Grower Summary

FV/PE 458

Lettuce: biology and management of Fusarium wilt caused by *Fusarium oxysporum f. sp. lactucae* race 4

Final Report 2020

Project title:	Lettuce: biology and management of Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lactucae* race 4
Project number:	FV/PE 458
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The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

John Clarkson

Reader

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Signature  Date: 18/04/2020

GROWER SUMMARY

Headline

Specific molecular diagnostic tests were developed for Fusarium wilt of lettuce and critical levels of pathogen inoculum and effects of temperature on disease defined. A range of disinfectants and heat treatments were identified that killed *Fusarium* spores.

Background

Fusarium wilt of lettuce caused by the soilborne fungal pathogen *Fusarium oxysporum* f.sp. *lactucae* (FOL) results in severe losses in production areas globally. Four races occur, with race 1 (FOL1) being the most widespread in the field and protected crops particularly in warmer parts of the world such as the USA and Italy. FOL was first reported in the UK in 2017, and identified as the recently emerged FOL race 4 (FOL4) first reported in the Netherlands in 2013. So far, FOL4 has been restricted to protected lettuce and has not yet been identified as causing disease in the field. Control of FOL4 is challenging, as like all *F. oxysporum* f.spp., it produces chlamydospores which survive for long periods of time in the soil which makes management using fungicides or biological control agents difficult. Currently there is no varietal resistance widely available for the lettuce types grown under protection although breeding companies have begun the limited release of new resistant or partially resistant cultivars. Consequently, FOL4 represents a significant threat to the UK lettuce industry. Crop hygiene is therefore very important to prevent further local and regional spread. This project was undertaken in response to an AHDB research call to investigate aspects of the biology and management of FOL4 which followed an extensive literature review to identify gaps in the knowledge base (AHDB project CP17/18-1006; Taylor & Clarkson, 2018).

The main aims of this project were to i) develop molecular tools for rapid FOL4 identification and quantification to help monitoring of further outbreaks and understand key aspects of pathogen biology such as infection, colonisation and survival, ii) understand key factors affecting FOL4 disease development including inoculum level and temperature and iii) identify hygiene (heat and disinfectant) approaches to limit pathogen spread. The specific project objectives were to:

1) Develop tools for molecular detection and quantification of FOL / FOL4
2) Determine the effect of temperature and inoculum level on FOL4 disease development and the impact of non-hosts / fallow on FOL4 survival.
3) Test hygiene measures to eliminate FOL4 inoculum

Summary

Develop tools for molecular detection and quantification of FOL/FOL4

Objective 1.1 Test LAMP assay for FOL / Objective 1.2 Develop and test qPCR for FOL

Genome sequencing of isolates of FOL1 and FOL4, identification of pathogenicity genes and comparison with other *F. oxysporum* genomes was carried out by NIAB-EMR to identify target genes for diagnostic tests. Primers gene specific to FOL (both FOL1 and FOL4) were then designed for use in both LAMP and qPCR assays while further sets of primers were designed for use in a qPCR assay to specifically detect FOL4. The new LAMP assay was highly specific to FOL, unlike a previously published test which resulted in some non-specific detection of other *F. oxysporum* f.spp. The qPCR primer pairs targeting g23490 and g19968 genes were highly specific to FOL4 and FOL (FOL1 / FOL4) respectively. Both the new LAMP and FOL4 qPCR assays also successfully detected FOL4 in artificially inoculated diseased lettuce plants as well as in soil infested with the pathogen. Both tests have also since been used to confirm presence of FOL4 in lettuce samples from growers as part of ongoing monitoring of FOL4 in the UK at Warwick. These molecular tools will be valuable not only for diagnostics and soil tests, but also for determining FOL4 dynamics and understanding key aspects of pathogen biology such as infection, colonisation and survival. The LAMP assay also detected FOL4 in diseased plant material in approx. 7 min following a crude 5 min DNA extraction allowing for very rapid disease diagnosis. The Genie II LAMP machine also has the advantage of being portable and could therefore potentially be used at grower sites to quickly identify FOL4. These tests could be made available to growers through commercial companies already specialising in molecular diagnostics such as FERA Science Ltd and Eurofins.

Objective 1.3 Assess FOL viability in relation to molecular tests

In a preliminary test, DNA from dead FOL4 spores was shown to rapidly degrade in a sandy soil in just a few days, suggesting that if molecular tests such as LAMP or qPCR were used to monitor pathogen levels in soil, then they would most likely only detect live spores. DNA survival can be enhanced through binding to clay minerals, larger organic molecules, humic acids and other charged particles and compared with clays, sand has been found less effective in binding DNA. Therefore, further work would be required to determine if there are differences in FOL4 DNA survival in different soil types.

Objective 2: Determine effect of temperature and inoculum level on FOL4 disease development and impact of non-hosts and fallow on FOL4 survival

Objective 2.1 Determine the effect of inoculum concentration on FOL4 disease development

A clear relationship was established between concentration of FOL4 inoculum and Fusarium wilt development in lettuce grown in both compost and soil with critical levels of 1 x 10^4 cfu g^{-1} and 1 x 10^5 cfu g^{-1} respectively, required to cause substantial disease. The greater amount of FOL4 inoculum required to cause disease in soil could be due to some suppressive activity of the resident microbial community. Further work is now required to directly relate soil FOL4 inoculum levels and disease development with qPCR results as has been recently done for *F. oxysporum* f.sp. *cepae* on onion (AHDB project P1908312; Clarkson, 2020). This will allow results of soil tests to be directly related to risk of Fusarium wilt development in lettuce.

Objective 2.2 Determine the effect of temperature on FOL4 disease development

Although there was little difference between growth of FOL1 and FOL4 isolates on agar at different temperatures, high levels of Fusarium wilt disease developed between 12 and 28˚C when lettuce plants were inoculated with FOL4 but this only occurred at 28˚C for FOL1 (Figure A).

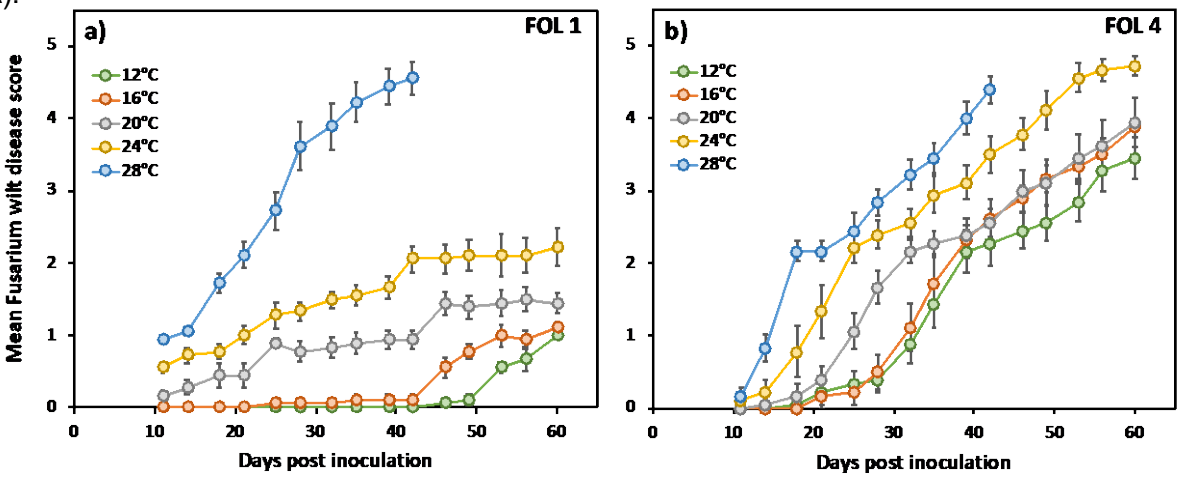

Figure A. Effect of temperature on Fusarium wilt disease development for lettuce plants inoculated with FOL1 and FOL4. Error bars represent the standard error of the mean (SEM).

However, the rate of FOL4 disease development in lettuce increased at higher temperatures and anecdotal evidence from growers in the Netherlands and UK does suggest that Fusarium wilt due to FOL4 is more damaging in Summer. Hence, affected growers have resorted to growing lettuce only in the cooler months of the year. Nonetheless, the ability of FOL4 to cause disease at low temperature has been observed in UK protected lettuce grown in Lancashire in December 2017 (transplanted in October) where air temperatures were 8°C (Taylor & Clarkson, 2018). This raises the possibility that FOL4 could cause disease in

outdoor lettuce, and hence growers should be vigilant, although the less intense production system with the rotations employed may help prevent build-up of FOL4 inoculum in the soil. Further work is required to confirm the effect of temperature on FOL1 and FOL4 disease development across a range of different isolates.

Objective 2.3: Evaluate the impact of fallow and non-host cropping on FOL4 survival

The survival of FOL4 in fallow soil from an artificially inoculated polytunnel was assessed. Immediately following a summer lettuce crop (Crop 1) with a high incidence of Fusarium wilt, FOL4 was detected at an elevated level in the soil by qPCR but this declined by 96% three weeks after harvest of a following winter crop (Crop 2) with low disease. Thereafter, FOL4 inoculum in the soil remained relatively stable up to 10 months and this was confirmed by parallel tests using dilution plating where levels were approx. 10^3 cfu g^{-1} soil for most of this period. This is in principal below the threshold required to cause substantial Fusarium wilt based on our experiment examining the effect of different FOL4 inoculum levels on disease development. Nonetheless, it would be anticipated that successive lettuce plantings would quickly increase this level of FOL4 inoculum allowing the critical concentration for substantial disease development to be reached. It is likely that survival of FOL chlamydospores will vary with soil type, moisture level and resident microbial community. The soil in the FOL4 infested polytunnel in our experiment was left to dry out during the fallow period which may have prolonged spore survival as it has been shown that chlamydospores of *F. oxysporum* f.sp. *melonis* can survive for 17 years in dry soil stored at 3-4 °C (McKeen & Wensley 1961).

The potential colonisation of rotation / alternative crop plants by FOL4 as assessed by qPCR was also investigated. Here, high levels of the pathogen were detected in roots of inoculated susceptible lettuce,1695-6483 picograms (pg) FOL4 DNA mg^{-1} root, with reduced levels in resistant cultivars (15-467 pg FOL4 DNA mg^{-1} root). Lower FOL4 levels were detected in the roots of all other crop plants tested, with mizuna, pak choi, chard and cucumber most conducive to pathogen colonisation (20-34 pg FOL4 DNA mg^{-1} root). This suggests that these other crops can sustain FOL4 populations. In the Netherlands, a grower reported that lettuce grown between pak choi crops showed no reduction in Fusarium wilt disease (Taylor & Clarkson, 2018), confirming the results here that this crop can be colonised by FOL4. However, initial observations in Lancashire showed that after two crops of pak choi, Fusarium disease incidence was greatly reduced with only around 5% losses observed (Taylor & Clarkson, 2018). Further work is therefore required to understand these reported inconsistencies. Our results also indicated that even when resistant lettuce cultivars are grown, FOL4 can still colonise roots and levels were higher than for the other crop plants. Overall therefore, growers should be cautious about how useful ‘break’ crops and resistant lettuce cultivars can be in reducing FOL4 inoculum.

Objective 3: Test hygiene measures to eliminate FOL4 inoculum

Objective 3.1 Test disinfectants for activity against FOL4 chlamydospores *in vitro*

All the disinfectants evaluated in this project (Jet 5, Unifect G, bleach, Huwa-San, Distel, Virkon and Disolite) effectively killed FOL4 chlamydospores at 100% and 50% of the manufacturers recommended rates in as little as 1 min exposure time and are therefore all potentially useful for maintaining hygiene and eliminating the pathogen from trays, equipment work surfaces etc and for foot dips. However, the efficacy of these products in the presence of large amounts of soil or organic matter which can limit efficacy was not assessed. Nevertheless, results largely confirm those from previous studies with other *F. oxysporum* f.spp. Overall, a range of products effective against FOL4 are therefore available to growers, allowing a choice to be made based on cost and situation. However, further work is needed to confirm their efficacy in the presence of soil and organic matter which potentially contaminates propagation trays and foot dips etc. Ideally however, growers should clean all equipment and surfaces before using disinfectants. Residues might also be an issue especially for quaternary ammonium compounds and it should be noted that the limit is 0.1 mg kg^{-1} for lettuce (Taylor & Clarkson, 2018).

Objective 3.2 Test heat treatments against FOL4 chlamydospores *in vitro*

Heat treatment is an effective way of killing FOL4 chlamydospores and our results showed that at 70˚C, spores in a suspension of water were killed within 5 min. The minimum heat treatment required to kill FOL4 spores was 60˚C for 15 mins although viability was only 4.7% after 1 min. Unlike chemical disinfection, heat treatments are unaffected by soil contamination and have no residue issues and would therefore be potentially useful for propagation trays.

Objective 3.3 Test disinfectant and heat treatments against FOL4 chlamydospores on polypropylene

Efficacy of all the disinfectants tested in this project at 50% of the manufacturers recommended rates was confirmed on polypropylene discs with FOL4 chlamydospores, confirming their utility for sterilising propagation trays. Heat in the form of steam was also effective at eliminating FOL4 from polypropylene but when using a jet of steam, it was demonstrated that it is important that the distance and duration of this treatment is sufficient to achieve >65°C for at least 1 min which will depend on the equipment used.

Conclusions

- Specific LAMP and qPCR based molecular diagnostic assays were successfully developed for FOL / FOL4 and utilised to identify and quantify the pathogen in diseased plants, roots and soil. These can now be employed to identify further FOL4 outbreaks and potentially carry out soil tests.
- Critical levels of FOL4 inoculum required to cause substantial Fusarium wilt in lettuce were determined in both compost and soil in artificially inoculated systems. Further work is now required to relate inoculum and disease levels to molecular quantification of the pathogen.
- FOL4 can cause substantial Fusarium wilt at temperatures as low as 12°C with more rapid disease development at higher temperatures up to the maximum of 28°C tested. In contrast, FOL1 only caused significant wilt at 28°C. Further work is required to confirm this across multiple isolates of FOL1 and FOL4.
- Following a lettuce crop with high levels of Fusarium wilt, there was an initial reduction in FOL4 inoculum in soil, but the pathogen then persisted for 10 months when the soil was left fallow. In this situation, it is likely that FOL4 disease would quickly increase if successive planting of lettuce crops were grown.
- A range of non-host crop roots can be colonised by FOL4 and of those tested, mizuna, pak choi, chard and cucumber were the most conducive to the pathogen. However, colonisation levels were variable.
- The disinfectants Jet 5, Unifect G, bleach, Huwa-San, Distel, Virkon and Disolite were all effective at killing FOL4 chlamydospores in solution, even at 50% of the manufacturers recommended rates and 1 min exposure time. However, efficacy in the presence of soil contamination still needs to be assessed.
- FOL4 chlamydospores in water were killed within 5 min when exposed to a temperature of 70˚C and within 15 min at 60˚C. Heat treatments are therefore an effective way of eliminating pathogen inoculum.
- All disinfectants applied at 50% of the manufacturers recommended rates killed FOL4 chlamydospores on polypropylene within 5 min. Steam jet treatments also eliminated FOL4 spores but temperatures >65°C for at least 1 min were required.

Financial Benefits

- None at this time

Action Points

Check lettuce plants for symptoms of FOL and cut suspect plants in half to look for typical vascular browning. If this symptom is observed, send intact plant samples FAO Alison Jackson, Warwick Crop Centre, University of Warwick, Wellesbourne, Warwick, CV35 9EF for confirmation. Early diagnosis is critical for limiting the spread of FOL.