Grower Summary

Downy Mildew & Late Blight Control

CP 184

Final report

Project title:	Downy Mildew & Late Blight Control
Project number:	CP 184
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The results and conclusions in this report are based on investigationc conducted over a one-year period in some cases. 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.

GROWER SUMMARY

Headline

Integrated management is now essential for the effective management of downy mildews on horticultural crops. Reported here is the final year of work on an ambitious project with several lines of investigation bringing together:

1) The development of new molecular tests to detect and quantify downy mildew pathogens in basil, spinach and column stock seed-lots;

2) Heat treatments were demonstrated to be effective at removing infections from contaminated seeds;

3) PMA-qPCR was 'road-tested' for testing seed for viable low-level downy mildew infections – whilst it works, it is very involved and only currently suitable as a very specialised laboratory method for detecting and quantifying low level infections;

4) Lettuce downy mildew race typing and fungicide resistance testing;

5) Updated information about the resistance of lettuce, spinach and column stock downy mildew populations to approved and widely used fungicides;

6) Reviews on the general biology and control of 14 horticulturally important downy mildews, the potential for using elicitors in integrated control and the current state of development of Decision Support Systems/Tools for the management of downy mildews maximise integrated management possibilities.

Background

The oomycetes are a large group of fungus-like organisms many of which have evolved to become pathogens of plants. A large and varied group of oomycete plant pathogens are spread by air-borne and/or water-splashed propagules and cause diseases primarily of the above-ground parts of plants are collectively known as the Aerial Oomycetes. Horticulturally significant pathogens within this group are the downy mildews (main genera in horticultural crops: *Peronospora, Hyaloperonospora, Pseudoperonospora, Plasmopara, Bremia*), stem rots, shoot diebacks and blight caused by *Phytophthora* spp. as well as shoot and leaf 'blisters' caused by *Albugo* spp.

Diseases caused by aerial oomycetes typically exhibit rapid epidemics, which if left unchecked under optimal environmental conditions have the potential to cause complete crop loss either directly by mortality, or by rendering foliar and fruit produce unmarketable. Disease control options are limited or under-utilised and currently management is heavily reliant upon the use of fungicides, often used prophylactically, as none of the available chemicals can reliably achieve curative control, and once disease is observable in crops it will often already have become established and difficult to manage. Unfortunately, the number of currently available fungicides is becoming very restricted as a result of product withdrawals and too few new introductions. The resulting reduction in the number of active ingredients being used in control programs greatly increases the risk of pathogen populations developing fungicide resistance. The use of resistant varieties, where available, is a good disease management option although their use puts huge selection pressure on oomycete pathogen populations for new races capable of overcoming host resistance. Cultural disease management methods (e.g. appropriate tilth management, removal/treatment of crop debris, manipulation of environmental conditions), often have a limited impact on disease when used alone but can greatly (even synergistically, e.g. control of {a non-oomycete with analogous epidemiology} *Botrytis* grey mould in ornamentals, O'Niell *et al*., 2002), increase the efficacy of chemicals and plant resistance in integrated management programs. Similarly, the use of rapid pathogen detection and disease simulation models can optimise the timing of fungicide applications and in some seasons reduce their number – increasing efficacy whilst reducing costs and potential environmental impacts.

The use of contaminated seeds is considered responsible for many outbreaks of downy mildews on basil and spinach, caused by *Peronospora belbahrii (Pb)* and *Peronospora effusa (Pe),* respectively. *Pe* is a seed-borne pathogen, producing heterothallic oospores in the seed coat (Kandel *et al.,* 2019) that cause systemic infection in the crop. The transmission of *Pb* on or in seed is less clear; only a single case of oospore production has been reported in basil (Cohen *et al.,* 2017) and most new disease outbreaks have been attributed to asexual aerial conidia and become evident only on relatively mature plants (Budge, Personal communication). *Pb* conidia have been observed in basil seed samples (Falach-Block *et al.,* 2019; Wood, 2021, personal communication) however, it is unlikely the propagules would remain viable for extended periods under unfavorable environmental conditions. Therefore, it is postulated that disease is propagated through mycelial infection inside the seed-coat (Jennings *et al.,* 2017).

Integrated pest and disease management (IPM) is an increasingly important and pertinent area of research for horticulture and this project aimed, through provision reviews and where possible new best grower practice information, consolidated current knowledge, ensuring that measures that can be taken up were quickly disseminated and potential barriers to uptake identified. The project built on current knowledge of several pathosystems (specifically downy mildew on lettuce, spinach and basil) to develop and validate the tools required for a long-term integrated approach to disease management. New tools for the genotypic analysis of

Bremia lactucae populations, linked to phenotypic characteristics such as 'race' and fungicide sensitivity, were developed to allow an understanding of population diversity to directly inform resistance deployment and breeding and fungicide stewardship to be greatly improved using an approach that has previously been highly successful for potato late blight (*Phytophthora infestans*, Ritchie *et al*., 2018). The other main strand of research focussed on identifying/verifying primary inoculum in spinach and basil by detection and viability-testing of seedborne infection to steer future integrated management both by improved quality screening and providing effective tools for assessing cultural controls. Here we report on the final year's progress, assessing pathogen races and progress with assessing molecular procedures for determining levels of viable downy mildew present in contaminated seed lots.

Summary

This project focussed on improving the possibilities for integrated management and developing best practice guides by: a) reviewing and collating information on potentially exploitable disease biology, on fungicides and elicitors still available (and any possibly in the future), and on disease forecasts and decision support tools (AHDB CP184 report 2019), b) developing and improving detection diagnostic procedures to screen seed for infection to help cut this significant source of disease (AHDB CP184 reports 2019, 2020 and here), c) developing molecular detection and quantitation of *Bremia lactucae* as well as consolidating and building on knowledge of markers for traits like fungicide resistance in *B. lactucae* populations (AHDB CP184 reports 2019, 2020 and here), and d) developing fungicide sensitivity test protocols to check pathogen populations for fungicide resistance (AHDB CP184 report 2019). The overall conclusions and findings of the project can be summarised as follows:

- Review has shown that more research is needed on elicitors, their interactions with specific pathosystems have to be further explored in time and space to maximise reliability and efficacy. Also, the impact that natural elicitors from various stresses have on crops impacts efficacy of applied materials (Walters *et al.,* 2013) – it is still uncertain whether elicitor applications provide consistent economic benefit when used on outdoor soil-grown crops exposed to natural elicitors (AHDB CP184 report 2019).

- Seaweed extracts benefit the plants in various ways, these benefits are small but can easily be used to help improve overall plant health. Phosphite has recently been registered in the EU as an active ingredient for plant protection having shown efficacy against oomycetes. It is still currently available as a component in many products that are sold as fertilisers or biostimulants, not as plant protection products. Chitin is another product that shows great promise as an elicitor, although it doesn't help to improve

nutrient uptake and as such will not be able to be included into the new EU fertiliser laws which cover biostimulants. Interestingly AMF have proven potential stimulating plant defences against soilborne pathogens, but their use against aerial oomycetes has not been explored (AHDB CP184 report 2019).

- Molecular testing was demonstrated capable of detecting and quantifying small amounts of basil, spinach and column stock downy mildew (DNA/RNA) both externally (in basil and spinach seed-lots) and inside contaminated seeds of basil, spinach and stocks (AHDB CP184 reports 2019, 2020 and here).
- LAMP primers have been designed for *Bremia* and have been successfully tested for specificity and efficacy in LAMP qPCR (AHDB CP184 report 2019).
- Seed-lots of basil, spinach and column stocks containing very low levels of downy mildew (DNA/RNA) still potentially pose a high disease risk to growers (AHDB CP184 reports 2019 and 2020 for basil and spinach, and here for column stocks).
- Across the seed-lots of basil and spinach tested, a greater quantity of downy mildew DNA was detected inside the seed coat compared to seed washings (AHDB CP184 report 2020).
- Steam-treated basil seed lots contained approximately 50% less downy mildew DNA than untreated samples from identical lots, indicating that steam-treatment reduces the pathogen load (AHDB CP184 report 2020).
- The downy mildew isolated from the column stock samples tested in this study was confirmed as *Pernospora matthiolae* by both morphological characters, and nucleotide sequence analysis, and basic phylogenetic study showed this to be more closely related to *Hyaloperonospora arabidopsidis* than *H. parasitica/H. brassicae* (AHDB CP184 report here).
- Quantitative PCR was conducted on commercial *Matthiola incana* seed lots suspected of containing downy mildew contamination, using a novel assay designed for the project. This is the first time a diagnostic assay has been described for the detection of *P. matthiolae* in seed (AHDB CP184 report here).
- Thirty-nine lettuce *Bremia lactucae* isolates were collected from 2019-2021 and assessed for race structure according to IBEB guidelines and protocols kindly supplied by Naktuinbouw, who also supplied seed of the 16 current accessions in the official lettuce differential set (Set C). Twenty-eight putative races were identified and of these, one (2020_BL4G) matched IBEB committee race description Bl:24EU, whilst four others (2019_BL2A, 2019_BL2B, 2021_BL11A & 2021_BL11C) matched IBEB race Bl:35EU.

Comparisons of this data with publicly available data will continue after the end of this project, whilst race testing results for all of the isolates tested from 2019 to 2021 were included in the IBEB EU Groslist 2021 (AHDB CP184 reports 2019, 2020 and here).

- Viability qPCR using Propidium Monoazide PMA has been demonstrated to be effective in detecting differences in the amount of DNA in live and dead cells from *P. belbahrii* and *P. effusa*. This procedure can be used effectively to determine the presence of viable/no-viable cells of *P. belbahrii* or *P effusa* in infected seed-lots, although research in this project as shown that control assays are needed to account for the internal effects of heat and of PMA addition which require the generation and use of live spores for every test, necessitating a lot more preparation than originally anticipated, and therefore higher costs when compared to reverse transcription qPCR methods that target RNA/active gene expression (AHDB CP184 reports 2020 and here).

- Fungicide resistance tests at JHI were restricted by the availability of fresh isolates from current epidemics. Nevertheless, tests were successfully carried out in 2019 and 2021 on *B. lactucae* isolates from lettuce, and on *P. matthiolae* from column stocks and in 2021 on *P. effusa* isolates from spinach (AHDB CP184 reports 2019 and here).

    - Fungicide resistance tests on *B. lactucae* isolates obtained from the industry in 2019 and 2021 showed that:

        - Mandipropamid (Revus) gave consistently high levels of disease control at field rate (99-100%) across all isolates tested.
        - Dimethomorph (95-100%) and Azoxystrobin (90-100%) also showed good control of lettuce downy mildew at field application rates.
        - These tests were carried out for the calculation of EC50 values for longer-term reference and use alongside pathogen race identifications, some large differences in the min and max values were noted for some isolates, particularly for Dimethomorph and Azoxystrobin. These individual values need to be treated with caution – their true value/meaning will only emerge with ongoing longer-term screening and EC50 calculations.

    - Fungicide resistance testing carried out on *P. matthiolae* isolates by JHI in 2019 and 2021 had the following outcomes:

        - Widespread resistance to Metalaxyl was found across a range of isolates in 2019 with no further testing therefore carried out

- Fosetyl-Aluminium gave moderate disease control (28-68%) in 2019 and 2021
- Mandiprompamid gave better control in 2019 (80-86%) than 2021 (52-82%)
- The tests suggest resistance to Dimepthomorph may be developing given the 80-90% control found in 2019 dropped to 10-15% in 2021
- Amectotradin/Dimethomorph and Mancozeb gave moderate and variable control of 34-66% and 15-65% respectively in 2021

- Fungicide resistance testing at JHI on P. effusa isolates in 2021 showed that:
  - When applied at field rate, Mandipropamid and Dimethomorph gave 100% control
  - Azoxystrobin applied at field rate gave excellent control between 99-100%.
  - Calculation of EC50 showed small differences between isolates' reactions to all 3 products tested but these differences were not shown to be statistically significant, showing control in the small population of P effusa tested so far to be consistent.

- Review of general downy mildew biology and of Decision Support Tools (DST) to assist for their integrated management in selected key horticultural crops shows that a range of options are currently available, from zero through simple risk rules to sophisticated simulations and forecast models as well as new possibilities of affordable molecular inoculum detection to further refine precision of risk assessments. Combinations of forecasts and inoculum detection are improving the accuracy and potentially the timing of risk warnings which is important as the range of fungicides available for control of downy mildews are predominantly of protectant action with the few curative chemistries available carrying medium to high fungicide resistance risks (AHDB CP184 report 2019).
- Review also indicates the possibility of applying simple rules-based DST or even some adapted forecast models to minor crops could be effective at supporting IPM and that the main influences on uptake of DST in general where the perceived high risks and more importantly the degree of 'user-friendliness' of the operational front ends of systems (AHDB CP184 report 2019).

Financial Benefits

Aerial oomycete infections significantly reduce crop yield, with those affecting plants in propagation, in particular, able to cause total crop loss, and those in ornamentals potentially causing the crop to become unmarketable (Wedgwood, *et al.,* 2016). Timely intelligence concerning prevalent phenotypes present in downy mildew populations has potential for significant financial benefits in terms both of managing fungicide resistance and the deployment of cultivars with suitable resistance genes. In addition, the detection and interception/treatment of infected seeds is likely to have a large impact on downy mildew incidence in crops such as basil, whilst the effective use of cultural controls and decision support systems could both reduce the frequency of spray applications, improve their efficacy and reduce the pressure selecting for new pathogen genotypes with fungicide resistance and/or capable of overcoming cultivar resistances.

Action Points

- Keep vigilant for the appearance of fungicide resistance and avoid use of metalaxyl for mildew control in column stocks
- Use Fosetyl-Aluminium and Amectotradin/Dimethomorph with caution especially in column stocks and as part of a spray program ‘ringing the changes’ with products such as Mandipropamid
- Consider use of Decision Supports to help with development of integrated control programs for downy mildew even if these are just based on simple ‘rules of thumb’
- Read the review summaries for basil, column stock, lettuce and spinach downy mildews for insights into how to update IPM strategies
- Consider requesting steam-treated seed to help manage the risk of infections starting from seed in basil
- Following the foundation work at James Hutton Institute from this project, race testing will be delivered commercially via an industry consortium and the genotypic profile of *Bremia* isolates could be monitored using the tools developed. This information will inform lettuce variety selection for more effective control of downy mildews. Contact JHI for further information on latest developments and testing possibilities..