Grower Summary

CP17/18-1006

Technical review on lettuce Fusarium wilt, caused by Fusarium oxysporum f. sp. lactucae

Final report, February 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.

<u>Actions for propagators</u>

- 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)	Applied directly to pots in a controlled experiment

		Field trials in USA showed no effect of fludioxonil against FOL race 1	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 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. 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*