Project title:	Leeks: White tip control (Phytophthora porri)
Project number:	FV 446
Project leader:	Erika F. Wedgwood, RSK ADAS Ltd.
Report:	Annual report, March 2017
Previous report:	None
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Date project commenced:	1 April 2016
Date project completed (or expected completion date):	31 May 2018

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Varieties, soil treatments and a wide range of foliar fungicides were examined for their effect on White Tip control. At this stage, no treatment combinations were observed that could be used for effective disease management in the field.

Background

Fungicide control of White Tip in leeks is limited by the number of approved foliar fungicide products and the number of applications permitted for each. Fungicide products for other oomycetes such as various downy mildews and potato blight could have potential for the treatment of *Phytophthora porri* on leeks and so require efficacy testing in order to increase the range of modes of action available to growers. Better targeting of the few applications permitted would be beneficial to the control of White Tip, as it is for potato blight, with published research indicating a link between heavy rainfall and the appearance of White Tip. Examination of weather data, and assessment of selected conditions which are correlated with leek infection is required to better understand disease incidence and the development of effective control methods.

Primary infection of the leek crop is through the splashing of *P. porri* resting spores (oospores) onto the leaves and in particular into the leaf sheaths which can remain moist for some time and therefore creating extended periods for white tip development. Oospores are released from infested plant debris, but although growers are aware of the need for crop rotation this is not always possible and so a method of soil treatment, pre-planting, would be beneficial. Chemical soil treatment options are very limited and expensive, but work elsewhere has shown reduction of oomycete activity following incorporation, separately, of two inexpensive waste products, Limex and Gypsum. Limex is already used by some growers to provide phosphate, magnesium and sulphur, while Gypsum (calcium sulphate dehydrate) has been applied in selected experiments for the reduction of *Phytophthora* root rots. Whether or not these products might be effective against *P. porri* oospores is not known.

There are indications that some leek varieties are more susceptible than others to White Tip, however comparison is needed of these alongside each other with a similar level of inoculum. Variety resistance is unlikely to give total disease control, however it is possible that the use of either a biostimulant, or a biofungicide with a mode of action including the stimulation of plant defence responses, could improve control.

It is likely that a combination of cultural and chemical measures will continue to be required by growers to reduce the incidence and severity of White Tip.

Summary

In WP 1, to determine by inoculated test if there are differences in leek cultivar susceptibility to infection by *P. porri,* ten varieties were sown into modules in June 2016. Inoculation tests with breeding lines have shown physiological resistance exists in leeks whereby a hypersensitive response by the plant stops lesion development, and that infection "escape mechanisms" related to shank height may not be as relevant. The varieties were from five different breeding companies and included Triton and Lexton, selected because of known susceptibility to White Tip. Cultivars that have been reported by growers to appear to have a lower susceptibility to White Tip, Pluston and Lancaster, were chosen for the experimental work. Other commonly grown varieties (Mancurian, Curling, O96, Belton, Krypton and Longton) with unknown susceptibility were included following grower consultation. Plots of 10 plants were arranged in a randomised block design outdoors under overhead irrigation at ADAS Boxworth. They were inoculated in a leaf sheath with a suspension of *P. porri* zoospores in September and November 2016. The experiment was still running at the time of this report, but up until the end of January 2017 no statistically significant differences in either incidence (mean 10% of plants with White Tip) or severity (1% of leaf area, principally at the tips) had been seen, although it was noted that no leeks of cv. Mancurian had exhibited symptoms.

In work package (WP) 2 novel conventional fungicides, biological control products and elicitors which may be of benefit against *P. porri* were reviewed. A number of the fungicides reviewed which had potential for off-label approval for foliar application to leeks were put into the WP5 field trial, as AHDB coded products, and others will be included for use at propagation [WP3]. Information was included on the potential for fungicide resistance and biostimulant effect of products used in this project, whereby host defence mechanisms may be elicited.

In WP4, to determine by inoculated tests whether treatments applied to soil may lead to subsequent reduction in White Tip on the plants, treatments of Limex (a by-product of sugar beet processing, containing mainly phosphate, magnesium and sulphur) and Gypsum (calcium sulphate) were incorporated into containers of soil-based growing-media. A measure of control of other soil-borne oomycetes (*Pythium* sp. and *Phytophthora* spp., respectively) had been reported by these products. In June 2016, oospores of *P. porri* were artificially produced and inoculated onto growing-media that had been treated with the equivalent of 5 kg/ha of either product three days earlier. Ten 6 to 7 leaf (three month old) module-grown leek plants were transplanted into each container two days after inoculation. The potentially susceptible variety Triton was planted in half the containers and cv. Pandora in the other untreated, Limex and Gypsum treatments. The containers were arranged outdoors in

randomised blocks at ADAS Boxworth and subject to heavy droplets of irrigation twice daily in order to mimic the splashing of spores from the soil by rain up into the leaf sheaths as naturally would cause White Tip in crops. By September 2016, a mean 30% of the plants had developed White Tip with no significant difference following either the Limex or the Gypsum treatment. Triton and Pandora were also equally affected by the disease, with only a minimal (0.3%) leaf area affected per plant by September, and no further symptom development by mid-October.

In WP 6, a field trial was carried out in the east of the UK (Nottinghamshire) to evaluate foliar applied plant protection products against *P. porri.* A randomised block experiment was set up in a commercial crop of cv. Pluston sown on land with a recent history of White Tip. Products either in use, or pending registration, against *Phytophthora* spp. or downy mildews on other crops, and with potential for off-label registration on leeks, were selected. There were eight experimental products and two products approved on leeks; Invader (mancozeb + dimethomorph) and Infinito (fluopicolide + propamocarb). Two treatments remained untreated to give a total of 12 treatments with four replications. Each product was applied experimentally thrice in succession at timings when the weather was forecast to become wet and so favourable to White Tip infestation of plants by soil-splash of soil-borne spores. Applications were made using an Oxford precision knapsack sprayer at 400 L/ha on 7 September, 9 November 2016 and 6 February 2017 to the four-row beds, with 5 m plot lengths for assessment. First symptoms were seen in August 2016, but did not progress until January 2017. By 1 February virtually all the leeks had White Tip, with a mean 7.2% of leaf area affected per plant in the untreated plots, rising to 13.6% by 1 March 2017. No significant differences were found in the incidence or severity of White Tip between the untreated and treated plots, indicating that none of the products had protected the crop from infection. Weather data including rainfall were recorded using a station in the nearby field margin which relayed readings back to the researchers. These readings will be examined in WP 7 together with recordings to be produced in 2017/18 in order to explore the potential for weather-based disease forecasting to assist spray application timings.

Further work packages of assessing the use of products in propagation against White Tip (WP 3), and a second year of evaluating foliar fungicides (WP 5) but located in the west of England will be carried out in 2017/2018.

Financial Benefits

Although leek white tip is a sporadic disease of mainly late crop leeks, when active it can cause very serious losses. Given a yield loss estimate of 7.5% this would equate to a financial ingress of £2.25 million annually for leeks were current fungicide control measures not able to be used. In a wetter than average winter the severe losses experienced late in the season can give losses of £3,250/ha when 50% of a crop is affected, (based on typical costs of production of such a crop of at least £6,500/ha).

Action Points

- Be aware of any history of White Tip in fields being considered for leek planting
- If possible leave at least three years between leek crops
- Removal of infested leek debris/trimmings from fields should reduce the amount of resting spores which will be deposited in the soil from leaf lesions
- Although not proven in the current work, the selection of certain varieties has the possibility of reducing the level of White Tip infection in a crop
- Target foliar fungicide application to periods of forecast heavy rain as this is when infection is most likely to take place
- Be aware that because fungicide applications against White Tip infection can be months apart protection can be lost before an infection event, and so inspection for symptom development will still be needed
- Consider precision spray application onto patches of plants with White Tip
- Be aware that irrigation that splashes soil onto the crop is more likely to encourage White Tip development than lighter droplets, particularly if the leaves then remain wet for a prolonged period in which *P. porri* spores can penetrate the tissue
- Be aware that White Tip lesions are enlarged by secondary invasion of fungi and so consider fungicide application against them as well
- Be aware that there are other causes of white tipping on leeks - various stresses such as drought and scorching by wind, frost or chemicals, but that fresh *P. porri* lesions tend to be a brighter white, often with a wetter/softer appearance (which can cause leaf tip dangling) and tending to have a sharp boundary with green tissue rather than a progression of yellowing
- It may be prudent to lift leek crops with White Tip earlier than intended to reduce the need for trimming and also to curtail disease development and oospore production.

SCIENCE SECTION

Introduction

Leek White Tip (*Phytophthora porri*) can give severe losses of up to 50% in a single leek crop late in the season, especially in wet winters. Although overall losses to White Tip in an average year can be <5%, the severe losses experienced late in the season can give losses of £3,250/ha when 50% of a crop is affected, taking into account typical costs of production of at least £6,500/ha. A severe loss of a large area of crop may also lead to extra costs in time and money, as replacement product has to be sourced from elsewhere to maintain programmes scheduled with retailers. Even if a crop affected by White Tip may be saleable, there will be extra costs at harvest due to the extra trimming required to remove the affected leaves. This also slows harvesting down, reducing the speed of output, and again adding to the costs of production.

Leek growers are aware of the need to apply fungicides for the control of White Tip, but the products available are very limited and the period required for protection of the overwintering crop is long. The pathogen has a long-lived resting spore stage in the soil from leek leaf debris and the disease can suddenly erupt in wet conditions. Unless controlled, the incidence and severity of white lesions developing on the foliage can reach epidemic proportions.

The pathogen is an oomycete, and research and development by the AHDB and globally on plant protection products, including biofungicides, and also stimulants and soil treatments has been carried out against other oomycetes such as *Phytophthora infestans* of potato and downy mildews of various crops. Information directly related to White Tip is limited and has focused on variety susceptibility and seeking to understand how weather conditions influence the entry and spread of the pathogen (Smilde, 1996; Declercq et al., 2012). This project aims to utilise this information in experiments on methods of White Tip control, and will consider a range of approaches to test for efficacy.

Epidemiology

The lifecycle of P. porri has been studied in the laboratory and field by Declercq *et al.* (2011). Oospores of *P. porri* are formed in infected leaves and enter the soil with leaf debris and survive beyond summer. In temperatures between 0°C to 24°C with free water available, the oospores germinate and form a germ-tube which develops one or more sporangia. The sporangia burst open, resulting in large amounts of zoospores (flagellate swimming spores) in free water and puddles in leek fields. The zoospores are then transferred to the leeks during a rain event as a result of rain splash onto the surface of the leaves and encyst. After an incubation period of about 12 hours the germ tube that is formed penetrates the leaf indirectly through the stomata. Once inside the leaf, the hyphae grow intra-cellularly between the epidermal cells, leading to the typical symptoms of *P. porri*. Lesions are formed on the leaves and sporangia can be formed on wet leaves that release 10-30 zoospores, although Declercq *et. al.* (2011) have queried the importance of this means of spread. Oospores are produced in old lesions and reach the soil when leaves decay (the oldest leaves can start to droop to the soil and decay while the plant is growing), and the oospores can survive in soil for at least four years (Declercq *et. al.*, 2011). A diagrammatic illustration of the probable lifecycle of *P. porri* causing primary infection from the soil and secondary spread from lesions on infected leek plants is shown in Figure 1.

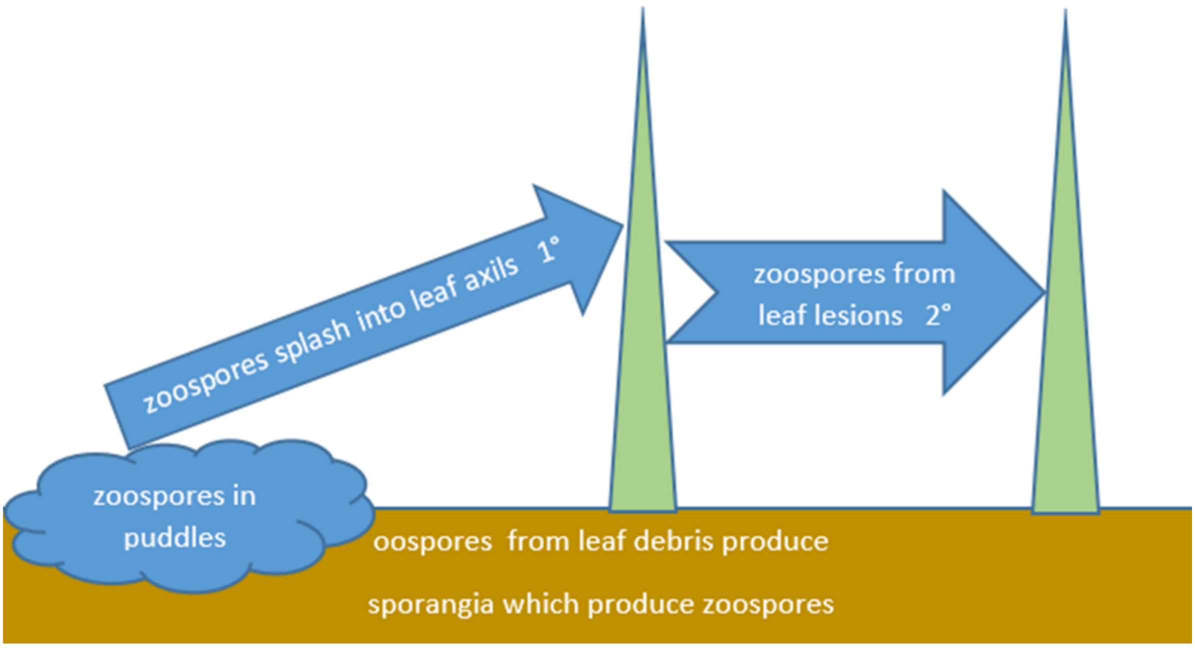

Figure 1. Sources of primary and secondary infection of leek plants (shown as triangles) by *Phytophthora porri*. Zoospores originate from sporangia formed from oospores in the soil then infest leaf axils via water splash. Sporangia can then develop in leaf lesions and release zoospores in wet weather to cause further leaf infection.

The epidemiology of *P. porri* was studied as part of a PhD by Smilde completed in 1996 and information on the relevance of rainfall quantity (Smilde *et al*. 1996a) and temperature (Smilde *et al*., 1996b) published and developed further by Declercq *et. al*. (2011). Smilde used a day-degree model for incubation periods at various temperatures and confirmed the White Tip increase is correlated with rain, this being greatest early in the season possibly as secondary infection by sporangia appeared not be so strongly rain driven. From the timing of appearance of fresh lesions in the field (in Lancashire between October and March) following rain events that were conducive to infection it was proposed that the period in which the disease remains symptomless in the plant lengths at lower temperatures (Locke, 1996). Disease progress has been shown to be correlated with average daily rainfall, with greatest correlation early in the season. This was hypothesised to be because secondary spread is less reliant on rain splash (Smilde, 1996). Declercq et al (2011) showed that leeks grown in infested soil do not become infected if they are experimentally covered by polythene frames.

Symptoms

White Tip causes severe foliar symptoms on leeks. Epidemics may destroy the crop before the crop would otherwise have been harvested between January and April. The initial symptoms are blanching of the leaf tips and water-soaked spots on the leaves (Figure 2). The leaves will then die rapidly from the tips and become white, dry and crisp. Leaves often also become distorted and twisted, with the infection travelling to almost half way down the leaf. Many infections can start in the leaf basin that is usually present near the leaf axils, though lesions are seen more at a distance above the water basin due to the growth of the leaves during the incubation period. Both mature and immature plants are affected, and severe infections can result in the leaves rotting off at the soil level. The symptoms are similar in onions, where white spots surrounded with green water-soaked patches are present all over the leaf. Severely damaged leaves will die off. Sometimes, *P. porri* will cause shanking in onions and shallots, where the base or bulbs will become water-soaked and soft.


Figure 2. White Tip lesions showing blanching of the leaf tip (left) as well as blanching by the leaf axil (right). Note secondary colonisation by *Cladosporium* sp. causing dark green sporulation on the leaf tip. In each case there is a water-soaked edge to the *P. porri* lesion. Nottinghamshire, 2017.

The initial infection develops from soil infestation as zoospores released from resting spores (oospores) splash onto foliage (there is no root infection by this *Phytophthora* species). Sporangia are produced on wet leaves. They do not detach from the stalk (Erwin and Ribeiro, 1996) and so must release zoospores which can then be splashed to other plants. Infection foci thus enlarge and epidemic development across the field can occur. Oospores develop in infested leaves and debris returned to the field provides inoculum to infect Allium crops on too short a rotation. There is no disease forecasting programme for White Tip for growers to guide fungicide application timing to leek crops. Other crops have had disease forecasting programmes developed to aid fungicide application timing, including other oomycetes such as potato blight and more recently downy mildew and white blister of brassicas (Gillies et al., 1996; Kennedy, 2005) and downy mildew on rose (Xu, 2011). The ready availability of in-field signal transmitting electronic weather stations and reception of information including weather forecasts through mobile phones is making spray timing decisions based on the probability of infection conditions a more realistic proposition for more growers. Optimum conditions are likely to include temperatures between 4 and 22°C to allow sporangia to be produced by oospores and then heavy rain to facilitate zoospore release into puddles and from there to splash onto plants,

Crop susceptibility

Leek White Tip mainly affects leek crops that overwinter, due to the increased risk of infection when it is wet and humid with a greater occurrence of standing water in the crop. Greater incidence of the disease is seen in varieties such as Triton (Syngenta), whereas varieties such as Pluston and Lancaster may have a lower susceptibility. More vigorous varieties have been reported to have a greater level of tolerance to the pathogen and appear to be less susceptible. There have been claims that some of the stockier varieties have more resistance to White Tip. Variety selection for disease resistance can be particularly useful in organic crops and they can work together with biofungicides to supress disease. Varietal resistance/reduced susceptibility could mean reduced reliance on fungicide applications; one problem with the latter being the difficulty in particular in locations in the UK, such as the Lancashire leek growing areas, of prolonged wet periods and not being able to spray when the weather and ground conditions are favourable for the spread of *P. porri.*

Fungicide treatment of *P. porri* should be made preventatively, and growers are aware that their crops show disease following rainfall. After seeking cultural control by crop rotation, the main control method used to control White Tip is to apply preventative fungicides such as Amistar Top (azoxystrobin and difenoconazole), Nativo 75WG (trifloxystrobin and tebuconazole) and Signum against pathogens in general. Invader (dimethomorph and mancozeb) is applied as an eradicant specifically targeted at White Tip.

There is strong evidence of physiological resistance to *P. porri* in leek varieties that is probably related to a hypersensitive response by the host to infection. In less resistant varieties the pathogen may still then be able to develop around the green margins of the lesion (Smilde, 1996). Hypersensitivity of the host to the pathogen causes plant tissue necrosis at the point of pathogen penetration, thereby halting pathogen progress to within the lesion and sporulation is also stopped (Smilde *et al*., 1995; Smilde, 1996). Inoculation tests in the glasshouse involving dripping *P. porri* zoospores into the leaf sheath pockets of different breeding lines of leeks were shown to correlate well with results of plant resistance following natural infection in field plots (Smilde, 1996). This indicated that field resistance was not based on an escape mechanisms such as increased shank length (Smilde, 1996). It does not, however, discount any benefit from having leaf sheath pockets (axils) at a greater height up the shank from the ground and so less liable to trap splashed spores from the soil in them.

P. porri does not infect via the roots, but zoospores are released from oospores in the soil to splash onto leaves. For this to happen the zoospores must be at the soil surface and so deep penetration of the soil by a treatment would not be required and control of spores on the leaves by a spray would be possible.

Fungicide control measures

Prophylactic application of foliar fungicides against white tip is currently carried out in autumn and spring based on grower knowledge of when white tip symptoms have most often been seen in the absence of control measures. Currently growers only have two conventional fungicides approved for the control of white tip. These are Invader (dimethomorph and mancozeb) and Infinito (Fluopicolide + propamocarb hydrochloride), with Infinito only gaining approval very recently in 2016 to give growers a further mode of action. Other fungicides are approved for use on the crop but these are less effective against white tip and applications of these are targeted towards the control of rust through the summer and autumn months. These products are Amistar Top (azoxystrobin and difenoconazole), Nativo 75WG (trifloxystrobin and tebuconazole) and Signum (boscalid and pyraclostrobin).

Integrated control measures

Crop rotation of four or more years is a key method of disease avoidance (Declercq et. al., 2011). Ideally the crop will be grown on a long rotation in order that the resting spores of *P. porri* lose their viability, however, commercial crops are becoming infested and so means of protecting the leeks are required. The effectiveness of a straw-covering around the base of the leeks to prevent rain splash was tested in Holland, but Locke (1996) in FV 172 found this ineffective as the straw degraded over time. Reducing compaction, improving field drainage and taking care with irrigation management to minimise the production of standing water will reduce the conditions favourable for zoospore infection.

A number of disease management measures may be needed to protect the crop at stages when the pathogen is most likely to infect. These could start with the selection of a less susceptible variety, treatment of plants prior to transplanting in order to increase their defence responses against pathogens, the treatment of soil pre-planting or sowing, and the introduction of different foliar applied fungicide products from the standards currently available. The targeting of foliar applications based on information relating infection risk to weather conditions (in particular rainfall) would also be beneficial because of the long cropping season and the limited number of fungicide applications possible because of product restrictions for resistance management.

A number of approaches for control of white tip are being investigated in this project, and in the 2016/17 season varietal resistance (WP1) and soil treatments (WP4) were tested in pot experiments alongside a field trial in the midlands screening a number of foliar fungicide treatments (WP6). Methods and results for each work package are presented below.

In 2017/18 propagation treatments (WP2), and a further field trial (WP5) will be carried out.

WP1. To determine by inoculated test if there are differences in leek cultivar susceptibility to infection by *P. porri.*

Materials and methods

Plant production and maintenance

Ten leek varieties were selected for the trial from across the seed houses following discussions with each, selecting mainly later harvested varieties that commercially would be in the ground overwinter and so more likely to receive infection by *P. porri*. A variety with greater susceptibility (Triton), and another with lower reported susceptibility (Pluston) were also included (Table 1).

Seeds were sown on 15 June 2016 into peat-based growing-media in module trays of cell width 78 x 65 mm and placed in a polytunnel. On 12 July they were moved outside into their positions in a randomised block design with four replicate blocks. These were all to be inoculated with *P. porri*. Ten plants (one per cell) were positioned close together in each plot, stood on capillary matting within latticed plastic trays (Figure 3), with a variety at opposite ends of the tray with a space in between. A temperature and humidity logger was set up in a ventilated screen stood in one of the trays. A further tray of 10 plants of each variety was set four metres to the side of the main trial to remain uninoculated for use as an aid in distinguishing any other form of white tipping that might occur from that resulting from *P. porri* inoculation.

Table 1. Leek varieties selected for the testing of resistance by inoculation with *P. porri* zoospores. ADAS Boxworth 2016/17

Treatment number	Variety	Breeder
1	Triton (susceptible)	Syngenta
2	Mancurian	Syngenta
3	Curling	Bejo / Elsoms
4	O96	Enza Zaden
5	Belton	Nunhems / Bayer
6	Krypton	Nunhems/Bayer
7	Lexton	Nunhems/Bayer
8	Longton	Nunhems/Bayer
9	Pluston	Nunhems/Bayer
10	Lancaster	Tozer Seeds

Overhead sprinkler irrigation surrounding the trays was calibrated to give a water volume of 150-200 ml in 15 minutes and turned on in the morning and afternoon for this time period, unless it had been raining. Plants were given weekly foliar applications of 20 ml Tomorite fertiliser in 4.5 L water as directed on the label. No experimental treatment applications were made to the plants and no routine fungicide or insecticides were needed. Weeding was carried out by hand.

Figure 3: Four replicate blocks of ten leek varieties (two varieties per tray), December 2016. ADAS Boxworth

Inoculation

Inoculation was carried out to mimic the mode of infection which is believed to occur in the field, in which zoospores are released by soil-borne oospores. These splash onto leaves and fall (or are washed by rain) into the leaf sheaths where water can be held for days. A method for testing the susceptibility of leek breeding lines was devised in the PhD by Smilde (1996) and was followed for the current experiment.

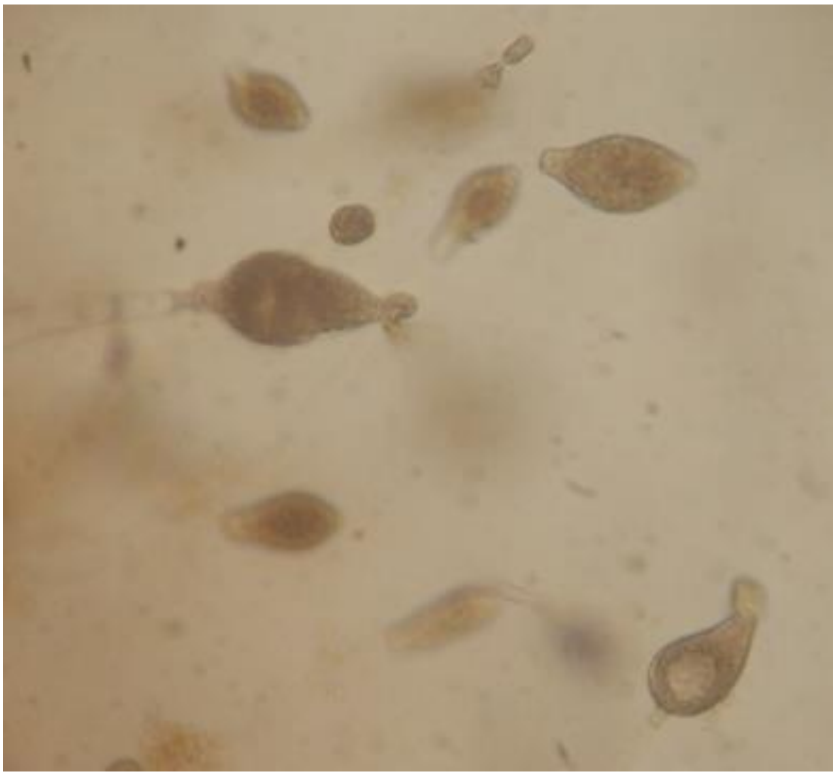

Figure 4. Pear-shaped sporangia of *P. porri* produced in soil water and releasing zoospores from their tips following chilling and warming in the laboratory at ADAS Boxworth

The isolate BX15/102 obtained from an unknown variety (a volunteer in a field) of leek in December 2015 was bulked up on 10% V8 agar plates for two weeks. Plugs of mycelium were then moved to incubation in soil water to produce sporangia within 48 hours. On the inoculation day on 29 September 2016, when plants had around six leaves, the plates were moved to a fridge for an hour and then returned to room temperature in order to trigger zoospore release (Figure 4). Zoospore concentration was estimated at between 10^4 and 10^5 per ml (the count cannot be accurate as the spores swim back and forward in the counting chamber). One ml of zoospore suspension was taken with an electronic pipette to inoculate each of the 400 plants into the third leaf sheath up from the bottom of the plant. Care was taken when handling the zoospore suspension to prevent them encysting as a result of a shock, and to keep them suspended throughout the inoculum by gentle movement of the liquid.

As a control, the plants to be left uninoculated were also given 1 ml of soil water into the same leaf sheath in case this non-sterile water used to induce sporangial production for the inoculated plots had any independent effect on the leeks.

The zoospore production and inoculation procedure was repeated after symptoms had not developed on the leeks a month after the September inoculation. Plants were re-inoculated on 14 November 2016 during a spell of warmer than average and drizzly weather that was anticipated to be favourable to disease establishment.

Assessments

Plants were examined for White Tip symptom development on 27 October 2016 when symptoms were first likely to be visible as white lesions, four weeks after the first inoculation in late September. When the plants were re-inoculated in mid-November they were re-examined to check for any symptom development resulting from the earlier inoculation. Plants were again examined on the 10 December 2016 and 9 January 2017 for any symptoms. A full assessment of the incidence and severity of White Tip on the leaves was made on 23 January 2017 when a number of varieties were showing symptoms.

At each assessment date, records were taken for plants in the same order (clock wise starting always from the top left) so that a track could be kept of the any symptoms development over time in individual plants. This also meant that if plants died from White Tip and rotted then they could be included in any analysis of the total number of plants infected. A pre-inoculation assessment was made for any potential symptoms and at each visit the uninoculated plants were compared with the inoculated. Crop vigour was recorded per plant with a measure of height to the uppermost leaf axil (where the youngest leaf was emerging). Disease incidence was scored as 0=for no incidence and 1=for White Tip presence per plant, the number of leaves with White Tip counted (as an indication of what trimming might be needed) and the % of the plant area affected. No chemical treatments were applied, but any scorching or distortion of the foliage (such as from wind or frost scorch) was looked for at each of the assessments and described in the trial diary. The experiment was maintained with further observation until mid-May 2017, which would be the finish of a late commercial crop (results to be reported in the Final Report).

Results

The plants were assessed individually on the 27 October. Some white tipping was present, but did not fully match the whiter, papery, symptoms with a distinct margin expected from *P. porri* and it was principally on older leaf tips (including the uninoculated) and probably physiological. No White Tip had developed.

Following the second inoculation (14 November), the plants were examined at intervals, but no White Tip was seen until the 23 January 2017. White Tip was then seen on the tips of the youngest leaves which had been inside the leaf sheaths at the time of the inoculations. Some blotchy White Tip lesions were also seen on the leaf blade of a few older leaves. However, there was no significant difference between the varieties (Table 2), with a mean of 10.6% of plants affected (P=0.167, L.s.d. 12.11), although no White Tip was seen on the variety Mancurian and the potentially less susceptible (from grower experience) Pluston had an incidence of 5%. The number of leaves per plant affected by White Tip was low and also did not differ significantly between varieties, with a mean 0.122 per plant (P=0.084, L.s.d. 0.137). The % of total leaf area affected by White Tip was only a mean of 1%, with no significant differences between the varieties (P=0.450, L.s.d. 2.867).

Table 2. Leek varieties inoculated with *P. porri* zoospores and tabulated in order of increasing mean % of plants affected, with their mean height to the uppermost leaf axil from ground-level, and the incidence and severity of White Tip on 23 January 2017.

Treatment number code	**Variety**	**Height to top of youngest leaf axil (cm)**	**White Tip symptoms**		
			Mean % of plants affected	**Mean number of leaves affected / plant**	**% leaf area affected**
2	Mancurian	12.03	0.0	0.0	0.00
9	Pluston	13.16	5.0	0.05	0.14
6	Krypton	12.41	7.5	0.10	2.00
1	Triton	10.70	10.0	0.13	1.20
8	Longton	13.99	10.0	0.13	0.19
4	O96	12.10	12.5	0.13	1.64
10	Lancaster	11.46	13.1	0.15	2.81
5	Belton	12.41	15.0	0.15	0.35
7	Lexton	14.19	15.0	0.15	0.39
3	Curling	14.60	17.5	0.25	2.04
Mean		12.60	10.6	0.12	1.08
L.s.d.		1.421	12.11	0.137	2.867
F. pr.		<0.001	0.167	0.084	0.450

On 23 January a highly significant difference was shown in the height of plants to the top of the youngest leaf sheath. The varieties Triton, Mancurian and O96 were two or three centimetres shorter than Curling, Lexton and Longton (probably due to their genetics).

Figures 5 and 6 show graphs of daily temperatures and relative humidities adjacent to the trial. The air temperature following the first inoculation on 29 September 2016 was a mean 11.9°C and fell steadily through autumn to 6.7°C by 29 October (with relative humidities of 84 and 98, respectively). By the second inoculation on 14 November it was a mean 5.5°C and after month, when infection should have been establishing, the mean temperature was 5.9°C on 14 December (with relative humidities 93 and 97, respectively).

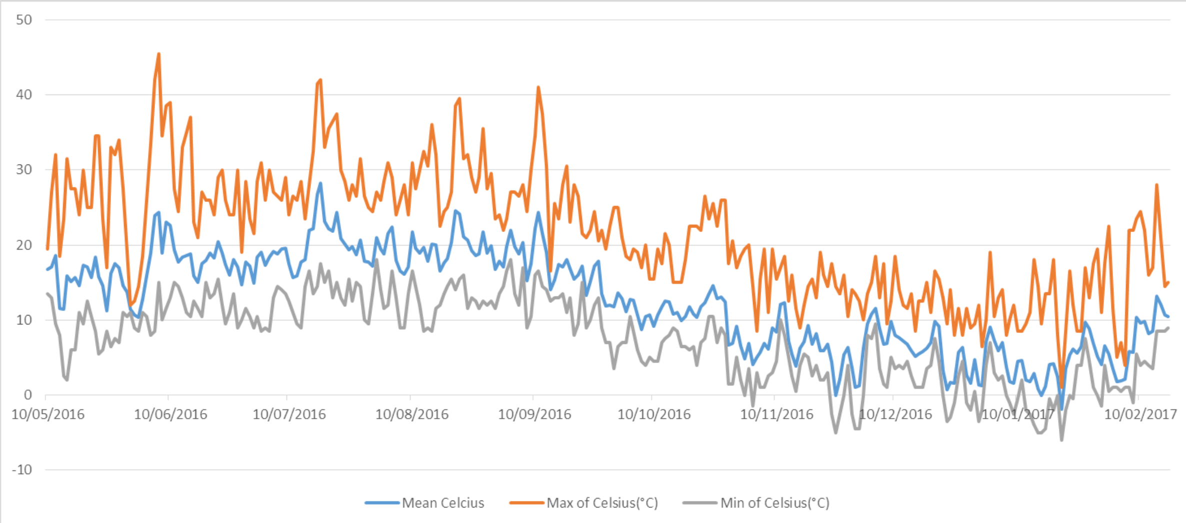

Figure 5. Mean, maximum and minimum daily air temperature adjacent to leek containers between May 2016 and February 2017. ADAS Boxworth hardstanding area.

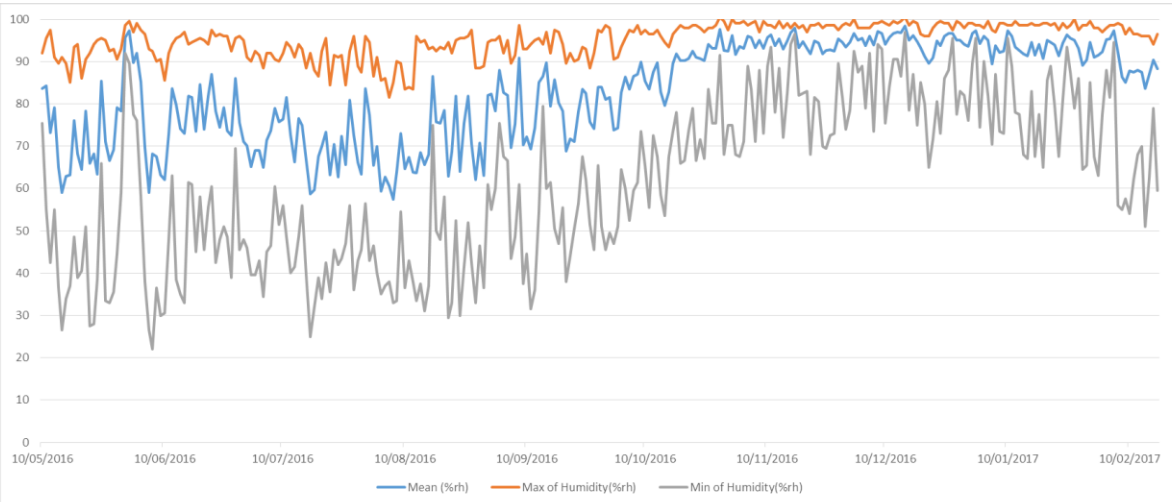

Figure 6. Mean, maximum and minimum daily relative humidity adjacent to leek containers between May 2016 and February 2017. ADAS Boxworth hardstanding area.

WP2. To review the novel conventional fungicides, biological control products and elicitors which may be of benefit against *P. porri.*

An overview of the mode of action on elicitors was given in a review of aerial oomycetes, CP 157 in 2016. Such products stimulate plant's own systemic defence responses against pests and diseases and so prime them in advance of any attack. While there are chemical substances such as plant extracts e.g. of *Reynoutria sachalinensis* that can act as elicitors there is also evidence that fungi and bacteria in biofungicides e.g. *Trichoderma asperellum* and *Bacillus subtilis* can also induce systemic acquired resistance in plants.

Fungicides targeted against oomycetes vary in the likelihood of resistance developing and this information was tabulated in a review of root infecting oomycetes, CP 126 in 2015 (Appendix 1). Active ingredients such as azoxystrobin and fenamidone from the QoI fungicide group, and metalaxyl from the phenylamide group have a high resistance risk. The active ingredient ametocradin from the Qxl fungicide group has a medium-high risk. Dimethomorph and mandipropamid from the CAA fungicide group have a low-medium risk, as does the carbamate propamocarb HCl, and the urea cymoxanil. However, the resistance risk of newer actives such as the acylpicolide fluopicolide are not known and it is important for all products that fungicide programmes are developed with alternating modes of action by utilising FRAC Groupings.

Information on products with potential activity against *P. porri* were reviewed as part of the current project to allow the selection of products for the experiments on the soil and foliar treatments. Treatments used in this project were those which the supplying companies agreed might be able to be used by growers in the UK in the near future were an Extension of Authorisation for Minor Use (EAMU) able to be obtained.

Conventional chemical plant protection treatments

Currently the strongest fungicide option for growers to control White Tip in leeks is Invader (dimethomorph + mancozeb) which was identified and approved for use after work on its efficacy in AHDB Hort project FV 172. Infinito (Fluopicolide + propamocarb hydrochloride) gaining approval in 2016 gave growers a further mode of action. Metalaxyl-M used to have an approval, and evidence of resistance to this had been identified in FV 172, as has also occurred in other crops e.g. downy mildew in lettuce. Many of the approved chemicals are protectant in activity, and once white lesions have been observed in the crop it is then stated it is too late to treat (Declercq, 2009). However, there is secondary spread from lesions to healthy tissue (Smilde, 1996) and so application of fungicide with curative and/or anti-sporulant activity should stop the enlargement of the foci of infection from the soil and prevent the development of an epidemic.

Approved alternative products to Invader are available and some have activity against White Tip, however they are broader spectrum and tend to be applied mainly to target the control of rust through summer. These are Amistar Top (azoxystrobin and difenoconazole), Nativo 75WG (trifloxystrobin and tebuconazole). Signum (boscalid and pyraclostrobin) is approved for use on leeks against White Tip under EAMU 2134 of 2010. There is little potential to use these products both in summer for rust and in autumn/winter for White Tip due to restrictions on the number of sprays permitted and concerns over resistance development. Therefore growers are keen to identify alternative products for control of White Tip, especially those with more specific activity against oomycetes.

There are a number of products available against potato blight, *Phytophthora infestans*, within Europe and rated in the Euroblight fungicide table as having a good curative and anti-sporulant effects based on field trials and in commercial crops **http://euroblight.net**. This includes Consento and Infinito containing propamocarb-HCl plus, respectively, either fenamidone or fluopicolide. Invader (dimethomorph + mancozeb) does not have as good curative action.

Phytophthora spp. are oomycetes (not fungi) and this grouping includes downy mildews. Work in SCEPTRE on downy mildew in Alliums has identified useful products; Cassiopeia (dimethomorph plus pyraclostrobin) and a product coded 197 and these would be tested for efficacy against *P. porri.* Cassiopeia was also effective against *Phytophthora cactorum* causing crown rot in strawberry.

Products such as Paraat (dimethomorph) and Fenomenal (fenamidone + fosetyl-Al) can be applied as drenches to control soil-borne pathogens such as *P. cactorum*. There is no evidence of infection via the roots by *P. porri*, but these products could be useful to control oospores and/or zoospores in the soil-borne phase of White Tip before the spores are splashed up to infect the leaves. Fenomenal is also said by the suppliers to stimulate the plant's natural defences and promote root growth. The high volume application of at least 1000 L/ha required for drenches is well above the standard 200-400 of water volume L/ha for field crops dictated by both the spray tank capacity and the increased time required for application, however if "hot-spot" spraying of infection foci is developed and taken up growers perhaps using precision farming imaging or in-crop sensor techniques coupled with global satellite positioning (GPS) as currently available for nitrogen application (N sensor) then targeted drenching is a future possibility. Drenching, is however feasible in propagation where seeds are sown in module trays of growing media, because total spray volumes would be less, and it might be possible to "prime" plants to resist infection. Seed treatments including systemic fungicides for early foliar disease control, and a phosphite stimulant, are available on cereals, but the small amount of product used may limit the duration and/or strength of protection against leek white tip to the period close to sowing. Foliar treatment would continue to be needed to protect against infection of plants later in the crop year. Only thiram is currently authorised on leek seeds, but it has no efficacy against oomycetes such as *Phytophthora* species.

Non-conventional plant protection products

Regalia is a product available in the USA which is an extract of the Giant Knotweed *Fallopia sachalinensis* (formerly *Reynoutris sachalinensis*), but this is not yet registered in the UK. According to a US Environment Protection Agency fact sheet, when sprayed on plants the extract causes the plants to activate an internal defence system that prevents growth of certain fungi, especially powdery mildew and grey mould. It gave moderate control of downy mildew *Plasmopara viticola,* powdery mildew, *Uncinula necator* and bunch rot *Botrytis cinerea* in a vineyard, although it was out-performed by standard fungicides (Schilder *et al.,* 2002). Research in SCEPTRE has shown this product to be effective against powdery mildews, with another chemical elicitor (coded and with uncertainty about marketing in the UK) effective against onion downy mildew (O'Neill, 2015).

Secondary colonisation of white tip lesions by bacteria and other fungi takes place and this can then allow further tissue collapse. It is possible that products or materials that strengthen the tissue could reduce damage. Some conventional fungicides with activity against downy mildews such as Amistar (azoxystrobin) and Signum (boscalid + pyraclostrobin) are also known to have elicitor activity and were tested against a bacterial species on onions in AHDB project FV 417.

Biofungicides may develop an antagonistic population of beneficial microbes and many are also said to stimulate plant defences. These include products such as Serenade ASO (*Bacillus subtilis* strain QST 713) with known anti-bacterial as well as antifungal activity and Prestop (*Gliocladium catenulatum* strain J1446) which has efficacy against fungi and oomycetes. These both have approvals for soil or compost application against oomycetes and so might have a role in control of the resting stage on the soil surface and leek leaf debris. Two microbial fungicides in SCEPTRE gave some *P. cactorum* (crown rot) control; Prestop (*Gliocladium catenulatum*) and a *Trichoderma* sp. coded product. Serenade ASO is authorised for use on outdoor leeks under EAMU 0306 of 2015 with *Phytophthora*, *Pythium*, and *Rhizoctonia* listed as being controlled. T34 (*Trichoderma asperellum* strain T34), and the plant growth promoter Trianum G (*Trichoderma harzianum* strain T22) are available in the UK against *Phytophthora*, *Pythium* and other root rots.

Materials other than plant protection products (plant strengtheners)

Plants use physical and chemical barriers to prevent infection. In addition, they use their innate responses to ward off pathogens (Rivière *et al.*, 2011), having evolved the ability to detect microbes. Pathogens can suppress this by secreting effectors. Plants can respond by effector-triggered immunity (ETI). ETI is associated with both localised hypersensitive response (HR) and the whole plant systemic acquired resistance (SAR) that is long lasting and effective against a broad spectrum of pathogens.

Natural compounds which activate various plant defence responses include chitosan, laminarin, salicyclic acid and the salicylic acid derivative acibenzolar-S-methyl (also known as BTH; benzo (1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester). BTH protects against a broad spectrum of pathogens (Rivière *et al.*, 2011) and it is available in the product Insimmo, which is registered (but not marketed) in the UK against Chrysanthemum white rust. Elicitors are recommended as supplements that allow fungicide application to be reduced.

There are some products sold as fertilisers including potassium phosphite products and calcium products that have been shown to indirectly reduce oomycete infection. Farmfos (potassium phosphite) was effective against shrub and herbaceous downy mildew in HNS 186 and the same chemical as Horti-Phyte applied as a soil drench to Camellia appeared to allow symptomless leaf regrowth from a *Phytophthora ramorum* infested bush (Wedgwood *et al*., 2013). Potassium phosphite tree injections are used on *Banksia* sp. and *Eucalyptus* sp. to protect them from *P. ramorum* (Scott et. al., 2012). The mode of action is reported to be direct antifungal activity of phosphonate toward mycelial growth and, perhaps, indirect stimulation of host defences. Phosphite is transported in the xylem and phloem and this was shown to be effective in the control of potato blight (Johnson *et al.,* 2004).

Limex (calcium) is a coproduct from British Sugar factories and when incorporated into *Pythium violae* (an oomycete) infested fields before drilling carrots fewer roots showed the cavity spot disease it causes (Boor, 2004). Gypsum (calcium sulphate) a waste-product from plaster-board manufacture has been shown to reduce *Phytophthora* root rots including *P. rubi* of raspberry when used as a pre-planting soil amendment, also increasing yield (Maloney *et al*., 2005) and has been tested against *Phytophthora* root rots of avocado (Messenger *et al*., 2000) and pawpaw (Vawdrey *et al*., 2002). Sporangial production of *Phytophthora cinnamomi* was reduced in gypsum-amended soil (Messenger *et al*., 2000). The active ingredients of Limex are phosphate, magnesium and sulphur (expressed as P_2O_5, MgO and SO_3) and

Gypsum is calcium sulphate dehydrate. These products are likely to be cost-effective to apply to leek fields before planting. Although a number of experiments are being carried out in AHDB projects, in particular on strawberry and ornamentals, with products such as biochar, plant growth promoting rhizobacteria, lavender waste, and brassica meal in order to increase the population of beneficial microbes around plant roots against soil borne *Phytophthora* and *Verticillium* species these would be more expensive products for use across whole fields.

WP4. To determine by inoculated tests whether treatments applied to soil may lead to subsequent reduction in White Tip on the plants

Materials and methods

Treatment application

Two treatments, Gypsum and Limex, able to be incorporated into soil were tested for pre-planting treatment of soil infested with oospores of *P. porri*. A rate of use of 5 kg/ha was selected for Limex based on work on carrots with cavity spot (Boor, 2004) and this rate was also selected for Gypsum based on the work on *Phytophthora* root rot of papaw (Vawdrey *et al*., 2002). The dose rate of 34.5 g per planting container of 320 mm x 230 mm was calculated based on the 690 cm^2 surface area of the containers. The containers were plastic washing up bowls with drainage holes in the base. Each container was designated as one plot. A John Innes soil-based No 2 growing-media was used rather than field soil so that there would not be any unwanted pathogens or pests introduced.

Table 3. Leek varieties, growing-media treatments and whether or not the growing-media was inoculated with *P. porri* oospores. Un-inoculated containers were stood away from the inoculated. ADAS Boxworth 2016

Treatment	Variety	Growing-media treatment 10 June 2016	Product per bowl (g)	Inoculated 15 June 2016
1	Triton	Untreated	0.0	No
2	Triton	Untreated	0.0	Yes
3	Triton	Gypsum @ 5 kg/ha	34.5	Yes
4	Triton	Limex @ 5 kg/ha	34.5	Yes
5	Pandora	Untreated	0.0	No
6	Pandora	Untreated	0.0	Yes
7	Pandora	Gypsum @ 5 kg/ha	34.5	Yes
8	Pandora	Limex @ 5 kg/ha	34.5	Yes

On 10 June 2016 the growing-media for four replicates of two containers (i.e. one plot per replicate for each of the two varieties to be planted later) were treated with Limex (T4 and T8) and another eight containers (T3 and T7) with Gypsum (Table 3), ensuring that the powders were well distributed through the soil by mixing each in a larger container and then turning the treated growing-media into the planting container. Each container was filled to within 40 mm of the rim with four litres of treated growing-media. A further eight planting containers were filled with untreated growing-media. Samples of the untreated and treated growing-media were sent for nutrient analysis (Appendix 2).

Inoculation

The containers were left for 72 hours after treatment incorporations on 10 June before the appropriate treatments were inoculated with a suspension of *P. porri* oospores (Table 3). The oospore suspension was produced by growing an isolate of P. porri (BX15/102, isolated in December 2015) on 10% V8 agar for a month to produce oospores and then adding 15ml of a 0.5% w/v solution of lysing enzymes of *Trichoderma harzianum* to each of 23 agar plates and leaving them at around 15°C in the dark for 17 days. The *P. porri* was then taken off each agar plate into a tube and centrifuged three times and the resulting plugs of oospores re-suspended in 10 ml sterile distilled water (discarding the supernatant). A spore count of 1×10^{6} / ml was obtained. This procedure followed methods used in PhD work by Smilde (1999) and Declercq et al. (2012).

On 13 June 2016 15 ml of oospore suspension was syringed over the growing media surface of each of the containers of T2-T4 and T6-T8, aiming to inoculate evenly across the whole surface. This resulted in 15 Million oospores in an area of 0.6 m^2. Four replicate containers for each of the two varieties were left uninoculated (T1 and T5) and kept two metres away from the inoculated. The containers were left for 48 hours after inoculation, keeping them moist, before leek plants were transplanted into them.

Planting

Leek plants for the experiment were sown in early March 2016. Two leek cultivars were selected; Triton (bred by Syngenta) which is a potentially White Tip susceptible variety and Pandora (bred by Elsoms-Bejo). The seedlings were initially glasshouse-grown in peat-based growing media in 70 mm deep, 80 x 80 mm-celled module trays, and moved to a polythene tunnel after 11 weeks, before transplanting outdoors into the containers at 14 weeks.

On 15 June 2016 a trowel was used to push apart the growing media to make holes in order to transplant the leeks into the soil so causing minimal disturbance of the growing media beyond each planting hole. The trowel was cleaned between treatments. Ten plants were spaced out in an oval shape around each bowl. The Triton had six to seven leaves and the Pandora had seven to eight leaves at the time of transplanting, three months after sowing.

The nutrients in the growing media were supplemented as required by foliar feeding with Miraclegrow fertiliser as directed on the label, and ammonium nitrate prills were scattered onto the growing-media surface once in July. No insecticide or fungicide treatments were applied to the plants. Leaves that died on the plants were left *in-situ*.

The experiment was terminated on 11 October 2016 as no further symptoms were developing, and there was also the potential for infection to spread from the lesions between plots and this would confound any treatment benefits against oospores in the growing-media.

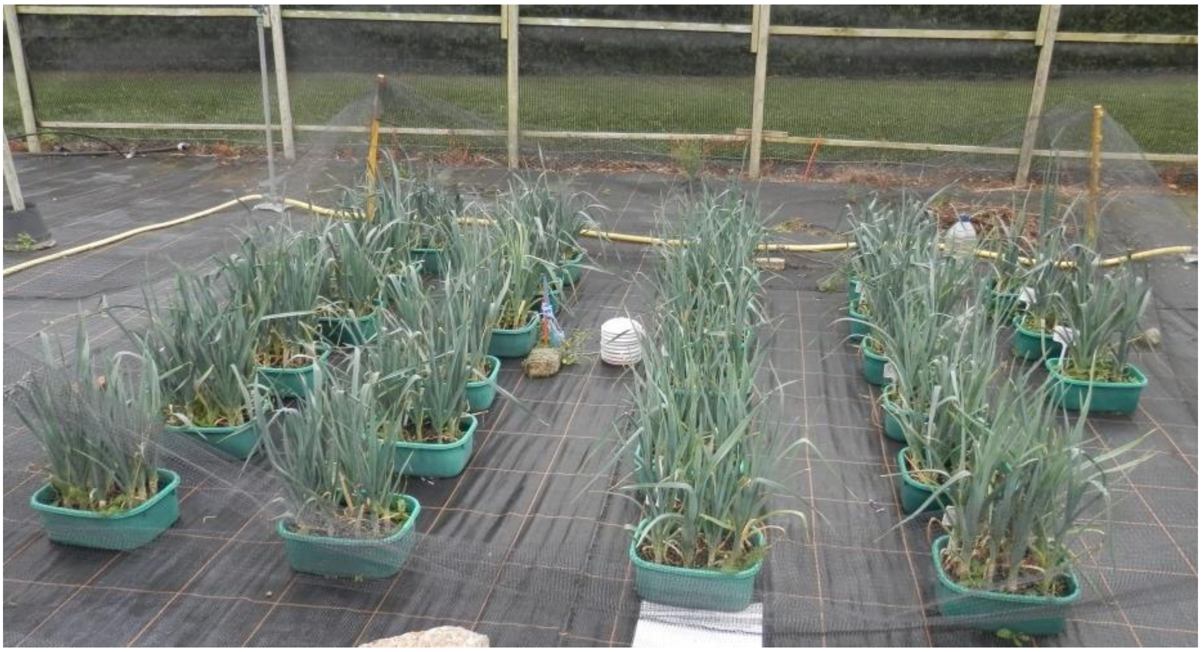

Figure 7. Leeks of varieties Triton and Pandora in containers, with four replicate blocks of the randomised six inoculated treatments. Four replicate blocks of uninoculated containers of each of the two leek varieties set apart from this layout are not shown in the photograph. ADAS Boxworth 9 September 2016.

Layout

Four replicate blocks of 32 randomized of inoculated plots were set up on 15 June 2016 on woven ground-cover matting outdoors in a standing area surrounded by windbreak mesh at ADAS Boxworth (Figure 7). Soil splash between bowls and leaf contact between treatments was avoided by spacing bowls 0.3 m apart within the blocks, with pathways at least 0.5 m wide. There were no leek crops in the surrounding fields. The uninoculated containers of treatments 1 and 5 were laid out in the same standing area as the inoculated, but two metres away in order to avoid any cross contamination from water splashing, but near enough to be able to compare their establishment with the inoculated plots.

Irrigation on the standing area was set up to achieve good water splashing in order for oospores to be propelled from the containers up onto the leaves and into the leaf sheaths to cause infection. Four overhead water sprinklers were placed at the four corners of the trial, calibrated to give average water volume of 150-200 ml per plot over a 15 minute irrigation period. Unless it had rained, an early morning and afternoon irrigation were given for about 15 minutes each by manual control.

A temperature and humidity logger was placed in a small ventilated screen between the bowls and a temperature logger was buried in the growing-media of one container. The ADAS Boxworth Meteorological station was sited within a kilometre of the trial area.

Assessments

At each of three assessment dates (on 1 July, 16 August, and 6 September 2017), records were taken for plants in the same order (clock wise starting always from the top left) so that a track could be kept of any symptom development over time in individual plants. A pre-treatment assessment was made for any potential symptoms. Crop vigour was recorded per plant, measuring height, number of leaves and leaf health. Disease incidence at initial low levels was scored as 0=for no incidence and 1=for White Tip presence per plant, with severity recorded in later assessments. Phytotoxicity such as scorching or distortion of the foliage was looked for at each of the assessments, described, and would be recorded using a 0 (zero) to 9 (severe) index.

Analysis was carried out using ANOVA with assessment for any interaction between either of the incorporated treatments and the two varieties. The uninoculated observation plots were not included in the analysis, but the inoculated untreated plots were included to give five variates with 15 degrees of freedom (d.f.).

Results

There were no significant differences in White Tip between either the treatments or the two varieties at any of the three assessment dates. Data for individual treatments and varieties for July, August and September is given in Appendix 4 together with the statistical analysis.

White tips were first observed on the 1 July, with 29 plants out of 120 having white tips across all treatments, mostly resulting in around 3% of the plant leaf area damaged, but with an overall mean 0.48% of the plants' leaf area with *P. porri* symptoms. No plants in the uninoculated soil had White Tip-like symptoms. Leaf samples with symptoms when put in soil water floats produced some non-septate (oomycete) mycelium, but no sporangia were produced to confirm the identity, and isolation of *P. porri* on agar was unsuccessful.

By the 16 August White Tip (Figure 8) was seen on three more plants in total, resulting in a mean incidence of 13.1%. Plant heights were measured and Pandora (mean 379 mm) was shown to be highly significantly ($P<0.001$) taller than Triton (337 mm). There was no significant difference ($P=0.075$, L.s.d. 22.6) between the plant heights of both varieties treated with Gypsum (mean 371 mm) compared with those left untreated (359 mm) or with Limex (345 mm). No plants in the uninoculated soil had White Tip, although there was some senescence of leaf tips which resulted in a gradual paleness just back from the tips (not the bright white and collapsed wet symptom typically present in the inoculated plots). There was also some of this creamy-yellow coloured tip senescence in the inoculated plots which was clearly distinct from symptoms of *P. porri.*

Figure 8. White Tip symptoms on leeks in early August following inoculation of the growing-media with oospores in mid-June 2016. ADAS Boxworth.

By 16 September the incidence of White Tip had only risen to a mean 16.3% of plants, so that White Tip severity remained low at a mean 0.29% of plant leaf area. There had, however been a small amount of breaking off of old infected white tips and, because only symptoms visible at each assessment were recorded, an analysis was also done of white tip incidence over the season. This was possible because records were kept for individual plants, however, although the mean incidence rose to 30.8% of plants which had shown symptoms at any time, no significant differences were shown between either treatments ($P=0.958$ L.s.d. 20.99) or varieties ($P=0.839$ L.s.d. 17.14), (Table 4). There were still no White Tip symptoms in any of the untreated plants growing in the uninoculated soil.

Table 4. Overall proportion of plants which had White Tip symptoms over the period July to September 2016 for each of the incorporation treatments and varieties. No significant differences P = for 0.958 treatments, P = 0.839 for varieties. ADAS Boxworth.

	Overall mean proportion of plants in inoculated soil showing White Tip symptoms		
Incorporation	Triton	Pandora	Mean % incidence for each treatment
Untreated	30.0	30.0	30.0
Gypsum	32.5	27.5	30.0
Limex	27.5	37.5	32.5
Mean % incidence for each variety	30.0	31.7	30.8

By the 10 October 2016 there had been no further disease symptom development. Sporulation was not observed on the leaf lesions at the time of any assessments and so infection by inter-plant spread was not thought to be occurring, but the infection originating from the oospores in the soil.

Temperature and relative humidity graphs on the ADAS Boxworth hardstanding area where the trial was located were shown in Figures 4 and 5. When the treatments were applied on 10 June the mean temperature was 22.6°C and at experiment termination on 11 October it was 10.7°C. The compost analysis (Appendix 2) showed a large increase in calcium and sulphate with the addition of the calcium sulphate (Gypsum). Magnesium content in the Limex treatment was above that in the untreated, but there were not any other marked changes from the original soil-based J.I. No 2 potting compost/growing-media.

WP6. To carry out a field trial in the east of the UK to evaluate foliar applied plant protection products against P. porri.

Currently growers only have two conventional fungicides approved for the control of white tip. These are Invader (dimethomorph and mancozeb) and Infinito (Fluopicolide + propamocarb hydrochloride), with Infinito only gaining approval very recently in 2016 to give growers a further mode of action. Invader is typically first applied in late August, with crops that are not being overwintered receiving only one application. Other fungicides are approved for use on the crop but these are less effective against white tip and applications of these are targeted towards the control of rust through the summer and autumn months. These products are Amistar Top (azoxystrobin and difenoconazole), Nativo 75WG (trifloxystrobin and tebuconazole) and Signum (boscalid and pyraclostrobin).

A number of candidate materials were selected for screening after a review of products trialled in SCEPTRE, other AHDB projects and representatives of the principal plant protection companies. Products were selected where there was evidence of activity against oomycetes, and where there may be a likelihood of approval (either full or extension of use) in future.

Materials and methods

Figure 9. Leek field on 18 August 2016 with plots marked out in the direction of the headland within each of four four-row beds (replicate blocks) of leeks, with discard strips between each plot. The weather station was positioned on the headland. Nottinghamshire.

The trial was carried out on a commercial leek crop cv. Pluston, at a site in Nottinghamshire within a field with a previous history of white tip (Figure 9). The crop was drilled in April 2016 and fungicide applications were applied as per commercial practice until September 2016. After this date, the crop was monitored for the appearance of white tip symptoms and when the first period of favourable weather was forecast (i.e. heavy rain showers) then the first application of the experimental treatments was made on 7 September 2016. Two further applications of the treatments were made on 9 November 2016 and 6 February 2017, again timed to be applied prior to a period of weather favourable to infection of white tip. Treatments as per table 5 and were applied with an Oxford Precision knapsack sprayer at 400 L/ha with

02F110 flat fan nozzles. Assessments were made along each 5 m plot length on 15 plants at random, recording in detail the % leaf area affected by White Tip and leaf layer affected. Records were made on the 25 November 2016 and then 18 January and 1 February 2017.

Table 5. Treatments applied to the field trial, Nottingham, 2016. Products without either on-label or an EAMU for foliar application to outdoor leeks are shown with AHDB codes.

Treatment number	Product or AHDB code	Active Ingredient (unless coded)	Rate (L or kg/ha)	Status
1 + 2	Untreated	-	-	n.a.
3	Invader (standard)	Mancozeb + dimethomorph	2.0	Approved (on label)
4	F212	-	-	experimental
5	F213	-	-	experimental
6	F214	-	-	experimental
7	Infinito	Fluopicolide + propamocarb hydrochloride	1.6	Approved (EAMU 1552/16)
8	F215	-	-	experimental
9	F216	-	-	experimental
10	F217	-	-	experimental
11	F218	-	-	experimental
12	F219	-	-	experimental

Results

Symptoms of white tip were first noted on 2 August 2016 after a period of heavy rain in late July, however the disease did not progress further at this point as the weather was not favourable to encourage spread of the disease after the initial infection. Sporangial production of *P. porri* was not seen in August from floating infected leaf pieces of Pluston in soil water, although some structures resembling encysted zoospores were seen. The next date when symptoms were noted again was early September, but the incidence of lesions did not increase further than one or two affected plants in selected plots until February.

In February, isolation of *P. porri* from samples of dry white lesions with a distinct boundary with healthy tissue of the leeks from the trial was unsuccessful. Isolates were obtained from aggressive wet/active lesions on some volunteer (old crop) plants of the variety Triton (known to be susceptible) in the neighbouring field.

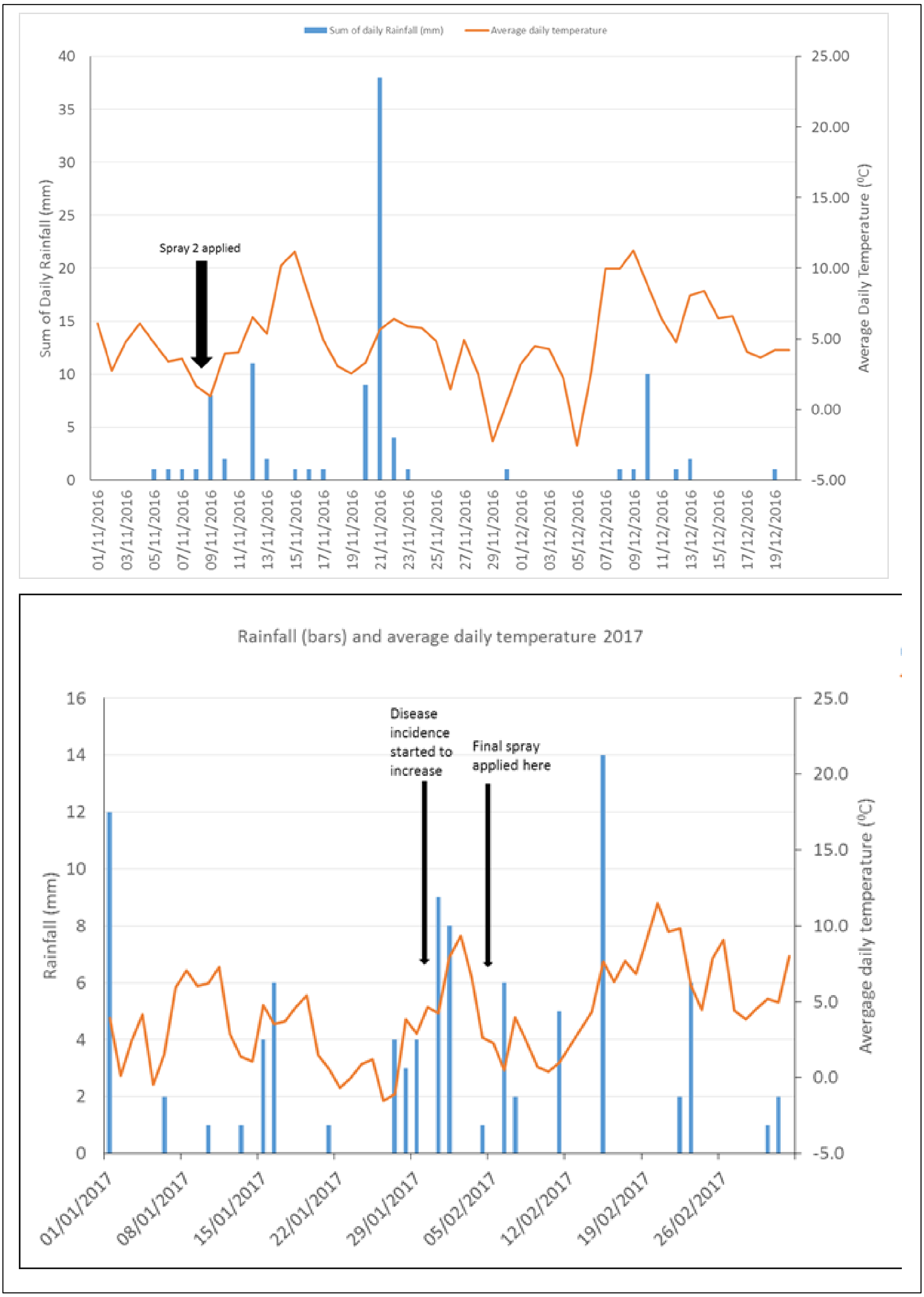

Figure 10. Spray application dates and daily rainfall (blue bars) and average daily temperature (orange line) between November 2016 and February 2017 when disease incidence had increased to nearly 100% in all treatments at the Nottinghamshire field site

Figure 10 shows when the two were sprays applied in relation to rainfall and temperature and the first symptoms of White Tip observed. Evaluation of weather data in relation to disease will be made when there is two years' data after the field trial to be set up in 2017.

At the assessments in February 2017 symptoms increased with most plants showing some white tip in all plots (incidence at nearly 100%) and severity at 7.2% area affected per plant on 1 February in the untreated plots (Table 6). This increased to 13.6 % area affected per plant a month later on 1 March. No significant differences were found between the untreated and treated plots or between fungicide treatments at any of the dates. The crop was deemed acceptable harvest quality at the end of March, but would require extra stripping. The white tip was not affecting quality of the shanks once the leaves were removed. Assessments are ongoing, and are planned to continue until the end of March.

Table 6. Severity of white tip on cv. Pluston as % of plant area affected, Nottingham 2017.

Treatment number	**Product / AHDB code**	**Severity % plant area affected by white tip on date shown**		
		1 February 2017	13 February 2017	1 March 2017
1 + 2	-	7.2	8.6	13.6
3	Invader	7.7	9.2	13.1
4	F212	6.8	7.7	12.8
5	F213	7.3	8.5	12.6
6	F214	5.8	7.9	10.8
7	Infinito	7.2	8.5	11.1
8	F215	7.1	9.4	12.7
9	F216	6.8	8.9	12.9
10	F217	6.9	8.1	13.1
11	F218	7.5	9.7	11.8
12	F219	6.7	8.2	11.2
F pr.		NS	NS	NS
LSD (33 d.f.)			1.383	2.775

Differences were seen across the leek beds (replicate blocks); on 13 February White Tip severity was significantly greater (P=0.016 L.s.d. 0.798) in replicate block one (9.4% of leaf area affected), with replicates two to four having 8.1% and 8.3% and 8.7%, respectively. When the youngest leaf layer showing White Tip symptoms were recorded on this date, there were highly significant (P<0.001 L.s.d 0.171) differences with most infection in replicate one and four occurring on average on leaf 3 (mean 3.5 and 3.7, respectively), rather than the leaf above in replicates two and three (means of 4.0, 3.9, respectively).

A further visit was made on the 15 March 2017 and the lesions did not appear to have progressed in the recent period of warm dry weather. In an adjacent field, leeks of the variety Triton (known to be susceptible to *P. porri*) were seen to have large white lesions causing the affected leaves to collapse. Leaf samples were taken to the laboratory from both the Pluston in the trial and the Triton, and under the microscope oospores were seen only in the Triton.

In 2017, under WP5 a second field trial will be established in the west of England and information from both WP5 and WP6 will contribute to WP7 and the examination of White Tip infection in relation to the weather prior to such symptoms being seen. The potential benefit of applying products to plants in propagation will be studied under WP3.

Soil analysis showed the soil to have 3.3% organic matter and a pH 6.7 with P:K:Mg index of 4:1:1 (Appendix 5).

Discussion

In the variety susceptibility experiment the White Tip symptoms were slow to develop although conditions for infection should have been good (Smilde, 1996) and a substantial quantity of zoospores were inserted into leaf sheaths. It was likely that the zoospores entered the tips of the leaves developing in the centre of the plant as the inoculation into the third leaf sheath drained downwards. The tips of leaves are particularly thin and have guttation openings which are likely to offer weak points for zoospore entry. It is reported by growers that White Tip is often more prevalent after frosts and it is possible that the exudates from damaged tissue and the broken epidermis then favour zoospore colonisation. When lesions develop in the centre of leaves these are often where there is a bend, and this damage may aid pathogen colonisation (or alternatively the break follows attack).

From observation by a grower of varietal susceptibility (Tim Casey, Bomber Produce) it seems likely that the blue-green modern hybrids are less susceptible than the older open-pollinators. It does not appear that longer shank length has much effect (through reducing the chance of zoospores splashing into the open top of the shank) as the out-pollinators are taller than the hybrids. Any resistance is more likely to be through other aspects of breeding or by generally being a stronger more vigorous plant. The out-pollinators are earlier autumn varieties where White Tip is least likely to occur and so the disease might be unlikely to have appeared during selection programmes and resistance would not have been a sought for trait.

In the experiment with Gypsum and Limex it was shown that at least 30% of plants could become infected as a result of recently produced *P. porri* oospores in the soil. The amount of oospores, was however very high, as 15 ml per bowl at the concentration produced resulted in 15 Million oospores in an area of 0.6m^2 . Oospore production can be abundant in oomycetes, with it possible for cells to be crammed with them as the pathogen uses up resources and switches to making survival structures, but information on the extent of oospore production under field conditions by *P. porri* requires further investigation. When inoculum is high then control measures have to have greater efficacy. Oospores are very resilient to heat, requiring at least five hours at 45°C in the soil before germination, and so infection, being reduced. Infection would have taken place across the range of 11°C to 23°C experienced by the plants and would be expected to take 4 to 11 days for symptoms to be seen (Smilde, 1996).

In the field trial of foliar applied treatments, although no significant differences were seen up to the early March assessment date, growers do not expect to see variations until symptoms increase later towards harvest at the end of March. White Tip symptoms were, however, present on virtually all plants, indicating that the disease has a significant impact on the crop and that protection was not gained by the products at the application timings used that had been selected on the basis of forecasted wet weather. A patchy distribution of White Tip had been expected rather than all plants assessed having some infection by February, as it was assumed that infested plants would have been in foci of infection from oospores in the soil. There was however difference in severity between replicate blocks across the field. It should be noted that distinguishing leaves damaged by *P. porri* from those damaged by various environmental causes of tip scorch / dieback is not easy particularly if low moisture conditions “dry up” the lesion and because damage of any sort is usually followed by secondary infection. Cladosporium sporulation was present on the dead tissue of samples examined from the field trial in August. Sporangial production of *P. porri* was not seen in August from floating infected leaf pieces of Pluston in soil water, although some structures resembling encysted zoospores were seen. In February, isolation of *P. porri* from Pluston from the trial was unsuccessful, but successful from volunteer plants of Triton. It is possible therefore that when a host with some resistance produces a hypersensitive response that this then “walls off” the green tissue at the “leading edge” of the lesion from where isolation onto agar in the laboratory is usually successful. Secondary spread in the field may thus be less likely from resistant varieties because sporangia will not be able to be produced in the green tissue beyond the white dry lesion. Oospores were only seen in Triton, but perhaps the pathogen was unable to feed on the plant for long enough to stimulate the stage of oospore production in the Pluston. If there is a reduced risk of oospore production this is of benefit to future leeks in the rotation.

All the 2016 work packages have been carried out as proposed, with infection seen in all three trials, but no particular varieties or treatments were identified as reducing White Tip.

Conclusions

- Leeks developed White Tip symptoms two months after being transplanted in June into irrigated oospore infested growing-media, with 30% of plants affected by early September
- Neither Gypsum nor Limex incorporated into artificially inoculated growing-media, at the rates and timings used, reduced the incidence of infection from oospores in the soil. It is not known whether any reduction in oospore viability took place.
- Neither Gypsum nor Limex had any phytotoxic effects on the plants transplanted into the treated growing-medium, but nor were any improvements in plant resistance to disease seen compared with the untreated
- There was no difference in the incidence or severity of White Tip between the cultivars Triton and Pandora growing in inoculated soil in the period July to November
- Development of White Tip symptoms took four months to appear following initial inoculation by zoospores into the leaf sheath, with only a mean 10% of plants affected
- No differences in White Tip symptom incidence or severity occurred up to January after ten varieties were inoculated in their leaf sheaths in September and November with zoospores of *P. porri*
- Varieties were confirmed to differ in their shank length
- In the leek field crop, symptoms following natural infestation of White Tip were not observed until early August following April sowing
- There was little increase in incidence and severity in the field until February when most plants had symptoms, perhaps because of more days with rain, with severity subsequently also increasing
- After three fungicide application dates to the field there was no difference in White Tip symptoms between either the standard and experimental programmes, or between these and the untreated plots
- No phytotoxicity was seen following any of the foliar fungicides at the rates used

Knowledge and Technology Transfer

One presentation is to be carried out as part of this project to summarise information from the two years, together with an AHDB Grower article.

Glossary

Oomycete – micro-organisms that used to be classified as fungi, but because they have swimming spores (zoospores) and a different cell wall material to fungi they are now considered more closely related to algae. Oomycetes include *Phytophthora* and *Pythium* species, downy mildew species and white blister. Fungicides active against fungi are often ineffective against oomycetes.

Oospore – the thick walled resting stage of many oomycetes. They are produced sexually. They can remain viable for at least five years. They germinate (usually following contact with host exudates) to release either many zoospores or a sporangium. These then produce an infection peg (following chemical signals) to penetrate the host. Water or high humidity is important to prevent desiccation after release from the oospore before host entry.

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Appendices

Appendix 1. Fungicides with activity against oomycetes such as *Phytophthora porri* and their resistance risk. Taken from CP 126 AHDB review by Tim Pettitt in 2014.

Fungicide Group	Active ingredient example(s)	Target site of action (Mode of action)	Products (examples only)[*]	FRAC Group	Resistance risk
Phenylamides	Metalaxyl	RNA polymerase I	Fubol Gold	4	High
Isoxazoles	Hymexazol	DNA/RNA synthesis[†]	Tachigaren	32	Low
Benzamides	Zoxamide	ß-tubulin assembly in mitosis	Electis	22	Low-Medium
Acylpicolides	Fluopicolide	Delocalisation of spectrin-like proteins	Infinito	43	Not Known
Q_oI (Quinone outside inhibitors) {Strobilurins, Oxazolidinediones & Imidazolinones}	Azoxystrobin	Inhibition of Complex III: cytochrome bc1 (ubiquinol oxidase) at Q_o site (cyt b gene)	Amistar	11	High
	Famoxadone		Tanos (mixture)		
	Fenamidone		Sonata		
Ureas	Cymoxanil	Unknown	Option	27	Low-Medium
Phosphonic acids	Phosphonates	Unknown[§]	Aliette Plant Trust	33	Low
	Phosphonic acid & salts	Unknown[§]	Various		
Pyridinamines	Fluazinam	Uncoupler of oxidative phosphorylation	Shirlan	29	Low
Dithiocarbamates	Mancozeb	Multi-site contact activity	Dithane	M3	Low
Chloronitriles	Chlorothalonil	Multi-site contact activity	Bravo 500	M5	Low
Sulfamides	Dichlofluanid	Multi-site contact activity	Elvaron	M6	Low
	Tolyfluanid		Euparen		
Quinones	Dithianon	Multi-site contact activity	Dithianon WG	M9	Low

[*] Note that not all products listed are necessarily currently approved for use in the UK. It is essential that you take specialist advice on product authorisation prior to use of a particular product to ensure you comply with all current legislation regarding pesticide application.

† proposed target site of fungicide action

Appendix 2i. Analysis of untreated John Innes No. 2 growing-media on 10 June 2016 (T1) used in Limex/Gypsum incorporation experiment (WP 4).

VASILIKI TZORTZI
ADAS BOXWORTH
BATTLEGATE RD
BOXWORTH
CAMBRIDGE
CB23 4NN

T515

Please quote above code for all enquiries

VASILIKI TZORTZI

XBM5594 COMP 13 06 2016

COMPOST ANALYSIS RESULTS

Sample Reference :

UNTREATED GROWING

Sample Matrix : COMPOST

Laboratory References	
Report Number	21111
Sample Number	98340

Date Received	14-JUN-2016
Date Reported	21-JUN-2016

The sample submitted was of adequate size to complete all analysis requested.

The sample will be kept at ambient temperature for at least 3 weeks.

ANALYTICAL RESULTS *on 'as received' basis.*

Determinand	Value	Units	Determinand	Value	Units
pH	6.8		Cond. at 20 C	769	uS/cm
Density	690	kg/m3	Ammonia-N	8.5	mg/l
Dry Matter	61.8	%	Nitrate-N	312.2	mg/l
Dry Density	426.4	kg/m3	Total Soluble N	320.8	mg/l
Chloride	44.5	mg/l	Sulphate	610.8	mg/l
Phosphorus	30.9	mg/l	Boron	0.08	mg/l
Potassium	259.0	mg/l	Copper	<0.01	mg/l
Magnesium	88.1	mg/l	Manganese	0.84	mg/l
Calcium	422.7	mg/l	Zinc	0.02	mg/l
Sodium	43.0	mg/l	Iron	0.46	mg/l

The extraction is performed by adding a weight of sample equivalent to 60mls volume to 300mls of deionised water (ref BSEN 13652:2001). Samples submitted under 1 litre will necessitate the use of scaled down equipment for density pH and Conductivity measurements are made at 20°C. I.S. = Insufficient Sample.

Released by ......*P G Taylor*...................... Date *21/06/16*......

NRM Coopers Bridge, Braziers Lane, Bracknell, Berkshire RG42 6NS
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Appendix 2ii. WP 4. Analysis of growing-media after mixing on 10 June 2016 with Gypsum (calcium sulphate) at the rate of 34.5 g Gypsum per 4 L John Innes No. 2 (T3)

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T515

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VASILIKI TZORTZI

XBM5594 COMP 13 06 2016

COMPOST ANALYSIS RESULTS

Laboratory References	
Report Number	21111
Sample Number	96338

Sample Reference :

T3

Sample Matrix : COMPOST

Date Received	14-JUN-2016
Date Reported	21-JUN-2016

The sample submitted was of adequate size to complete all analysis requested.

The sample will be kept at ambient temperature for at least 3 weeks.

ANALYTICAL RESULTS *on 'as received' basis.*

Determinand	Value	Units	Determinand	Value	Units
pH	6.4		Cond. at 20 C	3398	uS/cm
Density	658	kg/m3	Ammonia-N	7.6	mg/l
Dry Matter	62.5	%	Nitrate-N	308.6	mg/l
Dry Density	411.3	kg/m3	Total Soluble N	316.2	mg/l
Chloride	41.6	mg/l	Sulphate	11346.4	mg/l
Phosphorus	24.7	mg/l	Boron	0.06	mg/l
Potassium	288.6	mg/l	Copper	<0.01	mg/l
Magnesium	232.9	mg/l	Manganese	3.76	mg/l
Calcium	5019.2	mg/l	Zinc	<0.02	mg/l
Sodium	44.8	mg/l	Iron	0.13	mg/l

The extraction is performed by adding a weight of sample equivalent to 60mls volume to 300mls of deionised water (ref BSEN 13652:2001). Samples submitted under 1 litre will necessitate the use of scaled down equipment for density pH and Conductivity measurements are made at 20°C. I.S. = Insufficient Sample.

Released by P G Taylor Date 21/06/16

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Appendix 2iii. WP 4. Analysis of growing-media after mixing on 10 June 2016 with Limex (phosphate, magnesium and sulphur) at the rate of 34.5 g per 4 L John Innes No. 2 (T4).

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VASILIKI TZORTZI

XBM5594 COMP 13 06 2016

COMPOST ANALYSIS RESULTS

Laboratory References
Report Number 21111
Sample Number 98398

Sample Reference :

T4

Sample Matrix : COMPOST

Date Received 14-JUN-2016
Date Reported 21-JUN-2016

The sample submitted was of adequate size to complete all analysis requested.
The sample will be kept at ambient temperature for at least 3 weeks.

ANALYTICAL RESULTS *on 'as received' basis.*

Determinand	Value	Units	Determinand	Value	Units
pH	7.9		Cond. at 20 C	1008	uS/cm
Density	701	kg/m3	Ammonia-N	9.7	mg/l
Dry Matter	62.6	%	Nitrate-N	345.9	mg/l
Dry Density	438.8	kg/m3	Total Soluble N	355.6	mg/l
Chloride	49.5	mg/l	Sulphate	762.9	mg/l
Phosphorus	13.2	mg/l	Boron	0.05	mg/l
Potassium	276.7	mg/l	Copper	0.03	mg/l
Magnesium	109.3	mg/l	Manganese	0.32	mg/l
Calcium	627.4	mg/l	Zinc	<0.02	mg/l
Sodium	46.0	mg/l	Iron	0.56	mg/l

The extraction is performed by adding a weight of sample equivalent to 60mls volume to 300mls of deionised water (ref BSEN 13652:2001). Samples submitted under 1 litre will necessitate the use of scaled down equipment for density pH and Conductivity measurements are made at 20°C. I.S. = Insufficient Sample.

Released by *P G Taylor* Date *21/06/16*

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Appendix 4. WP 4. White Tip leaf symptoms (incidence and severity) and plant height after transplanting two leek varieties into growing-media with and without either gypsum or Limex powder prior to the addition of *P. porri* oospores. Boxworth July, August and September 2016.

Appendix 4i. Mean proportion of plants with White Tip leaf lesions on 1 July 2016, where 1.0 would be 100% of plants with any visible symptoms. No significant differences.

Treatment	Treatment mean	Triton	Pandora
UT	0.087	0.075	0.100
Gypsum	0.150	0.075	0.225
Limex	0.125	0.100	0.150
Variety mean		0.083	0.158

Source of variation	d.f.	F.pr.	L.s.d.
Block	3	0.552	0.1550
Treatment	2	0.617	0.1342
Variety	1	0.165	0.1096
Treatment x. Variety interaction	2	0.587	0.1898
Residual	15		

Appendix 4ii. Mean % leaf area with White Tip symptoms per plant, July 2016. No significant differences.

Treatment	Treatment mean	Triton	Pandora
UT	0.28	0.10	0.45
Gypsum	0.74	0.50	0.98
Limex	0.43	0.28	0.58
Variety mean		0.29	0.67

Source of variation	d.f.	F.pr.	L.s.d.
Block	3	0.851	0.671
Treatment	2	0.255	0.581
Variety	1	0.113	0.474
Treatment x. Variety interaction	2	0.947	0.822
Residual	15		

Appendix 4iii. Mean proportion of plants with White Tip leaf lesions on 16 August 2016, where 1.0 would be 100% of plants with any visible symptoms. No significant differences.

Treatment	Treatment mean	Triton	Pandora
UT	0.100	0.125	0.075
Gypsum	0.114	0.125	0.103
Limex	0.178	0.100	0.256
Variety mean		0.117	0.144

Source of variation	d.f.	F.pr.	L.s.d.
Block	3	0.113	0.1259
Treat	2	0.298	0.1090
Variety	1	0.516	0.0890
Treatment. x Variety interaction	2	0.127	0.1542
Residual	15		

Appendix 4iv. Mean plant height to the leaf axil of the youngest leaf (cm) on 16 August 2016. Pandora was 3 cm taller than Triton, but no significant treatment differences.

Treatment	Treatment mean	Triton	Pandora
UT	35.86	33.67	38.06
Gypsum	37.16	34.27	40.04
Limex	34.52	33.32	35.72
Variety mean		33.76	37.94

Source of variation	d.f.	F.pr.	L.s.d.
Block	3	0.001	2.611
Treat	2	0.075	2.261
Variety	1	<.001	1.846
Treatment x Variety interaction	2	0.307	3.197
Residual	15		

Appendix 4v. Mean proportion of plants with White Tip leaf lesions on 6 September 2016, where 1.0 would be 100% of plants with any visible symptoms. No significant differences.

Treatment	Treatment mean	Triton	Pandora
UT	0.150	0.125	0.175
Gypsum	0.150	0.250	0.050
Limex	0.188	0.200	0.175
Variety mean		0.192	0.133

Source of variation	d.f.	F.pr.	L.s.d.
Block	3	0.357	0.1675
Treat	2	0.819	0.1451
Variety	1	0.310	0.1184
Treatment x Variety interaction	2	0.203	0.2052
Residual	15		

Appendix 4vi. Mean proportion of plants with White Tip leaf lesions on 6 September 2016, where 1.0 would be 100% of plants with any visible symptoms. No significant differences.

Treatment	Treatment mean	Triton	Pandora
UT	0.225	0.250	0.200
Gypsum	0.331	0.600	0.063
Limex	0.312	0.375	0.250
Variety mean		0.408	0.171

Source of variation	d.f.	F pr.	L.s.d.
Block	3	0.042	0.3874
Treat	2	0.775	0.3355
Variety	1	0.084	0.2739
Treatment x Variety interaction	2	0.279	0.4745
Residual	15		

Appendix 5. WP 6. Soil analysis for Nottinghamshire field leek crop in October 2016.

Contact : MARTIN CROOKES
ADAS ENVIRONMENT GROUP
ADAS GLEADTHORPE
MEDEN VALE
MANSFIELD
NOTTINGHAMSHIRE
NG20 9PF
Tel. : 01623 848373

K708

Please quote the above code for all enquiries

Client : XBM5594 LEEKS

Distributor : XBM5594 LEEKS

Sample Matrix : Agricultural Soil

Laboratory Reference
Card Number 88305/16

Date Received	21-Oct-16
Date Reported	25-Oct-16

SOIL ANALYSIS REPORT

Laboratory Sample Reference	Field Details			Index			mg/l (Available)		
	No.	Name or O.S. Reference with Cropping Details	Soil pH	P	K	Mg	P	K	Mg
534502/16	1	**XBM5594** *No cropping details given*	6.7	4	1	1	54.8	89	46

If general fertiliser and lime recommendations have been requested, these are given on the following sheets.
The analytical methods used are as described in DEFRA Reference Book 427
The index values are determined from the DEFRA Fertiliser Recommendations RB209 8th Edition (Appendix 4).

MICRO NUTRIENT REPORT

DATE **25th October 2016**

SAMPLES FROM **XBM5594 LEEKS**

MARTIN CROOKES
ADAS ENVIRONMENT GROUP
ADAS GLEADTHORPE
MEDEN VALE
MANSFIELD
NOTTINGHAMSHIRE NG20 9PF
Tel: 01623 848373

Reference: 88305/534502/16 Field Name: XBM5594	Result	(*)	Deficient	Marginal	Target	Marginal	Excessive
Organic matter (LOI) %	3.3	1	*OM level data not available for this crop*				

Notes (*)

(1) NRM considers Organic soils to contain between 10-20% organic material with Peaty soils containing over 20% . The optimum ranges for Organic Matter which have been set are dependent on the soil type and the cropping but these must be viewed as guidance values only.

