Project title:	Leeks: Investigating control measures for White Tip.
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Project leader:	Erika F. Wedgwood, RSK ADAS Ltd.
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Key staff:	Angela Huckle, RSK ADAS Ltd. Erika Wedgwood, RSK ADAS Ltd. Aoife O'Driscoll, RSK ADAS Ltd. Vasiliki Tzortzi, RSK ADAS Ltd. Gail Bennett, RSK ADAS Ltd. Michael Morris, RSK ADAS Ltd.
Locations of project:	ADAS Boxworth, Cambridgeshire CB23 4NN. Farms in Nottinghamshire and Lancashire.
Industry Representative:	Tim Casey, Bomber County Produce, J & V Casey and Son Ltd., Coningsby, Lincs. LN4 4UY
Date project commenced:	1 April 2016
Date project completed :	31 June 2018

DISCLAIMER

The results and conclusions in this report are based on investigations 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.

Erika F. Wedgwood

Research Scientist

RSK ADAS Ltd.

Signature E.F. Wedgwood Date 30 June 2018

Angela Huckle

Agronomy Consultant

RSK ADAS Ltd.

Signature Date 30 June 2018

Report authorised by:

Dr Barry Mulholland

Director

RSK ADAS Ltd., Horticulture

Signature Date 30 June 2018

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GROWER SUMMARY

Headline

White Tip (*Phytophthora porri*) severity reduction is possible by variety selection and understanding that two days of heavy rain heighten infection risk. Application pre-Christmas of some novel fungicides can result in a greater number of healthy new leaves at harvest.

Background

Fungicide control of White Tip in leeks (Figure 1) 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 downy mildews and potato blight could have potential for the treatment of *P. porri* on leeks and so required efficacy testing to seek 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 and so examination in this project of weather data in relation to symptom development, and information on this from published research, could help to better understand the conditions leading to disease development.

Figure 1. White Tip on leek in March, caused by *P. porri*

P. porri oospores are released from infested plant debris and the zoospores released splash up into leaf axils. Crop rotation to deplete oospores is not always possible and chemical soil treatment options are limited. Work elsewhere has shown reduction of oomycete activity following incorporation of one or other of Limex and Gypsum (inexpensive waste products), and a small-scale trial was needed to see if these products might be effective against *P. porri*.

There are indications that some leek varieties are more susceptible than others to White Tip. Infection “escape mechanisms” related to shank height have been proposed, although a hypersensitive response as seen in leek plant breeding lines following inoculation tests may be involved in stopping lesion development. Comparison was needed of varieties alongside each other with a similar level of *P. porri* inoculum. Variety resistance is unlikely to give total disease control, but chemical fungicide use could be reduced, and biostimulants or biofungicides able to stimulate the plant's own defence responses could also be integrated in programmes to improve control. A combination of cultural and chemical measures is likely to continue to be required to reduce White Tip incidence and severity.

Summary

A number of work packages (WP) were carried out between 2016 and 2018 to improve the control of *P. porri* on leeks. In WP1, to determine by inoculated test if there are differences in leek cultivar susceptibility to infection by *P. porri,* ten varieties were sown into module trays in June 2016. The varieties were sourced from five different breeding companies and included Triton and Lexton, selected because of reported susceptibility to White Tip, and the cultivars Pluston and LV446, reported by growers to appear to have a lower susceptibility. Other commonly grown varieties (Mancurian, Curling, O96, Belton, Krypton and Longton) with unknown susceptibility were included. 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. Up to the end of January 2017 there was no statistically significant differences in either incidence (mean 10% of plants with White Tip) or severity (1% of leaf area, principally at the tips), although it was noted that no leeks of cv. Mancurian had exhibited symptoms. Some further plants had White Tip symptoms by late 24 April 2017 in all varieties (mean 34% of plants). The only significant difference between varieties was in their vigour, with Pluston and Longton significantly stronger growing than O96, LV446, Lexton and Krypton, with plants showing less than 3% of leaf area affected by White Tip compared with the mean 6.6%.

In WP2, novel conventional fungicides, biological control products and elicitors which could 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 two field trials (WP 5 and 6), as AHDB coded products, and others were to be included for use at propagation in WP3. Information was included on the potential development of fungicide resistance, and the effect of biofungicides/biostimulants 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. 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 half of the untreated, Limex and Gypsum plots. The containers were arranged outdoors in randomised blocks at ADAS Boxworth and subject to heavy irrigation droplets twice daily in order to splash spores from the soil into the leaf sheaths. 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 in comparison with the untreated. Triton and Pandora were equally affected, with only a minimal (0.3%) leaf area affected by White Tip per plant by September, and no further symptom development in any of the plants by mid-October.

In WP 6, a field trial was carried out in the east of the UK (Nottinghamshire) in 2016/17 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 three times 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 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 transmitting station nearby.

A second year of foliar fungicide evaluation (WP5) was carried out in 2017/18, but in Lancashire, using the same procedures as for the Nottinghamshire site but with the variety Triton. All but one of the experimental products of 2016/17 were tested again with Invader (mancozeb + dimethomorph) as the standard. Instead of F219, one treatment used two of the experimental products in a programme with Infinito (fluopicolide). Spray applications triggered by rain forecasts took place on 7 September, 30 October and 19 December 2017. White Tip was first confirmed on the final spray date and affected up to 13.8% of the leaf area on plants with symptoms, although less than 1% of plants were affected and there were no significant differences between treatments. By 8 February a mean 17% of plants had symptoms and affected plants had a mean 18.6% leaf area with White Tip, without any significant differences either between treatments or between them and the untreated. By 21 March 2018, the infection incidence had changed little, with a mean 19% of plants with White Tip and still no treatment differences. By March, most fungicide treatments had significantly more (P<0.001) central (newer) leaves without visible White Tip i.e. two more than the untreated. Plots receiving three applications of Invader (currently used by growers against White Tip) had a lower number of healthy leaves, equivalent to untreated plants (Figure 2).

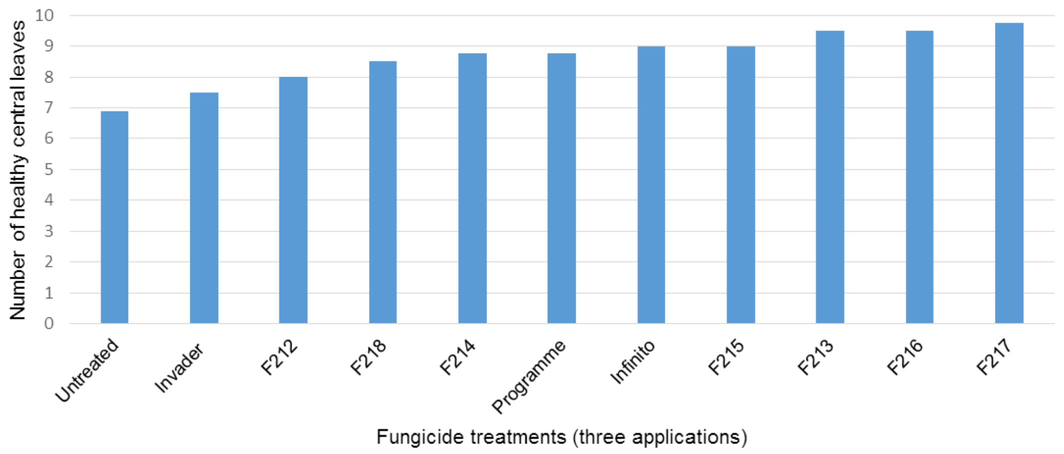


Figure 2. Lancashire. The modal number of central leaves without visible White Tip by 21 March 2018 following fungicide applications on 7 September, 30 October and 19 December 2017. Significantly more (P<0.001) leaves were healthy in F214, F215, F213, F216, F217, the programme (F212, Infinito, F218) and Infinito alone than following the use of Invader (mancozeb + dimethomorph) as the standard (L.s.d. 1.155).

In WP7, from in-field weather station readings from 2016/7 and 2017/18 when at least two wet days were followed by a day with over 35 mm rain (on 21 November 2016 and on 22 November 2017) then White Tip incidence increased. It was hypothesised that, as illustrated in Figure 3, wet days in mid-November 2017 provided conditions for zoospore release in the soil and, after a day with heavy rain splashing zoospores into leaf axils, first symptoms would have developed a fortnight later, i.e. the published interval for lesions to show at mean daily temperatures of 8°C.

When plant growth is slow the incidence of White Tip could be underestimated for perhaps a month or more because it is likely that the symptoms on leaf tips infected by zoospores in water inside the shank would not be visible until the new leaf emerged through the leaf axil. Therefore, although the apparent incidence of White Tip was low when fungicides were applied on 19 December 2017 there would have already been infection resulting from zoospore splash on 22 November. In order to give better awareness of the likelihood of crop infestation then knowledge will be required per crop field of the soil type/water penetration and the likelihood of puddle formation.

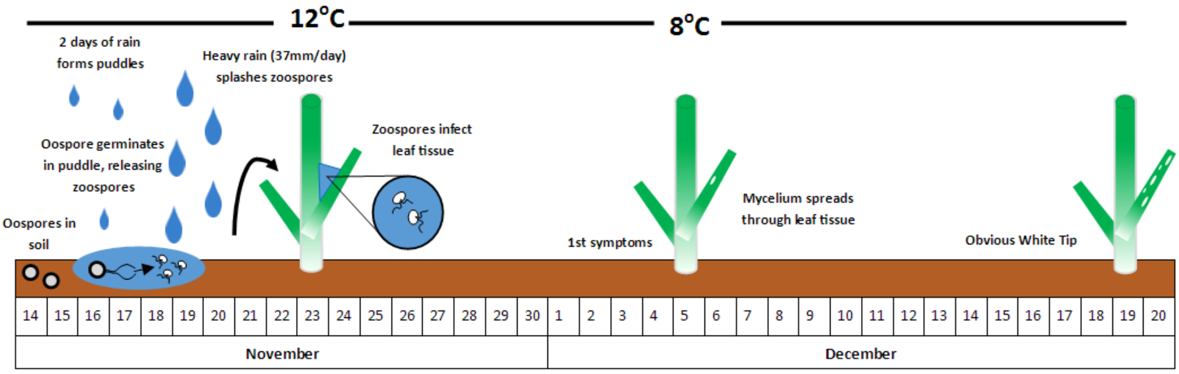


Figure 3. Timeline for leek infection from oospores and White Tip symptoms in the Lancashire field trial in 2017, showing required preceding puddle formation and heavy rain.

WP3, to assess the use of products in propagation against White Tip, was commenced but difficulties in multiplying-up the *P. porri* culture for inoculation, and in producing plants to produce open leaf axils suitable for receiving zoospores, meant that this work was not completed.

An additional work package (WP10) was started in 2017 to investigate any varietal susceptibility of field grown plants to White Tip from natural *P. porri* infection. Twelve varieties, principally those grown overwinter in the UK, were sown in 2.5 m long beds and given no fungicide treatments from August 2017. By 8 February 2018, White Tip incidence was higher than in the adjacent efficacy trial, with a mean 71% of plants visibly affected. With over 40% White Tip on affected plants significantly more (P<0.001) Krypton and Comanche plants were affected than Lexton, Pluston, a Triton seedstock, Galvani and Longton (Figure 4).

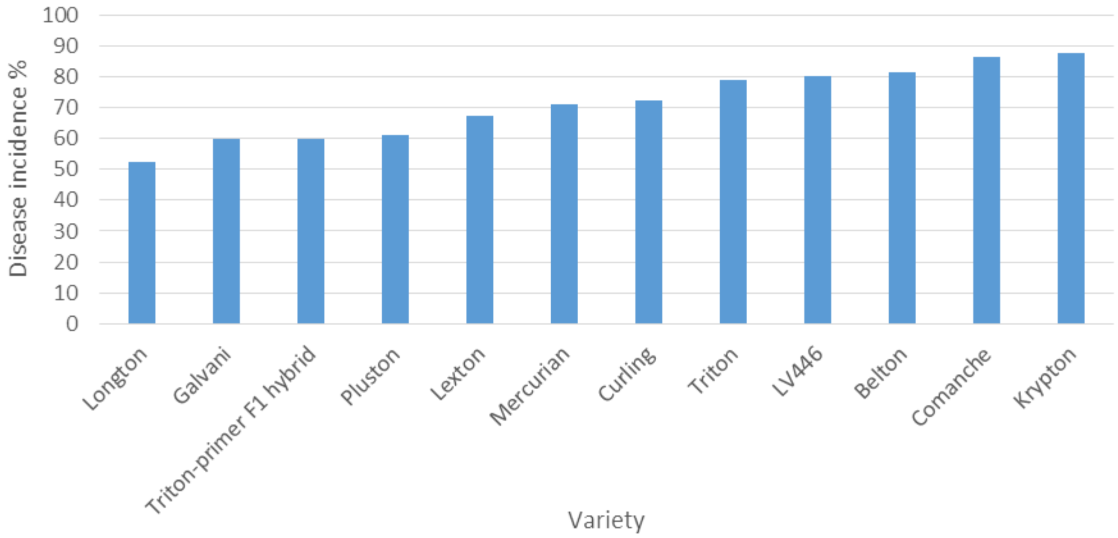


Figure 4. The proportion of leek plants in each variety with White Tip on 8 February 2018 (P<0.001, L.s.d. 16.24) following natural infection by *P. porri* at the Lancashire trial site. Longton, Galvani and Triton-primer had a significantly lower incidence than LV446, Belton, Comanche and Krypton with 80% or more plants affected.

By March 2018, no significant differences remained and the incidence in all varieties had decreased to a mean 18% because damaged leaf tissue was lost, leaving around five healthy central leaves. Shank height differed significantly ($P<0.001$) between varieties by March, with Galvani being the shortest and Lexton the tallest, but no correlation was found in March between variety height and the proportion of plants per variety with White Tip.

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 loss of £2.25 million annually for leeks where current fungicide control measures cannot be used. In a wetter than average winter the severe losses experienced late season can give losses of £3,250/ha when 50% of a crop is affected, (based on production costs of £6,500/ha).

Action Points

- Be aware of any history of White Tip in fields being considered for growing leeks, and if possible leave at least three years between leek crops
- No benefit was shown from the application of either Limex or Gypsum pre-crop
- The selection of certain varieties, such as Lexton, Pluston, Galvani and Longton could reduce the severity of White Tip infection. Taller shanks do not improve resistance.
- Zoospores are released and splashed from puddles, so avoid soil compaction, including in sprayer and irrigator wheelings, and improve drainage in problem areas
- Be aware that heavy irrigation that splashes soil onto the crop can spread White Tip
- There is potential for a nurse crop of cereals to provide soil cover and prevent soil splash in the early life of a leek crop until herbicide breakdown of the cereal plants
- Target foliar fungicide application to periods of forecast heavy rain, especially when the ground is already saturated so allowing puddle formation, as this is when conditions are right for the pathogen to splash onto leaves
- Application of products such as Infinito and certain experimental products pre-Christmas can increase the number of healthy new leaves produced by March
- Before deciding to spray, consider other potential physiological causes of white tipping on leeks. Fresh *P. porri* lesions tend to be a bright white, usually with a sharp boundary with green tissue, although lesions are soon invaded by secondary fungi
- It may be prudent to lift leek crops with White Tip earlier than intended, however without heavy rain the infection of further plants may be unlikely and outer infected leaves (due to be trimmed) are likely to die in Spring and healthy new leaves develop
- Removal of infested leek debris will stop *P. porri* resting spores entering field soil

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 (oospores) 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 six to 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 5.

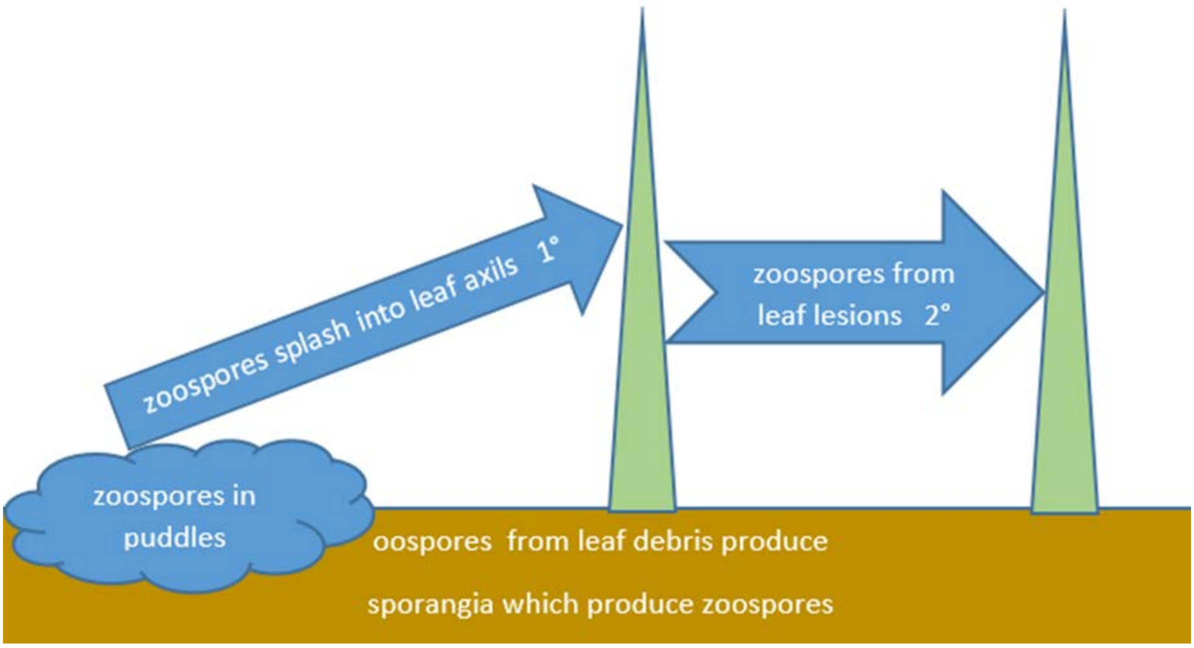


Figure 5. Sources of primary and secondary infection of leek plants (shown as triangles) by *Phytophthora porri*. Zoospores originate from sporangia formed from oospores in the soil then infest leaf axils via water splash. Sporangia may then develop in leaf lesions and release zoospores in wet weather to cause further leaf infection and oospores also form.

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 then 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, following rain events that were conducive to infection, it was proposed that the period in which the disease remains symptomless in the plant lengthens 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 that prevent rain splash.

Symptoms

White Tip causes severe foliar symptoms on leeks. Epidemics may destroy the crop before the overwintered 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 6). 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 6. 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 may be produced on wet leaves. The sporangia do not detach from the stalk (Erwin and Ribeiro, 1996) and so must release zoospores *in situ* 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 a period of rain to facilitate zoospore release into puddles and from there heavy rain to enable 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 has been reported in varieties such as Triton, whereas varieties such as Pluston and LV446 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 there is potential for them to work together with biofungicides to supress disease. Varietal resistance or 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.*

Observations (Tim Casey, Bomber Produce, pers. comm.) on varietal susceptibility suggest modern hybrids are less susceptible than older out-pollinators. The out-pollinators are earlier autumn varieties, when 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. Longer shanks could reduce the chance of zoospores lodging in leaf axils, but the out-pollinators are taller than the hybrids. Any hybrid resistance is more likely to be through other aspects of breeding or by generally being stronger more vigorous plants.

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 have four conventional fungicides approved for the control of White Tip. Of these the most effective are Invader (dimethomorph and mancozeb) and Infinito (fluopicolide + propamocarb hydrochloride), with Infinito only gaining approval under EAMU 1552 of 2016 recently to give growers a further mode of action. Other fungicides are authorised for use on the crop and contain azoxystrobin (Amistar, Azofin, Affix and 5504 with three applications/crop) with Amistar Top also containing difenoconazole (one application per 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. Signum (boscalid and pyraclostrobin) has EAMU 2134 of 2010 for the control of *P. porri* on leeks, with two spray applications permitted on the crop.

Prestop (*Gliocladium catenulatum*) can be applied to outdoor leeks under EAMU 2773 of 2015, but only as a soil drench/growing media incorporation with the targets including *Phytophthora* spp.. and other soil-borne pathogens. Serenade ASO (*Bacillus subtilis*) has an EAMU 0306 of 2015 for use as a root drench on outdoor leeks, but the target organisms are listed as White Rot and damping-off.

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 were investigated in this project and the objectives and work package numbers as given in the original proposal are listed below.

WP 1: To determine by inoculated test if there are differences in leek cultivar susceptibility to infection by *P. porri.* (Year 1)

WP 2: To review the novel conventional fungicides, biological control products and elicitors which may be of benefit against *P. porri.* (Year 1)

WP 3: To determine by inoculated tests whether treatments applied during propagation can lead to a subsequent reduction in White Tip on the plants (Year 2)

WP 4: To determine by inoculated tests whether treatments applied to soil may lead to subsequent reduction in White Tip on the plants (Year 1)

WP 5: To carry out a field trial in the west of the UK to evaluate foliar applied plant protection products against P. porri. (Year 2)

WP 6: To carry out a field trial in the east of the UK to evaluate foliar applied plant protection products against P. porri. (Year 1)

WP 7: To explore the potential for weather-based disease forecasting to assist spray timing applications for White Tip

WP 8: To communicate results, and carry out further knowledge exchange, of White Tip control with growers in order to improve sustainable control of the disease

WP 9: To prepare a factsheet to give grower-focused information on the lifecycle, epidemiology and cultural and chemical control measures of *P. porri*

In the 2016/17 season varietal resistance (WP1) and soil treatments (WP4) were tested in container experiments. Products with potential for the control of *P. porri* were reviewed (WP2).

A field trial was set up in Nottinghamshire screening a number of novel foliar fungicide treatments (WP6).

In 2017/18 treatments (WP3) were selected for testing on plants while in propagation modules, seeking to demonstrate if protection from *P. porri* might be maintained once older. A further efficacy field trial (WP5) was carried out, moving to Lancashire as the western, coastal, side of the UK is usually subject to wetter weather. A supplementary field trial (now listed as WP10) was also set up in 2017 to investigate whether differences in White Tip infection could be seen in leek varieties available to UK growers. Information on 2016/17 experiments as well as of 2017/18 are reported in full in this final report as, at the time of writing, the annual report has not had publication on the AHDB website and the discussion refers to all years.

A meeting with growers to present the work of this project will take place in 2018 (WP8). A factsheet on White Tip (WP9) may be commissioned for publication in 2019.

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 reported 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, positioned on capillary matting within latticed plastic trays (**Figure 7**), 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 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	LV446	

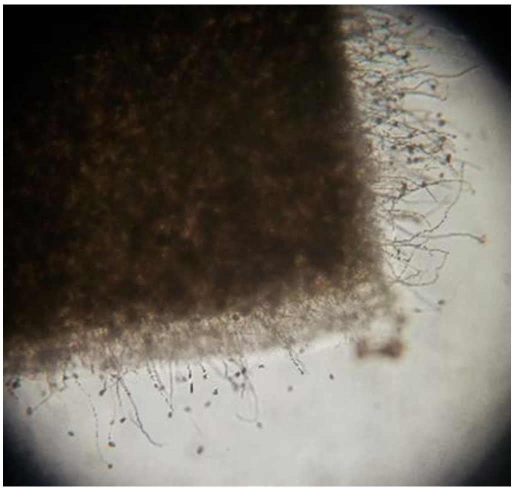
Overhead sprinkler irrigation surrounding the trays was calibrated to give a water volume of 150-200 ml in 15 minutes and turned on in the morning and afternoon for this time period, unless it had been raining. Plants were given weekly foliar applications of 20 ml Tomorite fertiliser in 4.5 L water as 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 7. Four replicate blocks of ten leek varieties (two varieties per tray). December 2016 at ADAS Boxworth following sowing on 15 June and inoculation of *P. porri* zoospores into leaf sheaths on 29 September and 14 November 2016

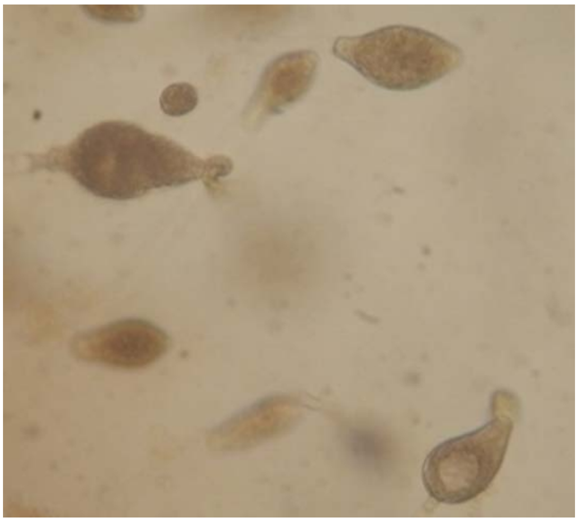
Inoculation

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.

i) Spiralling growth of hyphae on agar typical of *P. porri*



ii) *P. porri* sporangia from a square of agar with mycelium in soil water (a float)



iii) Sporangia releasing zoospores via a discharge pore into chilled then warmed water

iv) Sporangium of *P. porri.* The spheres inside are zoospores waiting to emerge

Figures 8i to iv. Mycelial growth on agar and pear-shaped papillate *P. porri* sporangia in soil water and releasing zoospores from their tips following chilling and warming in the laboratory.

The *P. porri* isolate BX15/102 obtained from an unknown leek variety (a volunteer in a field) 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 refrigerator for an hour and then returned to room temperature in order to trigger zoospore release (Figure 8). 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 by gentle movement of the inoculum 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 = no incidence and 1 = 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 the end of April 2017, which would be towards the harvest of a late commercial crop. Results from the final assessment were not available in time for the Annual Report. After the final assessment the accumulated number of plants affected by White Tip was calculated, this was needed because by keeping an individual record for each plant it was shown that infected leaf tips fractured off the plants and some plants had been lost to White Tip over the assessment period.

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, and White Tip was first seen on 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).

On 23 January a highly significant difference was shown in the height of plants to the top of the youngest leaf sheath (**Table 2**). The varieties Triton, Mancurian and O96 were 10 or 30 mm shorter than Curling, Lexton and Longton (probably due to their genetics).

When the final assessment was made on 25 April (**Table 3**) some plants with White Tip had died; with Curling, Belton, Triton and LV446 in particular having only the remains of a mean four out of the ten plants per plot, but this was not significantly more than for the other varieties. Another one or two plants had produced White Tip symptoms in most plots, but varieties shown no significant differences. The mean severity of White Tip had changed only a little since January (from a mean 1.1% to 6.6%), with no significant difference between varieties, although a number of LV446 plants were severely affected resulting in a mean severity of 15%.

Table 2. Leek varieties in modules inoculated with *P. porri* zoospores in September and November 2016 at ADAS Boxworth. Shank height on 23 January and for both 23 January and 25 April 2017 mean % of plants affected and the incidence and severity of White Tip.

			White Tip (*P. porri*) symptoms at each assessment					
Treat-ment code	**Variety**	**Shank height (cm)**	**Incidence: Mean % of plants affected**		**Mean number of leaves affected / plant**		**Severity: Mean % leaf area affected**	
		23.01.17	**23.01.17**	**25.04.17**	**23.01.17**	**25.04.17**	**23.01.17**	**25.04.17**
1	Triton	10.70	10.0	35.0	0.13	0.50	1.20	4.22
2	Mancurian	12.03	0.0	16.4	0.0	0.22	0.00	4.13
3	Curling	14.60	17.5	37.9	0.25	0.49	2.04	7.23
4	O96	12.10	12.5	28.0	0.13	0.31	1.64	5.24
5	Belton	12.41	15.0	45.3	0.15	0.57	0.35	10.10
6	Krypton	12.41	7.5	20.6	0.10	0.20	2.00	7.52
7	Lexton	14.19	15.0	30.6	0.15	0.62	0.39	7.48
8	Longton	13.99	10.0	23.6	0.13	0.29	0.19	2.30
9	Pluston	13.16	5.0	28.6	0.05	0.55	0.14	2.97
10	LV446	11.46	13.1	39.3	0.15	0.83	2.81	15.11
Mean		12.60	10.6	34.1	0.12	0.46	1.08	6.63
L.s.d.		1.421	12.11	25.06	0.137	0.244	2.867	10.810
F. pr.		<0.001	0.167	0.124	0.084	0.253	0.450	0.425

By adding up all the plants affected by White Tip from January to April (to include those whose tips had been shed or where plants had died) it was seen that Belton, LV446, Curling, Lexton and Triton had had a mean 4 plants (40%) with symptoms, but this was not significantly more than the other varieties. Plant vigour was assessed on a 0-9 scale, with dead plants given an index 0, small plants given index 5 and healthier plants (usually with more leaves and taller) received indices 7 or 8. Reduced vigour caused by White Tip was included within this assessment. Highly significant differences (P<0.001) were shown, with Pluston and Longton being more vigorous than O96, LV446, Lexton and Krypton. Curling and Triton were also significantly more vigorous than O96 and LV446 (**Table 3**).

Table 3. Leek varieties in modules inoculated with *P. porri* zoospores in September and November 2016 at ADAS Boxworth. Vigour index on 25 April 2017 (0 = dead, 9 = very good) which included the effects of White Tip on growth together with ranking of varieties by Duncan's test. The accumulated total proportion of plants with any White Tip symptoms between January and April 2017 whether or not visible by April.

Treat-ment code	**Variety**	**Mean Vigour index (0 – 9) 25.04.17**	**Duncan's multiple range test for vigour**	**Accumulated White Tip % incidence January to April 2017**
1	Triton	4.95	bcd	42.5
2	Mancurian	4.13	abc	15.0
3	Curling	4.50	bcd	45.0
4	O96	2.67	a	25.0
5	Belton	3.81	abc	47.5
6	Krypton	3.45	ab	22.5
7	Lexton	3.43	ab	42.5
8	Longton	5.19	cd	30.0
9	Pluston	5.70	d	27.5
10	LV446	2.71	a	43.9
Mean		4.05		34.1
L.s.d.		1.415		25.06
F. pr.		<0.001		0.124

Figure 9 and Figure 10 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).

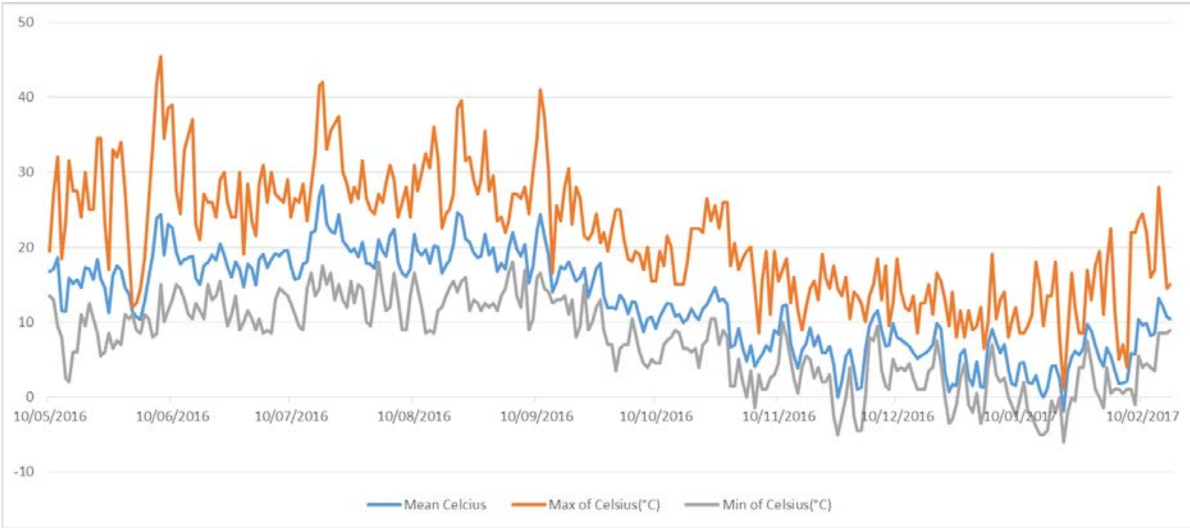


Figure 9. Mean, maximum and minimum daily air temperature adjacent to leek containers between May 2016 and February 2017. ADAS Boxworth hardstanding area.

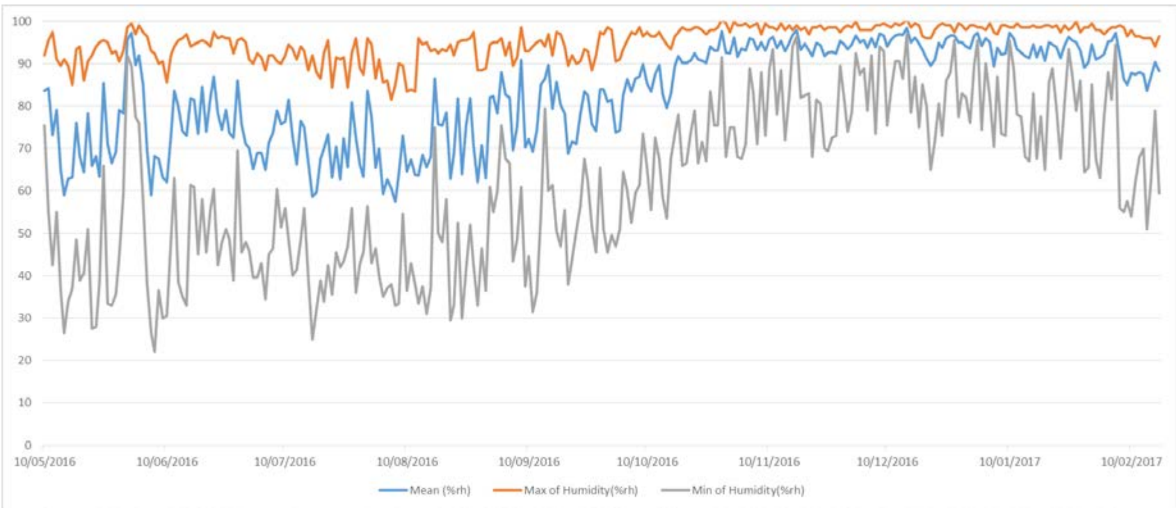


Figure 10. Mean, maximum and minimum daily relative humidity adjacent to leek containers between May 2016 and February 2017. ADAS Boxworth hardstanding area.

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 QxI 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 a number of products containing azoxystrobin and Amistar Top (azoxystrobin and difenoconazole). 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. It is registered in the UK for use against downy mildew on bulb onions, shallot and garlic.

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 by 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, which is an extract of the Giant Knotweed *Fallopia sachalinensis* (formerly *Reynoutris sachalinensis*), is a product available in the USA, but registration has been delayed 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 showed efficacy against powdery mildews, with another chemical elicitor 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. The product Amylo X WG containing *Bacillus amyloliquefaciens Plantarum* strain D747 has been registered for use in the UK as EAMU 0532 of 2017, but is only for use under full permanent protection (as is T34 Biocontrol).

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.

Limex (calcium) and Gypsum (calcium sulphate) have been shown to have the potential to individually reduce inoculum levels of oomycete pathogens when used as a pre-planting soil amendment. These products are likely to be cost-effective to apply to leek fields before planting.

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

WP4 was reported fully in the Annual Report.

Materials and methods

Treatment application and inoculation

Two treatments, Gypsum and Limex, with potential as soil incorporated products for the reduction of White Tip in leeks were tested for pre-planting treatment of soil infested with oospores of *P. porri*. On 10 June 2016 containers were filled with a John Innes soil-based No. 2 growing-media mixed with either of the products, with some plots also left untreated (**Table 4**). Samples of the untreated and treated growing-media were sent for nutrient analysis (Appendix 2). A rate of 5 kg Limex/ha was selected based on work on carrots with cavity spot (Boor, 2004) and the same rate used of Gypsum (calcium sulphate dehydrate) based on the work on *Phytophthora* root rot of papaw (Vawdrey *et al*., 2002). On 13 June an oospore suspension of 1 x 10 ^{6} / ml was syringed over the soil in most treatments, resulting in 15 million oospores per container surface area of 0.6 m^2 (**Table 4**). The suspension was produced utilising methods in PhD work by Smilde (1999) and by Declercq *et al.* (2012), after growing an isolate of *P. porri* (BX15/102, isolated in December 2015) on 10% V8 agar for a month.

Table 4. 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

Treat-ment	Variety	Growing-media treatment 10 June 2016	Product per container (g)	Inoculated 13 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

Planting and Layout of experiment

On 15 June 2016, ten 14-week old leeks were transplanted into each of the containers. Two leek cultivars were selected; Triton (bred by Syngenta) which is a potentially White Tip susceptible variety and Pandora (bred by Elsoms-Bejo). The Triton had six to seven leaves and the Pandora had seven to eight leaves.

Four replicate blocks of 32 randomized inoculated plots were set up outdoors at ADAS Boxworth under coarse overhead irrigation given twice daily (**Figure 11**). The containers were spaced to avoid soil splash between them, and on 11 October once leaf lesions were developed and risked spread between plants (i.e. secondary infection rather than primary infection from the soil) the experiment was terminated.

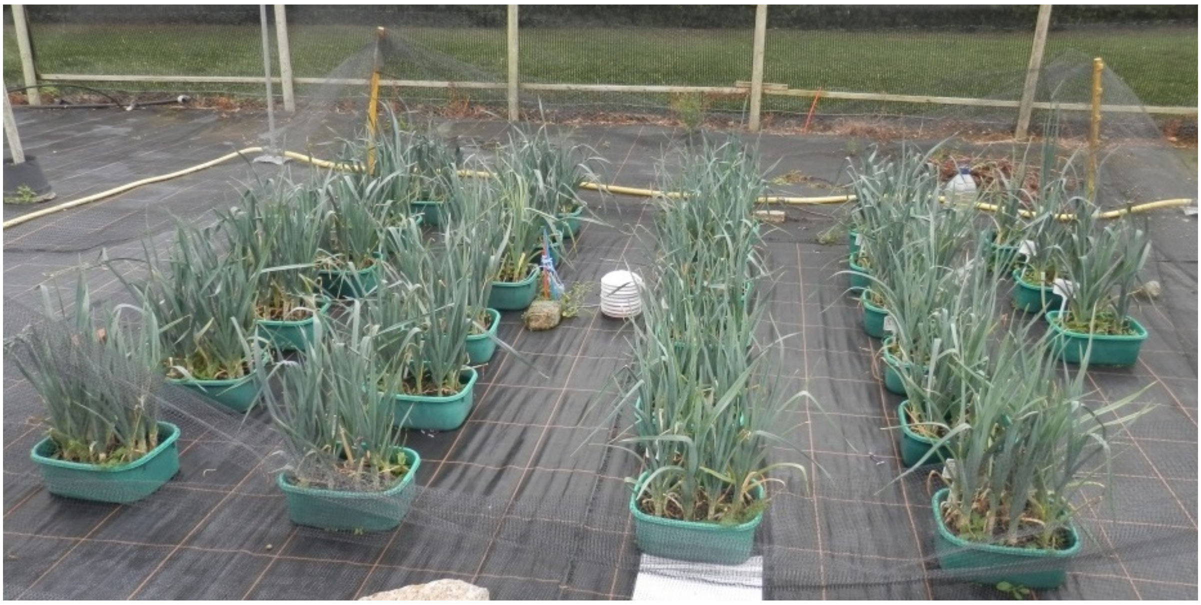


Figure 11. Leeks of varieties Triton and Pandora in containers, with four replicate blocks of the randomised six inoculated treatments. Four replicate blocks of uninoculated containers of each of the two leek varieties set apart from this layout are not shown in the photograph. ADAS Boxworth 9 September 2016.

Assessments

On each of three dates, 1 July, 16 August, and 6 September 2017, plants were individually recorded so that symptom development, and any loss of tissue with White Tip could be noted. Crop vigour was recorded 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 and any phytotoxicity recorded. Analysis was carried out using ANOVA with assessment for any interaction between either of the incorporated treatments and the two varieties.

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 3 together with the statistical analysis. No White Tip was seen at any time in the uninoculated containers.

White tips were first observed on 1 July, on 29 plants out of 120 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. Leaf samples with symptoms 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 12**) 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 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).

Figure 12. 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 able to be recorded, an analysis was also done of white tip incidence over the season using the records kept for individual plants. However, although the mean incidence rose to 30.8% of plants to have 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 5**).

Table 5. 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 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 secondary spread was not thought to have occurring at this time, rather the infection was originating from the oospores in the soil (primary).

Temperature and relative humidity graphs on the ADAS Boxworth hardstanding area where the trial was located were shown in Figure 9 and Figure 10. 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.

WP3. To test conventional and non-conventional fungicide products in leek propagation for their efficacy following subsequent exposure of plants of two varieties to *P. porri* zoospores.

Treatments were to be carried out to give protection of plants when commercially they would be in multicell propagation trays under protection and receiving any treatments by overhead spray or irrigation. This work was terminated early because of a series of challenges with both plant and inoculum production and extremes of temperature. The procedures carried out up to this time, and the treatments intended are, however, reported for future reference.

Materials and methods

Isolates of *P. porri* were obtained in 2016 and 2017 from plants with White Tip collected from commercial crops and abandoned areas of crop. Such plants had white mid-leaf lesions and leaf tips that were bright white and hung limply down when fresh. This differs from physiological cream coloured tip die-back progressing from chlorotic tissue (**Figure 13**).

Figure 13*.* Natural infection by *P. porri* causing a blotchy lesion and bleached thin white tip.

Leek seeds were sown on 11 September 2017 using two varieties of reputed differing susceptibility to White Tip; Triton (the more susceptible) from Syngenta and Pluston from Bayer. Multicell trays were set up in a polytunnel at ADAS Boxworth in a randomised block design with 10 treatments in four blocks and 10 plants of two varieties per plot. (**Figure 14**).

Figure 14. Module trays of ten leek plants per variety (two varieties per slatted tray) on 30 April 2018 arranged in four replicate blocks in the tunnel at ADAS Boxworth.

All products were to be applied as a spray at 1000 L/ha using an Oxford gas-assisted sprayer. There were to be two treatment timings tested that could be used by propagators (**Table 6**): 1) Established i.e. once seedlings had a fully emerged leaf (around a month from sowing, but dependent on growing conditions) and 2) Pre-transfer i.e. a week before plants would be transplanted in the field (about two months from sowing depending on growing conditions).

Table 6. Protectant treatments due to be applied once when established and usually again to plants at a stage for field transplanting. Application due to module-raised leeks in a tunnel at ADAS Boxworth, before inoculation with *P. porri.*

Trt no.	**Treatment**	**Dose rate/ ha**	**Application timing**	**Basis of rate (& any stated minimum application interval)**
1	Untreated control inoculated	Not applicable	Not applicable	No water to be sprayed (no mimic of wetting by a fungicide spray)
2	Serenade ASO (*Bacillus subtilis*)	10 ml/L water applying 1000 L / ha	**1.** Established **2.** Pre-transfer	EAMU 0306 of 2015: outdoor leek drench. EAMU 0706 of 2013: bulb vegetables spray (7 d)
3	Prestop (*Gliocladium catenulatum*)	5 kg/1000 L water applied over 1 ha	**1.** Established **2.** Pre-transfer	EAMU 2773 of 2015: leeks max 500 g / 100 L water (no guidance on area) (21 d)
4	T34 Biocontrol (*Trichoderma asperellum*)	5 g/1000 L water applied over 1 ha	**1.** Established	EAMU 1809 of 2016: under full protection herbs, brassicas. One spray trays / multipots 5 cm deep max dose 0.5 g /m²
5	Trianum P (*Trichoderma. harzianum*)	15 kg/ha applying in 1000 L water / ha	**1.** Established **2.** Pre-transfer	Authorisation for protected edibles. Maximum two applications in propagation (14 d)
6	Amylo X (*Bacillus amylolique-faciens*)	2.5 kg/ha applying in 1000 L water / ha	**1.** Established **2.** Pre-transfer	Authorisation for full protection salads, protected edibles & fruit. Max. 15 kg/ha per crop in up to 1000 L/ha (7 d)
7	Paraat (dimethomorph)	3.0 kg/ 1000 L water over 1 ha	**1.** NONE **2.** Pre-transfer	Label for outdoor strawberry kg/ha (3 g/1L) spray in 1000 L/ha. One application only. Experimental treatment
8	Fenomenal	2.5 kg/ha applying in 1000 L water/ha	**1.** NONE **2.** Pre-transfer	EAMU 0109 of 2016 spray leafy veg & herbs. In minimum 200 L /ha. Max two sprays/ crop(7-14 d)
9	Cassiopeia	2.5 L/ha applying in 1000 L/ha	**1.** NONE **2.** Pre-transfer	Label for onions, shallots, garlic (for downy mildew). Max three per crop (7-10 d). Foliar 200-500 L.
10	Untreated control NOT inoculated	Not applicable	Not applicable	No water to be sprayed (no mimic of wetting by spray)

Results

Plants were neither treated nor inoculated and so there are no efficacy results to report.

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

Materials and methods

Figure 15. Leek field on 18 August 2016 with plots markers visible looking down the wheeling within each of four four-row beds (replicate blocks) of leeks, with discard strips between each plot. The weather station was sited on the headland. Nottinghamshire.

The trial was carried out in Nottinghamshire, within a commercial leek crop that was available in a field having a recent history of White Tip (**Figure 15**). The crop of cv. Pluston was drilled in April 2016. Plots were each one 1.8 m bed wide, with four rows per bed and of 5 m length, with a metre of unsprayed discard left between each of the plots down the beds.

A number of candidate fungicides were selected for efficacy testing (**Table 7**) after a review of products trialled in SCEPTRE, other AHDB projects, discussions with representatives of the principal plant protection companies and AHDB staff. 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. Currently growers only have four conventional fungicides approved for the control of White Tip. The most effective are Invader (dimethomorph and mancozeb) and Infinito (fluopicolide + propamocarb hydrochloride), with Infinito only gaining Extension of Authorisation for Use 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, Amistar Top (azoxystrobin and difenoconazole) and Signum (boscalid and pyraclostrobin) approved for use on the crop are less effective against White Tip and applications of these are targeted towards the control of rust through the summer and autumn months.

A programme of fungicide applications against rust was carried out by the grower as per commercial practice up until September 2016. After this, the weather forecasts for the area were regularly checked for the probability of a wet period and when indicated 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 were applied with an Oxford Precision knapsack sprayer at 400 L/ha with 02F110 flat fan nozzles. Plots were monitored for the appearance of White Tip symptoms. Assessments were made on the 25 November 2016 and then 18 January and 1 February 2017 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.

Soil analysis showed the soil to have 3.3% organic matter, to be pH 6.7 with P : K : Mg index of 4:1:1.

Table 7. 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 under experimental code)	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, before experimental treatments were applied, 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 (ex. previous crop) plants of the variety Triton (believed to be susceptible) in the neighbouring field, and so it is possible that isolation needs to be carried out while mycelium is still advancing at the leading edge.

Figure 16 shows when the two sprays were applied in relation to rainfall and temperature and the first symptoms of White Tip observed.

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 8). 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.

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 were seen to have large white lesions causing the affected leaves to collapse. This variety is said to be particularly susceptible to *P. porri* and so it was not put by the grower in the trial field because of the field's recent history of White Tip and so poor chance of producing a crop. 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. 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.

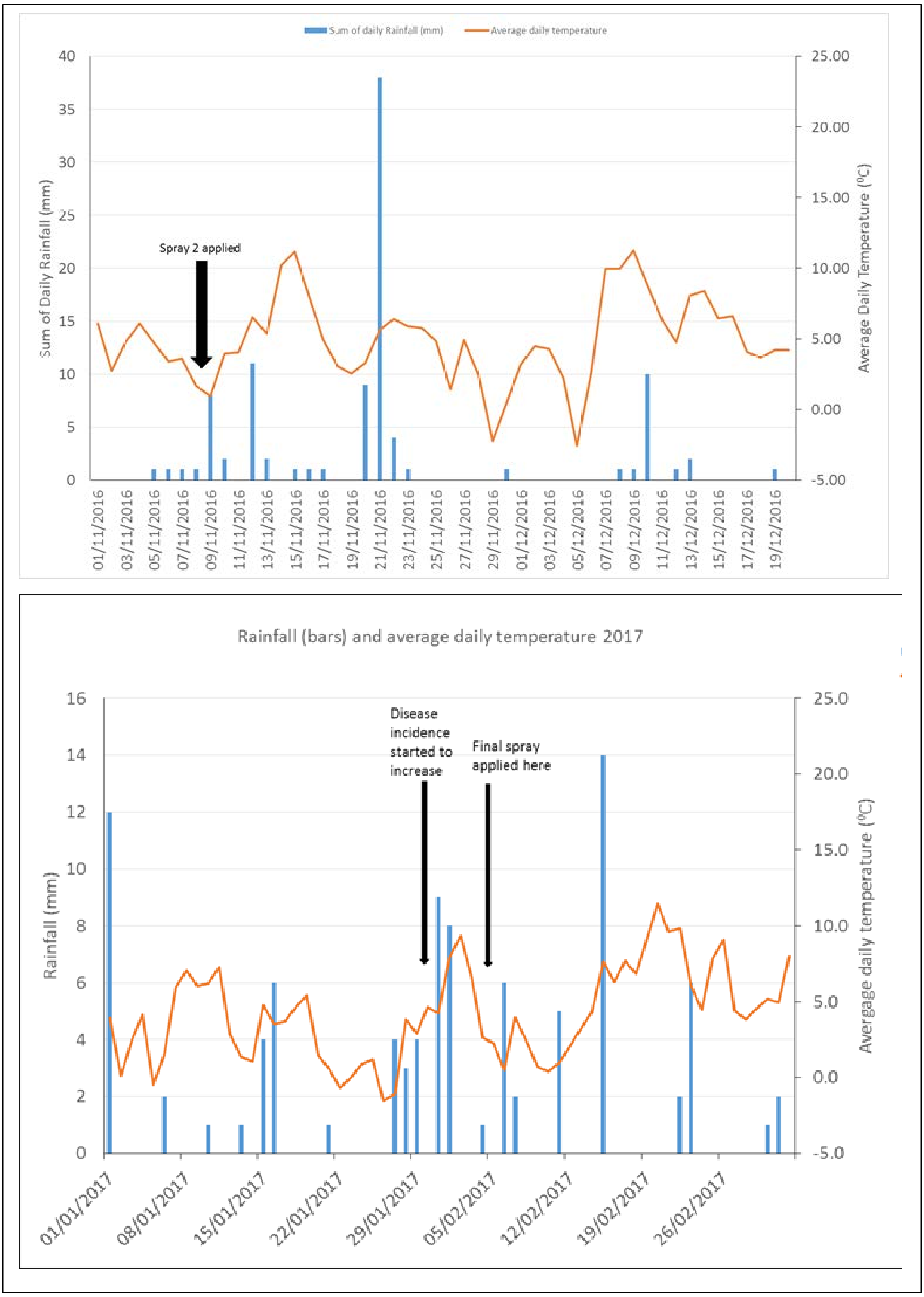


Figure 16. Spray application dates and daily rainfall (blue bars) and average daily temperature (orange line) between November 2016 and February 2017 when disease incidence had increased to nearly 100% in all treatments at the Nottinghamshire field site

Table 8. Severity of white tip on cv. Pluston as % of plant area affected, Nottingham 2017.

Treatment code no.	Product or AHDB code	White Tip Severity % leaf area affected 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
L.s.d. 33 d.f.			1.383	2.775

WP5. To carry out a field trial in the west of the UK to evaluate foliar applied plant protection products against *P. porri*.

Materials and methods

The trial was carried out on a commercial leek crop of cv. Triton, drilled in 2017 at a site in Lancashire. This was the second year of testing a range of fungicides in the field, but with a different variety and in weather conditions likely to be wetter than those at the former site sown in 2016 in Nottinghamshire. The field had no previous history of White Tip, however the grower said it was his experience that infection arrived in leek fields not recently planted with this crop and suggested the trial was aligned to take account of the prevailing south-westerly wind. Plots were 5 m long and a 1.8 m (four-row) bed wide, and treatments were randomised within each of four replicate blocks (**Figure 17**). The crop was drilled on the 15 May 2017 and fungicide applications were applied as per commercial practice against rust until September 2017. A discard area of one metre was left between the assessed 5 m of each plot. 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) the first application of the experimental treatments was made on 7 September 2017. Two further applications of the treatments were made on 30 October 2017 and 19 December 2017, again timed to be applied prior to a period of weather favourable to infection of White Tip. Treatments were carried out as per **Table 9** and were applied with an Oxford Precision knapsack sprayer at 400 L/ha with 02F110 flat fan nozzles. Plants were examined for any signs of phytotoxicity, such as stunting, yellowing or distortion, or any poor vigour on 20 September 2017, 9 November 2017 and 4 January 2018, with each plot assessed using a vigour index of 1 = strong green plants to 9 = crop dead.

Table 9. Lancashire 2017/18. Treatments selected to investigate the effect of fungicide treatment on White Tip development. Products without approval for foliar application to outdoor leeks either on-label or as an EAMU 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	F212 +Infinito + F218 in a 3 spray programme	-	-	Experimental
		Fluopicolide + propamocarb hydrochloride	1.6	EAMU 1552/16
		-	-	Experimental

Disease assessments were conducted on 30 October, 19 December 2017, and again on 8 February and 21 March 2018. At the assessments on 30 October, 19 December and 8 February disease incidence was low and so the number of disease plants per plot was recorded. Disease incidence was thus calculated as the number of individual plants affected by White Tip as a % of the total number of plants per plot (based on plant counts recorded on

31 August 2017). Disease severity was recorded as the % of leaf area affected on plants displaying symptoms and was in order to give a representation of the marketability of the affected plants (rather than the mean leaf area affected across both healthy and White Tip affected plants).

For the assessment done on 21 March, disease incidence was much higher and so was recorded as the overall % of diseased plants per plot. At this assessment, disease severity was not recorded; instead the number of central leaves most commonly still healthy and green on the plants was recorded (as an indication of how much of the plant would be left after trimming for market). The leaves were counted from the central leaf sheath of the plant outwards.

Figure 17. Leek field in Lancashire on 8 August 2017 showing four beds of four-row 5m long plots of leeks, with 1m discard strips between each plot. The weather station is visible to the rear, sited on the headland. The herbicide treated cereal “guard crop” is visible in plots.

Results

At the plant count on 31 August 2017 there was a mean 124 plants (no plots having less than 83 plants, or more than 162) within the four rows of each plot and the plants had between two and eight leaves and were on average 140 mm tall. On 20 September no yellowing or scorching had arisen from the fungicide applications on 7 September nor were any problems recorded in any plots on 9 November (after 30 October applications) and 4 January (after 19 December applications). On 9 November the untreated plots of T2 and the plots of T6 (F214) had a mean vigour index of 6 because plants were a little smaller than other plots with an index of 5. By January all plots had a similar, healthy, mean index of 3 (data not presented) where an index of 1 would be excellent growth.

Although four plants with white markings were seen in the trial on 20 October, and photographs taken, these were not typical of White Tip. True symptoms of White Tip were first noted on 19 December 2017, (although these was not checked for oomycete mycelial growth by means of laboratory leaf floats). By the second assessment on 8 February, symptoms were observed on all plots (mean % incidence 17%). This increased to 19.3 % area affected per plant 6 weeks later on 21 March. Leaf tissue was collected in both February and March in order to isolate *P. porri* onto agar, but isolates were unable to be obtained. Disease symptoms manifested themselves as bright white lesions, leading away from a chlorotic margin, then surrounded by green healthy tissue. White Tip was present not only at the tips, but also across the leaf blades and within the leaf sheath (Figure 18). Between treated and untreated and fungicide treatments, there were no significant differences in disease incidence or severity at any of the assessment dates.

There was however, a significant difference in the number of healthy green leaves on the plant following fungicide application. Application of F217 resulted in an average of 9.7 healthy green leaves which were marketable, and similarly F213 and F216 had an average of 9.5 healthy leaves per plant. This number healthy leaves was significantly more than for the untreated control plants, which only had an average of 6.9 leaves per plant (**Table 10** and Figure 2).

No significant differences between replicate blocks for either plant growth or White Tip were recorded at any assessment date.

Figure 18. White Tip symptoms on plants, including leaf tips, blades and leaf sheaths at the field trial site in Lancashire. Images were taken at the final assessment on 21 March 2018.

The daily rainfall graphs are presented in **Figure 19** for August and September 2017, **Figure 20** for October to December 2017 and **Figure 21** for January to March 2018 and show that a forecast period of wet weather followed the 7 September initial White Tip spray. The second application on 30 October would also have provided protection during the subsequent wet days in early November, but more rain fell towards the end of the month before the mid-December final application. Detailed discussion of rainfall in relation to White Tip infection is given within the work in WP7. Temperature and relative humidity charts are given alongside the rainfall charts in Appendix 4. Temperature was always suitable for White Tip infection. Problems were experienced with the MET station on two occasions with erroneous readings being detected remotely, but it was not possible to visit to inspect and remove the station and then return to replace it without gaps in recording between 1 and 24 August 2017, and again between 11 December 2017 and 5 January 2018.

Table 10. Comparison of mean % disease incidence and severity (n = 4 plots) of White Tip at the Lancashire site at assessment dates In December 2017, February and March 2018 for 10 fungicide treatments plus a double untreated. The mean number of central leaves still healthy was recorded at the last assessment on 21 March 2018 to give an indication of the amount of trimming that would be needed at harvest.

Treatment number	Product / AHDB code	19th December 2017		8th February 2018		21st March 2018	
		Incidence*	Severity**	Incidence*	Severity**	Incidence***	No. of central leaves still healthy****
1 + 2	Untreated	0.24	3.8	16.5	18.5	19.4	6.9 **a**
3	Invader (standard)	0.00	0.0	14.8	16.2	23.7	7.5 **ab**
4	F212	0.42	5.0	19.2	17.5	22.5	8.0 **bc**
5	F213	0.56	13.8	18.7	18.8	17.5	9.5 **d**
6	F214	0.00	0.0	14.4	18.8	8.2	8.7 **bcd**
7	Infinito	0.38	2.5	21.2	26.2	11.2	9.0 **cd**
8	F215	0.00	0.0	17.0	18.8	20.0	9.0 **cd**
9	F216	0.18	1.3	12.1	15.0	18.8	9.5 **d**
10	F217	0.00	0.0	18.8	18.8	23.7	9.7 **d**
11	F218	0.55	5.0	17.7	18.8	28.7	8.5 **bcd**
12	F212 +Infinito + F218 in 3 spray programme	0.59	5	17.1	17.5	18.7	8.7 **bcd**
Mean		0.263	3.3	17.0	18.6	19.3	8.5
L.s.d.		0.847	11.52	10.69	13.92	19.61	1.155
ANOVA F. pr.		0.726	0.434	0.897	0.964	0.669	< 0.001 Differences shown using Duncan's multiple-range test

* Disease incidence in December and February given as a % of the total number of plants per plot was calculated from the number of individual plants affected by White Tip per plot divided by the total plant counts recorded on 31 August 2017.

** Disease severity in December and February was recorded as the % of leaf area affected only on those plants displaying White Tip symptoms i.e. in order to indicate marketability of infected plants not an overall plot mean including healthy plants.

***Disease incidence in March was recorded as an overall assessment of the proportion of diseased plants within each plot.

**** The number of central leaves still usually healthy and green on plants was recorded, from the central leaf of the plant outwards.

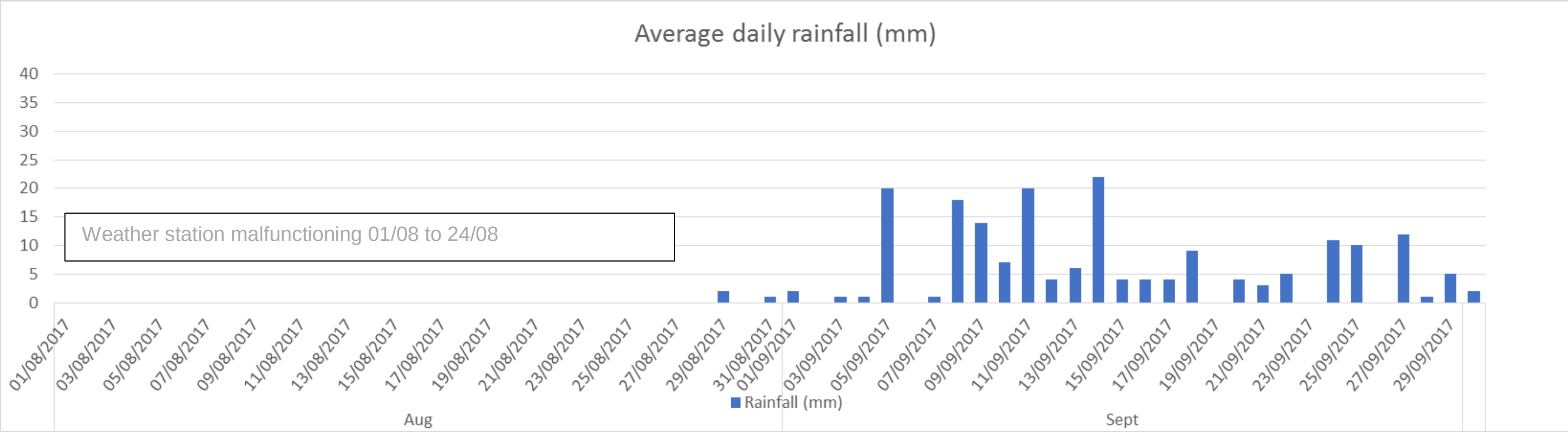


Figure 19. Daily rainfall in mm (blue bars) between 1 August 2017 and 30 September 2017 at the Lancashire field site.

First White Tip experimental fungicide application given on 7 September 2017 when a period of wet weather was forecast.

Note: Rainfall between 1 and 24 August was probably not recorded by the in-field MET station as the air temperature charted at this time remained above 20 °C at night and this is unlikely during this period suggesting station malfunctioning (as shown in Appendix 4).

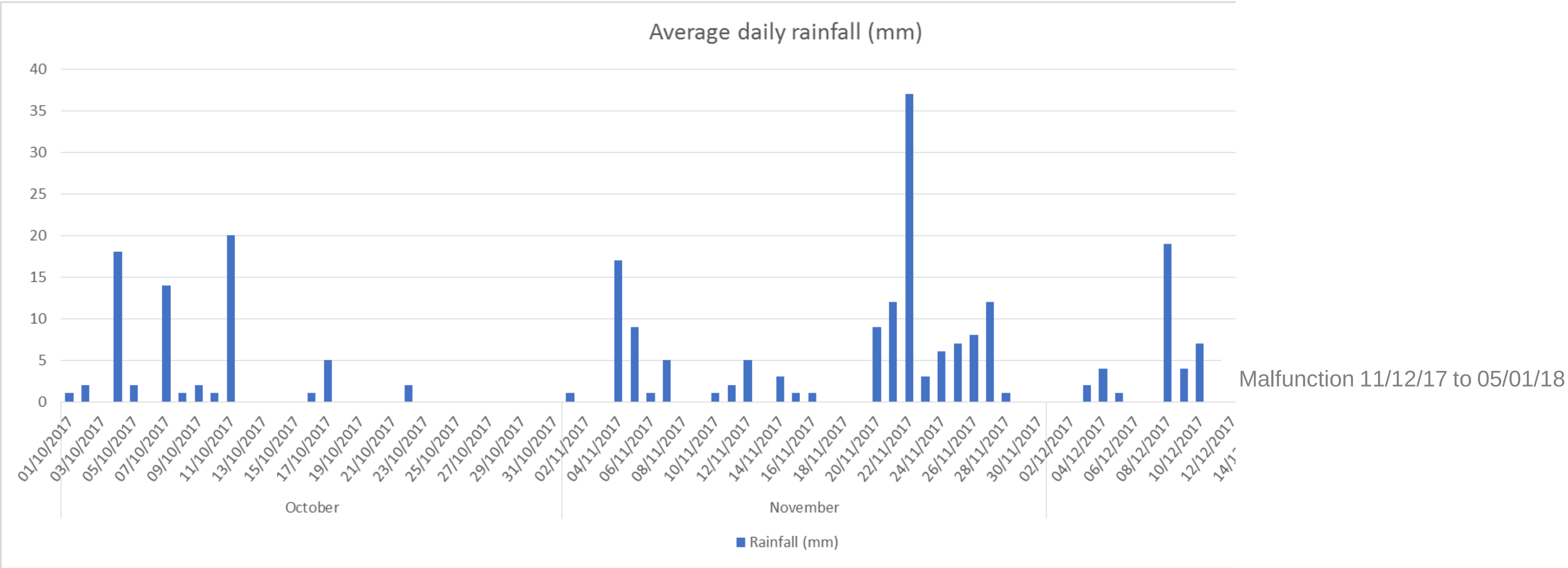


Figure 20. Daily rainfall in mm (blue bars) between 1 October 2017 and 11 December 2017 at the Lancashire field site.

The second experimental fungicide application was done on 30 October and the third and final on the 19 December.

The in-field weather station ceased recording accurately at 16:00 on 11 December and it was not possible to get it repaired and replaced it until 5 January 2018.

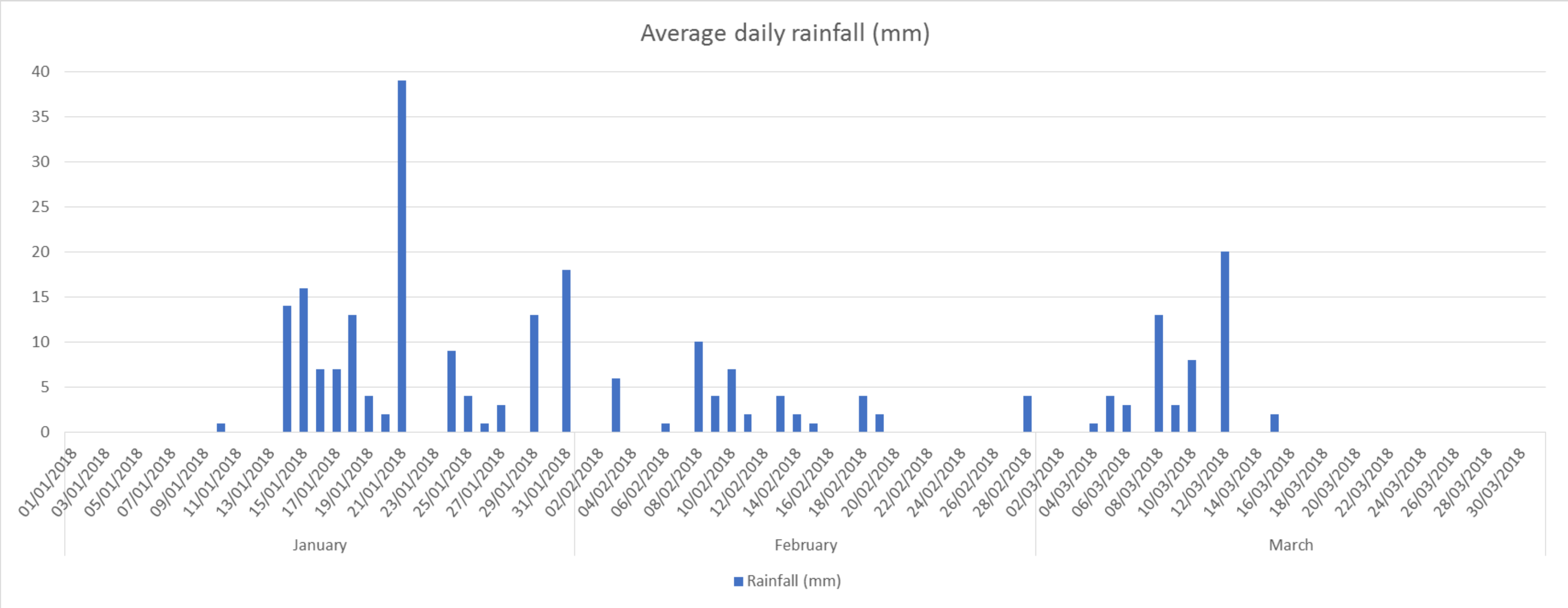


Figure 21. Daily rainfall in mm (blue bars) between 5 January 2018 and 31 March 2018 at the Lancashire field site.

Note: the MET station malfunctioned was not able to be replaced in the field before 5 January 2018. The high volume of rain recorded on 21 January may have been as a result of collected snow thawing as temperatures rose rapidly (see Appendix 4iv) and this would not have resulted in rain-splash.

WP10. To carry out a field trial to determine if there are varietal differences in leek cultivar susceptibility to infection by *P. porri.*

Introduction

Screening of varieties for resistance to *P. porri* could offer growers the option of planting material that is less likely to be affected by White Tip and so save both pesticide application and trimming time. Varieties which are resistant or tolerant would also be of interest for adoption in organic production as a cultural method of control. Grower and breeder observations have pointed to there being differences in the severity of White tip infection in different leek varieties, and these are noted below.

Philip Lilley (Glassford-Hammond Farming) indicated that White Tip can be a big problem, especially in the latest crops of Triton, as this variety is particularly susceptible. However, Triton is the only late leek variety that will harvest very late into the season. He also said that a wider range of product options with activity on White Tip is desirable to guard against resistance, as the current options are very limited with a lot of reliance on Invader

Tim Casey (J and V Casey, growers) reported trouble with White Tip, mostly in the later harvested crops that overwinter, especially when it is wet and mild. Control is normally acceptable, however it seems to be seen more in open pollinated varieties and the late, slow growing Syngenta variety Triton. The disease is seen less in the Nunhems varieties cv. Pluston and cv. Lexton (harvested after Christmas), perhaps as these tend to be a lot stronger growing / more vigorous than other varieties and therefore appear to be more resistant.

R. Murison (Nunhems Seed Company, with a 90% share of the leek production market in the UK) has said that the cultivars that are bred in Europe for Autumn are used to over-winter in the UK, due to milder temperatures. These cultivars tend to be softer growing types such as cv. Lexton. The company has a new hybrid cv. Pluston which has demonstrated good performance against White Tip, which gives some confidence to the company in providing another robust cultivar. Mr Murison agreed that architecture is a factor in the robustness of the cultivar against White Tip. The shorter, stockier cultivars are more resistant to wind chill and tangibly White Tip, than those with a taller shaft. Although the shorter leek cultivars are hardier against the elements and some diseases, growers are sowing higher volumes of tall-shaft cultivars due to the pre-pack market. More than 80% of the leek crops in the UK are drilled rather than transplanted, due to harvest timing and weed competition.

As a follow on to the variety trial in containers which was conducted at ADAS Boxworth in 2016, and supplementary to the original work programme, a replicated variety trial was prepared at T.W. Wilson’s farm in Lancashire, on the same field as the product efficacy trial.

Materials and methods

Plant production and maintenance

In 2017 twelve leek varieties were selected 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 become infected by *P. porri* (**Table 11**). A variety with greater susceptibility (Triton), and another with lower reported susceptibility (Pluston) were also included. The crop was drilled on the 15th May 2017 and fungicide applications were applied as per commercial practice until September 2017.

Table 11. Leek varieties selected to investigate the effect of variety on White Tip development at field site in Lancashire in 2017/18.

Treatment number	Variety	Breeder or supplier
1	Triton	Syngenta
2	Pluston	Nunhems/Bayer
3	Belton	Nunhems/Bayer
4	Lexton	Nunhems/Bayer
5	Longton	Nunhems / Bayer
6	Krypton	Nunhems/Bayer
7	Comanche	Enza Zaden
8	LV446	
9	Curling	Bejo/Elsoms
10	Mercurian	Syngenta
11	Galvani	Seminis
12	Triton Primer F1 Hybrid	Syngenta

Variety plots were drilled 2.5 m long (half the length of those in the efficacy trial) and the beds were a standard 1.8 m wide. Varieties were randomised with each of four replicate blocks and did not receive any of experimental fungicide applications.

Assessments

Plant establishment was assessed on 8 August 2017. Plants were examined for White Tip symptoms. For the assessments done on the 30 October, 19 December 2017 and 8 February 2018, when disease incidence was low the number of disease plants per plot was recorded. Disease incidence was thus calculated as the number of individual plants affected by White Tip as a % of the total number of plants per plot, measured on 31 August 2017. For the assessments done on 8 February and 21 March 2018, disease incidence was much higher and so was recorded in the field as the % of diseased plants per plot.

Disease severity was recorded in December 2017 and February 2018 as the % of leaf area affected on plants displaying symptoms. This was in order to give a representation of the marketability of the affected plants (rather than the mean leaf area affected across both healthy and White Tip affected plants).

Disease severity was recorded differently at the pre-harvest assessment on 21 March 2018, in order to allow visualisation of how much leaf trimming might be required, and so the mean number of central leaves still healthy and green on the plants in each plot was recorded. This was recorded from the central leaf sheath of the plant outwards. At this final assessment the mean shank height was measured and recorded at two positions in the plot (there being good uniformity within plots), the average of these two positions was then recorded for each plot across the whole trial area to get an overall average. Shank height was measured from the soil to uppermost leaf axil (where the youngest leaf was emerging).

Though no chemical treatments were applied, 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.

Analysis of data was carried out using ANOVA, with comparisons shown by Duncan's Multiple Range test whereby varieties with the same letter designation are not significantly different. A regression analysis was carried out to see whether there was any correlation between White Tip incidence and variety shank height (as this relationship had been suggested to exist).

Figure 22. View on 8 August 2017 along the leek White Tip variety trial in Lancashire, with herbicide treated cereal "guard crop" plants visible between leek plants

Figure 23. View on 1 September 2017 across the leek White Tip variety trial in Lancashire

Results

Distinctive bright white "moist" lesions of White Tip developed which caused the affected tissue to become papery surrounded by healthy green tissue. Samples were not, however, collected to confirm *P. porri* presence by laboratory culture. There was no significant difference in between varieties at the assessments conducted on the 19 December 2017 and the 21 March 2018, with a mean of 0.85% of plants (P=0.416, L.s.d. 2.649) and 18.5% of plants (P=0.16, L.s.d. 1.402) affected respectively on each assessment date (**Table 12**).

At the assessment conducted on 8 February 2018, there was a significant difference in disease incidence across varieties with a mean of 71.6% of plants affected (P>0.001, L.s.d. 16.24). At this assessment, Longton proved to be the least susceptible variety with the lowest incidence of disease (mean 52.5%). Longton was not significantly different from Pluston, Galvani and the Triton F1 Hybrid. Krypton and Comanche had the highest level of disease at this assessment time (**Table 12** and **Figure 4**).

Galvani had overall the shortest mean shank height of 74 mm (P>0.001 L.s.d. 26 mm); Triton, Curling and the Triton F1 hybrid were not significantly different from Galvani (all below 100 mm). Lexton (146 mm) and Krypton (127 mm) had the tallest shank height, not differing significantly from each other (**Table 12**).

The regression analysis of % plants with White Tip on 21 March 2018 (mean 18.5%) against their shank height (mean 104.4 mm) gave a P value of 0.982 (d.f. 46) and showed no relationship between these parameters (**Figure 24**).

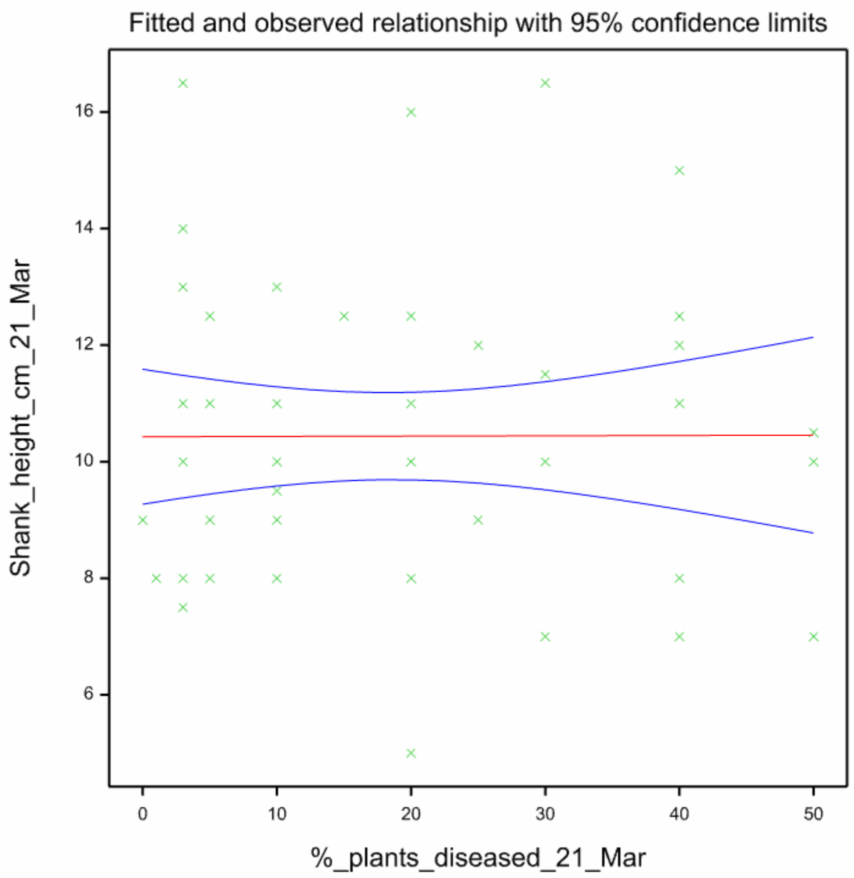


Figure 24. Regression chart showing no relationship on 21 March 2018 between the incidence of White Tip on the twelve leek varieties and their shank height. Lancashire field site 2017/18 cropping year.

Table 12. Comparison of mean % disease incidence and severity of White Tip at three assessment dates for 12 varieties at Lancashire site. The mean number of central leaves still healthy was recorded at the last assessment. Duncan's Range test shows differences when no letters are the same.

Variety code	Variety	19 December 2017		8 February 2018		21 March 2018		
		Incidence*	Severity***	Incidence**	Severity***	Mean shank height cm ****	Incidence**	No. of central leaves still healthy*****
1	Triton	0.00	0.00	78.75 **cde**	30.00	9.00 **ab**	17.00	4.50
2	Pluston	0.00	0.00	61.25 **abc**	18.75	10.75 **bc**	40.00	6.00
3	Belton	0.75	2.50	81.25 **de**	37.50	10.38 **bc**	4.75	5.25
4	Lexton	1.00	3.75	67.50 **abcd**	23.75	14.63 **d**	10.25	5.75
5	Longton	0.00	0.00	52.50 **a**	25.00	10.88 **bc**	15.00	5.50
6	Krypton	2.00	15.00	87.50 **e**	43.75	12.75 **cd**	20.75	5.25
7	Comanche	1.25	4.50	86.25 **e**	40.00	10.88 **bc**	23.75	5.25
8	LV446	1.00	6.75	80.00 **de**	35.00	10.50 **bc**	25.00	6.00
9	Curling	0.50	2.50	72.50 **bcde**	27.50	9.62 **ab**	16.25	5.75
10	Mercurian	0.50	2.50	71.25 **bcde**	27.50	10.50 **bc**	15.00	4.00
11	Galvani	0.25	3.75	60.00 **ab**	16.25	7.38 **a**	25.75	4.75
12	Triton-primer F1 hybrid	3.00	10.00	60.00 **ab**	21.25	8.00 **ab**	8.50	5.25
Mean		0.85	4.27	71.60	28.90	10.44	18.5	5.27
L.s.d.		2.51	9.587	16.24	15.62	2.649	21.09	1.402
F. pr.		0.416	0.096	<0.001	0.019	<0.001	0.129	0.16

* Disease incidence was recorded as the number of diseased plants and is shown as a % of the total number of plants per plot that had been measured on the 31st August 2017.**Disease incidence in February & March recorded in the field as % of diseased plants per plot *** Disease severity in December & February, in order to indicate marketability, recorded as the % of leaf area affected on those plants displaying symptoms i.e. how much leaf area White Tip covered on those plants affected, not the mean including healthy plants. **** Mean shank height was measured in March from the soil to uppermost leaf axil (where the youngest leaf was emerging) and recorded at two positions in each plot. ***** The number of central leaves still healthy and green most commonly present on plants in each plot was recorded from the central leaf sheath of the plant outwards.

WP7. To explore the potential for weather-based forecasting to assist spray timing applications for White Tip

Materials and methods

An initial, "look-see", study, utilising the weather and disease data from the two field trials of the current project, was made to elucidate whether developing a forecasting model might be feasible in future in order to give growers support in their decisions regarding when or whether to spray.

In-field meteorological stations with satellite communication of the data to allow download by ADAS were set up as near as possible to the trial area at the field site in each of the two years. Temperature, relative humidity and rainfall were recorded and charts produced to show the daily ranges of air temperature and relative humidity and the daily rainfall depth. The likely influences of temperature and rainfall on leek White Tip development were studied by Smilde (1996). Declercq *et al*. (2012) reviewed the information from Smilde and carried out further observations in order to put forward further ideas on the factors influencing the development of this disease. The current project examined how their observations aligned with the weather conditions and disease symptom expression at the two field sites.

Records were made of the incidence and severity of visible White Tip symptoms, but it was not possible to make the frequent number of visits required in epidemiological studies in order to produce detailed information on the timing of appearance of disease symptoms and their changes in severity over time (disease progress curves). However, work by Smilde (1996) and Declercq *et al*. (2012) has provided information on the time periods for various stages of the infection process of leek White Tip and symptom expression and this information was reviewed and utilised when examining the field records from the current project.

Declercq *et al*. (2012) noted that monitoring of White Tip disease progress was not straightforward as the dry white tips of infected leaves often either broke or rotted off and older leaves senesced over the period of up to a year before crops are harvested. In the current project the potential for incidence and severity decreases, was taken into account in the inoculated experiments as records of each plant in a plot were maintained separately together with the position of the symptoms present at that time.

The base temperature for *P. porri* symptom development was given by Smilde (1996) as below freezing, at - 3°C, and the upper as 30°C, as outside this range no lesions developed following zoospore inoculation. If zoospores were able to splash up and penetrate leaves before water froze then when the air temperatures fell to 0°C on a number of occasions between November 2017 and March 2018 then, according to Smilde, symptoms would still be expected within 36 to 56 days from penetration. Lesions would take 13-18 days to show at 5°C and 4-11 days to show at over 11°C. On average Smilde found a lesion appeared 120 degree-days after infection (a day degree being the average daily temperature above a base temperature of minus 3°C)

This requirement for a high amount of rainfall on at least one day prior to the onset of visible infection was identified by Smilde (1996). He found that in each of three years of field experiments in the Netherlands that the onset of White Tip epidemics in (late August, early August and mid- September, respectively) were preceded by over 15 mm of rain on a single day 8-12 days earlier. Subsequent peaks of disease were shown to be preceded 8-21 days earlier by over 20 mm rain within a period of two to three days. He saw that there were relatively short periods of explosive disease increase alternated with periods in which no new infections occurred. Smilde (1996) confirmed a statistical correlation with rain at the start of the season only, proposing that lesion and infected leaf loss later prevented subsequent correlation.

The practical implication for growers of knowing the time for lesion development is that if a fungicide was applied soon after there were wet conditions advantageous for zoospore spread, there would be a shorter wait in warmer weather to see if it had been effective i.e. requiring inspections of the crop within a fortnight of spraying rather than between five to eight weeks later in cold weather. Given humid conditions, it is possible for sporangia to be produced by confirmed lesions and so a further fungicide application could be timed to halt secondary zoospore spread.

Results

The development of primary infection

The affected Lancashire field trial plots in December 2017 were scattered across the field. This is a pattern seen with infection originating from the soil. With *P. porri* this may reflect widely dispersed oospores in the soil or the low probability of a zoospore successfully being splashed into a leaf axil. High infection from the soil was not anticipated by the grower at this site because of the rotation used, but he was certain that infection would take place. The four year break from leeks recommended is to allow the number of viable oospores to decline and the speed of this will depend on a variety of factors.

For *Phytophthora* species in general, sporangia may be driven long distances by wind and rain during thunderstorms (Davidson *et al*., 2005). Unusually high winds associated with rarer storm events may blow raindrops with spores, with sporangia of *P. capsici* shown to have moved 3 m in windblown rain (Davidson et al, 2005). There is some possibility that the zoospores could arrive in wind or mists, but unlike *Phytophthora* species such as *P. capsicum* the sporangia head of *P. porri* containing around 20 to 50 zoospores does not detach for dispersal and so zoospores would need to be released from sporangia and then survive independent travel.

Rainfall conditions August 2017 to March 2018 at the Lancashire Field site (2017/18) and White Tip development

Please refer to rainfall records for Lancashire in 2017 and 2018 in Figure 19, Figure 20 and Figure 21 and mean daily rainfall, temperature and relative humidity charts between August 2017 and March 2018 given in Appendix 4. The temperatures at the Lancashire field site were virtually always conducive to pathogen growth, based on Smilde (1996).

Assessment visits were made on 31 August and 20 September, but no potential White Tip was seen until 30 October (when four plants in the trial had white lesions, although not removed to confirm in the laboratory if P. porri was present). By 19 December there had been a notable increase in the incidence of White Tip, with symptoms visible in 10 out of 48 plots (over 20%), although only on two or three plants in these plots. By February 2018 many more plants were affected, a mean 17% of plants, with no significant treatment differences recorded in the trial.

i) August / September 2017

August was dry, but although September 2017 was wet White Tip was not seen. Infection could have been expected in early September as there were four days when rainfall was over the 15 mm threshold seen by Smilde (1996) and so first signs of infection should have been noticed in late September/early October (given the approximate 11 days for lesions to show after a rain event in Smilde's field records between September and November). However, the pattern of rainfall (whether the day's rainfall was spread out or fell within hours) seems to be important for splash dispersal.

Looking in detail at the rain records in the Lancashire field taken every 15 minutes (data not presented) on 5 and 11 September the rain fell over a period of four hours with one millimetre recorded every 15 minutes, while on 14 September two to four millimetres fell every 15 minutes to give 20 mm over two hours. However, it is most likely that zoospore splash-up may have followed the intense rain on 8 September when 13 mm fell in 45 minutes, particularly as this followed almost daily rain which would have left puddles. The first fungicide application on 7 September was thus ideally timed. It is hypothesised, however, that the rain was still not able to generate splash and this could be because herbicide-treated cereal “guard crop” plants sown to protect the leek seedlings still covered the soil in September.

Leek seedlings take at least three months for the plants to “thicken up” and produce leaves that do not cling tightly around the plant and provide a place for water to lodge. Young plants are erect and waxy so that water runs off. If zoospores are not trapped this is likely to explain why symptoms were not seen in the first few months of the crop and supports leek growers' withholding of the limited number of oomycete specific fungicides until later in the year.

ii) *October / November / December 2017*

Early October had some rain most days, with three days when 18 mm, 14 mm and then 20 mm of rain fell before the second fungicide application on 30 October, at which date white markings which could have been the early stages of White Tip were seen on four plants.

Following inoculation tests at various set temperatures Smilde (1996) determined that White Tip symptoms would have taken no more than 18 days (13 to 18 days) to develop at around 5°C, and no more than 11 days (4 to 11 days) at over 11°C. The temperature records at the site in November and December 2017 fluctuated around a mean 8°C, and so first symptoms around this mid-point of Smilde's temperature ranges might be expected to be appear around 14 days from infection.

Symptoms by mid-December were already obvious and it is thus likely they had first become visible at least a fortnight prior to assessment i.e. by 5 December. Taking 5 December as when first symptoms arose then infection 14 days earlier would have meant zoospore penetration around 21 November. This scenario was summarised pictorially in **Figure 3**.

For oospores to germinate and produce sporangia, which then release zoospores, Declercq *et al*. (2012) determined that they needed to be kept wet for seven days. Germination occurred across the tested range of 4°C to 22°C. In the current project, the field soil would thus need to be wet in the period 14-21 November 2017 (a week before the hypothesised zoospore penetration day) to allow oospore germination. It did rain on five out of the seven days during this period in November (**Figure 25**).

Following the proposed period of oospore germination the next day, 22 November, had 37 mm of rain, double that of any other day in November 2017. This fell as one or two mm every 15 minutes from 2:30h to 20:45h, but then a downpour of 6 mm occurred which is likely to have caused splash up (15 minute interval rainfall meter records not presented). To get zoospores up onto the plant requires heavy rain splash from puddles and, as the previous two days had a total of 17 mm, the soil is likely to have already been saturated (**Figure 25**).

The 21 November and 22 November were warmer than the preceding 20 days, around 12°C, peaking at 14°C, before becoming colder. The initial warm temperature is likely to have accelerated mycelial growth, aiding establishment within the host (**Figure 25**).

There was also rain during all six days after 22 November and this would have helped to keep water in the leaf axils so that zoospores could swim before encysting. However, this is unlikely to be necessary as Declercq *et al*. (2012) saw zoospores encyst and then germinate to produce hypha that entered stomata or penetrating tissue directly within six hours of zoospore arrival. It was therefore likely that the obvious lesions present by 19 December 2017 were as a result of a wet period four to five weeks previously followed by a day of rain totalling over 30 mm on 22 November.

In December, there was some rain at the start of the month, but the maximum of 19 mm on 7 December fell evenly over six hours (data not presented) and so was unlikely to splash up.

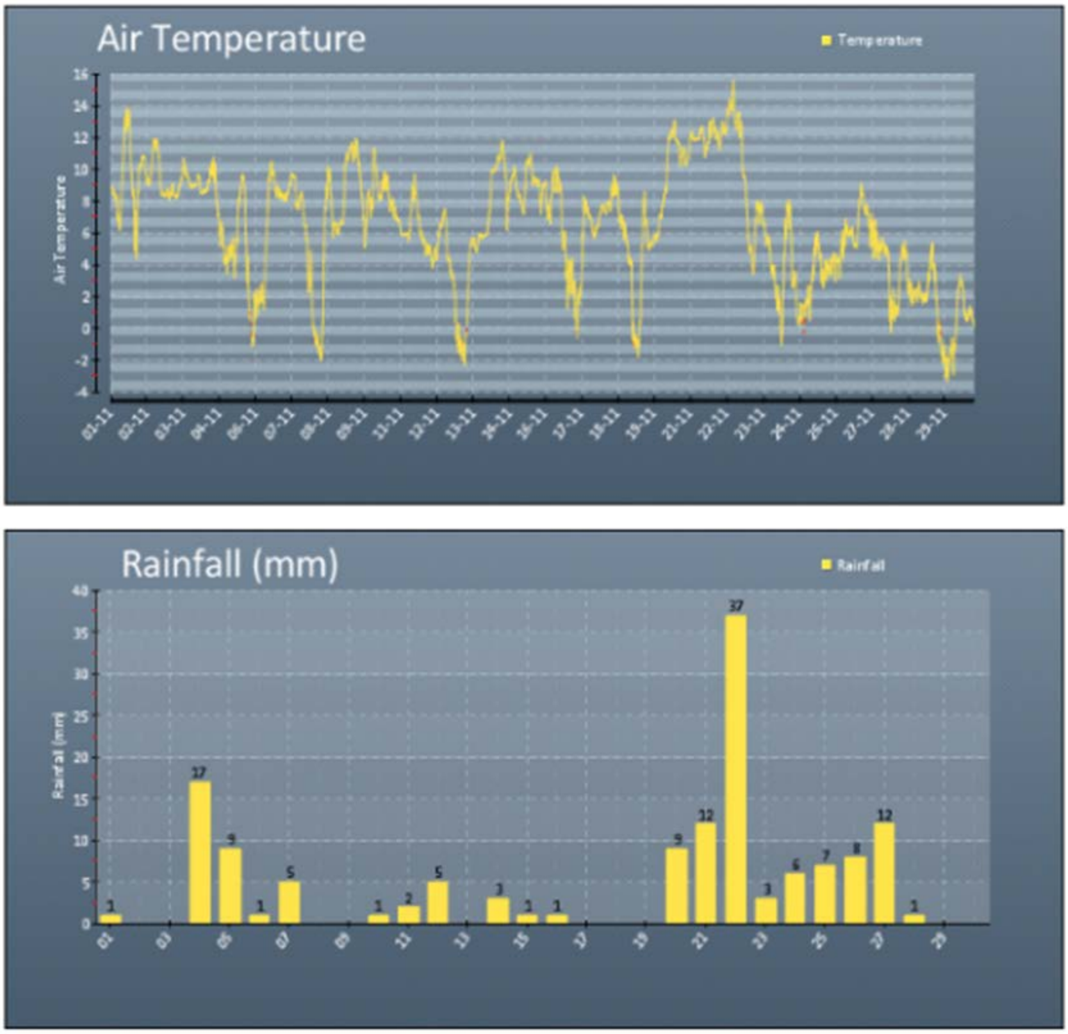


Figure 25. Lancashire field site weather station record for November 2017 showing heavy rain (37 mm) on the 22 November, preceded directly by two days totalling 21 mm. Wet conditions persisted for six days after 22 November. White Tip was first seen in December.

iii) January / February / March 2018

A very high rainfall of 35 mm was recorded on the 21 January 2018, with 19 mm within 15 minutes of 8.30h with air temperature of 0.2°C. On this day temperatures had fallen below zero overnight, but then rose to stay above zero from 07.45h. The MET office reported the Midlands and East were covered by snow and this was followed by floods as it melted swiftly. It is not possible to know from the automatic records whether the North West field site had exceptionally heavy rain rather than rapidly melting snow. As temperatures were above the -3°C of disease development, then infection could still have taken place following splash and could be the cause of the infection visible in all plots on the 8 February 2018 and temperatures above 5°C during the end of January would have allowed disease to develop within the 13 to 18 days determined at this temperature by Smilde (1996).

During February there was intermittent rain, but no heavy downpours and further disease progress was probably associated with lesion enlargement and lesions showing that had developed on leaf tips emerging from down within the central growing point.

By 8 February 2018 all plots had White Tip, with a range of seven to 36 plants showing symptoms in the plots (which had 95 to 124 plants). The increase in incidence from 20% of plots to all plots in the seven weeks between observations could have resulted from a number causes:

a) Further oospores germinating in the soil to produce sporangia that release zoospores and subsequent zoospore splash onto leaves

b) Oospores germinating from leaves/leaf tips with White Tip in December that had since decayed and released oospores to allow zoospore production in puddles.

c) the production of sporangia on the leaf surface from lesions produced by mycelium growing in the tissue and their release of zoospores to cause secondary infection (Smilde 1996 in the Netherlands reported spread from sporulation over 2 m distance, although Declercq *et al*. (2012) in Belgium reported no sporulation.

e) Further development of visible symptoms from (latent) infection in late November.

Latent (symptomless) infection is hypothesised to have followed the rain on 22 November 2017 and based on the prevailing temperatures, all infection should have started to become visible within about 14 days. However it is likely that white leaf tips were present on new leaves, but these leaves were still down inside the shank i.e. had not emerged up from the leaf axils to be visible. In the current project, following artificial inoculation into leaf axils, it was observed that in winter it took a couple of months for the youngest developing leaflet in the centre of the plant to emerge and that when visible it had a white tip. Declercq et al. (2012)

said infection occurs both through tissue penetration and via stomata, however another likely entry point is the hydathodes (guttation pores) at leaf tips that are connected to the plant's vascular system. Oomycete zoospores in soil are known to be attracted to roots by their exudates and it is a possibility that zoospores in the water pool in the centre of the plant would be attracted to exudation from leaf tip hydathodes and potentially enter the leaf through by this route.

In some plots the incidence and/or severity of visible White Tip infection decreased between assessments. Severity can decrease when new leaves are produced and either do not become infected or show that they have been infected. More leaves are produced in warmer weather so the area of the plant affected by White Tip lesions is reduced unless more symptoms develop. In colder weather plant growth is slowed, but as the disease takes longer to express when cooler then the leaves will appear to remain uninfected. It was noted by Smilde (1996) that visibly infected leaves can be short-lived, and during September to November he observed that the leaves were dead within 21 to 32 days. Some of this was through the invasion of fungi such as *Pleospora herbarium*. In the current project once a mid-leaf lesion developed, although initially bright white, it usually became invaded by a *Cladosporium* species that produced dark green spore covered spots over the affected area. Leaf tips were also noted by Smilde to be lost. White Tip affected leaf tips become paper-thin with a distinct margin with green tissue and in the current project it was observed that leaf tips desiccated in dry weather and were easily broken from the leaf.

Records in 2018 showed the outer (older) leaves to be those affected, not the most recently emerging six to nine central leaves, and the most likely explanation for this is that infection within the central leaf whorl did not happen in the recent months before these leaves emerged. Outer leaves are trimmed off, with the greatest amount of trimming for pre-packs so that also only the base of young leaves remains.

Conditions leading to infection at the Nottinghamshire Field site (2016/17)

In autumn 2016, there was earlier observation of White Tip than in autumn 2017, on 7 September rather than in October, with white lesions on one or two plants in some plots. Nothing more had developed by the second fungicide application on the 9 November 2016 and significant incidence was not recorded until February 2017

At the assessment on 1 February, White Tip was visible on most plants in all plots and with around 7% leaf area affected it was an indication that the disease was well established. Infection in February 2017 was preceded by the recording of an exceptional over 35 mm of rain on the 21 November which could have provided conditions for zoospore splash-dispersal from soil into wet leaf axils, particularly as the previous day's 8 mm of rain could have made puddles on the soil already wet from 12 days of rain within the previous 16 days. This period would also have provided the necessary wet soil conditions for the start of oospore germination leading to zoospore release.

The final fungicide application on 6 February 2017 was made after wet days following the earlier assessment and would be expected to protect newer leaves from infection. Infection had not developed much further by the 13 February, but disease already in the leaves was unable to be controlled by the fungicides.

By 1 March 2017, without further days of potential rain splash to start new infection, there was only a small increase in severity so that around 14% of plants' surface area had visible white mid-leaf lesions and white tips.

Discussion on weather and its impact on P. porri infection in general situations

The amount of rainfall needed before splash up is possible from puddles will depend on the soil type and how free-draining it is. Free-water in soil is also needed in the week leading up to oospore germination. Hence, crops on heavier or more-compacted soils are likely to be more susceptible to White Tip.

The volume of individual raindrops i.e. their mass is likely to be of importance in whether or not there is splash up from puddles. This is not directly measured by rainfall sensors, but indications can be gained from close recording intervals (15 minutes or less) as these will show if a lot of rain fell in a short period. What depth of water is produced in a set time could be measured by testing a rain gauge with a water sprinkler, but it is likely that in excess of 3 mm in 15 minutes would be produced by heavy rain. A steady rain over several hours producing perhaps 7 mm over five hours would be good for saturating the soil and not just running off and so allow puddles, but showery rain is unlikely to cause splash up.

It is possible that herbicide treated guard/nurse crop, cover crop plants or cereal volunteers around leeks can reduce the amount of soil/zoospore splash onto the crop. Heavier rain could thus be needed to splash through the cover to the soil. Living weeds or an intercrop will also cover soil and keep crop plants clean, but they could also raise humidity around the plants and provide favourable conditions for pathogens such as *Botrytis* spp.. The effectiveness of a straw-covering around the base of the leeks to prevent rain splash was tested in a project funded by AHDB Horticulture in 1995-6, but this proved ineffective as the straw degraded over time. This was a practice that was also trialled in the Netherlands and gave some positive results, which lead to about 10% of the Dutch leek acreage to be mulched on the surface with straw.

Showery rain, while providing ongoing moisture for pathogens such as rusts and allowing time for spore germination and penetration into leaves, is not important for *P. porri* when the zoospores are in leaf axil water puddles, but could allow infection of locations such as where leaves bend downwards or on leaf tips. Six hours of wetness are needed from zoospore arrival, through encystment and to allow hyphae then produced to enter the host. In general, however, when water lands on leek plants the water droplets run down into the leaf axils and, in the current project, pools in axils were seen to remain for at least three days, even in summer.

In winter, as in December 2017, when temperatures are around freezing there is a possibility that precipitation falls as snow. If the rainfall meter becomes submerged by snow this will affect readings, with the amount of water held in the snow only recorded on melting. Slow melting of snow would probably be recorded as a gentle rain over several hours, whereas a fast melt would appear as a deluge. The latter would be indicated by a fast temperature rise, particularly together with sunshine. Water from snowmelt will add to puddles but there is unlikely to be any splash up. If rain fall records are used as part of a warning that conditions have been suitable for White Tip infection, then people monitoring the results will need to be aware of snowfall. For both the recording of heavy raindrops and the falling of snow rather than rain, a webcam with recording could be checked for the weather periods of concern.

Unlike diseases such as botrytis, high relative humidity of the air is probably not important for *P. porri.* In many fungi the spores require humid conditions before they germinate. The humidity then needs to remain high to prevent desiccation of the germ tube (that is produced from the spore) before it enters the host. In oomycetes such as *P. porri* the zoospores swim with flagella and so require water around them.

Wind speed may also have a bearing on rain splash, as rain driven by strong winds is likely to have a greater impact into puddles and result in more water being thrown up. If sporulation does occur on leaves then wet windy conditions will facilitate plant to plant spread of zoospores. High winds may cause leeks to thrash about so that leaves become bent down (particularly if there is a White Tip lesion at the midrib causing weakness as has often been observed in the current project). Once leaves are bent down to touch wet ground they are more likely to start to rot. White Tip affected leaf tips touching the ground would then release the oospores formed in the leaf tissue and these have the capability of releasing zoospores to splash up and cause secondary infection of other leaves.

At the outset of the current project it was thought that the rainfall data could be used together with disease assessments in a window pane type analysis to determine when conditions were right for infection to have taken place. Following a review of the weather data and the disease records and discussion with an ADAS expert in disease data analysis (D. Ginsberg), it has been determined that it is not feasible to get more information from the data without knowing what the ground conditions were like at any one time in the crop (saturated soil being required for both for oospore germination and zoospore splash). In addition there needs to be available detailed frequent (at least weekly) observations of the crop to record when symptoms first showed. Information on when plants had matured enough to have leaf axils proud from the shank in order to be able to trap zoospores, also needs to be incorporated. For other diseases electronic measurement of leaf wetness is not unusual, but the need for not only moisture monitoring in soil but also knowing if there is surface water on the soil is a technical step further.

P. porri oospore and zoospore production and survival

In Declercq *et al*. (2012), tests with oospore germination showed it only occurred in non-sterile soil water extract. This supports that oospore germination occurs in the soil, rather than an alternative hypothesis that oospores are splashed onto the plant and germinate there to produce zoospore in the water pools in leaf axils (although soil splashed into leaf axils might be sufficient to provide the stimulus required).

There is a knowledge gap concerning the timing of oospore germination in the soil. It is not clear whether there is a staggered germination of oospores over the year, or whether germination simply takes place once it is wet enough, or whether it requires the production of leek root exudates. Oospores are known to persist in the soil for up to four years, as determined by growing leeks in successive years (Declercq *et al*., 2012).

Oospores are produced in leaves within six days of infection (Declercq *et al*., 2012), but according to Smilde (1996) they may require a period of maturation before being able to produce sporangia. A maturation period of four to five months was shown by Smilde, but he put the oospores in sterile distilled water (whereas soil extract water gives optimum zoospore production) and so further investigation is required. It is important to know whether zoospores are released from infected tissue in the standing crop (such as leaf tips dangling in puddles) or whether for example oospores forming in January are unlikely to be mature enough before the crop is harvested.

(Declercq *et al*., 2012) did not see sporangia form on lesions on leek leaves, and no sporulation was seen on any of the dry white lesions in any of the experiments in the current project. Smilde (1996) reported seeing sporangia in the pale green lesions sometimes present on leaves. Such lesions were occasionally seen in the current project, but neither external mycelium nor sporangia were seen under suitable magnification (although the dark mycelium and spores of *Cladosporium* sp. were frequently present in association with older lesions).

In the laboratory Declercq *et al*. (2012) showed zoospores once released from oospore sporangia could survive for at least seven weeks in water over the temperature range of 0°C to 24°C. It is possible that in the soil, even if it stays wet, that zoospores might be subject to bacterial attack or possibly preyed on by e.g. protists/protozoa so there would be a more limited window in which to splash the zoospores onto leaves i.e. a smaller risk period following soil saturation. This is a knowledge gap and there could be potential for enhancing biological control in the soil. Both oospores and zoospores could be reduced by biological control products.

Fungicides need to be persistent if application is preceded by dry weather as the zoospores are not expected to be on the crop until after there has been a week of saturated soil for oospore germination. Systemic fungicide activity is needed, or high volume application that causes droplets to run into the leaf axils where the zoospores will be trapped, as direct spray contact inside the leaf axils where most infection takes place is unlikely. Most fungicides are best applied preventatively, but Invader (dimethomorph + mancozeb) has eradicant activity and could be held in reserve for once conditions have been right for infection.

Disease forecasting on other crops

In celery a "Septoria Predictor" was devised and reported in AHDB Factsheet 13/15 The Septoria Predictor was developed for use in celery in Michigan in the 1990s. In this, before the celery canopy is large enough to close over the rows, fungicide is applied when leaves have been wet for more than 12 hours. Once the canopy closes, regular fungicide applications at 7 to 10 day intervals are recommended. Tomcast was developed to manage leaf blights on tomato, but used successfully in experiments in California to reduce Septoria blight in celery. When these programs were compared in trials in Ontario in 2006 the severity of Septoria from both was comparable to a 6 to 8 calendar spray program, but with only 5 to 7 with the Septoria Predictor and 4 to 6 sprays with the Tomcast. The Tomcast model utilises both leaf wetness duration and the mean temperature, taking into account that disease severity will be lower when conditions are less than optimum for these two measures. The Factsheet 13/15 provides a table that shows that a shorter period of wetness is needed for infection to take place with warmer temperatures up to 25°C and so a fungicide application needs to be put on quicker in such situations before the pathogen is unable to be controlled by the fungicide. For celery septoria leaf wetness duration recorded on in-crop loggers provides a useful indication of risk of Septoria development. Spraying only when conditions are conducive for disease development may also provide the opportunity to reduce spray applications and is a useful component of an IPM strategy. Reduction of spray frequency, or at least adjusting set spray intervals to better match infection periods, is currently being carried out with potatoes and the infection from air-borne sporangia of the blight pathogen *Phytophthora infestans* whereby growers logging online to "Blightwatch" get a report of

“Hutton Periods” based on as local as possible temperature and relative humidity readings and reporting the parameters of Tmin°C and rh90%hours. These measures are not relevant for Leek White Tip zoospore infection as they require water, not just high humidity, and the normal range of field temperatures has little bearing on the infection process.

With leek White Tip the only influence of temperature seems to be on the speed of symptom expression as the pathogen infects over a wide temperature range. As discussed by Smilde (1996), forecasting White Tip infection will be unreliable as long as rain forecasts are unreliable. Unreliability is particularly caused by the very localised rainstorms that have increasingly taken place in England over the last decade or so (perhaps related to climate change) and so in-field recording and transmitting weather stations may be required for each crop. The soil needs to be wet for one to seven days for zoospore production from oospores, there then needs to be splash-up to leaf axil pockets (perhaps 150 mm from the ground) and then water needs to be held on the plant for six hours for zoospores to encyst and produce infection hyphae. The work in Lancashire showed that over 30 mm in a day was needed for rain splash. If irrigation is applied in dry conditions puddle formation should be avoided. It is possible that an overhead boom will produce less splash-up than a rain-gun. Whether or not splash of *P. porri* zoospores is likely requires knowledge of the soil conditions in the field together with weather data in order to decide on spray application. Attention needs to be paid by growers to field drainage and reducing compaction in order to reduce the formation of standing puddles. The hazard from the splashing up of rain (or irrigation water) from wheelings when machinery passes has been illustrated to the author by the use of infra-red photography of a celery crop whereby the Septoria disease pattern adjacent to a deep rut in a wheeling was seen as a splash pattern emanating from this position.

The observations of rainfall in relation to symptoms at the two field sites therefore support the scenario that the soil needs to be made waterlogged by either a period of wet weather or a heavy downpour that quickly saturates the land. Clay soils or compacted soils (standing water was observed in the current project in tractor wheelings) will be more at risk of puddles than sandy / well-draining soils. A downpour of rain, such as can occur in a thunderstorm, is then needed sufficient to cause splash up of at least 100 mm into the lower leaf axils, or higher into the centre of the leek where young leaves emerge.

Discussion

In the variety susceptibility experiment at ADAS Boxworth 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 may offer weak points for zoospore entry. Growers have reported 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 (unless the break follows attack).

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, it is 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. Oospores are very resilient to heat, requiring at least five hours at 45°C in the soil before germination, so resulting in 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 Nottinghamshire field trial of foliar applied treatments in 2016/17, no significant differences were seen up to the early March assessment date. 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 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 initially infested plants would have been in foci of infection from patchily distributed oospores in the soil as a result of a likely uneven spread of infested debris across the field from the previous leek crop. It is not clear whether increased incidence over the season results from further splash-up of zoospores or that there was sporulation on lesions leading to secondary infection within the crop (as in the work by Smilde (1996), although not seen by Declercq *et al.* (2012)). Incidence in February 2017 was 100%, but only reached at most 40% (mean 18%) in February 2018 and this may be because the first field trial was done in a field with a more recent history of White Tip.

In the second efficacy trial, in 2017/18, when one product was withdrawn but otherwise the same list of products were tested, there were again no significant differences in incidence or severity between the chemical treatments after the first two spray application timings. However, when the new leaves only were assessed in March 2018 then products F217, F213 and F216 had more of new leaves without symptoms, although a similar number of plants were affected. This indicates that fewer young leaves on these plants had become infected and this could have been as a result of protection given by the application on 19 December 2017. Two of these products are registered for use against potato blight (*Phytophthora infestans*) and have systemic and protectant activity, but unfortunately have components the same as Invader and Infinito so limiting their potential future benefit in programmes alternating modes of action. Invader (dimethomorph + mancozeb) can be expected to have eradicant activity and growers already use this following the use of protectant products such as Signum (boscalid + pyraclostrobin) which has off-label approval against White Tip in leeks, and Amistar Top (azoxystrobin + difenoconazole), which has a qualified minor use against White Tip in leeks (Simon Jackson, Allium and Brassica Agronomy Ltd, pers. comm.).

In February 2017, isolation of *P. porri* from Pluston from the efficacy trial was unsuccessful, but successful from nearby volunteer plants of Triton. It is possible for plants to produce a hypersensitive response to pathogens resulting in the pathogen mycelium being “walled off” so that the green tissue at the “leading edge” of the lesion (from where isolation onto agar in the laboratory is usually successful) remains healthy. It is not known whether hypersensitivity to *P. porri* infection arises in leek leaves.

In the variety field trial, a very high level of disease (mean 72 % incidence and 29% severity per plot) was recorded at the 8 February assessment date. Longton, Galvani and Triton-primer had a significantly lower incidence (60% or less) than LV446, Belton, Comanche and Krypton (having 80% or more plants affected). The high level of White Tip in these untreated variety plots compared with 16% incidence and 18% severity for untreated Triton in the efficacy trial by February suggests that there was a multiplication of disease within the variety plots. This could be attributed to the spread of zoospores following the initial infection, but whether from sporulating lesions or from oospores released into the soil from decayed leaves of the same crop is not known.

There were no significant differences in White Tip between varieties by 21 March 2018, approaching harvest. Incidence had declined to 18% of plants and this reduction in disease followed the decay of diseased tissue, leaving a similar number of new healthy leaves per variety. There was a great deal of variability between symptom incidence (from 5% to 40% of plants affected) in the trial. Pluston ranked above Triton in White Tip incidence, although it was expected that Triton would be the more affected. Galvani, Triton primer F1 hybrid and Curling were shorter than Krypton and Lexton, but whether the 40 mm difference in height would affect the chance of zoospore splash entry from the soil into leaf axils at the top of the shank is debatable and may account for the absence of any correlation between White Tip and shank height at this time. At this stage in the crop there is also a dense leaf canopy hanging down over the soil that is likely to impede splash-up of water with zoospores.

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, in particular Cladosporium. In such situations the use of lateral flow devices available to detect *Phytophthora* spp. could be utilised by growers to aid spray application decisions.

Milestones

All the 2016 work packages (WP1, 2, 4 and 6) were carried out as proposed, with infection seen in all three trials, but no particular varieties or treatments were identified as reducing White Tip. Details are given in the Conclusions section.

The second fungicide efficacy field trial (WP5) was completed in 2018, but no reduction in White Tip incidence was shown from the treatments at the times applied, although three products had more of the top nine leaves healthy by March.

An additional work package (WP10) involving a randomised block design of variety plots adjacent to the efficacy trial in 2017/18 showed significant differences in White Rot incidence between twelve untreated varieties in early February 2017, but this difference had been lost by March 2018 following the decay of lesions and the loss of older infected leaves.

Work for WP3 in 2017 on propagation treatments was delayed by lower than anticipated infection of the plants to be used as *P. porri* spreaders, so requiring a delay in sowing the test plants. The inoculation method was changed to require leaf axil inoculation, but by the end of 2017 the test seedlings were still small and their leaves were too tight against the shanks to provide a pocket for the inoculum. Very cold weather in December, followed by problems with bulking-up the *P. porri* isolate, meant that inoculation had not taken place by April 2018. Exceptionally hot weather at the end of April then scorched the plants in the polytunnel causing physiological white leaf tips and the experiment was deemed best terminated.

Conclusions

<u>2016 to 2017 experiments</u>

WP1 – cultivar susceptibility following zoospore inoculation

- 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 (mean 34% of plants) or severity (mean 6.6%) had occurred either in January, or by late April 2017, after ten varieties

were inoculated in their leaf sheaths in September and November 2016 with zoospores of *P. porri*

- Varieties were confirmed to differ in their shank length by January, with Curling, Lexton and Longton 20 or 30 mm taller than Triton, LV446, Mancurian and O96
- Variety vigour differed significantly by April, with Longton and Pluston significantly more vigorous than O96, LV446, Lexton and Krypton. Vigour was probably reduced by White Tip

WP4 – soil applied treatments against P. porri oospores

- Leeks developed White Tip symptoms two months after being transplanted in June into irrigated oospore infested growing-media, with 30% of plants having been affected by early September
- The white tips of affected leaves were prone to break off and so at most 16.3% of plants had visible symptoms during the experiment, with only up to 3% of the leaf area showing symptoms of White Tip
- 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 October, with nearly a third of plants having shown symptoms
- 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

WP6 – fungicide spray efficacy against P. porri in field-grown leeks (Nottinghamshire)

- 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

2017 to 2018 experiments

WP3 - To test conventional and non-conventional fungicide products in leek propagation

- A number of products, including biofungicides, were selected for potential use as drenches to young leek plants in propagation to seek to give them protection from *P. porri* infection later in their life. However, this experiment was stopped after nine months because of the condition of the plants had become poor before inoculation

WP5 – fungicide spray efficacy against P. porri in field-grown leeks (Lancashire)

- 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
- Symptoms did not appear until December 2017 and were then at a low level, with not all plots affected
- By February the incidence was 17%, and was still similar, 18.6%, by March 2018
- By March, fungicide treated plots (except those with Invader) had significantly more new leaves (of the most recent 10 emerged) without White Tip compared with untreated plots
- No phytotoxicity was seen following any of the foliar fungicides at the rates used

WP7 – to explore the potential for weather-based forecasting to assist spray timing applications for White Tip

- In both November 2017 and January 2018 there was a day with a lot of rain (30 mm or more) preceded by at least two or three rainy days (totalling at least 15 mm) and it was hypothesised from lesion development size at the next visit that symptoms would have first been visible 11-14 days after the heavy rainfall
- In both efficacy field trials, there was no heavy rainfall up to the assessment dates in September 2017 and October 2018 and this probably explains to lack of White Tip
- Fields with infested puddles receiving hard rain have a high probability of getting White Tip, but this information cannot be fully gathered from an in-field weather station as the type of rain is not recorded, only the volume
- The infection of plants depends on zoospores splashing up into leaf axils from the soil and whether or not this takes place will depend firstly on the creation of puddles (and thus factors including soil type, compaction and a site-specific amount of rain), secondly on rain drops making enough impact to splash up (and thus the size of the

drops) and thirdly the probability of the infested raindrop entering a leaf axil (and thus the density of the planting and the structure of the plant)

WP10 – comparison of variety susceptibility to White Tip in field-grown leeks (Lancashire)

- There was a big increase in White Tip incidence and severity in the field in February 2018, when between 52 % and 87 % of plants had symptoms, perhaps because of more days with rain, with severity subsequently also increasing
- Krypton and Comanche had a high incidence of White Tip by February, Longton had the least with Pluston, Galvani and the Triton-primer F1 hybrid also below average incidence
- When measured in March, some varieties were confirmed to differ in their shank length ranging from 74 mm (Galvani) to 146 mm (Lexton), but the majority were around 150 mm tall
- No correlation was shown in March between variety shank height and infection incidence, although by this time there had been a reduction in incidence probably because of the decay of lesions

Knowledge and Technology Transfer

As part of WP8, one presentation is due to be carried out in October 2018 as part of this project to summarise information from the two years.

An AHDB Grower article by Erika Wedgwood "Win against white tip" was published in the July/August 2017 edition (pages 24 and 25), reviewing the current measures that leek growers can take against *P. porri* and looking at the treatments being tested.

WP9. An AHDB factsheet does not exist for White Tip of leeks, with project FV 172 in 1996 providing minimal background information on the disease for growers. More information on the epidemiology of the pathogen has since become available through studies in the Netherlands by Smilde (1996) and a group of researchers in Belgium (Declercq et al., 2011) and observations within the current project. Any factsheet would be produced in consultation with growers and would be anticipated to include the following sections:

1. Action Points for growers.
2. Background – with sub-headings; Symptoms, Sources of disease, Weather conditions favourable for White Tip development.
3. Integrated disease management strategies – Varietal resistance. Minimising the risk of disease development in the field (including in organic production). Minimising disease spread in infected fields. Fungicides against White Tip and their use in

fungicide programmes to reduce fungicide resistance.

Table: Currently Approved and EAMU plant protection products against White Tip, with active ingredients, FRAG group, diseases controlled and details on doses and application intervals.

Figures: A full colour lifecycle would be drawn specifically for the factsheet. Photographs of symptoms on leaves, whole plants and field areas.

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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	33	Low
			Plant Trust		
	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).

nrm laboratories

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	96340

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
Tel: +44 (0) 1344 886338 Fax: +44 (0) 1344 890972 Email: enquiries@nrm.uk.com www.nrm.uk.com

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)

nrm laboratories

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 :

T3

Sample Matrix : COMPOST

Laboratory References	
Report Number	21111
Sample Number	96338

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*

NRM Coopers Bridge, Braziers Lane, Bracknell, Berkshire RG42 6NS
Tel: +44 (0) 1344 886338 Fax: +44 (0) 1344 890972 Email: enquiries@nrm.uk.com www.nrm.uk.com

Appendix 2iii. WP4. 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).

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 :

T4

Sample Matrix : COMPOST

Laboratory References	
Report Number	21111
Sample Number	96339

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 3. WP4. 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 before addition of *P. porri* oospores. Boxworth July, August and September 2016.

Appendix 3i. 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 3ii. 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 3iii. 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 3iv. 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 3v. Mean proportion of plants with White Tip leaf lesions on 16 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		

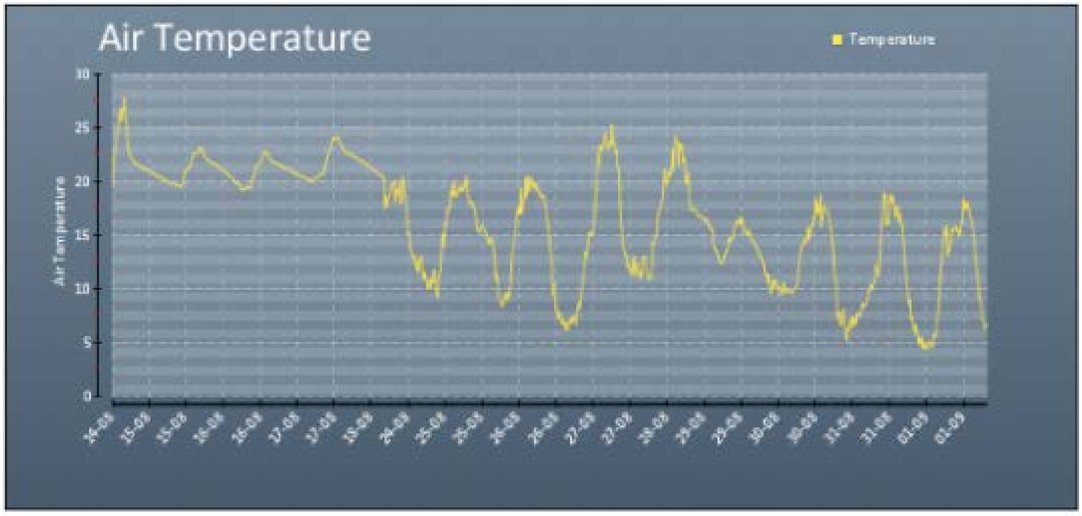
Appendix 3vi. Mean % leaf area with White Tip symptoms per plant, 16 September 2016. No significant differences.

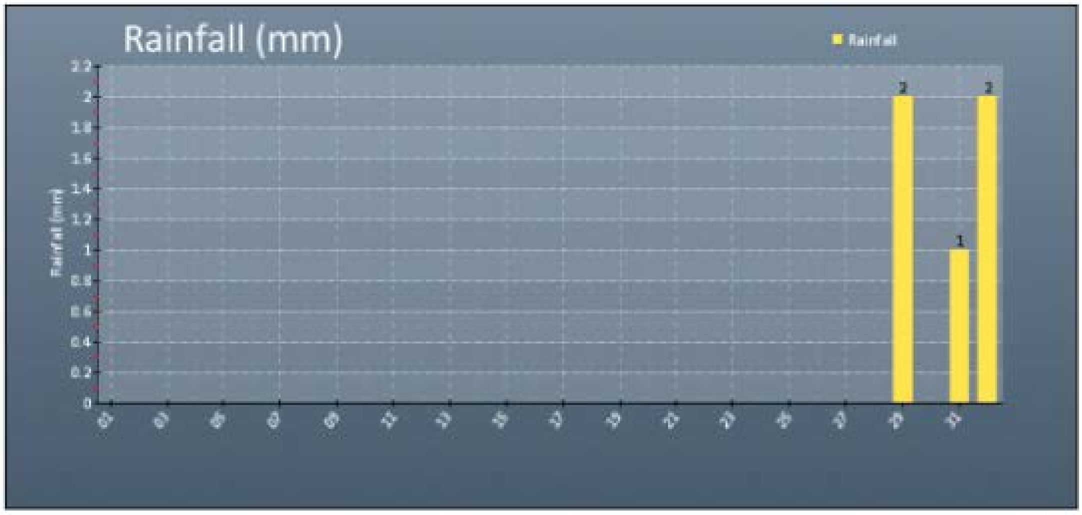
Treatment	Treatment mean	Triton	Pandora
UT	0.225	0.250	0.200
Gypsum	0.331	0.600	0.063
Limex	0.312	0.375	0.250
Variety mean		0.408	0.171

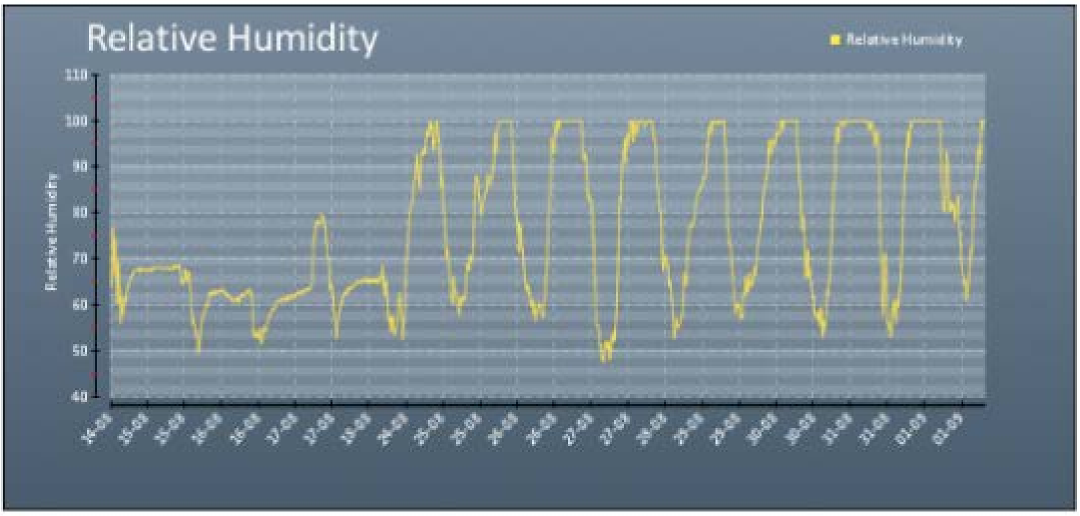
Source of variation	d.f.	F pr.	L.s.d.
Block	3	0.042	0.3874
Treat	2	0.775	0.3355
Variety	1	0.084	0.2739
Treatment x Variety interaction	2	0.279	0.4745
Residual	15		

Appendix 4i

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire from 24 August 2017 to 20 March 2018 (data prior to 24 August should be discounted as the station was malfunctioning).

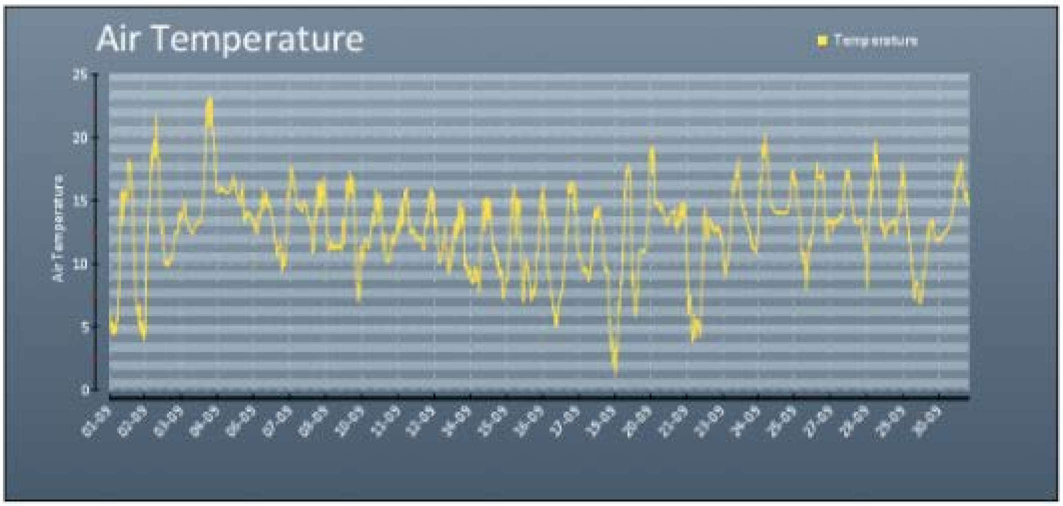


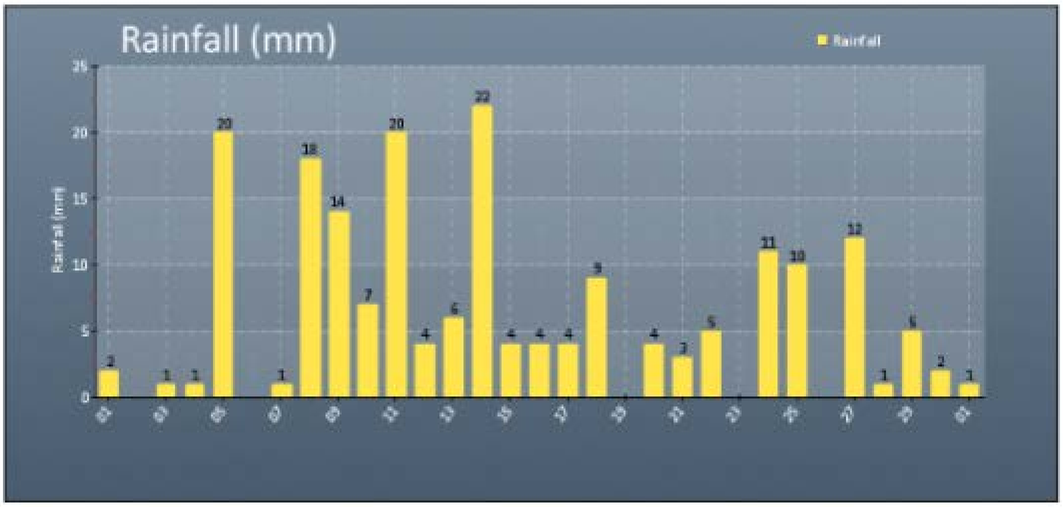


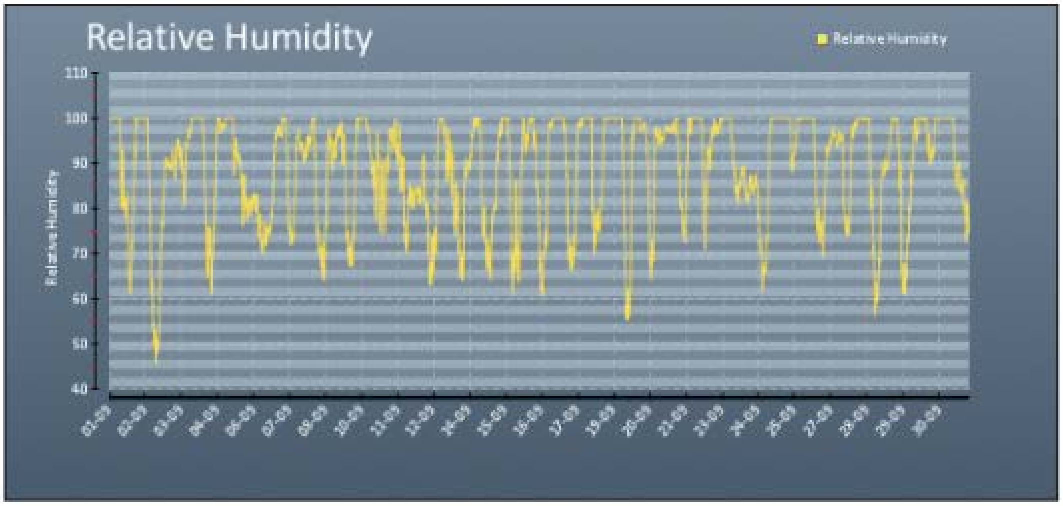


Appendix 4ii

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire in September 2017.

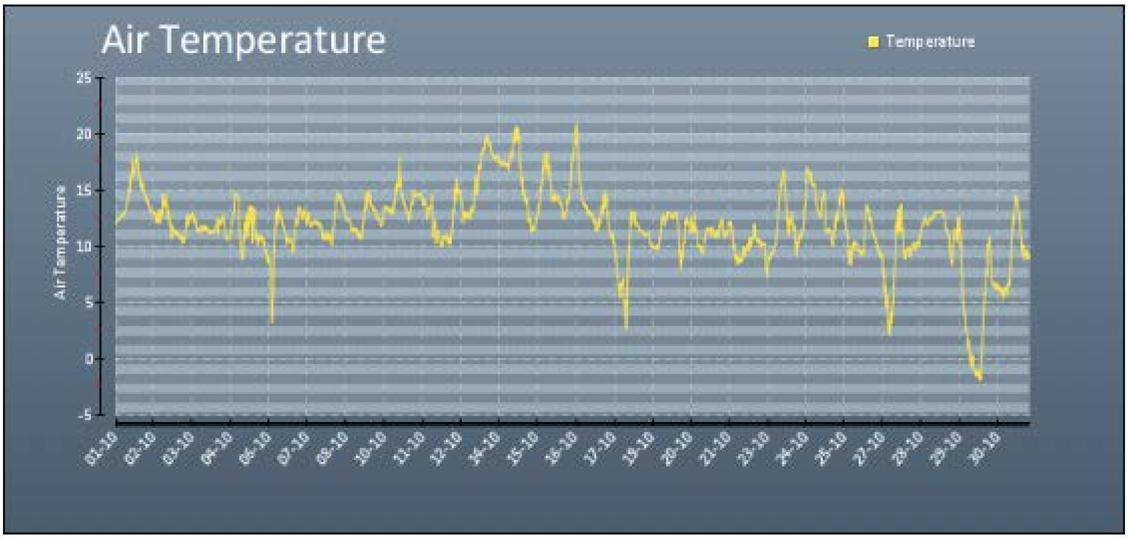


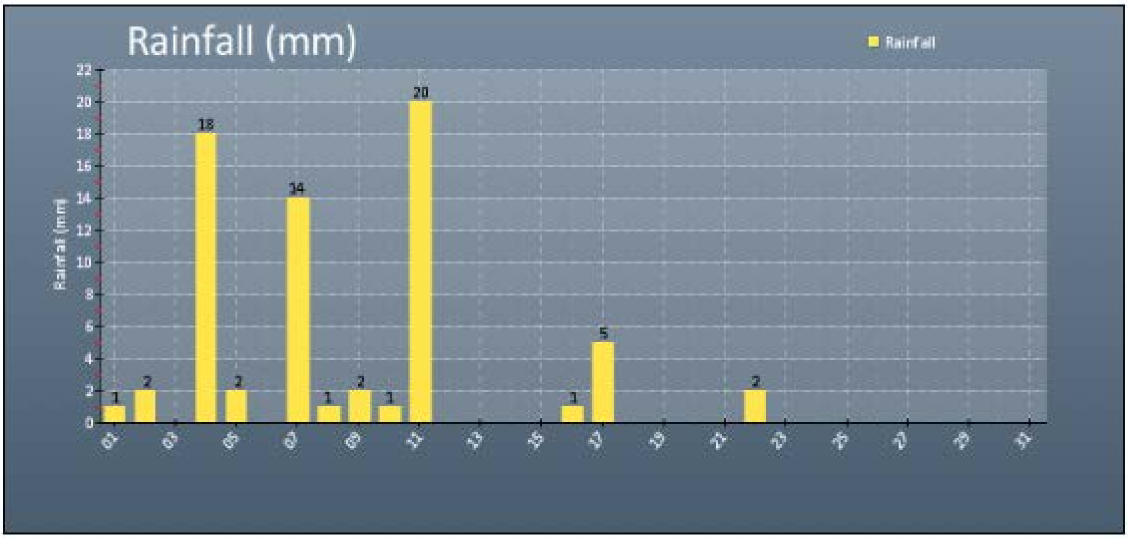


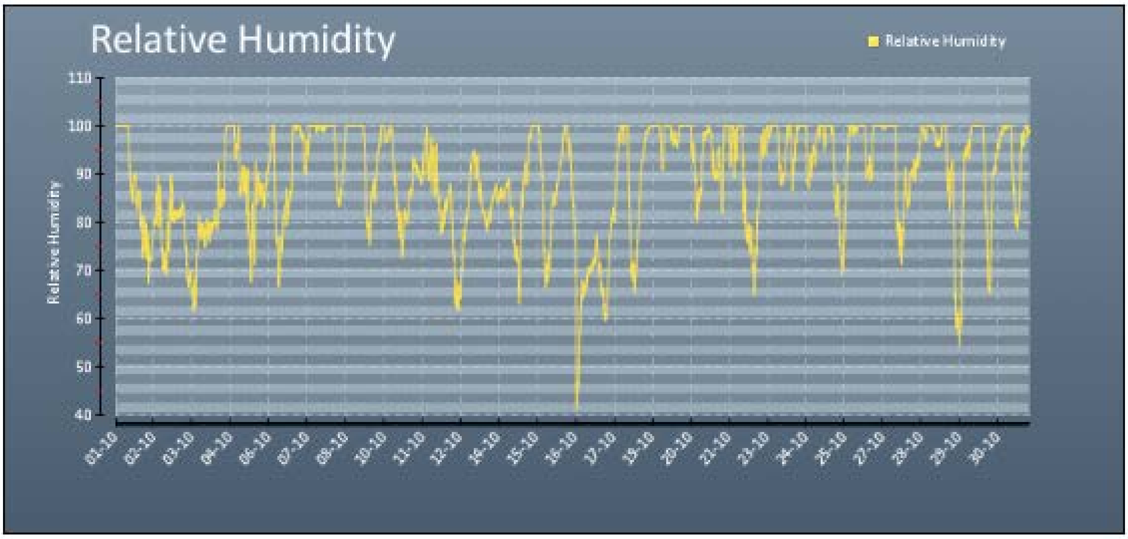


Appendix 4iii

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire in October 2017.

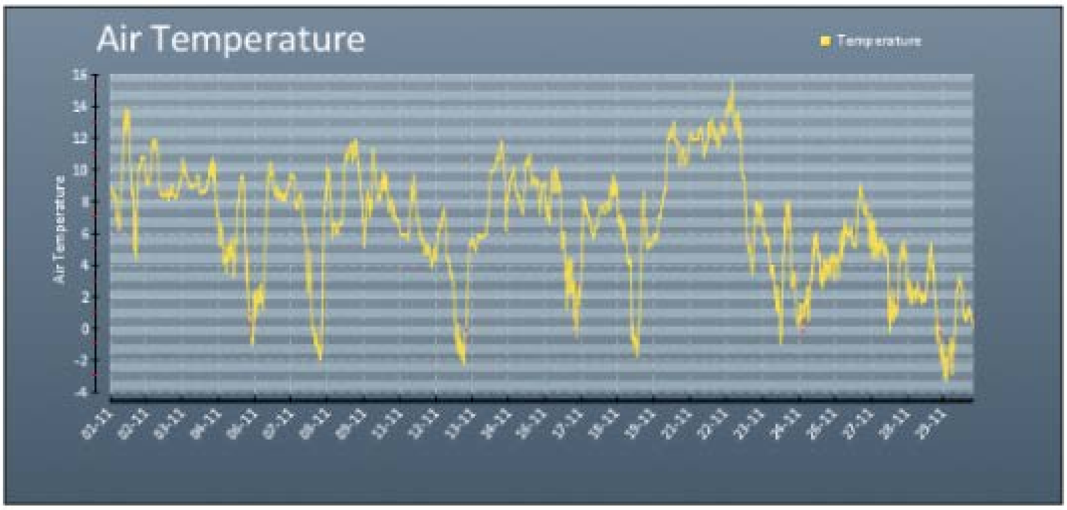


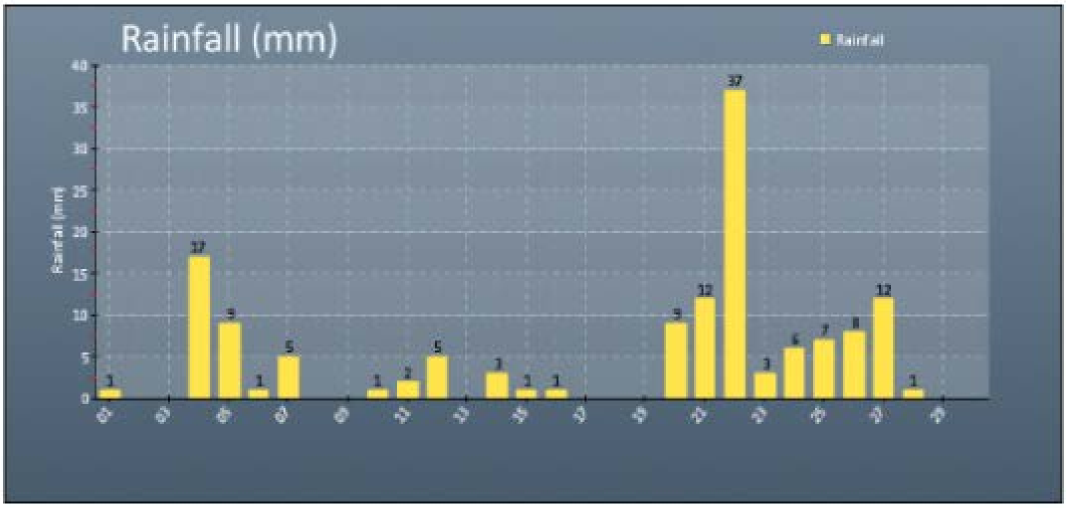


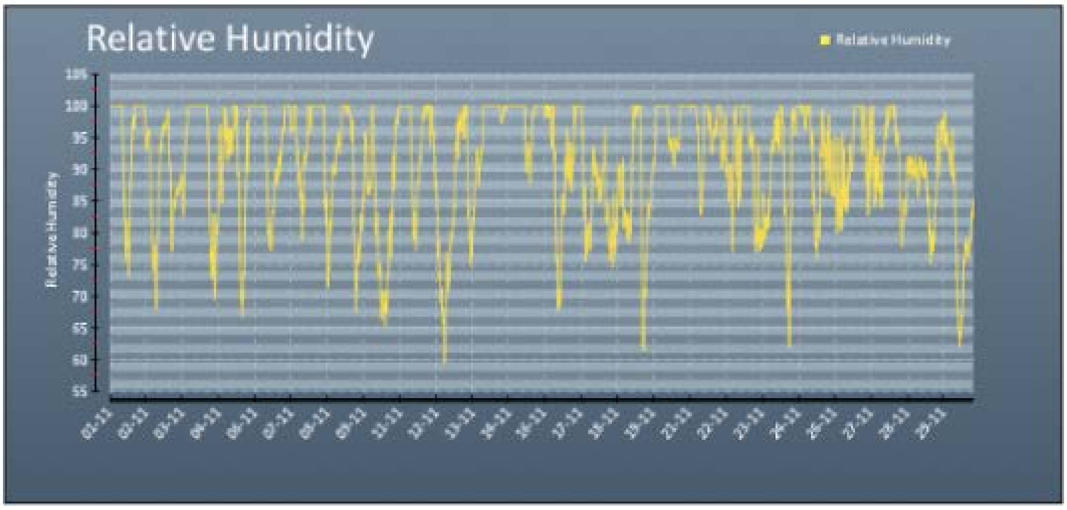


Appendix 4iv

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire in November 2017

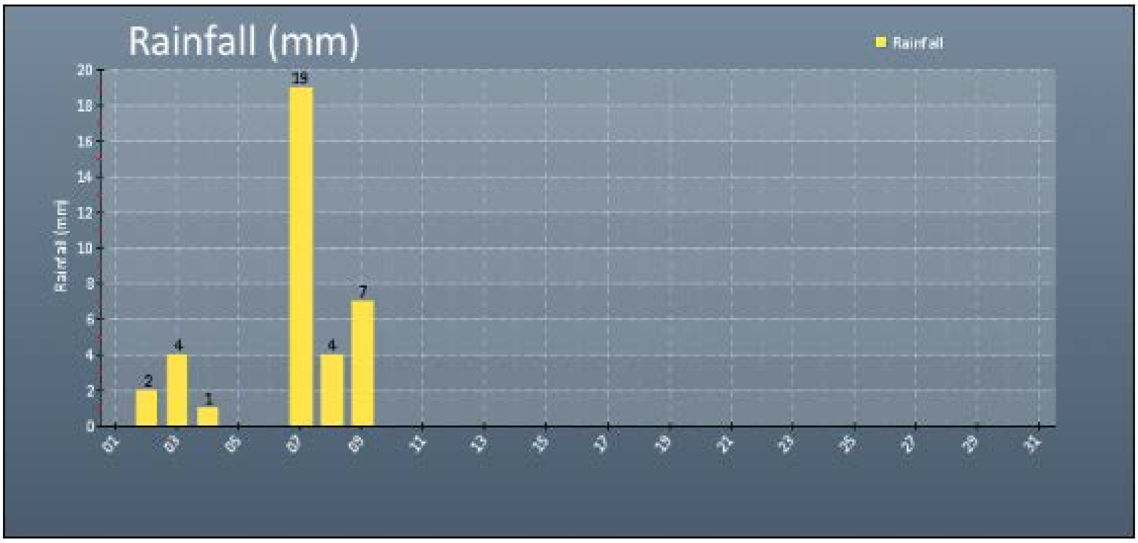


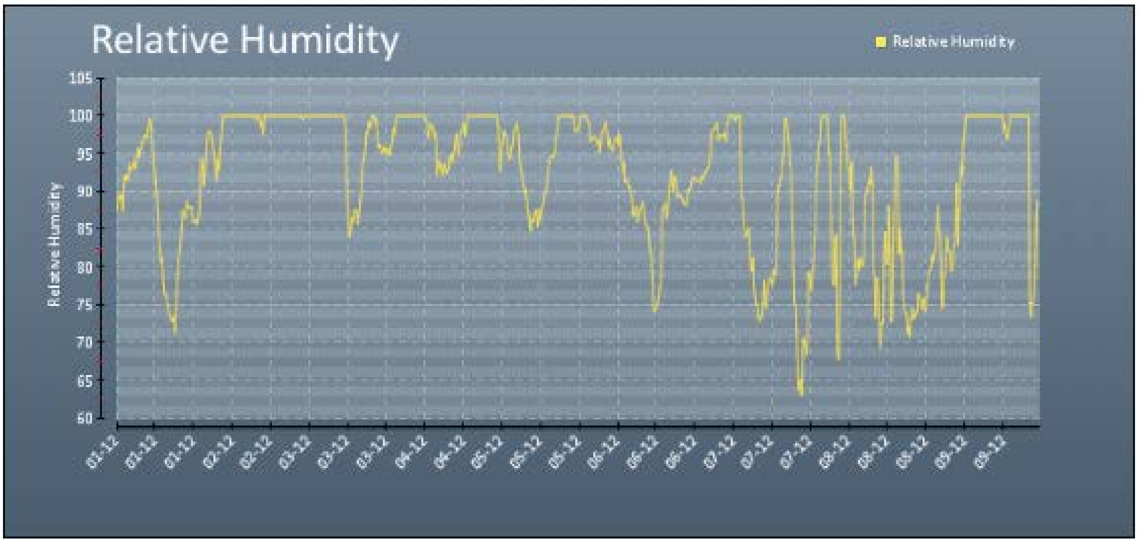




Appendix 4v

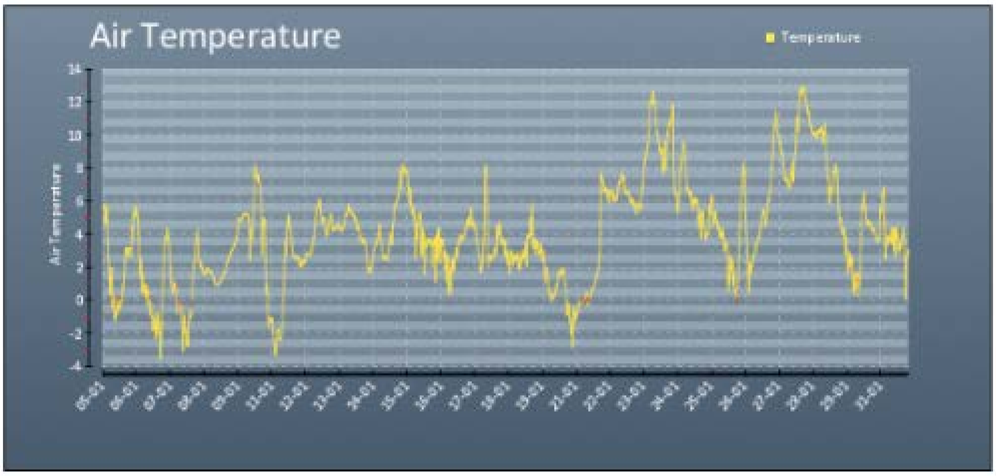
Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire In December 2017 (up until 10 December when the in-field station stopped recording).

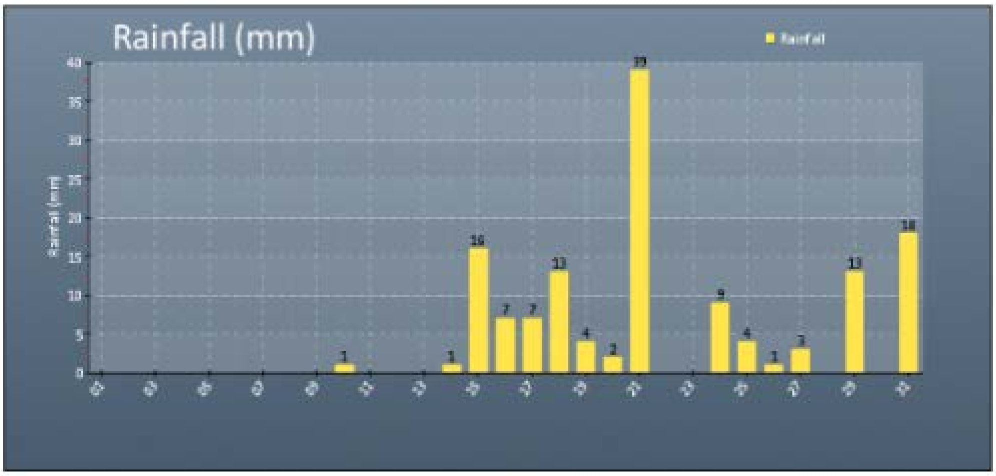


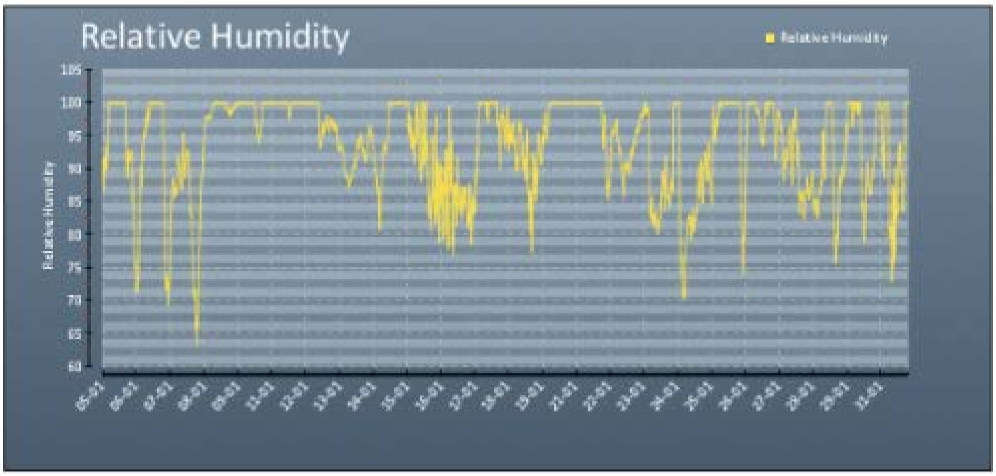


Appendix 4vi

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire in January 2018 (commencing 5 January when the MET station was replaced after repair)

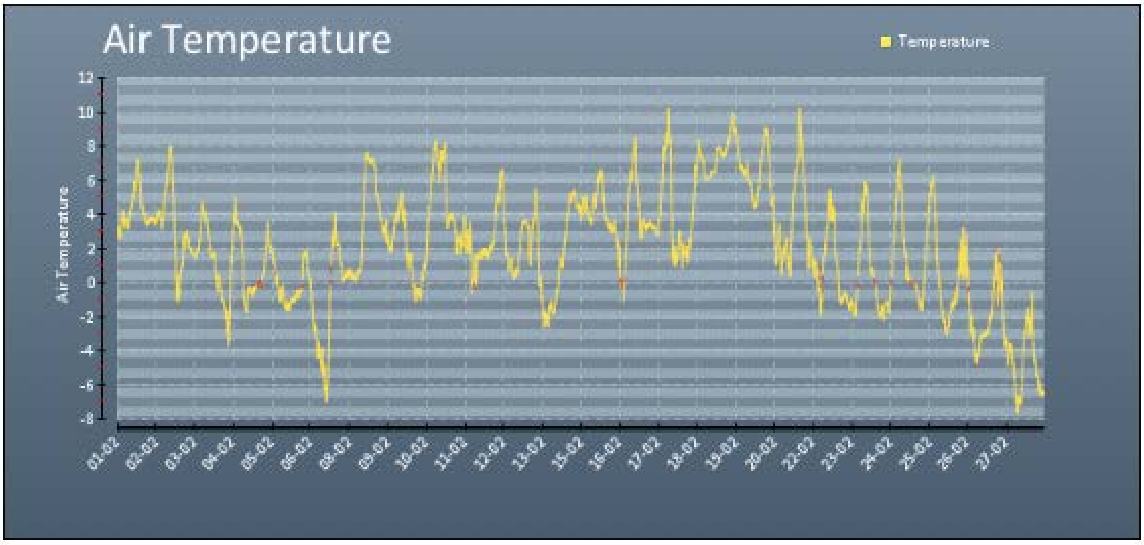


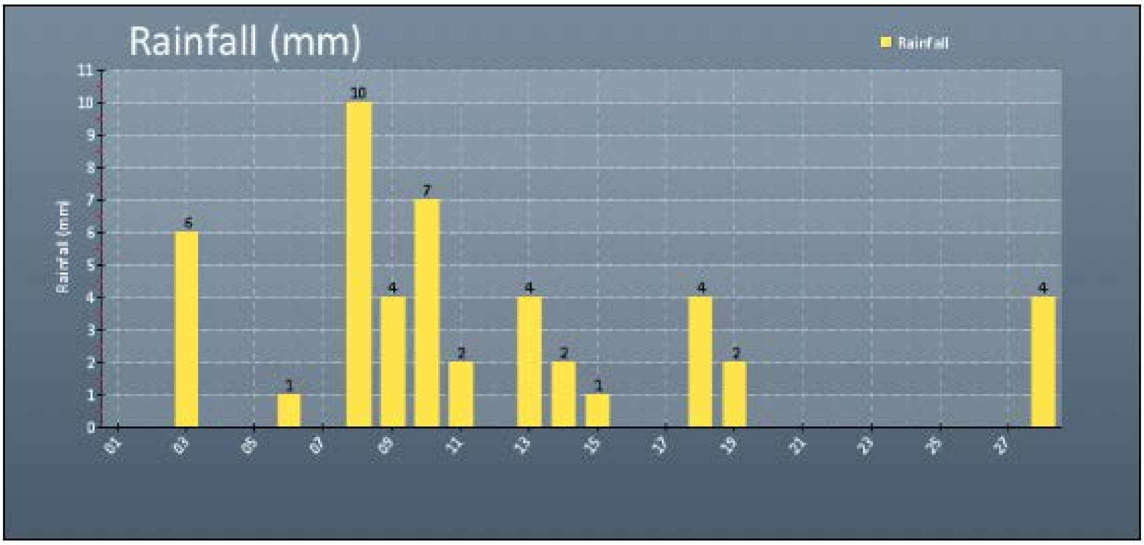


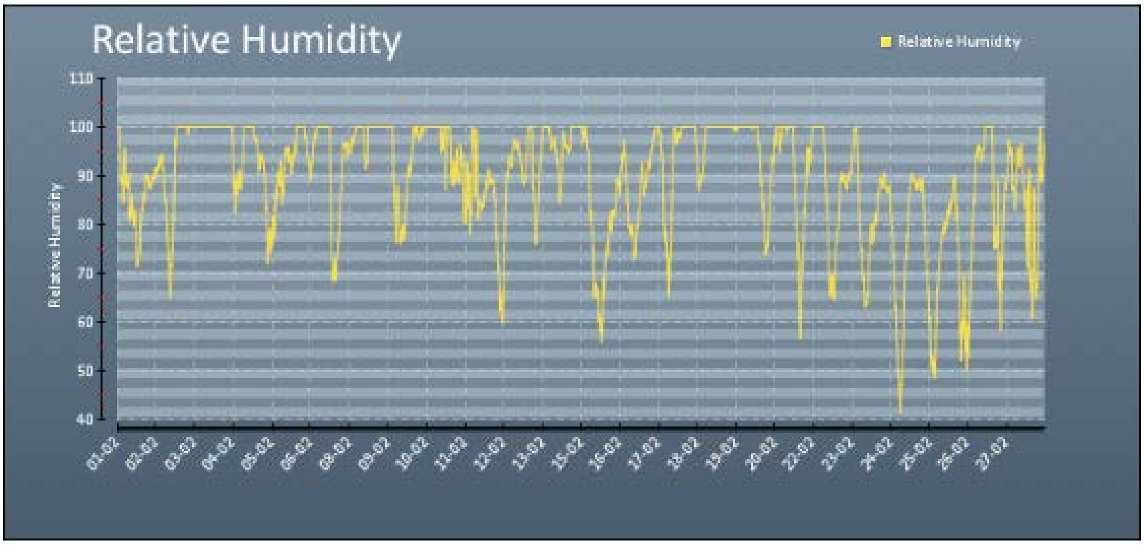


Appendix 4vii

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire In February 2018.







Appendix 4viii

Leek white tip trial air temperature, rainfall (mm) and relative humidity recorded at the leek efficacy and variety trial site in Lancashire in March 2018.