factors such as temperature and soil moisture affect the ability of this fungus to degrade *S. cepivorum* sclerotia (Clarkson *et al.*, 2004), as well as potentially integrating this biocontrol agent with the use of new onion accessions, fungicides, and composted onion waste (Clarkson *et al.*, 2006). However, strain S17A has not been commercialised. *T. atroviride* strain C52 was registered as Vegevax G for control of *Allium* white rot in New Zealand (Stewart *et al.*, 2004) and is now marketed as Tenet by Agrimm. The product is applied as granules and should be used as a preventative treatment at sowing into the planting furrow using rates between 25kg/ha and 50kg/ha depending on disease history. Other *Trichoderma* spp. are available as biocontrol agents worldwide, such as Tusal (*T. asperellum* strain T25 + *T. atroviride* strain T11) with activity against root pathogens including *Sclerotinia* in Spain and T34 Biocontrol (*T. asperellum*) and Trianum G / Trianum P (*T. harzianum* strain T22) for protected crops in the UK. However, the activity of some of these products has not yet been assessed against *S. cepivorum*.

Clarkson *et al.* (2004) found that although both *T. viride* S17A and *T. viride* L4 reduced sclerotial viability in all soil types, the efficacy of these strains in controlling disease in seedling bioassays was variable. For instance, *T. viride* L4 was the only isolate to prevent white rot symptoms developing in peat soil. Clarkson *et al.* (2006) reported that in seedling bioassays there was no increase in efficacy as a result of applying *Trichoderma* strains to growing media 12 weeks pre-planting as opposed to at the time of planting. Work carried out in Australia by Villalta *et al.* (2005) also suggested an effect of soil type on the level of control achieved using *T. atroviride*.

In two glasshouse-based trials, *T. atroviride* C52 (Tenet) gave 51 and 92% control of onion white rot when introduced into the soil as an infested solid-substrate formulation (Kay and Stewart, 1994; McLean and Stewart, 2000). Further trials in the field with different formulations indicated that isolate C52 was highly effective (57 - 81% disease control) under low to moderate (0–30% incidence in untreated control) disease pressure, but less so (40% disease control) under high (> 30% incidence in untreated control) pressure (McLean *et al.*, 2005; McLean *et al.*, 2012a). Treatment with this *Trichoderma* may therefore be a viable option at low disease pressures but is unlikely to be economically viable at high pressures. (McLean *et al.*, 2012a). Tenet may become available in Europe but further testing is required to assess its performance under UK conditions. McLean *et al.* (2012a) commented that whilst *T. atroviride* C52 has good potential as a biological control agent against *S. cepivorum*, the timing of the onset of disease may significantly influence its efficacy, providing reduced control if the disease occurred later in the season.

The formulation of biocontrol products also influences their efficacy; for instance, McLean *et al.* (2005, 2012a) found that a pellet formulation of *T. atroviride* C52 had better efficacy than a crude solid substrate formulation. Pellets consisted of *T. atroviride* air dried powder, bentonite clay, and barley grain chips and were applied at a rate of 50kg/ha (McLean *et al.*, 2012a). It was thought that the pellet formulation supported better growth of the biocontrol agent in the soil for longer and a combination of pellets and a soil drench formulation provided the best levels of control (McLean *et al.*, 2012a). Clarkson *et al.* (2002) also identified the importance of formulation and found that biological control agents applied as a wheat bran culture provided better control than when applied as a spore suspension.

More recently, HDC project FV 219b tested a range of commercially available biocontrol products, such as Prestop (*Gliocladium catenulatum* strain J1446) and Serenade ASO (*Bacillus subtilis* strain QST 713) with potential for control of *Allium* white rot (Noble, 2013). Products were applied either with an onion waste compost substrate, or as a drench, and the level of control achieved ranged from insignificant to ~80% in pot bioassays and from ~22% to ~75% in field trials (Table 2). Serenade ASO currently has off-label approval (EAMU 0706/13) for control of white rot on outdoor salad and bulb onions. *Coniothyrium minitans* available as Contans for control of *Sclerotinia sclerotiorum* is also known to be a mycoparasite of *S. cepivorum* sclerotia. In a study by McLean and Stewart (2000), *C. minitans* was as effective as some *Trichoderma* spp., against white rot in an inoculated seedling bioassay but further investigation in the field in various soil types is required.

By isolating organisms from the rhizospheres of healthy onion plants growing in close proximity to plants infected with white rot, Shalaby *et al.* (2013) identified numerous potential antagonists of *S. cepivorum*. Of particular interest were two *Bacillus subtilis* isolates (*B. subtilis* B4 and *B. subtilis* B5) and two *Trichoderma* species (*T. koningii* and *T. harzianum*) all of which resulted in reductions in both disease severity and incidence in field trials where onion transplants were immersed (bacterial isolates $0.8x10^8$ cfu/ml; fungal isolates $0.5x10^7$ spores/mL) in the respective treatments prior to planting. However, the field was naturally infected and no information was given about the infestation level of the field by sclerotia and, as illustrated by Stewart *et al.* (2004), this can significantly influence the level of control achieved by biological agents.

*In vitro* tests carried out by Ferry-Abee (2014), although often a poor predictor of field activity, identified a range of microorganisms antagonistic against *S. cepivorum* including isolates of two *Bacillus* sp. and three *Fusarium* sp. isolates which provided better suppression of mycelial growth than the UK registered biocontrol product Serenade ASO. As such they were identified as promising targets for further development.

Similarly, Castillo *et al.* (2011) showed *in vitro* using dual culture petri dish experiments that various strains of *Trichoderma* species such as *T. asperellum*, *T. harzianum* (including strain T22), and *T. atroviride* inhibited mycelial growth of *S. cepivorum* (from ~50-80%). An inoculated greenhouse study (100g sclerotia/kg soil) by Dilbo *et al.* (2015) found a range of *Trichoderma* spp. to be effective at controlling white rot of garlic (information on strains not provided), used individually, in combination, or used with a synthetic fungicide. Disease pressure was extremely high with 94.4% disease incidence in the uninoculated control and 100% in the inoculated control. The synthetic fungicides, which included tebuconazole, provided limited control with 88.9% disease incidence with the best control achieved with a fungicide seed treatment (mefenoxam / difenoconazole / thiamethoxam) + *T. hamatum* + *T. viride* (0% incidence) and *T. viride* on its own (11.1% incidence).

Given the increased demand for biological fungicides by growers, and the increasing number being registered in the EU, there may now be further impetus for developing and marketing biofungicide products as part of an integrated strategy for management of *Allium* white rot.

**Table 2. Summary of recent studies resulting in control of *Allium* white rot with different microorganisms**

| **Microorganism and code given in report** | **Pesticide status for UK onion** | **Efficacy against *S. cepivorum*** | **Experimental details** | **Reference** |
|---|---|---|---|---|
| *Coniothyrium minitans* | Contans WG approved in UK for soil incorporation prior to onion planting | 36% reduction on browning of tomato stem pieces | | Gerlagh *et al.*, 1996 |
| *Trichoderma viride* isolates S17A and L4 | No product | ~30% and 35% incidence reduction in field trial<br>>70% and up to 90% incidence reduction achieved in seedling bioassays<br>Best control when applied at sowing | <u>Field trials with bulb onions</u>:<br>Applied at sowing as pellets – natural infection<br>Fluid-drilling – applied at sowing as wheat bran culture incorporated into soil | Clarkson *et al.*, 2002;<br>Clarkson *et al.*, 2006 |
| *Trichoderma atroviride* isolate C52 | Not approved in UK; marketed in NZ as (Tenet) | 57 - 92% control in glasshouse trials | | Kay and Stewart, 1994;<br>McLean and Stewart, 2000 |

| **Microorganism and code given in report** | **Pesticide status for UK onion** | **Efficacy against *S. cepivorum*** | **Experimental details** | **Reference** |
|---|---|---|---|---|
| *Trichoderma atroviride* isolate C52 | Not approved in UK; marketed in NZ as (Tenet) | ~70% incidence reduction in field trial under low disease pressure<br>~40% incidence reduction in field trial under high disease pressure | Field trials with bulb onion:<br>Soil incorporation with pellets plus a drench treatment (Waikuku complex soil or peaty loam. | McLean *et al.*, 2012a |
| *Bacillus subtilis* strain QST713<br>(F43) | Serenade ASO (off-label) approved for use on onion (outdoor) | ~77% reduction under low disease pressure | Field trial with bulb onions:<br>Applied as a drench treatment using 2 g/m² after sowing & 10 wks later. Silty soil. | Noble, 2013 |
| *Gliocladium catenulatum* strain J1446<br>(F42) | Approved for use on onion as Prestop (off-label) | ~70% reduction in disease incidence under low disease pressure (control had 6.5% incidence) | Field trial with bulb onions: Applied as a drench at 5g/m2 after sowing & 10 wks later but ineffective at 2.5g/m² (low disease pressure). | Noble, 2013 |
| F37 (coded product, fungus) | Registered in UK on range of protected crops – does not include onions | Reduced disease incidence by ~55% compared with untreated soil control | Inoculated pot bioassays with bulb onions: high disease pressure ($1.6x10^4$ sclerotia/kg soil). Applied as a drench after planting | Noble, 2013 |
| F39 (coded product, fungus) | Registered in UK on range of protected crops – does not include onions | ~54% average reduction in disease incidence over first two years tested, insignificant control in third year tested | Inoculated pot bioassays with bulb onions: high disease pressure ($1.6\ x10^4$ sclerotia/kg soil).<br>Drenched after planting | Noble, 2013 |
| F40 (coded product, fungus) | Registered in UK on range of protected crops – doesn't include onions | 50% reduction in disease incidence. Only tested in one year. | Inoculated pot bioassays with bulb onions: high disease pressure ($1.6x10^4$ sclerotia/kg soil). Applied as granules | Noble, 2013 |
| F39 (coded product, fungus) | Registered in UK on range of protected crops – | 0% - 71% reduction in disease incidence | Naturally infested field trials. Treatment applied | Noble, 2013 |

| **Microorganism and code given in report** | **Pesticide status for UK onion** | **Efficacy against *S. cepivorum*** | **Experimental details** | **Reference** |
|---|---|---|---|---|
| | doesn't include onions | | as drench ~one month after sowing/transplanting sets. | |
| *Bacillus subtilis* isolates B4 and B5 | No products | B4 Reduced incidence in crop by 79% and severity by 78%, whereas<br>B5 reduced incidence in crop by 69% and severity by 84% | Naturally infested field. 60 day old transplants immersed for 12h at room temperature. One month after transplanting, a booster dose (5 mL) was added around plants | Shalaby *et al.*, 2013 |
| *Trichoderma koningii* | Not approved in UK | Reduced incidence in crop by 83% and severity by 84% | Naturally infested field. 60 day old transplants immersed for 12h at room temperature. One month after transplanting, a booster dose (5 mL) was added around plants | Shalaby *et al.*, 2013 |
| *Trichoderma harzianum* | Trianum-P approved in the UK for protected crops | Reduced incidence in crop by 86% and severity by 86% | Naturally infested field. 60 day old transplants immersed for 12h at room temperature. One month after transplanting, a booster dose (5 mL) was added around plants. | Shalaby *et al.*, 2013 |
| *Bacillus subtilis* isolates 303 and 307 | No products | 34% and 38% reduction in colony diameter on PDA | Petri dish study | Ferry-Abee, 2014 |
| *Fusarium sp.* isolates 206, 207, 212 | No products | Isolates ranged from 35% to 45% reduction in colony diameter on PDA | Petri dish study | Ferry-Abee, 2014 |
| *Trichoderma harzianum, hamatum, oblongisporum, viride* | No products | Reduced incidence by 44-89% reduction in disease incidence | Inoculated pot bioassays with garlic cloves: high disease pressure (100<br>g of sclerotia/kg) | Dilbo *et al*, 2015 |

### ***Sclerotial germination stimulants***

Soil inoculum can be reduced if *S. cepivorum* sclerotia can be triggered to germinate in the absence of host plants as they exhaust their nutrient reserves in the absence of an *Allium* host. Natural and synthetic germination stimulants such as diallyl disulphide (DADS) have been extensively researched (see Table 3 for details and levels of efficacy) 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). Germination of sclerotia is triggered by DADS as it mimics the stimulatory activity of *Allium* root exudates and it was available commercially in some countries (Coley-Smith and Parfitt, 1986; Koike *et al.*, 2007). However, effectiveness of this treatment relies on thorough application to soil as well as suitable temperature and moisture conditions; not all sclerotia will germinate in response to DADS so the remaining inoculum still poses a disease risk. The product Alli-Up (Product PC-129087) was risk assessed by the USA Environmental Protection Agency (EPA) in 2003 for use by direct soil injection (shanking system). It was determined that at the low use rate proposed and given the rapid breakdown and volatility of the active ingredient that environmental exposure was low. The IR-4 project new products list includes Alli-Up as a biopesticide for use against White Rot registered by UAP/Platt. However, this product was noted to have increased in price to cost over $200 and acre and subsequently it appears to have been withdrawn from the market (Ferry-Abee, 2014). Other possible product sources have yet to be identified.

Experimentally DADS has been used to stimulate germination of *S. cepivorum* sclerotia *in vitro* and assess their dormancy status and ability to infect plants (Clarkson, unpublished; Defra project HH3230SFV). Davis *et al.* (2007) found that 0.5ml/m$^2$ DADS was able to reduce sclerotial inoculum density in field trials (initial densities approx. 150 sclerotia/kg soil) by ~90%. 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 while effluent from a garlic processing plant was only able to achieve a 45% reduction. Despite the significant reductions in viable sclerotia resulting from the use of DADS and garlic powder, the level of white rot disease in subsequent garlic crops was still high, further illustrating that low levels of soil inoculum can still cause significant losses (Davis *et al.*, 2007). Ferry-Abee (2014) also found DADS to be effective at controlling white rot disease in a naturally infested field, reducing viable sclerotia by up to 80% and increasing marketable yield by 40% (Table 3). Garlic oil was also tested in the study and this reduced numbers of viable sclerotia by 50%, it did not have a significant effect on the marketable yield compared to the untreated control. In this study, DADS and garlic oil were applied to the field one year before planting onions during which time the field was cropped with wheat (Ferry-Abee, 2014).

Multiple applications of DADS may be able to provide near total eradication of sclerotia from soil but the economic feasibility of this practice needs evaluating, taking into account that the survival of low levels of sclerotia could still cause significant disease. In future experiments, records should be made of the density of sclerotia in soil in relation to disease levels to allow comparison of information between trials.

Other Allium products such as garlic granules and ‘garlic juice’ may also have potential as stimulants of sclerotial germination and some initial tests were carried out in Australia by Villalta *et al.* (2005) but no statistical analysis is presented and the results are inconclusive due to a low disease incidence in the untreated control. A new product formulated from garlic called NEMguard DE, a nematicide produced by ECOspray Ltd. and marketed by Certis is also currently approved in the UK and has off-label approval (EAMU 1838 of 2015) on several crops including on outdoor onion, leek, shallot, and garlic, with a maximum individual dose of 20 kg/ha. There is no MRL and it can be used on organic crops. NEMguard DE contains polysulphides, the active ingredient of ‘garlic concentrate’, and therefore most likely contains the appropriate chemical compounds for sclerotial germination. The product is a slow release granule formed by creating a honeycomb structure of diatomaceous earth which acts as a carrier matrix for the active ingredient. As this product is marketed in the UK it is more readily available to growers than DADS or onion compost and hence may be beneficial to evaluate. In addition, a liquid product containing the polysulphide active used in NEMguard would also be available for experimentation with a view to future commercial use (Alan Horgan, Certis, pers. comm.).

The use of sclerotial germination stimulants such as DADS and garlic powder for control of *Allium* white rot has potential but attention must be paid to whether specialist machinery is required and available for incorporation, and the associated cost of these applications. The timing of treatment is also an important factor to consider as this needs to fit between crops and during periods where sclerotial dormancy is likely to be low, although the factors influencing dormancy are still largely unknown.

Composted onion waste can also stimulate germination of *S. cepivorum* sclerotia when incorporated into the soil and this treatment has shown to be effective in repeated field trials with control levels up to 80-90% (Whipps and Noble, 2001; Coventry *et al.*, 2002; Coventry *et al.*, 2005; Coventry *et al.*, 2006; Whipps *et al.*, 2009; Noble, 2013). During composting, a temperature of at least 48°C needs to be maintained for a minimum of 3 days in order to kill any sclerotia of *S. cepivorum* in the waste material (Coventry *et al.*, 2002). Whipps *et al.*

(2009) demonstrated that composted onion waste reduced sclerotial viability by >95% and almost completely prevented subsequent disease development although this level of efficacy was not consistent in other studies (Table 3). Coventry *et al.* (2005) showed that although composted onion waste can reduce sclerotial viability, this may not always result in a reduction in white rot disease, again most likely due to low numbers of sclerotia initiating disease. The level of control achieved by incorporation of composted onion waste also appears to be influenced by soil type, with peat soil being the most compatible, and silt soils largely incompatible (Coventry *et al.*, 2005). It is important also to note the limited availability of onion waste compost in the commercial quantities required to treat all infested land and the associated transport costs.

**Table 3. Efficacy of *S. cepivorum* sclerotial germination stimulants in various studies**

| **Germination stimulant** | **Reduction in sclerotial viability** | **Type of study** | **Experimental detail** | **Reference** |
|---|---|---|---|---|
| DADS | ~90% | Field trial (naturally infested) | Application rate of 0.5ml/m$^2$<br>DADS was diluted with water and applied with a pressurized pesticide applicator. Application followed by disking. | Davis *et al.*, 2007 |
| DADS | ~80% (increased marketable yield by 40% in onion crop grown the following year) | Field trial (naturally infested) | Shank-injected into soil when soil at least 30% soil moisture and between 10-15°C. Cropped with wheat for one year prior to onions. | Ferry-Abee, 2014 |
| Garlic granules/ powder | ~90% | Field trial (naturally infested) | 112 kg/ha and 224 kg/ha<br>mixed with sand to facilitate application with a tractor-pulled fertilizer applicator or hand held fertilizer applicator. Application followed by disking. | Davis *et al.*, 2007 |
| Effluent from garlic processing plant | ~45% | Field trial (naturally infested) | 500L/ha<br>Application followed by disking. | Davis *et al.*, 2007 |
| Garlic oil | ~50% reduction in viability but did not affect marketable yield from an onion crop the following year | Field trial (naturally infested) | Shank-injected into soil when soil at least 30% soil moisture and between 10-15°C. Cropped with wheat for one year prior to onion crop. | Ferry-Abee, 2014 |
| Composted onion waste | ~55% | Pot experiments (one field experiment showed lower efficacy ~ 15-20%) | Seedling bioassays at 50%w/w incorporation rate (varies with soil type) | Coventry *et al.*, 2002; Coventry *et al.*, 2005 |
| Composted onion waste | ~90% disease control | Pot experiments | Seedling bioassays at 50%v/v incorporation rate | (Clarkson *et al.*, 2006) |
| Composted onion waste | >95% disease control when buried for 16 months in compost-amended soil; ~80% disease control when buried for 12 months. | Field trial | Applied as a 3.75cm layer and power harrowed into 15cm to give 25% v/v incorporation rate.<br>Subsequent onion crops had reduced disease/later onset resulting in increased yields. | Whipps *et al.*, 2009 |

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## ***Soil disinfestation***

### *Fumigation*

Historically, soil disinfestation with chemical fumigants such as dazomet, metam sodium and chloropicrin on heavily infested soils has been effective for control of white rot although the fungus is not always completely eradicated (Koike *et al.*, 2007). However, these treatments are relatively expensive and require specialist equipment, which has precluded widespread use. The continued approval of these chemicals for use as soil fumigants is also under scrutiny from European and national regulatory authorities and currently in the UK only dazomet is approved for use prior to planting *Allium* crops.

A new soil fumigant dimethyl disulphide (DMDS, naturally present in alliums), is available in Italy (as Paladin EC) and registration is being sought in the UK with a target date of availability in 2019 (A Horgan, Certis, pers. comm.). This fumigant, although primarily a nematicide, is reported also to have activity as a fungicide against the sclerotia of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* (Zanon *et al.*, 2014) and so may also have activity against *S. cepivorum*. Gómez-Tenorio *et al.* (2015) demonstrated that DMDS significantly reduced the inoculum density of *Fusarium oxysporum*, but did not prevent disease symptoms developing. Biofumigation with *Allium* and *Brassica* wastes also generates DMDS and other fungitoxic compounds ((Coventry *et al.*, 2002; Arnault *et al.*, 2013). DMDS may therefore have a role to play in the management of *Allium* white rot, although it was observed to be less effective against *Pythium ultimum* and *F. oxysporum* than on nematodes (Cabrera *et al.*, 2014). Residual vapours of DMDS may also reduce seed germination rates (Gómez-Tenorio *et al.*, 2015).

### *Biofumigation*

The use of certain crops, for example *Brassica* sp., have a biofumigation effect due to the conversion of glucosinolates to isothiocyanates (ITCs) following maceration and incorporation into the soil. In a recent study on potato crops, Larkin and Halloran (2014) showed that disease suppression of soil borne plant pathogens was better for biofumigation crops (mustard, rapeseed, and Sudan grass) compared to standard green manures. Smolinska (2000) demonstrated that *B. juncea* and *B. napus* residues reduced the number of *S. cepivorum* sclerotia recovered after 60 days as well as their viability compared to an untreated control. Treatment with *B. juncea* in one soil reduced recovery by >80% and reduced viability by 100%. 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

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as another mechanism in addition to ITCs which likely contributed to the reduction in sclerotial number and viability (Smolinska, 2000). Villalta *et al.* (2010) found that treatment with a mustard oil product (Voom®) at 5% v/v in lab experiments was able to achieve a 100% kill rate of mycelium and sclerotia of *S. cepivorum*. However, when Voom® was applied via shank-injection to raised beds containing sandy soil eight weeks before growing spring onions, only ~25% reduction in disease incidence was observed compared to the untreated control (untreated control, 13% disease incidence). The study also showed there was apparently no difference in efficacy whether or not the plots were sealed. Similarly, Dhingra *et al.* (2013) showed that an essential oil of mustard (93% allyl isothiocyanate) at 100 µl/L for 7 days resulted in 100% mortality of sclerotia of *S. rolfsii* with similar efficacy against *S. sclerotiorum* further illustrating the potential of cruciferous based substances against sclerotial pathogens. In the same study, no sclerotia of *S. rolfsii* were recovered from field plots treated with allyl isothiocyanate at 9ml/m$^2$ and then covered; while a significant number of sclerotia survived in uncovered plots suggesting the need to contain such volatile compounds to maintain efficacy. More work is required to see whether biofumigation or the use of biofumigant crop products is a useful component of an integrated control strategy for white rot.

### *Soil solarisation*

The efficacy of soil solarisation for control of white rot has been demonstrated in several countries including Australia, Spain and New Zealand (Porter and Merriman, 1983; Melero-Vara *et al.*, 2000; McLean *et al.*, 2001). However due to the temperate climate of the UK, this approach is unlikely to be highly effective or consistent in its efficacy against *S. cepivorum*. In one study, temperatures of 40°C for ~10 hours were required to kill 50% of *S. cepivorum* sclerotia (Adams, 1987) while temperatures of >50°C for 7 days were recommended to kill sclerotia when composting onion waste (Coventry *et al.*, 2005). However, exposure of sclerotia to sub lethal temperatures has been showed to increase their colonisation by other soil microorganisms (Entwistle and Munasinghe, 1990), suggesting that solarisation in the may have the potential to weaken sclerotia (McLean *et al.*, 2001). Ulacio-Osorio *et al.* (2006) in Mexico found reductions of 74% to 84% white rot incidence in a garlic crop when solarisation was combined with the prior incorporation of broccoli than without solarisation. Soil solarisation may therefore have a role in an integrated programme to increase the efficacy of various biocontrol agents or resident microorganisms against *S. cepivorum*.

*Steaming*

Soil steaming is generally a very effective method of soil disinfestation for soilborne pathogens including sclerotial fungi. Low temperature/short duration steaming was demonstrated to kill sclerotia of *S. cepivorum* in just 3 minutes at 50°C while steaming at 45°C had no effect (van Loenen *et al.*, 2003). Alternative methods of soil heating have been examined in recent years, such as hot air treatment and using microwaves (Runia and Molendijk, 2009; Gilardi *et al.*, 2014), but the cost of these practices may still preclude their widespread use. However, precision farming techniques which allow the mapping (via drone and satellite data) of patchy distributions of soilborne diseases in the field are likely to become more frequently utilised and so could help target the control input required.

*Flooding and anaerobic soil disinfestation*

Coley-Smith *et al.* (1990) showed in pot experiments that soil flooding could effectively reduce the numbers of viable sclerotia of *S. cepivorum* with an efficacy of 74-100% after 18 months, depending on soil type and age of sclerotia with older sclerotia being more resilient. Despite the evident ability of flooding to reduce the viability of sclerotia, there was no effect within the first 3 months and a limited effect within the first 6 months, although viable sclerotia were identified as weakened. However, in the field, Crowe and Debons (1992) found that it only required 3 months of flooding to reduce sclerotial viability by 99% at water depths of 12 cm-1m. In further 'bucket' experiments, Crowe *et al.* (2005) found that flooding for 12-18 months resulted in 99-100% mortality of sclerotia. However, following one year in non-flooded, but irrigated soil, a subsequent year of flooding resulted in only a 20% reduction in sclerotial survival compared to non-flooded buckets. This suggested that more mature sclerotia have increased resilience to soil flooding compared to newly formed sclerotia (Crowe *et al.*, 2005).

Anaerobic soil disinfestation (ASD) involves the homogenous incorporation of an organic feedstock (e.g. a green manure crop) into the topsoil followed by light compaction and irrigation of the field which is subsequently mechanically covered with air tight virtually impermeable film (VIF) to restrict oxygen supply and create anaerobic conditions. The soil is left covered for a period of time in the summer during which the anaerobic conditions facilitate the formation of toxic fermentation products (Lamers *et al.*, 2010). ASD was highly effective against sclerotia of *Sclerotinia sclerotiorum* (Lamers *et al.*, 2010) and in the USA an ASD treatment also reduced disease caused by *Sclerotium rolfsii* (Shennan *et al.*, 2014). In another study using both pot and field trials it was also observed that sclerotia of *S. rolfsii* were colonised by naturally occurring *Trichoderma* spp. following ASD (Shrestha *et al.*, 2013). Specific products incorporated into the soil to assist in the production of toxic chemicals by the microorganisms present in the soil as a precursor for ASD (such as 'Herbie' products produced by Thatchtec in the Netherland's) have caused a divergence of opinion between UK and EU pesticide regulatory bodies as to whether or not they are to be classed as pesticides and hence require registration, with only the UK considering that they do. However, such products contain no active ingredients and their breakdown likely leads to the production of the same antimicrobial chemicals as would be produced from organic amendments such as digestate or cover crops incorporated into soil and subsequently metabolised by the microorganisms present.

Some of these alternative methods of soil disinfestation may have a part to play in integrated management of *Allium* white rot either in particular situations (e.g. where flooding is a practical option) or if combined with knowledge on sclerotial distribution and infestation density in a field, e.g. to eliminate small foci of white rot in a field.

### *Improving soil health*

Soil health can be interpreted in numerous ways but was recently defined by Larkin (2015) as "the capacity of soil to function as a vital living system to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health". Larkin (2015) also identified that practices which promote good 'soil health' often lead to suppression of soil-borne disease. These include the use of crop rotations, green manures, cover crops, and organic amendments. These practices can facilitate the suppression of pathogens via a number of mechanisms as a result of increasing soil microbial biomass, activity and diversity. Disease control is probably due to a combination of complex mechanisms including general suppression, caused by an overall increase in microbial biomass and activity, and by specific antagonism caused by specific interactions between pathogens and various species of soil microorganisms.

Increasing organic matter content by utilising organic amendments such as manure, compost etc. has been shown to be suppressive to *Sclerotinia* spp. and other sclerotial pathogens (e.g. *Verticillium* spp. and *Rhizoctonia solani*; Bonanomi *et al.*, 2007; Noble, 2011; Bonilla *et al.*, 2012; Larkin, 2015). Organic amendments led to significant disease suppression in about 50% of cases (Termorshuizen *et al.*, 2006; Bonanomi *et al.*, 2007; Larkin, 2015). Bonanomi *et al.* (2010) also identified that the microbial component of organic matter as a key component to providing disease suppression as control was lost when the organic matter was sterilized. Although disease suppression can result from the incorporation of organic amendments into soil, results can be variable and dependent on numerous factors (Litterick *et al.*, 2004; Noble and Coventry, 2005; Termorshuizen *et al.*, 2006; Bonanomi *et al.*, 2010; Larkin, 2015).

Cover crops used as green manures, although not their primary purpose, can also provide a level of disease suppression. Disease suppression caused by green manures of non-biofumigant crops (e.g. pea, rye, barley) was attributed to positive changes in the microbial flora of the soil microbiome (Wiggins and Kinkel, 2005; Larkin, 2015). Ulacio-Osorio *et al.* (2006) in Mexico planted a broccoli crop and rotavated it into the soil after four months and then half the plots were covered with polythene for 90 days of solarisation. The subsequent garlic crop had a 74% reduction in white rot compared to the non-solarized treatment. However, as with organic amendments, green manures are not consistent at suppressing disease (Larkin, 2013). For example, Ulacio-Osorio *et al.* (2006) found that incorporation of a carrot crop did not significantly reduce *S. cepivorum* inoculum density or viability, while Banks and Edgington (1989) found that incorporation of a carrot crop reduced the number of sclerotia from 0.45 to 0.17 sclerotia $g^{-1}$ of soil.

In attempts to manipulate soil health for disease control, each individual practice may only have a small effect but these can be significant when multiple practices are combined.

### *Integrated control*

Control of *Allium* white rot is challenging and no single or predominant solution, other than growing on new land free of the pathogen (or at least a 20 year rotation), has been developed during several decades of research. Integrated disease management, where several disease management components effective against various stages in the pathogen lifecycle are combined into an overall disease management strategy, offers the most promise for improved control and partially effective treatments may have greater benefit when combined in appropriate ways. A number of research studies have therefore attempted to combine different treatments to provide an integrated approach. UK studies have examined combining the biological control agent *T. viride* with green waste (pre-colonised), composted onion waste, tebuconazole fungicide treatments and onion accessions with partial resistance to white rot (DEFRA, 2005; Clarkson *et al.*, 2006; Whipps *et al.*, 2009).

In experiments investigating combining *T. viride* S17A and green waste compost, one pot-based seedling bioassay showed no difference between treatments of green compost alone, *T. viride* S17A alone, and composts inoculated with *T. viride* S17A in terms of subsequent white rot incidence compared to an inoculated control (Whipps *et al.* 2009). However, in a second seedling bioassay, Whipps *et al.* (2009) found a combination of 40% green waste and *T. viride* S17A was the most effective treatment with a subsequent white rot incidence of ~30%, whereas 40% green waste alone and *T. viride* alone had disease incidences of ~50% and ~55% respectively compared to an incidence in the inoculated control of 62%. Treatments of *T. viride* S17A and green waste in the field were more successful. In an artificially inoculated field trial (0.2g sclerotia/m$^2$) green waste compost and *T. viride* S17A resulted in 100% control of white rot and was more effective than either treatment on its own (neither of which provided any degree of control), and a chemical fungicide (Folicur; a.i. tebuconazole). The treatment was effective under high disease pressure, increased marketable yield and provided control over two years. Data also indicated that the green waste compost allowed good survival and proliferation of the *Trichoderma* in soil enabling the high levels of disease control observed over the two years. In a more recent project, other microbial agents were combined with green waste in pot experiments (Noble, 2013) and another fungal biocontrol agent (coded product) gave significant disease control of white rot ranging from 35-79% control over 3 years. In field trials, this combination treatment gave some control, but to a lesser degree than in the pot experiments (<70% under low disease pressure and <64% under moderate disease pressure).

In glasshouse seedling bioassays Clarkson *et al.* (2006), tested two strains of *T. viride* (L4 and S17A) applied in combination with a tebuconazole seed treatment which resulted in significant reduction in white rot disease, generally greater than either treatment on its own. For example, in one experiment, with treatments applied 6 weeks pre-sowing, *T. viride* L4 + tebuconazole gave 93% reduction in disease incidence compared to the inoculated control (71% disease incidence), compared to 68% and 64% reduction by *T. viride* L4 alone and tebuconazole alone respectively. The only case where a combination did not provide additional control was where treatment with the *Trichoderma* isolates at the time of sowing provided extremely good control on their own (~90% reduction in disease), although the L4 / tebuconazole combination with tebuconazole provided complete control. When the two *Trichoderma* strains and onion compost were combined in a further seedling bioassay (Clarkson *et al.*, 2006), additive effects were not obvious due to the high level of control achieved by each treatment individually. However, in five out of eight of the combinations, 100% control was achieved. In the field, experiments assessing the effect of the same two *Trichoderma* strains and tebuconazole in combination with different bulb-onion accessions and cultivars found no additive effect on disease incidence compared to tebuconazole on its own (Clarkson *et al.*, 2006). Previous studies have also shown that other biological control agents are compatible with fungicide treatments for control of white rot (Budge and Whipps, 2001; McLean *et al.*, 2001; Stewart *et al.*, 2004; Dilbo *et al.*, 2015) suggesting this is a realistic approach for an integrated strategy.

In New Zealand, an integrated disease management plan has been developed consisting of DADS soil treatment pre-planting, *Trichoderma atroviride* strain C52 (Tenet) applied to the planting furrow, onion seed treated with fungicide (captan and/or procymidone granules), and follow up foliar fungicide sprays (Stewart *et al.*, 2004). Additionally, as the *T. atroviride* C52 was identified as being sensitive to nitrogen, no nitrogen applications should be made until at least four weeks after application of the biocontrol agent. However, no data on the efficacy of this integrated programme however was reported.

DADS and fungicides were also identified as an effective combination in an Australian study where DADS combined with procymidone gave 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). Villalta *et al.* (2005) also tested the compatibility of *T. atroviride* with DADS; *in vitro* DADS was antagonistic to mycelial growth of *T. atroviride* but *in vivo* the application of DADS to soil in a pot trial two weeks prior to application of the biocontrol agent had no adverse effect on mycelial growth of the beneficial fungus.

These studies show that integrated management of *Allium* white rot is an effective approach but research is required to develop similar strategies that are suitable for the UK, utilising current or potentially available disease control components. Practices might include an initial soil disinfestation treatment, followed by a germination stimulant, application of a biocontrol agent at time of planting compatible with fungicide treated seed, and a foliar fungicide programme. Maintaining or increasing soil health through application of organic matter might also be considered as a long-term approach to reducing disease pressure.

## Conclusions

This review has comprehensively examined the range of control measures that have been researched for *Allium* white rot. The following are suggested by the review team as the most promising approaches to potentially evaluate as components of an integrated control strategy under UK conditions. A summary is also provided in Table 4 with associated estimated costs.

1) **Chemical control:** tebuconazole has been widely used for white rot control including in the UK. The review has highlighted alternative chemistry with good activity including azoxystrobin, boscalid, penthiopyrad and procymidone. Effective control with these fungicides is dependent on targeted seedbed sprays just after sowing with follow-up foliar sprays. A selection of these fungicides should therefore be evaluated. Estimated costs per application vary between £18 and £41/ha.
2) **Biological control:** despite extensive research only one microbial product (Tenet) is registered specifically for use against white rot in New Zealand and Australia. However, a recent AHDB project FV 219b demonstrated that some UK registered biocontrol agents such as Serenade ASO and Prestop may reduce disease. Application as in-furrow treatments is the most effective option for delivering these products to the root zone. A selection of the most effective biological control products currently available in the UK

should therefore be evaluated. Estimated costs per application vary between £150 and £2604/ha depending on product.

3) **Sclerotial germination stimulants:** *Allium*-derived products/plant material can initiate germination of *S. cepivorum* sclerotia in the absence of a host and reduce inoculum. There are no current commercial suppliers of the key stimulant diallyl disulphide (analytical grade version is £2/g) but other products based on garlic being are developed for other crop protection applications, or for the food industry should be evaluated. The estimated cost for garlic granules is approx. £700/ha while the Ecospray product NEMguard DE which contains garlic-derived polysulphide actives costs approximately £180/ha.
4) **Soil disinfestation:** *S. cepivorum* sclerotia can be killed by chemical and bio-fumigation, soil solarisation, steaming, flooding and anaerobic disinfestation. Of these, promising approaches include dimethyl disulphide (DMDS) which is undergoing registration in the UK (price not yet available), biofumigant-derived products such as mustard meals and oils which allow immediate application without the requirement for growing a crop and anaerobic disinfestation (£45-£125/ha depending on organic material incorporated). A selection of these approaches should therefore be evaluated.
5) **Improving soil health:** application of organic amendments such as green manures, manures and composts may have a general beneficial effect on soil health through increased microbial activity and lead to general suppression of soilborne diseases including sclerotial pathogens. However, there is little evidence that this approach is particularly effective for control of *S. cepivorum*.
6) **Integrated control:** no single management practice will result in complete control of *Allium* white rot, and generally combining different approaches enhances control. Therefore, an integrated control approach based on individual treatments identified as having some efficacy against white rot should be evaluated.

**Table 4.** Treatments with potential to reduce *S. cepivorum* when applied pre-planting and during cropping. Treatment rates based on label rates (or experimental rate if different) and the list price cost (excl. VAT) of the product for a single application per ha.

| **Treatment** | **Treatment rate** | **Product cost (excl VAT)** | **Cost per ha** | **Comments** |
|---|---|---|---|---|
| **Pre-planting ASD treatments (require covering)** | | | | |
| Cover crops | 10 kg/ha | £45 / 10 kg grass/clover mix | £45 | Various types of grass/clover crop buried, and anaerobic conditions created by polythene sheet or Virtually Impermeable Film (VIF). |
| | 10 kg/ha | £89 / 10 kg Phacelia | £89 | This crop can survive mild winters. |
| | 7.5 kg/ha | Tillage radish | £67 | Deep tap root. Overwinter survival. |
| Anaerobic digestate | 50t /ha (max) | £2.50 /t + (transport £1.50 / t) | £125 | Maize feedstock most consistent & use permitted on all crops (unlike food waste). (Liquid would cost £4 /t) Vegetable-only waste also available e.g. Chambers (potato), Allpress (leek+onion) and soon from British Sugar (beet). |
| Green waste | Depends on Nitrogen content of soil | | Around<br>£4000 | Not biologically active (unless not properly made), Very variable content seasonally and especially if from a municipal waste source. |
| Herbie 72 or 82 (anaerobic for 2-8 weeks) | 1.2 to 3.8 kg/m² | £0.84 /kg | £100 to £320 | Effective for Verticillium control. Not Approved in UK so crop destruction would be needed. No approval as a pesticide needed elsewhere in Europe. |
| **Pre-planting fumigation treatments (require covering)** | | | | |
| Basamid (dazomet) | New lower rate 500 kg/ha | £42 / 5 kg | £4200 | From Certis. Current (2016) rate 750 g/ha. One use in every third year permitted. Cress test for phytotoxicity is needed before planting. |
| DMDS | Not declared | Not decided | - | From Certis. Not available yet in the UK, only in USA and Italy as Paladin |
| BioFence granules (defatted seedmeal of *Brassica carinata* | (1.6g /kg soil experimental). 2.5 t/ha recommended | To be determined | ? | Fumigant effect of isocyanate. Rate of 2.5 t/ha given by Plant Solutions Ltd, and equivalent to150 kg N/ha. |
| **Pre-planting germination stimulants** | | | | |
| DADS | 5 L/ha | n/a | n/a | Only an analytical grade product is available at £2 per gramme |
| Garlic granules | 112 kg/ha | £117 for 20 kg | £700 | Mix with sand to aid application. |

| Treatment | Treatment rate | Product cost (excl VAT) | Cost per ha | Comments |
|---|---|---|---|---|
| Garlic powder | Not reported | £62 for 25 kg | - | |
| NEMguard DE | 20 kg/ha | £9/kg | £180 | Approval for outdoor bulb (not salad) onions & shallots. Does not need sheeting. |
| | | | | |
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| | | | | |
| **Biological control agents applied at planting** | | | | |
| Trianum G *Trichoderma harzianum* T-22 | 15 kg/ha into furrow at drilling, 25 kg/ha if transplanting (protected baby leaf crops rates) | Trianum G £74 / 5 kg | £222 to £370 | Trianum G (granules) has UK approval in protected crops. |
| Trianum P *Trichoderma harzianum* T-22 | 15 kg/ha | Trianum P £74 / 500 g | £2220 | Trianum P (powder) **only has UK approval in protected crops.** |
| Serenade ASO *Bacillus subtilis* QST | 2 g/m² in 2L water (experimental); label 10 L/ha | £149 / 10 L | £149 label rate | 77% reduction using Noble 2013 rate, but Serenade is a liquid formulation. Serenade Soil no better than Untreated of F-Abee 2014. EAMU for outdoor crops but no EAMU for onions. Serenade Soil use at 2-6L/ha for vegetables proposed. |
| Prestop *Gliocladium catenulatum* | 5 g/ m² in 2L water<br>Label 0.6 g/m² | £48 / kg | £288 label rate | Rate as Noble 2013 (in 10 L water per 5.4 m² plot). Label rate for strawberry foliar spray 6kg/ha, EAMUS for outdoor crops but no EAMU for onions. |
| T34 Biocontrol *Trichoderma asperellum* | (3g / L water using 7ml / set experimentally).<br>0.5 g/m² growing media | £79 / 250 g | £1580 label rate | Application by spray to growing media, or in irrigation of container-grown crops. **Only Approved for use in permanent protected structures**. Drench of onion sets at planting, Noble 2013) |
| **Biological control agents applied over crop / drench** | | | | |
| Serenade ASO *Bacillus subtilis* QST | (2 g/m² in 2L water experimental)<br>Label 10 L/ha | £149 / 10 L | £149 label rate | EAMU for outdoor crops but no EAMU for onions. |

| **Treatment** | **Treatment rate** | **Product cost (excl VAT)** | **Cost per ha** | **Comments** |
|---|---|---|---|---|
| Prestop *Gliocladium catenulatum* | 5 g/ m² in 2L water<br>Label 0.6 g/m² | £48 / kg | £288 label rate | 10 weeks from sowing. Use outdoors permitted, but no EAMU for onions. |
| Trianum P *Trichoderma harzianum* T-22 | (3 g/ m² experimentally) 15 g/ 1000 plants | £74 / 500 g | £1443 label rate | Drench 1 month after sowing seed or transplant sets to reduce disease incidence using 10 L water per 5.4 m²plot (Noble, 2013). Not permitted outdoors in UK.<br>Assuming 65 plants / m² i.e. 650,000 ware crop onions / ha requires 9750 g/ha |
| **Fungicides applied over crop / drench** | | | | |
| Folicur (tebuconazole) | 1 L/ha | £90 /5 L | £18 | Various products with tebuconazole as the active have EAMUs for use on bulb onions & onion sets against white rot |
| Intellis (penthiopyrad) | 1.5 L/ha on cereals | £131 / 5L | £39 | No approval or EAMU on onions |
| Amistar (azoxystrobin) | 1 L/ha | £41 / L | £41 | Amistar on of the products with approval for downy mildew on onion control |
| Signum (boscalid + pyraclostrobin) | 1.0 kg/ha carrots and cauliflower | £157 / 2.5 kg | £63 | EAMU for use on bulb onions against white rot |

## Knowledge and Technology Transfer

A presentation will be made at the next BOPA meeting to discuss the findings and implications of this review.

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