



Grower Summary

**Integrated decision support tools
for management of downy
mildew in onions**

CP 184

Final report 2022

Project title: *Integrated decision support tools for management of downy mildew in onions*

Project number: CP 184

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Report: Final report, December 2022

Previous report: N/A

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Date project commenced: 01-04-2020

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

AUTHENTICATION

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

Paul Lansell

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Signature Date

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GROWER SUMMARY

Headline

Data collected during the project confirms that impaction spore samplers are as effective at trapping onion downy mildew spores as suction traps and could be used as a lower cost method for spore trapping, reducing equipment costs and time in kit maintenance and sample processing.

Evaluation of results derived during the study validate the use of LFD's to detect spore presence, and show the trend in spore numbers over time, however there are still concerns around the user friendliness of the LFD in its current form.

Results from the two years of sampling data indicate that where onions had previously been grown or were grown almost year-round, ODM spores were present at levels which could cause infection from early in the season.

Background

Downy mildew (*Peronospora destructor*) is a common disease that can result in major yield losses in bulb and salad onions and in onion seed production (Develash and Sugha, 1997) and crops may receive fungicide treatments as frequently as every 10 days. It is therefore crucial to have accurate and timely information around infection risk. Although the CropMonitor Pro (CMP) Platform by Fera Science Ltd on behalf of the Crop Health and Protection Centre now includes an onion downy mildew risk prediction module based on the MILLIONCAST algorithms (Gilles *et al.*, 2004), this module cannot predict the onset of spore presence. This means that although CMP can help guide spray timings once spores are present, currently growers may use in-field spore sample detection methods to pinpoint the onset of the epidemic. This spore sampling is expensive and time intensive so any cost savings that can be made would enable increased uptake of these methods.

Summary

This project aimed to validate the performance of two rotation impact samplers as a low-cost alternative to the more sophisticated suction traps for use in conjunction with a lateral flow device for detection of onion downy mildew (*Peronospora destructor*) spores to aid early detection of the pathogen entering onion crops. In year one, the three types of traps were tested in onion trials at four locations provided by G's. In year two, the SporeStick rotation sampler was tested at three locations provided by G's, with an additional site located at Fera

trailing all three traps. Further validation was undertaken by growers evaluating the ease of use of the different samplers as decision aids in the field. Results show both types of rotation impact samplers are as effective at trapping ODM spores as the Burkard suction trap. Since the initiation of the project, the OptiSense SporeStick sampler is no longer commercially available, however the Rotorod and GRIPS impact samplers are still able to be purchased. There remains a concern that in some cases the difference in spore numbers found on the two replicate spore sticks from either the Rotorod or GRIPS was found to be larger than the differences seen between the Rotorod and the GRIPS sampler. This could be a result of either inconsistencies in the sample collection or inability of the LFD to provide a quantitative measure of spore presence.

Data collected from field samples was intended to be used in modelling work to define spore thresholds more clearly for disease development in the crop, however the data generated meant this was not possible. All trial sites were hosted on an updated version of the CropMonitor Onion downy mildew module and results updated in real-time for use in local disease management decisions. Results from the trials sites were used for knowledge transfer activities to the wider industry, including presenting at relevant industry events (e.g. BOPA meetings) across the 2022 season.

During the early stages of the project concerns were raised about the suitability of the Global Access Diagnostics (GAD) (formerly MOLOGIC) Lateral Flow Device (LFD) assay for detection of *P. destructor* spores collected using rotation impact samplers due to potential interference with the coatings (Vaseline or silicone grease) used on the spore collection sticks. This led to the project being extended to also validate the LFD for use in this setting whilst simultaneously developing Loop-Mediated Isothermal Amplification (LAMP) and real-time Polymerase Chain Reaction (PCR) assays, which could be used as alternative detection methods if the LFD is proven to not be fit for purpose.

Results derived during the study validate the use of LFD's to detect spore presence with the limit of detection somewhere between 10 and 50 spores, with no effect of the media into which the spores were spiked, however at lower spore concentrations readings are more variable. Some concerns remain around the spore numbers detected by the LFD's from field samples, in particular the high counts seen from the Fera site in 2022 where numbers were higher than would be expected from the beginning of sampling. However, the peaks of spores detected did align with incidences of infection and sporulation which gives confidence in the LFD in detecting trends in spore numbers, if not with accurate spore counts.

There are still concerns around the user friendliness of the LFD in its current form. The LFD is provided as a dipstick rather than a fully housed test kit which could lead to problems with

contamination. The differences seen in cube readings if the LFD is analysed before or after the specified 15 minutes is also of concern.

Validation of the PCR and LAMP assays has been completed with primer sets developed for both assays capable of detecting a minimum of 10 spores in spiked samples. Results from testing on samples extracted from different media were highly variable by both testing methods with LAMP results showing little differentiation in time to a positive result between spore counts. When field samples were compared by both LFD and TaqMan® PCR, the LFD consistently recorded much higher spore counts. Disease sightings suggest that the LFD results are accurate and the PCR results are lower than would be expected. This may be due to the DNA extraction method chosen and could possibly be remedied by trialling alternative methods.

Results from the two years of sampling data indicate that where onions have previously been grown or are grown almost year round, ODM spores seem to be present in relatively high numbers from early in the season, at levels possible to cause infection. The conditions required for infection may explain why several sites in 2021 and 2022 did not record any disease despite having numerous occasions when spore numbers were high enough to produce infection. Monitoring of spore counts can give an indication of when spore numbers start to increase and peak against background levels (which, as seen in 2022 may already be high), giving an indication of increasing risk of infection should environmental conditions be suitable for infection.

Financial Benefits

The exemplar sampler which is the Burkard cyclone spore trap has a price point of £2,547 excl. VAT (excluding batteries), whereas the alternative samplers have a much lower price point with the SporeStick coming in at £600 excl. VAT (excluding batteries) and the GRIPS-99M coming in at £525 (+ shipping & excluding batteries).

In addition to the alternative samplers being cheaper to purchase they are believed to be easier to handle given that the weekly changeover of eight Eppendorf tubes in the Burkard cyclone would be replaced by changing over two matchsticks (or plastic sticks) coated in grease, resulting in a significant weekly time saving (approximately 20 minutes time saving per changeover event per trap).