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Key staff:	Andrew Taylor John Clarkson
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Industry Representative:	Liz Johnson, LJ Technical Consultancy. John Jackson, Seven Oaks Salads Ltd.
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AUTHENTICATION

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

Andrew Taylor

Research Fellow

University of Warwick

Signature:  Date: 09/02/2018

Report authorised by:

John Clarkson

Reader

University of Warwick

Signature:  Date: 09/02/2018

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GROWER SUMMARY

Headline

An outbreak of lettuce Fusarium wilt was reported in the UK in 2017. Control is challenging and growers / propagators are advised to review hygiene procedures. Monitoring and early diagnosis is critical and soil disinfestation may be required where infection has occurred. Potential chemical and biological control options for lettuce Fusarium wilt will be trialled under the AHDB SCEPTREplus project in 2018.

Background

An outbreak of lettuce wilt, caused by *Fusarium oxysporum* f. sp. *lactucae,* was reported in the UK and Ireland for the first time in October 2017 although earlier observations of the symptoms had been made in August 2017 in Lancashire and summer 2016 in Ireland. The pathogen was identified as race 4 of F. *oxysporum* f. sp. *lactucae* (FOL4), a particularly aggressive strain of the fungus with no known treatment or varietal resistance available. The disease is reported as a serious constraint to lettuce production in mainland Europe and FOL4 has previously been identified in the Netherlands and Belgium. The UK leafy salads industry and plant propagators are extremely concerned about the potential impact of the disease on UK production both under protection (soil and soilless systems) and outdoors, due to lack of effective control measures. The AHDB therefore commissioned this technical review to collate information on the biology and management of lettuce Fusarium wilt with the aims of i) informing industry of current best-practice guidelines for disease management, ii) to identify relevant research findings and knowledge gaps and iii) to guide future work that could minimise the impact of lettuce Fusarium wilt in the UK.

Summary

A comprehensive search of available literature was carried out and the review compiled following liaison with growers, propagators, agronomists, agrochemical manufacturers and seed companies in the UK and the Netherlands as well as academics from USA, Italy, the Netherlands and Belgium where the disease has been previously reported. Knowledge gaps were identified and are outlined in the Science Section to guide future research.

The soil-borne fungus *Fusarium oxysporum* is the most important and economically damaging *Fusarium* species for horticulture and can be a major constraint to the production of many food

crops including lettuce, rocket, onion, leek, tomato, spinach and several others. Once soil becomes infected, control is challenging and the fungus produces long-lived spores (chlamydospores) that can survive in the soil for at least 17 years. There are more than 100 pathogenic forms of *F. oxysporum*, known as *formae speciales* (f. spp.), as well as a range of non-pathogenic strains that are commonly found in soil. Importantly, each f. sp. is highly specific to its host and will not infect other plants. *F. oxysporum* f. spp. may also be further divided into races, which evolve to overcome a resistant crop cultivar. These can be identified based on their ability to infect a differential set of resistant / susceptible cultivars or, in some cases, through laboratory-based molecular tests.

Fusarium wilt of lettuce caused, by *Fusarium oxysporum* f. sp. *lactucae* (FOL), was first described in Japan in 1967 and has since been identified in many lettuce producing areas around the world. Four races of FOL have been identified with race 1 being the most widespread (particularly prevalent in southern Europe and the USA) while race 2 and 3 are confined to Asia. In Europe, FOL race 1 was first reported in Italy in 2001, while the more recently emerged race 4 (FOL4) was first observed in the Netherlands in 2013. This race has since spread to Belgium and was identified in Ireland in 2016 (not confirmed until 2017) and Lancashire (2017). At the current time, the confirmed UK outbreaks of FOL4 are limited to protected lettuce production in Ireland (two sites) and Lancashire (two sites).

Symptoms of lettuce Fusarium wilt include stunting and yellowing (often at leaf margins), ultimately leading to plant death (section 2.2). As well as wilting and leaf yellowing, a key characteristic symptom of the disease is a brown/black/red discolouration of the vascular tissue of the stem/taproot which can be observed when plants are cut longitudinally (Figure i). The main mode of FOL transmission appears to be infested soil which can be spread on farming equipment, trays, pallets and footwear. Therefore, hygiene measures are crucial to prevent initial entry of FOL and subsequent local spread (section 3). Seed transmission is also possible, but the significance of this route of transmission has not yet been proven and does not explain the rapid spread of FOL4 across the Netherlands and Belgium. Here, and also in the UK and Ireland, FOL4 may well have spread through transmission of infested soil. It is likely that a very low level of FOL4 inoculum was introduced into the UK / Ireland initially, leading to low or undetectable levels of infection of lettuce. Subsequent cropping of lettuce in the same area would then lead to a build-up and spread of inoculum until sufficient spores were present to cause economically damaging levels of disease. This is supported by the observation that all reported outbreaks have been on sites where lettuce has been produced very intensively over a number of years.

Figure i: Internal symptoms of lettuce Fusarium wilt.

As for most *F. oxysporum* f. spp., higher temperatures generally lead to more severe FOL outbreaks as observed previously for FOL race 1. Preliminary evidence from the Netherlands suggests that FOL4 may have a similar preference for higher temperatures; hence some growers have resorted to growing lettuce only in the cooler months of the year while growing crops such as fennel, pak choi and endive in the warmer summer months. However, FOL4 may still be able to cause substantial disease at lower temperatures as a high level of Fusarium wilt was observed in protected lettuce grown in Lancashire in December 2017 (transplanted in October) with air temperatures of 8 °C. Similarly, in the Netherlands, it has been reported that losses of up to 70% can still occur in December. Although FOL race 1 is prevalent in outdoor lettuce in the USA and other countries including some in southern Europe, FOL4 has not yet been reported in outdoor lettuce. However, based on the initial observations that FOL4 may be active at lower temperatures, outdoor growers should also be vigilant and consider hygiene measures, particularly if raising their own transplants, and should prepare risk assessments to cover possible routes of infection (e.g. seed, planting material, lettuce product imports, packhouse waste, soil from visitors, footwear etc). The fact that outdoor production is less intensive and often involves some rotation may be enough to prevent build-up of FOL inoculum in the soil. Dutch lettuce growers who have recently switched to

hydroponic production following problems with Fusarium wilt in soil-based systems have not so far have had any further problems with FOL although the disease has been reported in hydroponic production in Asia.

There are a range of potential disease management approaches for FOL but it is clear that no single measure will result in complete control. Hence a combination of measures is required to reduce the impact of the disease. Hygiene is critical to minimising the spread of FOL4. Soil disinfestation may be required where a severe outbreak has been observed. Other control options include reducing the intensity of cropping, biological / chemical control and soil amendments (e.g. Biofence). Treating lettuce seed with thiram (approved in the UK) may contribute to disease management. Whilst there is some resistance to FOL4, particularly in outdoor lettuce types, no current indoor butterhead cultivars are resistant although breeding work is in progress. Resistant cultivars would offer the best control option for FOL. The control options for FOL4 are summarised with respect to growers, propagators and seed companies in the action point section.

Action Points

Actions for the whole industry

Hygiene

- Limit the number of visitors to production areas and ensure they follow hygiene procedures. Overshoes should be worn to prevent spread of FOL from other areas.
- Treat plant trays, pallets and equipment with disinfectants. Research has shown that quaternary ammonium compounds (e.g. Unifect G) are the most effective disinfectants for *F. oxysporum* (section 3). However, many disinfectants are less effective in the presence of soil. Ensuring that trays and pallets are clean is imperative and should be the responsibility of both growers and propagators. For instance, soil / plant material should be removed before trays are returned to propagators.
- If any plants become infected, they should be removed along with surrounding soil and disposed of by bagging-up then taking to land-fill or burning. Soil disinfestation should then be considered for the whole area.

Monitoring

- Regularly check lettuce plants for any symptoms of FOL and cut suspect plants in half to look for typical vascular browning. If this symptom is observed, send intact plant samples to Andrew Taylor, Warwick Crop Centre, University of Warwick, Wellesbourne, Warwick, CV35 9EF for free confirmation. Early diagnosis is critical for limiting the spread of new outbreaks. FOL4 cannot currently be accurately diagnosed from soil samples. Sample results will be anonymised but will enable information on the distribution of FOL in the UK to be monitored for the benefit of the whole industry.
- Diagnosis of lettuce FOL to race level can also be requested via your seed company

Actions for growers

Preventing entry of FOL

- Follow hygiene and monitoring procedures as outlined above.
  Contact seed suppliers and request details of seed production practices, hygiene and handling, as well as information on seed testing for FOL or any other pathogens.
- Speak to propagators to understand what hygiene procedures they have in place and if transplants have been treated with any chemical / biological agents.

Cultural control

- Consider changing cropping practice and diversifying crops grown. Reducing the intensity of cropping and / or introducing rotation crops will reduce any potential build-up of FOL in the soil. Research has shown that spinach is a bad choice as a preceding crop as it is well colonised by FOL. Initial results suggest that pak choi may be a better choice of rotation crop.
- Consider not growing lettuce in the warmest summer months when disease risk is at its highest.
- Increasing the pH of soil may reduce disease incidence although this has not been tested for FOL.
- Take any measures possible to minimise plant stress, any stress factor (e.g. nutrient imbalance, herbicide damage, drought, soil compaction) will increase susceptibility of plants to wilt.
- Leaving soil fallow may be a good option to reduce FOL inoculum. As FOL will colonise the roots of a range of other plants, weeds must be removed. Research has shown a

large reduction in FOL inoculum after 10 months, and the level was below the threshold required to cause disease after 34 months.

- Remove all loose plant material from glasshouses and consider using a propane burner to eliminate remaining material. Any plant material left behind may be colonised by FOL.

Soil treatments

- If there is an outbreak of FOL, disinfest soil using steam or application of Basamid (see section 7). Both these methods of disinfestation have been shown to reduce disease incidence by >90%. Repeat treatments are required, with at least one treatment per year. Anaerobic soil disinfestation may be an alternative option although this has been less rigorously tested.
- Biofence (*Brassica carinata* pellets) can be incorporated as a soil amendment prior to transplanting; it is not approved in the UK as a plant protection product. Research data indicated that Biofence applied 14-60 days prior to lettuce transplanting gave up to 80% reduction in disease severity for FOL race 1.

Fungicides and biological control

- Review options for fungicides and biological control (Table i). Fungicides and bio-fungicides should be used as part of an integrated management strategy. It is recommended that products are applied either prior to, at or immediately after transplanting. Repeat applications may improve efficacy of biological control agents.
- Consider using thiram treated seed as research has shown efficacy against FOL race 1.
- The most promising fungicides appear to be azoxystrobin, fosetyl-aluminium and fluopyram + trifloxystrobin although these have not been tested against FOL4. Mancozeb may also have some efficacy but this has only been tested as a seed treatment which is not approved for lettuce in the UK.
- The most promising biological control agents for FOL control appear to be Trianum-P, T34 Biocontrol and Prestop. Mycostop may also provide some control although research on FOL race 1 showed no significant efficacy in three out of the five trials conducted. Serenade and Amylo X may provide some control although this may be limited as these products are only approved for foliar application on indoor lettuce.

Actions for propagators

- Follow hygiene and monitoring procedures as outlined above; particularly note use of disinfectants for cleaning trays and the need to remove soil and plant material prior to treatment. Heat may also be an option for sterilising trays as this can effectively kill *F. oxysporum* spores and will not be affected by presence of soil; for instance, a 60°C treatment for as little as 2 min killed most chlamydospores of *F. oxysporum* from daffodil with 100% kill after 15 min.
- Propagation on concrete (as some propagators already employ) may be beneficial as surfaces can be readily cleaned and disinfected in between crops.
- Ask growers to return trays free of soil / plant material and identify growers with FOL infection so that these trays can be thoroughly disinfected.
- Biological control agents could be applied to transplants to potentially provide some protection against FOL infection (Table i). The most promising biological control agents for FOL control appear to be Trianum-P, T34 Biocontrol and Prestop although AHDB-funded work is planned to investigate these options in more detail.
- There is some moderate resistance to FOL4 in commercial cultivars (section 4). However, there are not currently any resistant indoor butterhead lettuce cultivars. Breeding for resistance is in progress and resistant cultivars would provide the best option for controlling FOL4.

Actions for seed producers

- Review procedures for seed production, handling and hygiene to ensure risk of seed infection or external contamination is minimised
- Make customers aware of procedures in place for seed production, handling, hygiene and pathogen testing.
- Consider testing all lettuce seed for FOL.
- Change footwear / wear overshoes when visiting different growers / propagators.

Table i) Fungicides and biological control agents currently approved for use on lettuce in the UK that have been shown to have activity against *F. oxysporum* in published studies.

Active	Products available	Efficacy against *F. oxysporum*	Application method tested
CHEMICAL CONTROL			
Azoxystrobin	Amistar and others	56% reduction in disease severity (FOL race 1) 80% reduction in disease incidence as a seed treatment (FOL race 1)	Foliar spray in a high volume of water (before transplanting) Seed treatment
Fosetyl-aluminium	In Previcur Energy, Avatar or Pan Cradle (mixed with propamocarb hydrochloride). In Fenomenal (mixed with Fenamidone)	58% reduction in disease severity (FOL race 1)	Foliar spray in a high volume of water prior at 7 day intervals to transplanting
Mancozeb	Karamate dry flo Newtec In Fubol Gold (mixed with metalaxyl-M)	Up to 84% reduction in disease (FOL race 1)	Seed treatment
Thiram	Agrichem Flowable Thiram or Thyram Plus (approved as a seed treatment)	44-72% reduction disease incidence (FOL race 1)	Seed treatment
Fluopyram + trifloxystrobin	Luna Sensation	Trifloxystrobin shown to control Fusarium wilt of carnation (up to 77% reduction in number of dead plants) but was less effective on cyclamen and Paris daisy	Applied as a drench after transplanting
Cyprodinil + fludioxonil*	Switch	69% reduction in Fusarium wilt of tomato (fludioxonil) Field trials in USA showed no effect of fludioxonil against FOL race 1	Applied directly to pots in a controlled experiment Applied at seeding
Boscalid + pyraclostrobin	Signum or Insignis	Field trials in USA showed no effect against FOL race 1	Applied at seeding
BIOLOGICAL CONTROL AGENTS			
Prestop	*Gliocladium catenulatum* strain J1446	Slight effect reported against FOL4 60-85% reduction in mortality (*F. oxysporum* from cucumber) 81% reduction in disease severity (*F. oxysporum* from pepper)	No details given Suspension applied to cucumber seeds Applied directly to base of 7 week old plants
Trianum-P	*Trichoderma harzianum* strain T22	Up to 83% reduction in disease index (FOL race 1) 57-78% reduction in disease severity (FOL race 1) 'Slight effect' reported against FOL4	Applied as a liquid before transplanting Seeds sown directly in substrate containing T22. Applied 1 week after sowing with 600 g in 100 litres of water per are (100m2).
T34 Biocontrol	*Trichoderma asperellum*, strain T34	50% reduction in disease severity and	Cuttings transplanted into growing medium mixed with liquid T34.

		33% reduction in disease incidence (*F. oxysporum* on carnation) Up to 95% reduction in disease severity (*F. oxysporum* on tomato)	Additional T34 drench applied 47 days after planting Mixed into growing media as a liquid spore suspension prior to transplanting
Mycostop	*Streptomyces griseoviridis* strain K61	Up to 62% reduction in disease index (FOL race 1, no significant efficacy in three out of the five trials conducted) 29-35% reduction in disease (FOL race 1)	Applied as a liquid before transplanting Applied as a seed dressing
Serenade ASO	*Bacillus subtilis* strain QST713, only approved as a drench for outdoor lettuce, approved for foliar application on protected lettuce	31% reduction of disease severity (FOL race 1), extra applications improve control 43-54% reduction in disease incidence (FOL race 1)	Applied as a foliar spray at 7 day intervals with a high volume of water prior to transplanting Applied as a seed dressing
Amylo X WG*	*Bacillus amyloliquefaciens,* strain D747	**63% reduction in disease incidence and 70% reduction in disease severity (*F. oxysporum* on tomato) **65% reduction in disease incidence (*F. oxysporum* on tomato)	Mixed directly into soil Mixed into growing media and applied as a foliar spray

*the approval for Amylo X on lettuce currently only covers foliar application which may limit its ability to control FOL

**these studies used a different strain of *B. amyloliquefaciens*

SCIENCE SECTION

Introduction

An outbreak of lettuce wilt, caused by *Fusarium oxysporum* f. sp. *lactucae,* was reported in the UK and Ireland for the first time in October 2017 although earlier observations of the symptoms had been made in August 2017 in Lancashire and summer 2016 in Ireland. The pathogen was identified as race 4 of *F. oxysporum* f. sp. *lactucae* (FOL4), which is an aggressive strain of the fungus with no known treatment or varietal resistance available to date. The disease is reported as a serious constraint to lettuce production in mainland Europe and FOL4 has previously been identified in the Netherlands and Belgium. The UK leafy salads industry and plant propagators are extremely concerned about the potential impact of the disease on UK production both under protection (soil and soilless systems) and outdoors, due to lack of effective control measures.

The key aim of this project was to provide a thorough, concise and timely review on Fusarium wilt of lettuce to help UK growers understand the problem and to inform the industry of best practice approaches to manage the disease. The core objectives were:

1) Summarise available published and technical literature on lettuce Fusarium wilt
2) Liaise with relevant industry contacts (growers, propagators, seed producers, researchers) to determine the impact of lettuce Fusarium wilt and to identify current best-practice measures for disease avoidance and management.
3) Identify knowledge gaps and also research outputs that could be relevant to the UK and provide recommendations for knowledge exchange activities or research that could be implemented in the short, medium or longer term in order to minimise the impact of lettuce Fusarium wilt in the UK.
4) Deliver findings from the technical review to the UK leafy salads industry.
5) Collect diseased lettuce plant samples, isolate FOL and confirm identity using molecular techniques

Materials and methods

1. Summarise available published and technical literature on lettuce Fusarium wilt

Literature searches were carried out using leading web resources (mainly Web of Science, Scopus and Google Scholar) using appropriate keywords (e.g. lettuce AND *Fusarium*) to ensure that each aspect of the review scope (general background on *F. oxysporum*, disease occurrence, impact, disease progress in the UK, host range, symptoms, disease biology, relevant research in other crops, disease management etc.) was covered in sufficient detail. Searches encompassed peer reviewed journals, conference proceedings as well as technical reports (including AHDB projects) and grower factsheets. Authors of key publications were contacted in order to obtain the most up to date information. As the review progressed, more specific searches were carried out in order to obtain any missing information.

2. Liaise with relevant industry contacts

Industry representatives (and researchers) were contacted by e-mail or telephone to gather further information and tailor the review to UK needs. Three UK lettuce growers and one propagator were visited in December 2017. In January 2018, a trip to the Netherlands was undertaken including a visit to Enza Zaden in Enkhuizen, a meeting with a Dutch grower who has been affected by FOL4 and a crop advisor. In total, ten UK growers (protected and outdoor), one Irish grower and three UK propagators were contacted regarding lettuce FOL; some of the growers were also able to provide a perspective on Spanish production. Details of other contacts can be found in Appendix 1.

3. Identify knowledge gaps and provide recommendations for knowledge exchange activities or research

Once collated, the information on FOL was critically reviewed, and a summary made of knowledge gaps, further research and knowledge exchange that may be required to manage FOL effectively in the UK. This was done in collaboration with a selection of key industry contacts.

4. Deliver findings from the technical review to the UK leafy salads industry

A presentation was given at the Lettuce Fusarium Wilt Technical Workshop in Skelmersdale on 14th December 2017. Further presentations will be given at the Leafy Salad Technical days

on 1st and 20th March 2018. An article is currently being prepared for the AHDB Grower magazine.

5. Collect diseased lettuce plant samples, isolate FOL and confirm identity using molecular techniques

To provide added value to the review, we requested samples of Fusarium diseased lettuce plants from industry in order to carry out isolations, confirm the presence of FOL and verify identity and race using established DNA-based methods. Samples were received from two sites in Lancashire and two in Ireland. Isolations were carried out as part of the remit of project FV POBOF 452 and the identity of putative *Fusarium* isolates confirmed by sequencing part of the translation elongation factor gene. Race identity was determined using published race specific PCR assays (Pasquali *et al*., 2007; Gilardi *et al*., 2017b) which was validated using race typed FOL isolates (race 1 and 4) obtained from the Gullino lab (Turin, Italy).

Results

1. General introduction to *Fusarium oxysporum*

The soil-borne fungus *Fusarium oxysporum* is the most important and economically damaging *Fusarium* species for horticulture and can be a major constraint to the production of many food crops including lettuce, rocket, onion, leek, tomato, brassicas, asparagus, cucurbits, peppers, coriander, spinach, basil, beans, peas, strawberry and watermelon as well as non-food crops such as carnation, column stocks and narcissus (Michielse *et al.,* 2009). *F. oxysporum* was recently identified as the fifth most important plant pathogenic fungus based on its economic and scientific impact worldwide (Dean *et al.,* 2012). The *F. oxysporum* complex comprises non-pathogenic isolates which are common in soil as well as a large array of more than 100 pathogenic forms known as *formae speciales* (f. spp.), each of which are adapted to infect different crop and plant hosts (Gordon 2017). These f. spp. are highly specific and will not infect other hosts, despite their genetic similarity. Distinguishing between pathogenic and non-pathogenic isolates and also between the different f. spp. is very difficult and can only be done through pathogenicity testing on different hosts, which is both time-consuming and expensive. However, with advances in molecular technologies such as whole genome sequencing, along with an understanding of the genes associated with pathogenicity on different hosts, it is becoming possible to distinguish f. spp. using DNA-based molecular tests (Lievens *et al.,* 2009; van Dam *et al.,* 2016). *Formae speciales* of *F. oxysporum* may also be further divided into races, which evolve to overcome a resistant crop cultivar. These are therefore identified based on their ability to infect a differential set of resistant / susceptible cultivars.

F. oxysporum is distributed throughout the world and pathogenic f. spp. are most commonly associated with vascular wilt and root rot symptoms (Leslie & Summerell, 2006) but may also cause crown rots (Gordon, 2017). Initial symptoms can be seen as chlorosis or stunting and wilt symptoms often progress down one side of the plant initially before eventually causing plant death (Fig. 1). Splitting the stem of an infected plant longitudinally will often reveal characteristic browning / reddening of the vascular tissue (McGovern 2015). The above-ground infection is predominantly contained within the plant but in some cases (e.g. *F. oxysporum* on stocks, Fig. 1f) spores (white/pink in appearance) develop on the outside of stems following plant death, thus increasing the potential spread of the pathogen. This has also been reported for *F. oxysporum* on tomato (Katan *et al*., 1997).

F. oxysporum characteristically produces three spore types; macroconidia, microconidia and chlamydospores and only reproduces asexually (Leslie & Summerell 2006). Whilst micro- and macroconidia are produced readily during growth and proliferation of the pathogen, chlamydospores are produced under more unfavourable conditions (related to factors such as absence of a host, nutrient depletion or adverse environmental conditions) and are considered to be the primary source of infections (Smith 2007). Conidia can infect roots and have occasionally been reported to be spread short distances through the air (McGovern 2015). The primary role of macroconidia is thought to be for survival of the pathogen as they can convert to chlamydospores (Egel & Martyn 2013). Microconidia do not survive for long periods of time but may cause some secondary infection. Chlamydospores are thick-walled structures that allow the fungus to survive in the soil for many years, even in the absence of a host (Gordon 2017). For instance, studies from Fusarium wilt of melon have shown that chlamydospores can survive for 17 years in soil stored at 3-4 °C (McKeen & Wensley 1961). However, it should be noted that the chlamydospore viability will decline rapidly over time, especially if soil is left fallow. A study from lettuce showed an 86% reduction in the number of viable chlamydospores after just 12 months fallow (Gordon & Koike 2015). *F. oxysporum* can also survive and even proliferate on roots of non-host plants and weeds (Hennessy *et al.,* 2005; Leoni *et al.,* 2013) although this has been little researched.

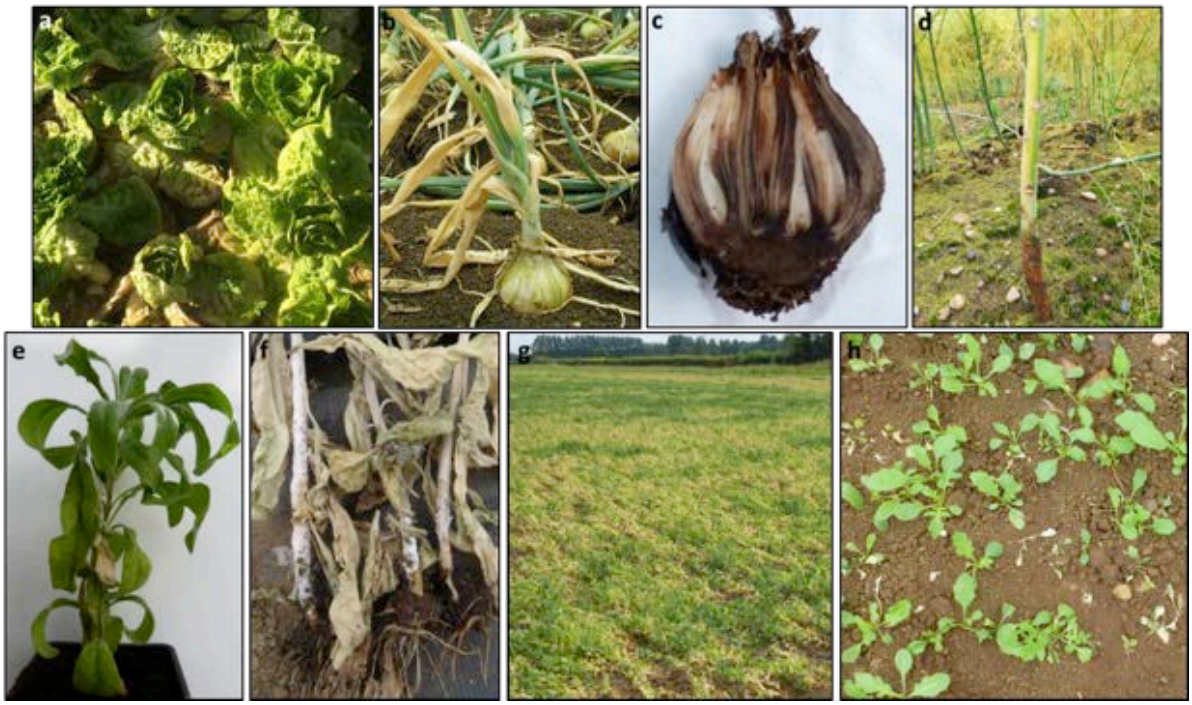

Figure 1: Typical symptoms of *Fusarium oxysporum* infections on lettuce (a), onion (b), daffodil (c), asparagus (d), stocks (e & f), pea (g) and rocket (h).

F. oxysporum chlamydospores germinate in response to root exudates from a host plant (Lockwood 1977). Whilst there is no evidence that response to root exudates conveys host specificity, there are now several studies showing that *F. oxysporum* spores have a higher rate of germination in response to a susceptible cultivar compared to a resistant cultivar (Gordon 2017). Once germinated, spores must be in within approximately 1 mm of the root in order to initiate infection (Huisman 1982). These spores produce hyphae which, once in contact with a plant root, will form a network of mycelium, enabling multiple infection points (Gordon 2017). The extent to which the fungus then progresses through the layers of root tissue (Fig. 2) determines whether it is pathogenic or non-pathogenic on that particular host. Pathogenic *F. oxysporum* forms will progress through the root cortex and into the vascular tissue. In response, the plant produces tyloses (invaginations in vascular cells), which block the xylem vessels hence inhibiting water uptake and leading to wilt (Egel & Martyn 2013). The pathogen also breaks down cells in the vascular bundle, leading to the production of gums which also cause xylem blockage. Non-pathogenic *F. oxysporum* forms will often still colonise roots but be restricted to either the root epidermal cells (outermost layer) or root cortical cells. However, it has been shown that on some hosts, non-pathogenic forms can still penetrate as deep as the vascular tissue (Scott *et al.,* 2014, see section 8 for details). In fact, vascular tissue can still be colonised when a pathogenic isolate confronts a resistant variety so it seems that plants can defend themselves against *F. oxysporum* at different cell levels and the basis of host resistance will differ between plants.

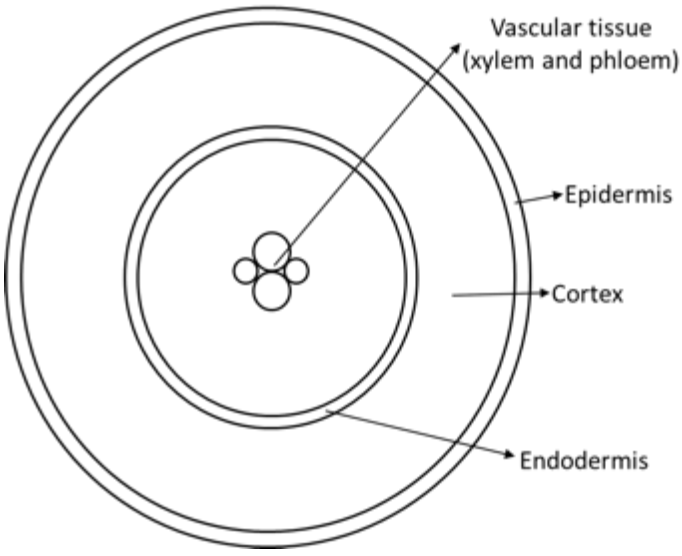

Figure. 2: Representation of a root cross section.

F. oxysporum may be transmitted on seed, in soil (e.g. via contaminated farm equipment), in water and even by some insects (McGovern 2015) and can also survive in plastic and within greenhouse structures. The main routes of transmission (particularly over long distances such as between countries) are thought to be seed and contaminated soil. Over short distances, *F. oxysporum* is often spread by contaminated farm equipment or irrigation water. There is minimal evidence for airborne spread. This is discussed in greater detail in relation to lettuce production in the next section. There is also a possibility that pathogen isolates may evolve 'locally' from non-pathogenic isolates but there is little direct scientific evidence so far to prove this.

2. *Fusarium oxysporum* f. sp. lactucae (FOL)

2.1 History and host range of FOL

Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f. sp. *lactucae* (FOL), was first described in Japan in 1967 (referred to as root rot rather than wilt) where it still causes serious problems (Matuo & Motohashi 1967) and since then has been identified in many lettuce producing areas of the world (Table 1). The pathogen was next reported in field-grown lettuce in California in 1990 (Hubbard & Gerik 1993) and is now widespread across all lettuce growing areas in the USA (Gordon & Koike 2015). Outside of Europe, the disease has been identified in Iran (Millani *et al.,* 1999), Taiwan (Huang & Lo 1998), Brazil (Ventura & Costa 2008), Argentina (Malbran *et al.,* 2014) and Korea (Kim *et al.,* 2008). It was first observed in Europe (Italy) in 2001 (Garibaldi *et al.,* 2002) when wilting plants were observed in plastic greenhouses, particularly when temperatures were higher. It has subsequently spread around Europe and has now been reported in Portugal (Pasquali *et al.,* 2007), tunnel grown lettuce in France (Gilardi *et al.,* 2017c), field lettuce in Spain (G. McCambridge, G's, personal communication), glasshouse lettuce in Belgium (Claerbout *et al.,* 2017) and glasshouse lettuce in the Netherlands (Gilardi *et al.,* 2017b). In the summer of 2017, wilting lettuce plants (butterhead and little gem types) were observed in protected lettuce crops in both Ireland (County Dublin area) and Lancashire. Following some initial confusion (first symptoms in Ireland actually observed in 2016) over the cause of these disease symptoms, they were subsequently confirmed as being due to FOL race 4 (FOL4) by both plant differential (details below) and molecular testing (see section 2.7). To date, two outbreaks have been confirmed in Lancashire and two sites confirmed in Ireland (two further sites suspected).

There are four races described for FOL with race 1 being present in Asia, USA, Europe and South America (Gilardi *et al.,* 2017b) while races 2 and 3 are confined to Japan / Taiwan. FOL4 was first reported in lettuce grown under glass from two different growers in the Netherlands with losses of around 50% reported (Gilardi *et al.,* 2017b) and identified through resistance / susceptibility testing using a set of differential lettuce lines (Gilardi *et al.,* 2017b). The start of the outbreak was much earlier than this publication, with the first symptoms observed in 2013 (J. van Kuijk, Enza Zaden, personal communication). New races of *F. oxysporum* evolve in response to the widespread deployment of a resistant cultivar and commonly occurs through the mutation or loss of a gene that is recognised by a corresponding resistance gene in the plant (Takken & Rep 2010). This allows the pathogen to avoid detection by the plant defences and cause infection. All FOL isolates identified in UK lettuce so far have been confirmed as race 4 (A. Taylor, A. C. Jackson and J. P. Clarkson, unpublished; M. Pel, Enza Zaden, personal communication; J. Schut, Rijk Zwaan, personal communication) and outbreaks have been confined to protected lettuce with none identified in outdoor production. Likewise, confirmed outbreaks in Ireland are due to FOL4 under protection. Preliminary data from Enza Zaden also suggests that there may be a FOL race 5 (based on lettuce differentials), which is closely related to race 1, (M. Pel, Enza Zaden, personal communication) but this has not been identified in Northern Europe.

As mentioned previously, whilst *F. oxysporum* will infect a wide range of plants, f. spp. of *F. oxysporum* are highly specific to their respective hosts. Therefore, FOL will only infect lettuce and *F. oxysporum* isolates causing wilts of other salad crops such as spinach and rocket will not infect lettuce. FOL can infect all lettuce types although butterhead types seem to be particularly susceptible.

In 2011, Defra published a Rapid Pest Risk Assessment for FOL (Sansford 2011) where it was decided that no statutory action would be taken following an outbreak in the UK due to the following reasons; i) FOL may already be present due to the lack of controls on the movement of plants, ii) other member states were not taking statutory action and the pathogen has been present in Europe for a number of years, iii) imported seed comes from the USA where the pathogen has been present for a long period, iv) detection is difficult, v) eradication is unlikely to be feasible in field crops and would be very demanding in protected crops, vi) there are other pathways (e.g. infected soil) which would be very difficult to control. FOL was added to the plant health risk register in 2014 with the recommendation that it should be managed by industry. It should be noted that none of the Defra documentation refers to FOL4 and only refers to FOL in general. Currently, they still support their original conclusion of no statutory action. In 2009, the European and Mediterranean Plant Protection Organisation

released an alert for FOL (EPPO, 2009). However, as FOL was included in EPPO Alert List for more than 3 years with no international action, the pathogen was deleted from the Alert List. This alert was issued prior to the emergence of race 4. The EPPO alert suggests that FOL will also infect lambs lettuce. However, there is no published evidence for this and Fusarium wilt of lambs lettuce has been shown to be caused by *F. oxysporum* f. sp. *conglutinans* (Gilardi *et al*., 2008).

Table 1: A history of outbreaks of lettuce wilt caused by *Fusarium oxysporum* f. sp. *lactucae*

Country	Year identified	Lettuce type	Production system	Race (s)	Reference
Outside Europe					
Japan	1967	Various	Outdoor	1,2,3	Matuo & Motohashi 1967; Fujinaga *et al.,* 2001; Fujinaga *et al.,* 2003
USA	1990	Various	Outdoor / Glasshouse	1	Hubbard & Gerik 1993
Taiwan	1998	Unknown	Outdoor	1 and 3	Huang & Lo 1998; Lin *et al.,* 2014
Iran	1999	Unknown	Unknown	1	Millani *et al.,* 1999
Brazil	2000	Unknown	Outdoor	1	Ventura & Costa 2008
Korea	2008	Unknown	Unknown	Unknown	Kim *et al.,* 2008
Argentina	2011	Butterhead	Glasshouse	1	Malbran *et al.,* 2014
Europe					
Portugal	2004	Unknown	Unknown	1	Pasquali *et al.,* 2007
Spain	2012?	Unknown	Outdoor	1?	Unpublished
The Netherlands	2013	Butterhead	Glasshouse	4	Gilardi *et al.,* 2017b
Belgium	2015	Unknown	Glasshouse	4	Claerbout *et al.,* 2017
France	2016	Batavian	Polytunnel	1?	Gilardi *et al.,* 2017c
Ireland	2016	Butterhead / little gem	Glasshouse	4	Unpublished
England	2017	Butterhead / little gem / curly	Glasshouse	4	Unpublished

2.2 Symptoms of lettuce Fusarium wilt and possible confusion with other disorders

The symptoms of lettuce Fusarium wilt are similar to those of many other Fusarium wilts and all races of FOL appear to produce identical symptoms. Initial symptoms observed are stunting and yellowing (often at leaf margins) which first become visible on older leaves (Hubbard & Gerik 1993; Garibaldi *et al.,* 2002; Matheron & Koike 2003, Fig. 3). When plants are cut

longitudinally, a brown/black/red discolouration of the vascular tissue can also be observed (Fig. 4). As FOL travels through the plant and blocks the vascular tissue, the wilt symptoms progress through the plant ultimately leading to plant death. Infected plants can retain an intact root system and often symptoms are not visible on the outer roots (Gordon & Koike 2015). Whilst root systems often remain white, browning may occur in the very advanced stages of infection. Whilst symptoms are most often observed in mature plants, FOL can also cause seedlings to wilt and die (Hubbard & Gerik 1993). No sporulation has been reported on either the leaves or at the stem base.

There are a range of other pathogens which may also cause lettuce leaves to wilt (Table 2, see O'Neill & Stokes 2005 for images and further details), but the key feature of Fusarium wilt is the vascular discolouration. However, *Verticillium dahliae* (images available at https://apsjournals.apsnet.org/doi/10.1094/PDIS-01-11-0075) can also cause a vascular discolouration of lettuce (Gordon & Koike 2015), although this pathogen is not currently a problem in UK lettuce production. *Phoma exigua* may also cause some slight vascular browning but can be distinguished from FOL as browning can be seen to originate from a lesion at soil level and does not tend to discolour the entire vascular system (Koike *et al.,* 2006). In addition, the base of affected plants and the roots are often very rotten (visible from the outside) following infection by *P. exigua*. It should be noted that *Pythium trachephilum*, a pathogen that is rare in the UK, can also cause vascular wilt symptoms that are similar to FOL (images available at http://ephytia.inra.fr/fr/C/5906/Salades-Pythium-tracheiphilum). The symptoms caused by this pathogen could easily be confused with FOL, but has some subtle differences. *P. tracheiphilum* is spread by soil splash and initial symptoms are a patchy leaf blight (Kumar *et al*., 2007). As the disease progresses, vascular browning is observed and a root rot symptom is evident. The root rot is likely to be more severe than FOL and visible on external root surfaces.

Initial FOL infection can easily be mistaken for a range of other disorders. Examples may be electrical conductivity / salt stress, water stress, pesticide (particularly herbicide) damage, heat stress or a nutrient imbalance. However, these problems are unlikely to cause a vascular browning so splitting plants and examining the vascular tissue is the key feature for correct diagnosis of FOL. It is also important that plant samples are sent promptly to an appropriate laboratory for confirmation. Samples can currently be sent to Andrew Taylor, Warwick Crop Centre, University of Warwick, Wellesbourne, Warwick, CV35 9EF.

Figure. 3: Symptoms of Fusarium wilt, caused by *F. oxysporum* f. sp. *lactucae*

Figure 4: Internal symptoms of Fusarium wilt, caused by *F. oxysporum* f. sp. *lactucae*

Table 2: Comparing the symptoms caused by common pathogens of lettuce. Adapted and expanded from Gordon & Koike, 2015. Fus – *Fusarium*, Vert – *Verticillium*, Sclero – *Sclerotinia*, Bot – *Botrytis*, Rhizo – *Rhizoctonia*, Pythium – *Pythium* root rot, Pt – *Pythium tracheiphilum,* Bact – bacterial rots.

Symptoms	Fus	Vert	Sclero	Bot	Rhizo	Pythium	Pt	Phoma	Bact
Stunting	**YES**	**YES**	**YES**	**YES**	NO	**YES**	**YES**	**YES**	NO
Plants collapse	**YES**	**YES**	**YES**	**YES**	NO	RARE	**YES**	**YES**	NO
Initial foliar symptoms on older plants	NO	**YES**	NO	NO	**YES**	NO	NO	NO	NO
Vascular discolouration in taproot and crown	**YES**	**YES**	NO	NO	NO	NO	**YES**	PARTIAL	NO
External crown and root tissue brown or rotted	RARE	NO	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**	**YES**
Fungal mycelium and sclerotia on crown or soil	NO	NO	**YES**	**YES**	**YES**	NO	NO	NO	NO
Leaf blight symptoms (patchy browning)	NO	NO	NO	NO	**YES**	NO	**YES**	**YES**	**YES**

2.3 FOL Transmission

The biology and transmission of FOL is predominantly discussed in relation to knowledge of races 1, 2 and 3. It is assumed that FOL4 has a very similar biology, particularly considering its genetic similarity to race 1 (see section 2.7). The life-cycle of FOL (Fig. 5) is similar to that of other *F. oxysporum* f. spp., as described previously.

Although it has been suggested that lettuce seed can provide a route for spread of FOL to new areas, there is only one publication that reports evidence of infection in commercial seed lots (Garibaldi *et al.,* 2004a). In this study, 27 commercial seedlots from Italy were tested

(500-1500 seeds per lot) and three were found to be infected with FOL. In all cases, only one infected seed was found (out of 500 or 1500), relating to an infection rate of 0.07-0.2%. The epidemiological significance of this is unclear. Only one of the three isolates came from disinfected seed, suggesting the infection is largely external. A subsequent publication from the USA showed that, following artificial inoculation of partially resistant lettuce plants, FOL could be routinely isolated from lettuce flower stalks and at a very low level (0-0.3%) from seed (Mbofung & Pryor 2007). However, no FOL was recovered from surface sterilised seed, suggesting that infection is largely external. The same study also showed that FOL could be isolated from *Fusarium* free lettuce seed mixed with infested plant debris, again suggesting external contamination rather than internal infection. A later publication from the USA found no evidence of seed infection by FOL in 88 commercial lettuce seedlots (Mbofung & Pryor 2010). Currently, lettuce seed is tested for a range of diseases by the seed industry. Enza Zaden, a major producer of lettuce seed for the UK, have never isolated *F. oxysporum* from any lettuce seed (G. Hiddink, Enza Zaden, personal communication). However, there is a significant body of evidence supporting seed transmission of other *F. oxysporum* f. spp. including stocks (O'Neill 2007), rocket (Garibaldi *et al.,* 2004c), watermelon (Petkar & Ji 2017), cotton (Davis *et al.,* 2006), tomato (Ajilogba & Babalola 2013), basil (Vannacci *et al*., 1999) and chickpea (Haware *et al.,* 1978).

Although seed transmission is possible for FOL4, and could be responsible for initial infection in a new country, other possible routes of entry exist. These include contaminated soil, potentially transported on the footwear of a visitor or infected soil / plant material introduced on imported lettuce that may be re-packed by growers close to their glasshouses. Local evolution from another f. sp. of *F. oxysporum* cannot be ruled out although little scientific evidence exists to support this. The subsequent rapid spread of the disease in the Netherlands and Belgium cannot be explained by seed infection. One possible explanation for the rapid spread is through infested soil. Panama disease of banana (*F. oxysporum* f. sp. *cubense)* was spread between countries by infected transplants (rhizomes and suckers) and their adhering soil (Nel *et al.,* 2007). However, local spread occurred due to infected irrigation water, farm equipment, vehicles and footwear (Ploetz 1994). Research in the USA has shown that FOL race 1, once present in an area, can be disseminated through movement of soil and contaminated farm equipment (Gordon & Koike 2015). There is also evidence that *F. oxysporum* can be spread via infected plant trays, as was found to be the case for Fusarium wilt of sweet basil (Guirado Moya *et al.,* 2004). This is a critical area to consider as plant trays and pallets are re-used many times and will travel between growers, propagators and supermarkets. Also, growers may exchange lettuce plants, providing another potential route for spread. Some growers may import packed lettuce from the Netherlands providing another

route for spread via infected plants or contaminated packaging. If plants are then re-packaged on site, this increases the risk of spreading FOL. There are also reports that fresh produce (including lettuce) importers in the UK may dispose of unwanted products on land adjacent to pack-houses, thus providing a further potential source of inoculum and risk to neighbouring lettuce growers. FOL4 could have been introduced to the UK either on infected or contaminated seed (at a very low level) or by infested soil either on the footwear of a visitor or from infected plant material / packaging. Local spread is likely to have occurred through infested soil. The UK isolates of FOL4 appear to be genetically very similar to those from the Netherlands (see section 2.7), suggesting a common origin and supporting the notion of the disease being introduced to the UK from Europe rather than evolving locally in the UK.

2.4 FOL inoculum build-up and survival

It is likely that a very low level of FOL4 inoculum was initially introduced into the UK, initially leading to low or undetectable levels of infection on lettuce. Subsequent cropping of lettuce on the same area would then lead to a gradual build-up and spread of inoculum until sufficient spores were present to cause economically damaging levels of disease. This is supported by the observation that all reported outbreaks have been on sites where lettuce has been produced very intensively over a number of years. As indicated previously, chlamydospores are generally the principal means of *F. oxysporum* survival between crops and hence also represent the primary inoculum source. A study from the USA has shown that chlamydospores of FOL race 1 can remain viable in fallow soil for at least 2.5 years although the number of viable spores decreased by 86% after 12 months (Scott *et al.,* 2012; Gordon & Koike 2015). However, it is likely that they will survive for many more years as 2.5 years was the maximum period of time examined. After 2.5 years, the level of FOL in the soil was 17.5 colony forming units (cfu) per gram. This level of FOL would likely pose a low risk for infection as preliminary work from the USA has shown that soil adjacent to infected plants contained 50-300 cfu/g whereas soil adjacent to healthy plants, but close to the infected area, contained 5-27 cfu/g (Gordon & Koike 2015). Other research on FOL race 1 has also shown that disease levels are directly related to number of spores in the soil although this work was carried out using conidia rather than chlamydospores (Hubbard & Gerik 1993). Even if a low level of FOL chlamydospores are present in the soil, successive lettuce planting will increase the number until the critical concentration for infection is reached. In addition, FOL race 1 has been shown to colonise (but not infect) spinach, broccoli, cauliflower, tomato, melon and cotton (Hubbard & Gerik 1993; Scott *et al.,* 2014, see section 8 for details). The proposed life cycle for FOL is

shown in Fig. 5 and is likely to be similar for all races. Current evidence suggests that FOL is contained within the plant and sporulation does not occur on the outside of the above ground parts of the plant. This means that airborne spread is highly unlikely and plant to plant spread during a single infection event will be limited. Fusarium wilts are generally thought to be monocyclic (Egel & Martyn 2007) with no plant to plant spread during a cycle although this has not been proven for FOL. Mycelium and conidia that are spread locally during infection will most likely be converted to chlamydospores which will accumulate for future infection.

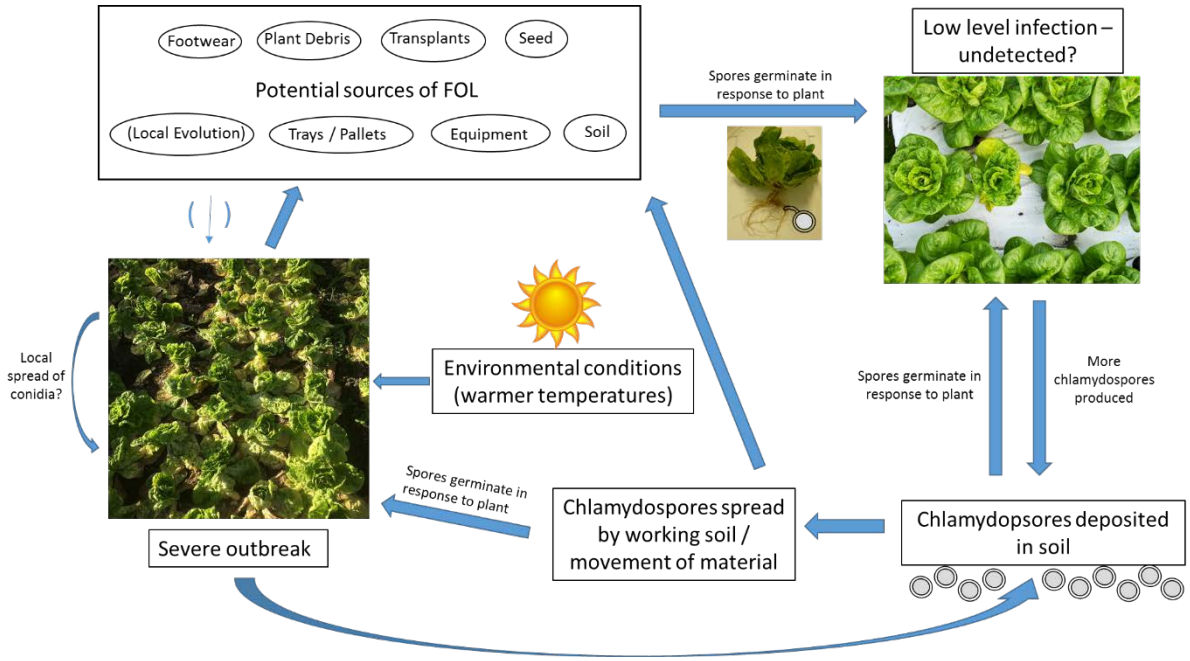

Figure 5: Proposed life-cycle of *F. oxysporum* f. sp. *lactucae*

2.5 Effect of environmental factors on disease development

As with many *F. oxysporum* f. spp., higher temperatures are an important driver of infection. For a FOL race 1 isolate from Italy, it was shown that a temperature range of 22-26°C increased disease severity by around 25% compared to 18-22°C (Ferrocino *et al.,* 2013). This is supported by work on FOL race 1 from the USA where disease incidence was recorded as 74-92% when the mean soil temperature was 26°C compared to 1-15% when the mean soil temperature was 14°C (Matheron *et al.,* 2005). Subsequent work from the USA further supported this where disease severity was worse when day temperatures were 33°C compared to 28°C or 26°C (Scott *et al*., 2010a). Preliminary evidence from the Netherlands suggests that FOL4 may have a similar preference for higher temperatures; hence some

growers have resorted to growing lettuce only in the cooler months of the year while growing crops such as fennel, pak choi and endive in the warmer summer months (R. Scheepers, Versland, personal communication). However, this assumption should be considered with some caution as a high level of Fusarium wilt was observed in protected lettuce grown in Lancashire in December 2017 (transplanted in October) with air temperatures of 8°C (soil temperatures not measured). Similarly, in the Netherlands, it has been reported that losses of up to 70% can still occur in December (J. van Kuijk, Enza Zaden, personal communication). Therefore, it appears that FOL4, whilst favoured by warmer temperatures, can still cause substantial disease at lower temperatures. Other *F. oxysporum* f. spp. have also been shown to cause disease at low temperatures; for instance *F. oxysporum* f. sp. *narcissi* causing narcissus basal rot has a minimum temperature for chlamydospore germination and subsequent growth of mycelium of 8-10°C (Price 1977). Whilst studies on FOL race 1 have shown that it can grow in culture between 8-32°C (Hubbard & Gerik 1993), the critical temperature for chlamydospore germination is not known for any race of FOL. If FOL4 is active at lower temperatures, then the risk to outdoor production should be taken seriously. Recorded summer soil temperatures in Kent for 2017 showed a range from 15 – 27°C (Table 3, C. Wallwork, Agrii, personal communication), highlighting the risk in the outdoor growing season.

Table 3: Soil temperatures (°C) recorded in Kent between 14th June – 12th July 2017 (C. Wallwork, Agrii).

Site	Minimum	Maximum	Mean
Sandwich	14.6	26.7	20.8
Marden	17.4	25.5	21.0
Chalton	16.4	26.3	20.4
Canterbury	15.9	23.2	19.4

Fusarium wilts of many plants have been shown to be more severe at lower soil pH (Opgenorth & Endo 1983, McGovern 2015). This may be due to the increased activity of antagonistic bacteria in neutral or alkaline soils which leads to disease suppression. In fact it has been shown that increasing the soil pH with agricultural limestone (equivalent to 97% $CaCO_3$) can reduce Fusarium wilt of spinach (Gatch & du Toit 2017). The best results were obtained after three successive annual applications of limestone at 4.48 tons/ha, leading to a 20% reduction in wilt incidence. It should be noted that the effect of pH on lettuce Fusarium wilt has not been tested and it has been found that the soils of Arizona, where FOL race 1 is prevalent, are

alkaline (Matheron & Koike 2003). Many Fusarium wilts are also more severe in light and sandy soils but again this has not been tested for FOL (Egel & Martyn 2007). Organic management of soils can lead to a soil that is more suppressive of Fusarium wilt, as was shown to be the case for *F. oxysporum* from cucumber under glasshouse conditions (van Bruggen *et al.,* 2015). This is likely due to an increased diversity of the microbial population and an increased stability of the microbial community in organic soils.

The effect of soil moisture on Fusarium wilt is a little more complex. Soil moisture is required for chlamydospore germination, and work on banana has shown that this can occur in a range of 20-80% field capacity (Peng *et al*., 1999). There is therefore no scope to limit watering to reduce the impact of Fusarium wilt. However, due to its aerobic nature, *F. oxysporum* does not survive well in soil close to saturation, suggesting the potential of anaerobic disinfestation as a control method (see section 7.3). Humidity has also been shown to have a strong effect on spore germination and subsequent proliferation of *F. oxysporum*. Work on *F. oxysporum* f. sp. *vasinfectum*, causing wilt of cotton, showed that germination of macroconidia was 77% at 100% humidity compared to 0% at 80% humidity and that spore production was 33 times higher at 100% humidity compared to 80% humidity (El-Abyad & Saleh 1971). This supports the evidence that soil moisture is required for infection and disease development; as humidity is always likely to be high in the soil during cropping, humidity changes are only likely to affect situations where *F. oxysporum* growth and sporulation occurs on plant stems (e.g. stocks) which does not apply to FOL.

Any factor that increases plant stress may lead to increased susceptibility to Fusarium wilt. Factors such as heat stress, lack of water, herbicide damage and soil compaction will all increase the susceptibility of plants to Fusarium wilt. Nutrient stress may also increase susceptibility to FOL and a grower in the Netherlands who has experienced the disease suggested that it is important to control micronutrients in the soil (E. de Winter, personal communication).

2.6 Impact of FOL on lettuce production

Following the recent outbreaks in Europe, it is clear that FOL can cause major economic losses for lettuce growers. Initial infection is usually seen as a small patch, leading to <10% losses. However, if left untreated, losses become much greater in subsequent crops. Reports from France (caused by either race 1 or race 4) and the Netherlands (race 4) have commonly observed 50% yield loss (Gilardi *et al.,* 2017b; Gilardi *et al.,* 2017c) while a grower involved in

the first FOL outbreak in Ireland, now reports 100% yield loss in some glasshouses. In the Netherlands, eight out of the ten year-round former protected lettuce growers no longer grow lettuce because of FOL, one is using a hydroponic system and the remaining one has reduced his output from six crops a year to five and still incurs losses of up to 20% (J. van Kuijk, Enza Zaden, personal communication). Due to the patchy nature of infection, it is difficult to estimate the scale of losses in the field. The first report of FOL race 1 in outdoor lettuce production in the USA described scattered symptoms across the whole field with one field containing two areas of 10 x 30 m^2 where severe symptoms were observed (Hubbard & Gerik 1993).

2.7 Laboratory and molecular identification of FOL

Identification of *F. oxysporum* isolates has historically been carried out by culturing and morphological identification. However, this is time-consuming, requires an expert in morphology and does not distinguish f. spp. or non-pathogenic isolates. DNA-based molecular tests have been developed in order to rapidly and accurately identify FOL. This involves isolation of the pathogen from infected plants, DNA extraction, PCR and sequencing. PCR amplification and sequencing of the translation elongation factor 1-alpha gene (TEF) can be used both to distinguish *F. oxysporum* from other *Fusarium* spp. and also identify different genetic lineages of different isolates within *F. oxysporum* (Taylor *et al.,* 2016). These sequences can then be used to produce a phylogenetic tree, inferring the evolutionary origins and relatedness of each isolate. Applying this approach to FOL isolate TEF sequences, including those generated recently at Warwick from UK and Irish isolates, it was observed that FOL races 1 and 4 appear to be closely related while races 2 and 3 are clearly from different evolutionary origins (Fig. 6). However, TEF sequencing does not allow different f. spp. of *F. oxysporum* to be distinguished from each other or non-pathogenic isolates as some may share the same evolutionary origin (Taylor *et al.,* 2016; Gilardi *et al.,* 2017b). For instance, an isolate of *F. oxysporum* f. sp. *mathiolae* from stocks, which is presumed to be non-pathogenic on lettuce, shares the same TEF sequence (Fig. 6). Recently, specific PCR assays have been developed for different races of FOL, thus allowing rapid race-specific molecular identification (Gilardi *et al.,* 2017b). Using these newly developed tests at Warwick, in combination with TEF sequencing, we have identified that all *F. oxysporum* isolates from UK and Irish lettuce were FOL4 (Fig. 7). Furthermore, following testing against six other *F. oxysporum* f. spp. (*cepae*, *cubense*, *lycopersici*, *mathiolae*, *narcissi*, *pisi*), and a non-pathogenic isolate (Fo47) we observed that the published race 4 test (Gilardi *et al.,* 2017b) also gave a positive result

for *F. oxysporum* f. sp*. mathiolae* indicating potential cross-reaction with other f. spp. Hence, although the test can be used effectively for *F. oxysporum* isolates derived from the inside of infected lettuce tap roots, it may not be accurate if used to detect FOL in soil samples which may contain a mixture of other *F. oxysporum* f. spp. All the new UK FOL isolates in the Warwick collection were also tested using a previously published FOL race 1 specific test (Pasquali *et al.,* 2007). All isolates were negative (confirming the race 4 result) while a positive result was obtained for an Italian race 1 isolate control (Fig. 7). The identification of all UK FOL isolates as race 4 is in agreement with the results of lettuce differential testing carried out by Enza Zaden in the Netherlands (M. Pel, Enza Zaden, personal communication). Ideally, FOL isolates from lettuce should be race-characterised using both plant differential tests and molecular tests.

For some pathogens, a soil test may provide useful information on presence / absence. However, this is not the case for FOL as conventional culturing techniques will not distinguish races of FOL and will not even distinguish FOL from other f. spp. or non-pathogenic isolates. It is hoped that quantitative molecular techniques, applicable to DNA extracted from soil, will be developed in the near future but the current molecular assay is not specific enough to use on soil and not designed for quantitative PCR.

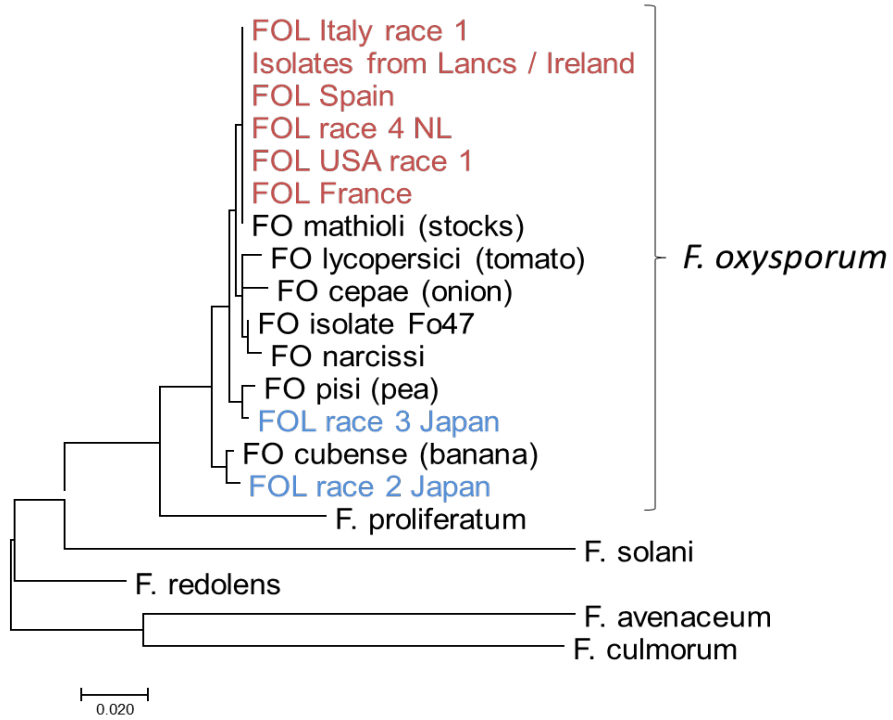

Figure 6: Phylogenetic tree showing the evolutionary relationships among *F. oxysporum and other Fusarium* spp. isolates, as inferred by TEF sequences.

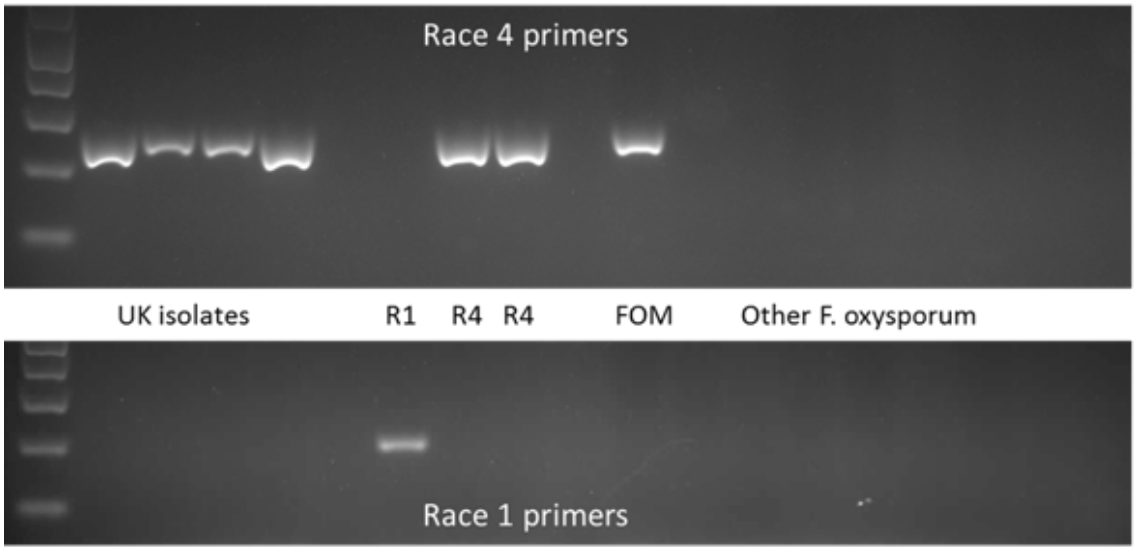

Figure. 7: Molecular characterisation of FOL isolates using race 4 and race 1 specific PCR tests. R1, race 1 isolate from Italy; R4, race 4 isolates from the Netherlands; FOM, *F. oxysporum* f. sp. *mathiolae* (stocks). 'Other *F. oxysporum'* are isolates of *F. oxysporum* f. sp. *cepae*, *cubense*, *lycopersici*, *narcissi*, *pisi*, and a non-pathogenic isolate (Fo47).

3. Hygiene and disease avoidance

3.1 General hygiene practices

The most important approach for mitigating against the risk of introducing FOL to an uninfected site, and to minimise further local spread within infected sites, is the establishment of appropriate hygiene protocols. These are well described in a recent publication from FUNSLA in Belgium, available on the AHDB website (Vandevelde *et al.,* 2017). Some key points to consider, combining the information in this document and personal observations are:

- Contact seed suppliers and request details of seed production practices / hygiene. Request information on seed testing for FOL or any other pathogens.
- After cropping, remove all plant material as FOL can survive and proliferate on loose leaves.
- It may be useful to use a propane burner to flame the soil surface between cropping as this can help to kill the pathogen in debris at the soil surface and possibly a few cm down

- Disinfect glasshouse structures and watering systems between crops
- Clean and disinfect all equipment (see section 3)
- Use separate tools and clothes for each compartment
- Do not let anyone go from a dirty to clean area
- Use foot dips or disinfection mats where possible
- Only use cleaned plant trays / pallets
- Check all incoming plant material and remove any suspicious plants
- Limit access to glasshouses unless absolutely required and restrict site visitors including contractors. All visitors should wear overshoes to prevent spread of contaminated soil
- Always walk the crop in the same direction
- Closely monitor crop, remove plants with Fusarium wilt symptoms and split stems to look for characteristic vascular browning. Send to lab to confirm diagnosis.
- If infection occurs, remove infected plants, neighbouring plants and the surrounding soil. Dispose of these away from site (by burning or to land-fill) and clean hands, clothing, footwear, transport and equipment afterwards.
- In recirculating hydroponic systems, disinfect by heating, UV or ozone,

3.2 Heat sterilisation and disinfectants

The choice of disinfectant method is crucial to achieving an effective kill of FOL. Steam sterilisation of equipment and propagation trays can be very effective but may be prohibitively expensive. Heat has been shown to effectively kill *F. oxysporum* spores; for instance a temperature of 60°C for as little as 2 min killed almost all chlamydospores of *F. oxysporum* from daffodil with 100% kill after 15 min (Lillywhite 2016). This is in agreement with other work where treating plastic pots in a hot water tank at 60°C for 10 mins was sufficient to achieve 100% kill of all tested fungal (*Fusarium* not tested in this study), nematode and insect pests (Lole 2007). Unlike chemical disinfection, heat treatments should be unaffected by soil contamination.

Chemical disinfectants may also be used to sterilise trays, equipment and work surfaces etc. However, to ensure efficacy it is extremely important that all soil is removed prior to disinfection and label instructions are followed carefully, paying attention to the temperature range stated. Research carried out on *F. oxysporum* from stocks (summarised in Table 4) has shown that the disinfectants Disolite and Unifect G were the most effective against both spores and mycelium, even in the presence of small amounts (0.1%) of peat contamination (Wedgwood

2015b). In addition, these products were effective on a range of surfaces including glass, plastic, aluminium, concrete and woven ground-cover. Both products were effective with a contact time of only 5 min.

Table 4: Efficacy of different disinfectants against *F. oxysporum* from stocks (adapted from (Wedgwood 2015b)

Product	Company	Active	Full rate	Kills spores and mycelium	Works with peat contamination	Effective on all surfaces
Jet 5	Certis UK	Hydrogen peroxide + peroxyacetic acid	0.8%	Yes (50-70% control)	No	No
Disolite	Progress Products	Phenolics	2%	Yes	Yes	Yes (40% control in woven ground cover)
Unifect G	Aromany	Quaternary ammonium compounds + glutaraldehyde	4%	Yes	Yes	Yes
Domestos extended germ kill	Unilever	Sodium hypochlorite	2.4%	Yes	Yes	No (only worked on glass)

A study on the effect of a range of disinfectants on spore germination of *F. oxysporum* from banana showed that Sporekill® (International Chemicals), a quaternary ammonium compound like Unifect G, was the most effective product, giving a 100% reduction in spore viability with only a 30 sec exposure time (Meldrum *et al.,* 2013). This product was also 100% effective at a quarter rate with a 5 minute contact time. The other products tested were Farmcleanse® (Castrol), which only reduced spore viability by 55%, even with a 15 minute contact time and Domestos® (sodium hypochlorite) which provided the same control as Sporekill®. When all these products were stored in a banana plantation for 6 months and then re-tested, Sporekill® was still 100% effective whereas the efficacy of Domestos® was reduced to 40% after 1 month and was completely ineffective after 3 months. The data on Sporekill® is supported by earlier studies on *F. oxysporum* from banana where it reduced spore viability by 100% with only a 30 sec exposure time (Nel *et al.,* 2007). However, both of these studies used microconidia in the experiments rather than chlamydospores which are more robust and the primary long-term survival structures of *F. oxysporum*. Another study from the USA investigated the effect of a range of disinfectants on viability of spores (both conidia and chlamydospores) of *F. oxysporum* from cotton under laboratory conditions and found that, even when used at a 1:100 dilution and with only a 5 min contact time, six products were still 100% effective (Bennett *et*

al., 2011). Of these, four were quaternary ammonium compounds – Formula 409 Orange Cleanser Degreaser (Clorox), Formula 409 Antibacterial All Purpose Cleaner (Clorox), Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent (Reckitt Benckiser) and Simple Green d Pro 3 (Sunshine Makers). The other two were sodium hypochlorite and Trewax Nature's Orange (Beaumont Products). In contrast to the results for Unifect G, these four products had no efficacy when soil contamination was introduced. However, this involved a much larger percentage of soil (10%) compared to the UK studies (Wedgwood 2015b). Under these high soil contamination levels, only sodium hypochlorite was effective, with 100% kill (Bennett *et al.,* 2011). Studies on *F. oxysporum* from narcissus have shown that Boot, another quaternary ammonium compound, is also effective against chlamydospores, even at low doses and with a 5 min contact time (Lillywhite 2016). This level of control however was reduced in the presence of organic matter (narcissus scales as opposed to soil) but a 70-85% reduction in spore viability was still achieved at higher concentrations of Boot. In conclusion, quaternary ammonium compounds are particularly effective for disinfecting trays, pallets, work surfaces and farm equipment. A product such as Unifect G may also benefit from the synergistic effect of the glutaraldehyde which is also present in this product. Quaternary ammonium compounds also tend to be non-corrosive and have low environmental impacts (Meldrum *et al.,* 2013). If used as disinfectants, it is unlikely that residues of quaternary ammonium compounds would be detected in lettuce crops but it should be noted that residues are tested for with a limit of 0.1 mg/kg. In the pesticide residues in food monitoring programme very few residues have been found in fruit and vegetable surveys (Defra, 2017).

4. Resistance to FOL

Plant resistance would be highly desirable for control of FOL, and whilst commercial resistant cultivars are available for race 1 in the USA (Scott *et al*., 2010b), breeding programmes for race 4 have yet to be initiated, although some resistance has already been identified through screening existing lettuce cultivars / breeding lines. Initial work from Italy showed that three cultivars which are susceptible to race 1, Banchu Red Fire (Butterhead), Lattuga Gentilina (Batavian) and Riccetto (Loose leaf) were resistant to race 4 (Gilardi *et al.,* 2017b). Whilst these cultivars are not adapted to UK conditions, it does at least provide some promise that resistance breeding is possible. Currently, there is no high-level resistance to FOL4 in UK adapted butterhead lettuce cultivars and only partial resistance has ever been identified in any butterhead types. In contrast, resistance to race 4 can be identified in romaine, Batavian, lollo

rosso and oak leaf lettuce types. A report from Belgium, incorporating information from Rijk Zwaan (Table 5), identified some lettuce cultivars that showed partial resistance to FOL4 (Vandevelde *et al.,* 2017). It should be noted that the butterhead types are outdoor adapted and suited to Southern Europe. Extensive resistance screening is currently in progress at both Enza Zaden and Rijk Zwaan with some promising results for some indoor little gem types (M. Pel & J. Johnson, Enza Zaden, personal communication) and partial resistance in an indoor butterhead type (J. Schut, Rijk Zwaan, personal communication). Seed for the butterhead type is expected to be available in the near future.

Table 5: Commercial cultivars that are partially resistant or susceptible to FOL4. These are all Rijk Zwaan cultivars unless stated (adapted from Vandevelde *et al.,* 2017)

Lettuce type	**Cultivar (s)**
Partially resistant	
Outdoor butterhead	42-120 RZ, Emilina, Sandalina
Lollo bionda	Lugano, Livorno, Limeira
Lollo rossa	Satine, Athmos, Soltero (Bayer)*
Multileaf	Codex, Haflex, Triplex, Extemp
Oak leaf	Kitonia, Xerafin
Romaine	Actina (Syngenta)
Batavian	Yacht
Crunchy	41-673 RZ, 41-692 RZ
Susceptible	
Butterhead	All indoor cultivars
Lollo bionda	Lozano
Multileaf	Vicinity
Oak leaf	Cook, Rouxaï, Saturdaï, Eventaï, Soupiraï, Xandra
Romaine	Maximus
Batavian	Othilie, Vessel, Bobal

*Soltero is an old variety which can be used indoor and outdoor but is no longer suitable for the market and lacking full downy mildew resistance (B. Penaloza, Bayer, personal communication).

Resistance to FOL race 1 (Garibaldi *et al.,* 2004b; Tsuchiya *et al.,* 2004a; Tsuchiya *et al.,* 2004b; Matheron *et al.,* 2005; Scott *et al.,* 2012; Cabral & Reis 2013) and race 2 (Tsuchiya *et al.,* 2004a; Tsuchiya 2009; Aruga *et al.,* 2012) has been reported previously and genetic markers linked to race 2 resistance have also been identified, potentially enabling marker-assisted breeding (Aruga *et al.,* 2012). However, no resistance to race 3 has been reported to date (Lin *et al.,* 2014). Iceberg lettuces show some variation in susceptibility to FOL race 1 but are not fully resistant (Gordon & Koike 2015) while high levels of resistance can be found in leaf and romaine types. In the USA, a strategy of only planting susceptible cultivars at the

coolest times of year and utilising resistant cultivars in the warmer months has been employed to reduce the impact of FOL in the field (Gordon & Koike 2015). However, a high level root colonisation is still observed even on highly resistant cultivars so whilst plants may remain asymptomatic, FOL may still accumulate in the soil (Scott *et al.,* 2014).

5. Chemical control

A range of chemical control measures have been tested against *F. oxysporum* (summarised in Table 6) and whilst some reduction in disease levels can be achieved, it should be noted that complete control is unlikely due to the soil-borne nature of the pathogen. The control options listed here should be considered as preventative measures and are very unlikely to work as curative treatments. In the past, carbendazim was used and provided good control of *Fusarium*, but this chemical is no longer approved in the UK or the EU. When tested against FOL race 1, azoxystrobin and fosetyl-aluminium produced 56% and 58% reductions in disease severity respectively in glasshouse tests (Gilardi *et al.,* 2016a). Fosetyl-aluminium is known to induce plant resistance. Three commercial fosetyl-aluminium products available in the UK (Previcur Energy, Avatar (for propagation only) and Pan Cradle) with on-label approvals for protected and outdoor lettuce also contain propamocarb hydrochloride for the control of oomycetes such as *Bremia lactucae* and *Pythium*. There are also off-label approvals for Fenomenal (fenamidone + fosetyl-aluminium) which is also used for controlling oomycetes. Azoxystrobin is a broad-spectrum fungicide with a wide range of commercial products available for lettuce (e.g. Amistar). Data from tests against Fusarium wilt of stocks (with high inoculum levels) showed that Amistar (applied 14 and 28 d after planting) reduced disease severity but did not reduce the proportion of plants affected at harvest (O'Neill 2007).

The efficacy of a range of other chemicals has been tested against FOL when applied as seed treatments (Gilardi *et al.,* 2005). The most effective of these (under low disease pressure), were mancozeb (84% reduction in incidence), azoxystrobin (80%) and prochloraz (87%). A subsequent trial found that treating seed with prochloraz led to a 92% reduction disease incidence and 95% reduction in disease severity under low disease pressure (FOL race 1, Lopez-Reyes *et al*., 2014). These fungicides are not currently approved for the treatment of lettuce seeds in the UK but some of these may still be effective as a drench. The only mancozeb product approved for both outdoor and protected lettuce is Fubol Gold where it is combined with metalaxyl-M, predominantly for downy mildew control. Mancozeb is also approved for use on outdoor lettuce only as Karamate dry flo Neotec. Prochloraz is a broad-

spectrum fungicide and in the form of Octave has also been shown to control Fusarium wilt of hebe (O'Neill 2009); however it is not currently approved for use on lettuce. There are no known approvals for treating lettuce seed with prochloraz in any EU member state (L. Matthews, BASF, personal communication).

The efficacy of thiram as a seed treatment against FOL has also been tested. Whilst initial trials showed no statistically significant effects, disease incidence was reduced by up to 44% (Gilardi *et al*., 2005). A subsequent study produced more convincing results where treating seed with thiram led to a 72% reduction in disease incidence and a 75% reduction in disease severity, under low disease pressure (Lopez-Reyes *et al*., 2014). As thriam is currently approved as a treatment for lettuce seed in the UK, it may be an option for improving control, particularly at the early stages of plant development. Treating seed with thiram has been shown to control *F. oxysporum* on other crops including cucumber where an 88% reduction in plant mortality was reported (Rose *et al*., 2003).

Luna Sensation (fluopyram + trifloxystrobin), which is approved for both indoor and outdoor lettuce, may also have potential activity against FOL. Whilst fluopyram is relatively new chemistry, trifloxystrobin, similar to azoxystrobin, has been shown to control Fusarium wilt of carnation (Gullino *et al.,* 2002). The product Switch (cyprodinil and fludioxonil), which is also approved for lettuce and provides good control for ring spot (*Microdochium panattonianum,* Gladders 2008), is marketed as providing Fusarium control and whilst fludioxonil has been shown to control Fusarium wilt of tomato (Amini & Sidovich 2010), field trials from the USA showed no efficacy against FOL when applied at seeding (Matheron 2015). In addition, Switch had no efficacy against Fusarium wilt of stocks (O'Neill 2007). Similarly, Cercobin WG (thiophanate-methyl) is approved for the control of Fusarium wilt of tomato and has been shown to control *F. oxysporum* on other plants including pepper (Cerkauskas 2017). However, field trials from the USA showed no efficacy against FOL race 1 when applied at seeding (Matheron 2015).

Prothioconazole (not approved for lettuce but approved for a range of other outdoor crops) may also be a useful product for future control of FOL as it has been shown to control Fusarium wilt of watermelon (Everts *et al.,* 2014) and initial trials from the UK show promising results for controlling *F. oxysporum* on onion (T. Lacey, Bayer, personal communication). However, approval for lettuce is unlikely in the near future (T. Lacey, Bayer, personal communication). Tebuconazole is approved for a range of outdoor and protected crops and was the most effective chemical for controlling Fusarium basal rot of daffodil. It has also been shown to control *F. oxysporum* on other crops including strawberry (Lin *et al.,* 2009). However, this active is not likely to be approved on leafy salad crops because of potential phytotoxicity.

Finally, two other fungicides approved for use on lettuce in the UK, are Signum and Insignis (boscalid + pyraclostrobin). However, this combination of chemicals had no efficacy against FOL race 1 in field trials in the USA (applied at seeding, Matheron 2015) and similarly preliminary glasshouse trials in Belgium showed no efficacy against FOL4 (I. Vandevelde, Proefstation, personal communication).

Another product which induces plant defence, acibenzolar-S-methyl, was shown to reduce severity of lettuce wilt by 60% (Gilardi *et al.,* 2016a). This chemical is not approved for lettuce, although approved products exist for ornamentals (Inssimio) as well as barley and wheat (Bion). However, the approval for these products will lapse next year and will not be renewed (B. Palle Neve, AHDB, personal communication).

Table 6: Fungicides with potential activity for controlling F*. oxysporum* f. sp. *lactucae*.

Active	Pesticide status for UK lettuce	Efficacy against *F. oxysporum*	Experimental Details	Reference
Approved for use on lettuce in UK, tested against FOL				
Azoxystrobin Amistar and others	Approved for use on outdoor or protected lettuce	56% reduction in disease severity (FOL race 1) Up to 80% reduction in disease incidence under low disease pressure (FOL race 1)	Applied as a foliar spray (0.19 g L-1) with a high volume of water (1500 L ha-1), prior to transplanting Applied as a seed treatment	Gilardi *et al.,* 2016a Gilardi *et al.,* 2005
Fosetyl-aluminium In Previcur Energy, Avatar or Pan Cradle (mixed with propamocarb hydrochloride). In Fenomenal (mixed with Fenamidone)	Approved for use on outdoor and protected lettuce	58% reduction in disease severity (FOL race 1)	Applied as a foliar spray (1.6 g L-1) at 7 day intervals (prior to transplanting) with a high volume of water (1500 L ha-1)	Gilardi *et al.,* 2016a
Mancozeb Karamate dry flo Newtec (outdoor only) In Fubol Gold (mixed with metalaxyl-M)	Extension of use for outdoor and protected lettuce	Up to 84% reduction in disease incidence under low disease pressure (FOL race 1)	Applied as a seed treatment	Gilardi *et al.,* 2005
Boscalid + pyraclostrobin Signum or Insignis	Approved for use on outdoor and protected lettuce	Field trials in USA showed no effect against FOL race 1	Applied at seeding in field	Matheron 2015
Thiram Agrichem Flowable Thiram or Thyram Plus	Approved for use on lettuce seed	Up to 44% reduction disease incidence (not statistically significant) under low disease pressure (FOL race 1) 72% reduction disease incidence and 75% reduction in disease	Applied as a seed treatment Applied as a seed treatment	Gilardi *et al*., 2005 Lopez-Reyes *et al*., 2014

		severity under low disease pressure (FOL race 1)		
Approved for use on lettuce in UK, not tested against FOL, efficacy against *F. oxysporum* on other plants				
Fluopyram + trifloxystrobin Luna Sensation	Approved for use on outdoor and protected lettuce	Trifloxystrobin shown to control Fusarium wilt of carnation (up to 77% reduction in number of dead plants) but was less effective on cyclamen and Paris daisy	Applied as a drench after transplanting	Gullino *et al.,* 2002
Cyprodinil + fludioxonil** Switch	Approved for use on outdoor and protected lettuce	69% reduction in Fusarium wilt of tomato (fludioxonil)	Applied directly to pots in a controlled experiment	Amini & Sidovich 2010
Tested against FOL but not approved for use on lettuce in UK, approved for use on other crops				
Prochloraz Mirage and others	Not approved for lettuce, products exist for cereals	Up to 87% reduction in disease incidence under low disease pressure (FOL race 1) 92% reduction disease incidence and 95% reduction in disease severity under low disease pressure (FOL race 1)	Applied as a seed treatment Applied as a seed treatment	Gilardi *et al.,* 2005 Lopez-Reyes et al., 2014
Acibenzolar-S-methyl Bion or Inssimo	Not approved for lettuce, has approval for ornamentals, barley and wheat	60% reduction in disease severity (FOL race 1) 59% reduction disease incidence and 61% reduction in disease severity under low disease pressure (FOL race 1)	Applied as a foliar spray (0.025 g L-1) at 7 day intervals (prior to transplanting) with a high volume of water (1500 L ha-1) Applied as a seed treatment	Gilardi *et al.,* 2016a Lopez-Reyes *et al*., 2014
Thiophanate-methyl Cercobin WG	Not approved for lettuce, approved for Fusarium control in protected tomato	No control of FOL reported in field trials.	Applied at seeding	Matheron 2015

		89% reduction in disease severity (*F. oxysporum* on pepper)	Applied directly to base of 7 week old plants	Cerkauskas 2017
Efficacy against *F. oxysporum* on other plants but not approved for use on lettuce in UK, approved for use on other crops				
Prothioconazole Rudis and others	Not approved for lettuce, approved for a range of other crops (outdoor)	Up to 80% reduction in wilt** *(F. oxysporum* from watermelon)	Applied as a drench at planting in the field	Everts *et al.,* 2014
Tebuconazole Orius and others	Not approved for lettuce, approved for a range of other crops (outdoor and protected)	Up to 62% reduction in disease severity under high disease pressure (*F. oxysporum* on daffodil)	Bulbs incubated in a water bath containing fungicide before planting in heavily infested compost	Clarkson 2014

*fludioxonil was tested on its own against FOL in field trials in the USA and shown to have no efficacy when applied at seeding (Matheron 2015).

**3-4 weeks after application. The authors noted that the effect was lost unless successive applications were carried out and applied the chemical 3 times through drip irrigation in subsequent trials

6. Biological control

No single biological control agent can fully control Fusarium wilt in lettuce, but studies with a range of microorganisms have demonstrated partial control (summarised in Table 7) and in most cases, products need to be applied as early as possible to maximise their efficacy. *Bacillus* species have been shown to both induce host resistance and promote plant growth (Kloepper *et al.,* 2004). Research from Italy showed that *B. subtilis* strain QST713 (marketed in the UK as Serenade), applied as either a foliar drench prior to transplanting or as a seed treatment, significantly reduced lettuce wilt (Gilardi *et al.,* 2016a) but initial trials with the same product in Belgium against FOL4 did not demonstrate any efficacy (I. Vandevelde, Proefstation, personal communication). Whilst Serenade is approved for soil application on outdoor lettuce, approval for indoor lettuce only allows foliar application, potentially limiting its activity against FOL4. *B. amyloliquefaciens* (Amylo X WG) whilst not tested against FOL, has been shown to reduce tomato Fusarium wilt by up to 70% (Maung *et al.,* 2017; Pane *et al.,* 2017). However, this product is only approved for foliar application on lettuce in the UK and as such its activity against FOL4 may be limited.

Gliocladium catenulatum (Prestop) has been shown to control a range of soil-borne diseases including *F. oxysporum* on pepper and cucumber (Rose *et al.,* 2003; Chatterton & Punja 2009; Cerkauskas 2017). However, the level of control may be strongly dependent on disease pressure and a field trial against *F. oxysporum* on onion showed limited efficacy (Noble 2013). Whilst there are no publications evaluating Prestop for FOL control, initial trials from Belgium have shown little efficacy against race 4 (I. Vandevelde, Proefstation, personal communication).

Some of the most widely tested biological control agents are *Trichoderma* species which often have multiple modes of action. It has also been shown that they colonise plant roots and can enhance root growth (Harman *et al.,* 2004). Among the different *Trichoderma* products studied, *T. harzianum* strain T22 (marketed in the UK as the product Trianum) has been widely investigated and shown to have an effect against *F. oxysporum*. Tests against FOL have produced promising results with up to an 83% reduction in disease if the product is applied before sowing or transplanting (Gilardi *et al.,* 2007; Innocenti *et al.,* 2015). However, as with many of these products, the level of control achieved was variable. Application of T22 for control of *F. oxysporum* in stocks and Lisianthus did not show any significant reduction in Fusarium wilt (O'Neill 2007). Initial trials from Belgium have shown a small effect against FOL4 (Vandevelde *et al.,* 2017). Another widely studied species is *Trichoderma asperellum*, strain T34 (marketed as the product T34 Biocontrol). Whilst there is currently no data for FOL,

good efficacy has been reported against other *F. oxysporum* affecting other plants including tomato and carnation (Cotxarrera *et al.,* 2002; Sant *et al.,* 2010). An additional *Trichoderma* product, currently not registered for use in the UK, may also have some efficacy against FOL4. A protected lettuce grower from the Netherlands reports that adding the product OCCU fungus (containing *Trichoderma hamatum*, *Trichoderma asperellum*, *Trichoderma harzianum* and a *Gliocladium* species; supplied by Royal Brinkman) to the soil before each lettuce crop had some small but potentially beneficial effects against Fusarium wilt (E. de Winter, personal communication).

Streptomyces griseoviridis strain K61 (marketed in the UK as Mycostop) has also been shown previously to control *F. oxysporum* in carnation, brassicas and cucumber (Kortemaa *et al.,* 1994). However, results against FOL have so far been unconvincing. One study showed a reduction in disease index of up to 62% in one trial but no significant effect was observed in three out of the five trials conducted (Gilardi *et al.,* 2007). When applied as a seed treatment, no statistically significant reduction in disease was obtained (Gilardi *et al.,* 2005).

A potential future option for biological control of FOL is the use of non-pathogenic *Fusarium* strains which can colonise roots rapidly and can outcompete pathogenic strains. For instance, non-pathogenic strain Fo47 has been shown to reduce disease caused by *F. oxysporum* f. sp. *lycopersici* on tomato and also has activity against a range of different *F. oxysporum* f. spp and other soil-borne plant pathogens (Alabouvette *et al.,* 2009). Whilst initial tests (using alternative non-pathogenic strains) against FOL have produced promising results (Gilardi *et al.,* 2005; Gilardi *et al.,* 2007), no commercial product currently exists in the UK.

Overall, it is clear from the literature that biological control agents have some potential for control of FOL but results can be variable, depending on time and method of application, and disease pressure, and hence these products should be used as part of an integrated control strategy. Many of these products also need more rigorous testing, specifically against FOL4. Many of the chemical and biological control agents discussed here have been tested against Fusarium from stocks in the UK (Mason 2013). The only treatment which provided significant control in this study was Octave (prochloraz, 85% reduction in infection). The following products showed no efficacy: Signum (pyraclostrobin + boscalid), Switch (cyprodinil + fludioxonil), T34, Serenade, Prestop and Trianum. This should not discount the possibility of an effect against FOL4. The method and timing of treatments (propagation and/or production, protectant or curative) will have a profound effect on efficacy and future trials should assess both application methods, timings, modes of action and rates. Future trials should also be conducted under conditions of high disease pressure.

Table 7: Biological control agents with reported activity for controlling *F. oxysporum* f. sp. *lactucae*.

Microorganism	Pesticide status for UK lettuce	Efficacy against *F. oxysporum*	Experimental details	Reference
Approved for use on lettuce in UK, tested against FOL				
Serenade ASO *Bacillus subtilis* strain QST713	Off-label approval as a foliar spray for protected lettuce and as a soil application for outdoor lettuce	31% reduction of disease severity (FOL race 1), extra applications improve control	Applied as a foliar spray (0.58 g L^{-1}) at 7 day intervals with a high volume of water (1500 L ha^{-1}) prior to transplanting	Gilardi *et al.,* 2016a
		43% reduction in disease incidence under low disease pressure (not statistically significant, FOL race 1)	Applied as a seed dressing	Gilardi *et al.,* 2005
		54% reduction disease incidence and 54% reduction in disease severity under low disease pressure (FOL race 1)	Applied as a seed dressing	Lopez-Reyes *et al*., 2014
Prestop *Gliocladium catenulatum* strain J1446	Approved for use on protected lettuce (extension of use covers outdoor lettuce)	Slight effect reported against FOL4	None given	Vandevelde *et al.,* 2017
		60-85% reduction in mortality (*F. oxysporum* from cucumber)	Suspension applied to cucumber seeds	Rose *et al.,* 2003; Chatterton & Punja 2009
		81% reduction in disease severity (*F. oxysporum* from pepper)	Applied directly to base of 7 week old plants	Cerkauskas 2017
		No affect in a field trial against *F. oxysporum* on onion (effective in pot test)	Sprayed at drilling (2.5g/m^2)	Noble 2013
Trianum-P *Trichoderma harzianum* strain T22	Approved in the UK for protected lettuce (not outdoor)	Up to 83% reduction in disease index (FOL race 1), significant reduction in disease in 4 out of 5 trials	Applied as a liquid before transplanting	Gilardi *et al.,* 2007
		57-78% reduction in disease severity in water stress conditions (FOL, presumed to be race 1)	Seeds sown directly in substrate containing T22.	Innocenti *et al.,* 2015
		'Slight effect' reported against FOL4	Applied 1 week after sowing with 600 g in 100 litres of water per are (100m2).	Vandevelde *et al.,* 2017

		No significant effect on Fusarium wilt of stocks or Lisainthus	Applied as a drench before transplanting or as a soil amendment after steaming	O'Neill 2007
Mycostop *Streptomyces griseoviridis* strain K61	Approved for use on protected lettuce	Up to 62% reduction in disease index (FOL race 1) 29% reduction in disease incidence under low disease pressure (not statistically significant, FOL race 1) 35% reduction disease incidence and 41% reduction in disease severity under low disease pressure (FOL race 1)	Applied as a liquid before transplanting Applied as a seed dressing Applied as a seed dressing	Gilardi *et al.,* 2007 Gilardi *et al.,* 2005 Lopez-Reyes *et al*., 2014
Approved for use on lettuce in UK, not tested against FOL, efficacy against *F. oxysporum* on other plants				
T34 Biocontrol *Trichoderma asperellum*, strain T34	Extension of use covers protected lettuce (not outdoor)	50% reduction in disease severity and 33% reduction in disease incidence (*F. oxysporum* on carnation) Up to 95% reduction in disease severity (*F. oxysporum* on tomato)	Cuttings transplanted into growing medium mixed with liquid T34 (10^4 cfu/ml) additional T34 drench applied 47 days after planting Mixed into growing media as a liquid spore suspension prior to transplanting	Sant *et al.,* 2010 Cotxarrera *et al.,* 2002
Amylo X WG *Bacillus amyloliquefaciens,* strain D747	Approved for use on protected lettuce as (only as a foliar spray)	*63% reduction in disease incidence and 70% reduction in disease severity (*F. oxysporum* on tomato) *65% reduction in disease incidence (*F. oxysporum* on tomato)	Mixed directly into soil Mixed into growing media and applied as a foliar spray	Pane *et al.,* 2017 Maung *et al.,* 2017
Efficacy against *F. oxysporum* on other plants but no approved product exists in UK				
Non-pathogenic *F. oxysporum*	No UK approval	Up to 77% reduction in disease incidence under low disease pressure (FOL race 1) Up to 85% reduction in disease index (FOL race 1)	Applied as a seed dressing Applied as a liquid before transplanting	Gilardi *et al.,* 2005 Gilardi *et al.,* 2007

*these studies used a different strain of *B. amyloliquefaciens*

7. Soil disinfestation

7.1 Chemical fumigation

One option for soil disinfestation is the use of chemical fumigants. However, these treatments are expensive, often need specialist equipment and their approval for future use is not guaranteed. Historically, methyl bromide was used as a fumigant but its use is now outlawed throughout Europe. In the UK, one soil fumigant that is approved is dazomet (marketed by Certis as Basamid). This product has the advantage that it controls a range of soil fungi, pests, nematodes and weeds. Recent work from Italy has shown that dazomet, applied at 49.5 g/m^{-2}, can reduce Fusarium wilt of lettuce by up to 91% (Gilardi *et al.,* 2017a), although the level of control obtained was found to be dependent on cultivar. Effective control was also maintained in a following lettuce crop and Certis now recommend that Basamid is used once in every three lettuce crop cycles. As noted on the product label, soil moisture must be at least 50% water holding capacity and soil temperature should be above 7°C. A possible disadvantage of Basamid is that it has to be incorporated thoroughly as a granule, so there is scope for incorporating the pathogen to depth and further in soil. In addition, there needs to be a gap before the next crop to allow full release of gas. The only other fumigant approved for lettuce in the UK is metam sodium (marketed by Taminco as Metam 510). Metam sodium may provide some control for FOL, with field trials from the USA showing that Fusarium wilt incidence in lettuce was reduced by 44% at crop maturity following a pre-plant treatment with metam sodium (Matheron 2015). A new soil fumigant, dimethyl disulphide (DMDS), is now available in Italy (marketed as Paladin EC) and Certis are attempting to get it registered in the UK (Alan Horgan, Certis, personal communication). Work from Italy has shown that the efficacy of DMDS against FOL was significantly better than dazomet, with reductions in wilt of up to 97% when applied by shank injection into the soil (details in Gilardi *et al.,* 2017a) and DMDS also provided control in a second lettuce crop. Moreover, DMDS can be used at a lower dose (achieving the same control) if combined with metam sodium. In the Netherlands, where FOL4 was first identified, no chemical fumigants are approved so soil disinfestation is predominantly achieved by steaming (J van Kuijk, Enza Zaden, personal communication).

7.2 Steaming

Soil steaming is another efficient way of sterilising the soil and reducing the impact of FOL, although this has the disadvantage of being expensive in terms of energy costs. An advantage is that cropping can occur sooner after treatment compared with chemical fumigation. The approach was shown to be very effective at controlling Fusarium wilt of stocks where a range of different steaming methods was evaluated in AHDB project PC 213 (O'Neill 2007). Using sheet steaming, where soil is prepared and steamed for 10 h under a thermal fleece, a >93% reduction in viability of *F. oxysporum* was observed and the treatment was equally effective at a depth of 3.5-33.5 cm. Large scale indoor lettuce growers in the Netherlands also currently using sheet steaming in July / August, and dropping from six crops a year to five, to help control FOL4 and report good reductions in levels of Fusarium wilt in three successive crops (E. de Winter, personal communication; R. Scheepers, Versland, personal communication). However, levels of disease increased in a fourth successive crop (also coincides with warmer temperatures) reaching 20% losses in a fifth crop and hence repeat treatments (minimum once per year) are required. Sheet steaming may also be less effective in certain soil types; a 67% reduction in viability was achieved when testing against *F. oxysporum* from Lisianthus in a medium sandy loam soil (O'Neill 2007). In these conditions, vacuum steaming may be preferable where slit-perforated plastic pipes, covered with woven plastic mesh, can be used with suction applied using a small pump. This has been shown to be a more effective method of heating soil than sheet steaming (O'Neill 2007). In the same study, a steam plough method was also tested where soil is steam injected at 31 cm and covered by a 4 m plastic sheet following the plough. This gives the soil around 20 min steaming at each point and using this method in stocks resulted in a >95% reduction in viability for *F. oxysporum* which was equally effective at a depth of 3.5-33.5 cm (O'Neill 2007). Finally, sandwich steaming, where steam is led under a hood through hollow pins that penetrate to 25 cm soil depth may also be an option and is considered to be the most energy efficient method (Möschle-Seifert-Dämpftechnik 2018). Whilst the utility of this method against *Fusarium* is untested, the soil temperatures achieved should be sufficient to kill spores (a temperature of 60°C for as little as 2 min kills almost all chlamydospores, see section 3.2).

7.3 Anaerobic soil disinfestation

Anaerobic soil disinfestation (also known as biological soil disinfestation) involves incorporating an organic substrate (e.g. cover crop), flooding the soil and tightly covering with a plastic sheet (Huang *et al.,* 2015). The soil is left covered for a number of weeks and the anaerobic conditions lead to the production of compounds which are toxic to pathogens such as *F. oxysporum*. It has been shown to be effective against a range of pathogens including *F. oxysporum* f. spp. infecting tomato, spinach and banana and asparagus (Blok *et al*. 2000; Huang *et al.,* 2015).

A recently developed, plant-based product Herbie 72® (Thatchtec BV) was tested in combination with anaerobic soil disinfestation against *F. oxysporum* on tomato and showed some excellent preliminary results in field trials where *Fusarium*-infected wheat kernels were buried in the soil prior to treatment. Treatment with Herbie resulted in a 90-100% reduction in viability of *F. oxysporum*, showing the potential of this product for *Fusarium* control (Minito *et al.,* 2017). The product is thought to increase the total bacterial load and diversity in the soil, hence potential enhancing pathogen suppression (H. Meints, Thatchtec BV, personal communication). A summer pot test using Herbie 14.3 showed a lower but significant effect on *F. oxysporum* from stocks with up to a 59% reduction in the viability of *F. oxysporum* on infected, buried stems (Wedgwood 2015a). However, a winter pot trial showed no significant effects, suggesting that this treatment is only likely to be effective when temperatures are higher. Extensive further testing of Herbie is required in order to assess the utility for controlling FOL and the product is not currently registered for use in the UK though it is approved for use in the Netherlands and Germany.

7.4 Soil solarisation

In the USA and countries with warmer temperatures, soil disinfestation can be achieved through soil solarisation. This involves saturating soil with water and covering with 1 mm thick clear polyethylene (containing a UV stabiliser) for a period of 1 month (Matheron & Porchas 2010). Trials in the USA have shown a reduction of up to 91% in Fusarium wilt of lettuce following solarisation in combination with soil flooding. However, outdoor temperatures in the UK are unlikely to be high enough for a sufficient duration to achieve effective solarisation. The utility of soil solarisation in a UK glasshouse is untested and warrants further investigation.

7.5 Effects on the soil microflora

Although the target organisms for soil disinfestation are pathogens, beneficial organisms may also be adversely affected. Whilst some organisms, including *Trichoderma* spp. and *Gliocladium* spp., may be less sensitive to fumigants, there is still a high risk that the 'biological void' will be filled by a pathogen (Spadaro & Gullino 2005). Therefore, incorporating a biocontrol agent after disinfestation may be advantageous. This has been shown to be effective for controlling various pathogens including *Rhizoctonia solani* on beans and carrots, where *Trichoderma harzianum* was added either before or after methyl bromide treatment leading to a greater reduction in disease compared to methyl bromide alone (Strashnow *et al*., 1985). Biological control agents can also be applied after steam disinfestation (Gullino 1992). Whilst initial research from *F. oxysporum* on stocks showed no added benefit of incorporating *Gliocladium catenulatum* or *T. harzianum* after steaming, this area should be further investigated for controlling FOL4.

8. Crop Rotation

Crop rotation may also be considered as an option for managing FOL although the knowledge of which crops are the best options for use in a rotation is lacking. Work from the USA has shown that spinach is a bad choice as a preceding crop as both the roots and vascular tissue are colonised by FOL (Scott *et al.,* 2014). In this study, the vascular tissue of 50% of the spinach plants was colonised compared to 7.4% for cauliflower and 0% for broccoli. For all three non-hosts, around 50% of the plants had some FOL root colonisation. Previous research from the USA has also demonstrated that FOL can colonise the roots of tomato, melon and cotton (Hubbard & Gerik 1993). A protected lettuce grower in the Netherlands has changed their production system to limit the impact of FOL by growing pak choi and fennel in the summer when disease pressure is greatest due to higher temperatures, and butterhead lettuce in the winter and spring (R. Scheepers, Versland, personal communication). The grower reported that test plantings of lettuce between pak choi crops showed no reduction in disease. However, initial observations on an infected site in Lancashire showed that, after two crops of pak choi, disease incidence was greatly reduced with only around 5% losses observed (J. Johnson, Enza Zaden, personal communication). Even when lettuce cultivars resistant to FOL are grown, roots and vascular tissue (71% of plants tested) are still colonised, potentially leading to build-up of FOL in the soil (Scott *et al.,* 2014). The utility of crop rotation is therefore an area of some debate given the longevity of *F. oxysporum* chlamydospores and

the lack of knowledge concerning the range of crops FOL colonises. Work on a related disease, Verticillium wilt of strawberry in the USA, has shown that rotating strawberry with broccoli or Brussels sprouts leads to reduced pathogen load in the soil and a subsequent decrease in disease severity (Subbarao *et al.,* 2007).

Another related approach for management of Fusarium wilt in lettuce is leaving an infected area fallow following a disease outbreak. The area must be kept weed free as FOL may proliferate on wild hosts. Work from the USA using this approach has shown that the number of viable FOL spores in the soil was reduced by 71% after six months and 86% after 12 months (Scott *et al.,* 2012; Gordon & Koike 2015). After this time, the decline appears to slow down as a low level of FOL could still be detected in the soil after 34 months. This would be below the threshold level required to cause disease (section 2.4) but would potentially allow build-up to begin again in subsequent lettuce crops.

9. Biofumigation and soil amendments

The effect of biofumigation and addition of various soil amendments has also been investigated for FOL (Table 8). Biofumigation involves growing a particular cover crop (usually a brassica species such as a mustard) which contains high levels of glucosinolate compounds. When the plant material is crushed and incorporated into soil with adequate moisture, isothiocyanate compounds are released which are toxic to a wide range of plant pathogens. Products derived from such biofumigant crops such as mustard meals and liquids are also available for immediate application. In relation to FOL, a study from Italy has shown that the biofumigant product Biofence (*Brassica carinata* pellets), mixed into soil 14 days before lettuce transplanting resulted in a 56% reduction in Fusarium wilt severity (Gilardi *et al.,* 2016a) but when applied seven days before transplanting, there was no effect. A well replicated pot test by the same research group showed that Biofence and to a slightly lesser extent Biofence 10 (a milled version), applied 30 days prior to transplanting (60 days produced the same results), led to significant reductions (up to 80%) in disease severity (Gilardi *et al.,* 2016b). When a second lettuce crop was planted in the same soil, similar levels of disease reduction were obtained (compared to the untreated controls). No phytotoxic effects were reported on either lettuce or rocket. Biofence has also been shown to decrease the incidence and severity of Fusarium wilt of tomato by around 55% (Pane *et al.,* 2017). The potential use of the biofumigant *B. juncea* (brown mustard) was also investigated. Here, *B. juncea* plants were grown to 80% flowering (when glucosinolates in plant tissue are at their maximum) and incorporated into the soil 30 or 60 days before planting (Gilardi *et al.,* 2016b).

The effects of this treatment (no significant difference between incorporation 30 or 60 days before transplanting) however were highly variable and any control of FOL was often reduced in the second cycle of lettuce. This may have been due to incomplete maceration of the plant material or a delay in incorporation, both of which would have reduced the final concentration of isothiocyanates produced in the soil and hence the biofumigation potential. In the same study, some significant reductions in Fusarium wilt were observed following addition of cattle manure, chicken manure or compost amendments. However, effects were quite variable, not as pronounced in the second lettuce crop and in some cases an increase in disease was sometimes observed.

Table 8: Effect of biofumigants / soil amendments for control of *F. oxysporum* f. sp. *lactucae* on lettuce. Data shown are mean percentage reduction in disease index and the range, compared to an untreated control treatment (adapted from Gilardi *et al.,* 2016b).

Treatment	Product	Disease reduction cycle 1 (%)	Disease reduction cycle 2 (%)
B. carinata pellets	Biofence	**68** (53-80)	**62** (42-84)
Brassica flour	Biofence 10	**51** (43-61)	**48** (26-72)
B. juncea plants	ISCI99	**39** (0-71)	**27** (0-50)
Cattle manure	-	**35** (0-65)	**15** (0-46)
Chicken manure	-	**25** (0-43)	**24** (0-35)
Compost	-	**51** (38-67)	**31** (11-42)

Phosphite-based fertilisers have also been tested for their effect on FOL. Work from Italy has shown that addition of potassium phosphite (applied as a foliar spray in a high volume of water) resulted in a 69% reduction in disease severity under glasshouse conditions (Gilardi *et al.,* 2016a) and an increase in yield. Phosphite based products can induce plant defences, as described for fosetyl-aluminium, a phosphite-based product sold as a fungicide. The role of phosphites in disease control has been documented and control has previously been reported for a range of pathogens including *F. oxysporum* (Guest & Grant 1991). If combined with an effective fungicide, it is likely that disease control will be greater than with the phosphite alone, as was shown to be the case for controlling stem rot of rice (Martínez 2016). However, the use of such products is controversial as phosphites do not supply phosphorous to the plant, do not have a beneficial effect on healthy plants and should not be applied when phosphorous levels are sub-optimal. In addition, a negative impact on lettuce growth was reported when phosphite was added to a hydroponic system (Thi Bich Thao *et al.,* 2009).

10. Risk of FOL to outdoor and hydroponic production

Although FOL race 1 is prevalent in outdoor lettuce in the USA and other countries including some in southern Europe, FOL4 has not yet been reported in outdoor lettuce. However, this risk must be taken seriously as there is some initial evidence that FOL4 is active at lower temperatures than FOL race 1 and infection has been observed under glass in December in both the UK and the Netherlands (see section 2.5). Hence, outdoor growers should consider hygiene measures as discussed in section 3, in particular preparing risk assessments to cover possible routes to infection (e.g. seed, planting material, lettuce product imports, packhouse waste, soil from visitors, footwear etc). The fact that outdoor production is less intensive and often involves some rotation may be enough to prevent build-up of FOL inoculum in the soil.

Dutch lettuce growers who have recently switched to hydroponic production following problems with Fusarium wilt in soil-based systems have not so far have had any further problems with FOL (J. van Kuijk, Enza Zaden, personal communication) but the disease has been reported in hydroponic production in Asia (Chinta *et al.,* 2014; Thongkamngam & Jaenaksorn 2017). Treatment with a non-pathogenic *F. oxysporum* (as a root dip) in this system can decrease incidence and severity by 60-80% (Thongkamngam & Jaenaksorn 2017) although there is no such registered product in the UK. Success of disease-free hydroponic systems relies on good sanitation and water treatment protocols.

Knowledge gaps / suggestions for future research

Pathogen Biology

- The level of inoculum and temperature range at which FOL4 causes disease is unknown. Simple experiments could be carried out to determine the effect of inoculum dose and soil temperature on disease development.
- Limited information is available on how FOL colonises other crop plants. A study on the colonisation of rotation crops relevant to the UK and subsequent effects on soil inoculum levels would therefore be beneficial.

Monitoring / Detection

- Ongoing monitoring of FOL outbreaks and race identification (using both plant and molecular tests) of FOL isolates in the UK will be required throughout 2018 and beyond. It is assumed that all further outbreaks will be caused by FOL4 but this requires confirmation.
- It is currently unknown if lettuce plants become infected with FOL4 at the propagation stage; hence some testing of seed and transplants may be beneficial. In addition, it would be useful to investigate the potential efficacy of biocontrol agents if applied at the propagation stage prior to planting in infested soil
- The development of a FOL4 specific quantitative PCR test would be greatly beneficial for soil and plant testing.

Hygiene

- Whilst the utility of disinfectants can be inferred from work on other *F. oxysporum* f. spp., it may be beneficial to test some disinfectants (particularly quaternary ammonium compounds) against FOL4, if possible using chlamydospores.
- The efficacy of different methods for sterilising trays warrants further investigation.

Seed health

- Seed is not always tested for FOL. Whilst the significance of seed infection on disease epidemics is unclear, a large-scale test of a range of commercial seedlots would be beneficial.

Control during propagation and production

- Screening for resistance to FOL4. Whilst some initial resistance has been identified, a comprehensive screen of genetically diverse lettuce lines is required. Host resistance would provide the best control option for FOL4.
- Development of a reliable plant infection assay for testing control options. The best products / lines showing could then be tested at an infested site. Artificial inoculation of a greenhouse / polytunnel is also possible as developed for other diseases at Warwick.
- The majority of control options in this review have been tested against FOL race 1 or other *F. oxysporum* strains. A comprehensive test of all potential control options against FOL4 is still required. For chemical and biological options, timing, rate and mode of application need further investigation. Control options will be tested as part of the AHDB SCEPTREplus programme.
- Any test of control agents should include treatment at the propagation stage to see if this can protect plants that are transplanted into soil infested with FOL4.
- The utility of combining soil disinfestation with biological / chemical control should be tested.

Conclusions

Crop monitoring, early diagnosis and treatment are vital aspects of effective FOL disease management. Currently, FOL4 has only been reported in glasshouse-grown lettuce, but the risk to outdoor lettuce production must be taken seriously as race 4 is known to be active at lower temperatures. Conversion to a hydroponic growing system dramatically reduces the risk of Fusarium wilt assuming that good hygiene levels are maintained, but occasional problems are still possible and such a drastic conversion may be impractical and too expensive for long-established growers. Unless options for effective soil disinfestation are improved, the long-term sustainability of high intensity lettuce production in soil where FOL has been introduced is questionable, especially as high-level resistance has not yet been identified in butterhead lettuce types. Sources of FOL resistance are available in other lettuce types (e.g. romaine/little gem) and should be introgressed into the butterhead types in the future, hence providing an additional component to an integrated control programme. Regardless of whether the disease has been seen, changes in cropping practices and diversification may help reduce the risk of future infection. Reducing the intensity of cropping and / or introducing rotation crops should also mitigate against build-up of FOL in the soil. Growers should consider not growing lettuce in the warmest summer months when disease risk is at its highest.

There is no single solution to managing Fusarium wilt of lettuce and essentially there are different approaches that can be adopted depending on whether the disease is present or not. Growers who have not so far seen any signs of infection should review hygiene protocols for their business, be vigilant for disease symptoms (section 2.2) and follow strict hygiene procedures to keep the disease out (section 3), bearing in mind that infested soil is the most likely source of inoculum. Here, disinfection (heat or chemical) of equipment and structures is an effective if costly solution. If disinfectants are to be used then soil must be removed first as this greatly reduces efficacy; evidence suggests that quaternary ammonium compounds (e.g. Unifect G) are the most effective products for controlling *F. oxysporum*.

Seed / transplant health is another crucial component for avoiding Fusarium wilt. Therefore, treating seed with fungicides may provide some insurance against any potential infection. However, lettuce seed for UK production is not routinely treated with fungicides. Research has shown that treating lettuce seeds with thiram, which is approved in the UK, can help control FOL (race 1) and this should be considered as a viable option. Treatment of seeds with biological agents (pending future approvals) may also be possible although their efficacy is lower than for chemical treatments. Some seed companies will test seed for FOL and other

pathogens and growers / propagators should now be asking seed companies to provide details of any routine testing. Whilst FOL can be transmitted on seed, the epidemiological significance of this is unclear and contaminated soil is likely to be the cause of spread within a country.

Where Fusarium wilt is already present (and identification of FOL confirmed) an integrated management strategy may provide the most effective control using a range of approaches (sections 4-9). Following high levels of infection, soil disinfestation will be required to substantially reduce FOL inoculum in the soil between crops. This can be achieved through chemical fumigation, steaming or biological disinfestation. Using a combination of chemical fumigants, biological control agents and potentially soil amendments, such as *Brassica carinata* (Biofence), may be beneficial although such combination treatments are largely untested. Increasing soil pH may also reduce disease incidence although this has not been proven for FOL. FOL infection is worse in the summer months and as such, growers may need to leave a badly infected glasshouse fallow at this time of year because the alternative of growing a rotation crop may not sufficiently reduce the levels of FOL in the soil. Use of chemical and biological treatments as protectants either at propagation or following transplanting is unlikely to be effective alone but may contribute to overall disease management. While this review has highlighted a range of products that show potential, there is a need to test these for efficacy against FOL4, investigating variables such as timing and mode of application, and background inoculum levels.

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Appendix 1

Table of contacts in addition to growers and propagators

Name	**Company**
Martin Kyte	Rijk Zwaan
Gerard van der Hut	Rijk Zwaan
Johan Schut	Rijk Zwaan
Daniel Ludeking	Rijk Zwaan
Brian Penaloza	Nunhems (Bayer)
Alan Cresswell	Enza Zaden
John Johnson	Enza Zaden
Matthieu Pel	Enza Zaden
Jan van Kuijk	Enza Zaden
Gerbert Hiddink	Enza Zaden
Liz Johnson	L J Technical Consultancy
David Balshaw	County Crops (Procam)
Stephen Alexander	Teagasc
David Norman	Fresh Produce Consultancy Ltd
Alan Horgan	Certis
Steve Williams	Aromany
Henk Meints	Thatchel
Lynne Matthews	BASF
Tim Lacey	Bayer
Giovanna Gilardi	University of Torino
Maria Gullino	University of Torino
Thomas Gordon	UC Davis
Isabel Vandevelde	Proefstation (Belgium)
Tim O'Neill	ADAS Associate Plant Pathologist
Michael Matheron	University of Arizona