Downy Mildew Control

Supplementary information to project CP 184

Fourteen downy mildew diseases were selected for review in the AHDB project CP 184. Two of these diseases have been studied in some detail: *Bremia lactucae* downy mildew in lettuce and *Peronospora destructor* downy mildew in onions. Of the remaining twelve diseases, some detailed information was only available for mildews in spinach, basil, rose and tomato (*Phytophthora infestans*; not actually a downy mildew, but selected for consideration in this project as an aerial oomycete), and to a lesser extent, peas, stocks, aquilegia, impatiens and hebe, whilst very little specific biological information of use for disease management (IPM/DSS) was found for downy mildews of geum, viola and parsley (see Table 1 in CP 184 yr1 report, Pettitt, Lees, Wood, Wedgwood, 2020, page 6).

The overall project was focussed on the downy mildews of lettuce, basil, spinach, and column stocks. Peas and tomato were originally considered but no worthwhile fungicide resistance work could be done in peas because of severe limits on available active ingredients and no isolates of *P. infestans* in tomatoes were sent in from the industry for assessment after the 'unusual' 2018 epidemic (Pettitt *et al*., 2019 Atypical late blight symptoms following first recorded infections by Phytophthora infestans genotype EU_39_A1 in UK vine tomatoes (ndrs.org.uk)). Column stocks were substituted instead since i) a range of fungicides were still appropriate for testing on stocks and ii) suitable seed-lots of stocks could be made available for DM contamination testing by PCR and box tests.

This document provides crop-based summaries of information emerging from CP 184 which updates on previous AHDB guidance for Downy Mildew management in Basil, Spinach, Column Stocks and Lettuce along with a summary of current decision support systems and future options to assist with crop management for downy mildew control.

Downy Mildew of Basil (*Peronospora belbahrii*)

Since publication of AHDB Factsheet 18/11 further information has become available:

Downy mildew species are generally highly specialised, only affecting one or a limited number of host species. Whilst generally favoured by 'cooler' temperatures, high humidity and plant surface wetness, individual Downy mildew species do still vary in their speed of infection and temperature and moisture requirements which can mean that the amount of practically useful information available for the management of individual species can be limited, especially those affecting minor crops. Nevertheless, species can be grouped according to their response to environmental factors. Basil DM has similar requirements to Lettuce DM (*Bremia lactucae*), including a similar capacity to rapidly proceed from sporulation to infection in a single day cycle given the right sequence of conditions. Baring this in mind, it may be worthwhile considering adaptation of a disease forecast system developed for lettuce such as BEMCAST for use in decision support with Basil crops.

Table S1: Comparison between Basil and Lettuce Downy Mildews of effects of environmental factors on infection and downy mildew disease

	Survival of spores (conidia)	Latent period	Infection		
			Leaf wetness duration	Optimum Temperature	Minimum RH%
Basil DM (*P. belbahrii*)	<3 days	5-10 days	≥4h	5-28°C	85%
Lettuce DM (*B. lactucae*)	>12h	7-9 days	>2h	5-23°C	85%

- Basil DM spread via seedborne infection is considered to be highly likely
- In simple 'box tests' of seed, 1 seed in 13000 produced spores and this level of contamination is capable of rapidly initiating widespread disease in a crop
- Symptoms are now reasonably straightforward to detect and identify, but by the time symptoms are seen it is too late for effective disease management
- Basil DM inoculum is readily detected in seed by PCR techniques – although proportions of contaminated seed present are generally very small (0.02 – 1.4%) they are still able to initiate serious outbreaks
- No tough oospores detected in seed so far, so inoculum in/on seed is potentially relatively fragile (and therefore possibly treatable)
- Standard PCR tests are unable to distinguish between viable/non-viable inoculum but a new PMA-PCR technique appears capable of viable pathogen detection, so it may be possible to test efficacy of seed treatments. Nevertheless, the technique requires a lot of work and internal controls that make it unwieldy and potentially costly for routine screening purposes
- Sensitivity of Basil DM spores to drying show that night-time drying cycles could have big impact on spore survival and help slow/stop outbreaks.

Downy Mildew of Spinach (*Peronospora effusa*)

Information gathered about P. effusa has largely originated from review as well as fungicide resistance tests and detection of seedborne infections by DNA-based tests.

- *Peronospora effusa* in spinach has recently been the subject of a scientific review (Kandel *et al*., 2019 Spinach Downy Mildew: Advances in Our Understanding of the Disease Cycle and Prospects for Disease Management | Plant Disease (apsnet.org)) and whilst there is much generic information presented, this review shows that there is still very little basic data (such as optimum infection temperatures) available specific to *P. effusa*

- The presence of oospores on spinach seeds was demonstrated in the 1980s, but recently has received more serious scrutiny as a potential source of primary infections and new disease outbreaks

- Little is published about sporulation of *P. effusa*, although the optimum sporulation conditions for the closely related species *P. farinosa* recorded on sugar beet as 12°C and RH of ≥85% (for a review on *P. farinosa* check AHDB report CP 157 pp 56-57)

- Fungicide resistance tests were carried out on UK *P. effusa* isolates in 2021. The fungicides tested were Mandipropamid, Dimethomorph and Azoxystrobin. All three fungicides gave high levels of control when applied at 'field rates'; Mandipropamid (100%), Dimethomorph (100%) and Azoxystrobin (99-100%). Calculation of EC_{50} 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.

- As with P. belbahrii in basil, standard PCR tests on spinach are unable to distinguish between viable/non-viable inoculum but a new PMA-PCR technique appears capable of viable pathogen detection, so may be possible to test efficacy of seed treatments. Nevertheless, the technique requires a lot of work and internal controls that make it unwieldy and potentially costly for routine screening purposes.

Downy Mildew in Column Stocks (*Peronospora matthiolae*)

Since publication of Factsheet National Cut Flower Centre/ AHDB Horticulture Information Sheet 11 (Maintaining successful control of downy mildew in protected crops of cut flower column stocks VB2318 Downy mildew fact sheet web.pdf (windows.net) also check out Downy mildew control recommendations for column stocks reinforced | AHDB), further information has become available much of it verifying or supporting the findings in this publication:

- No pre-existing DNA-based assays were available for mildews in Matthiola. Species seen in material sent to NIAB appeared more like *Peronospora matthiolae* than *Hyalospora parasitica* based on lesion appearance and sporulation characteristics. Disease DNA was sequenced confirming *P matthiolae* identification.
- The sequence data used to confirm *P matthiolae* identification was also used to design primers for *P. matthiolae*-specific PCR and these were used in project CP184 to develop a new sensitive qPCR test for *P. matthiolae*. This test was successfully used to test six Matthiola seed-lots. The assay detected low levels of *P. matthiolae* DNA in all six seed-lots, all of which were later verified as having disease arising from them.
- There is still a problem with determining whether DNA detected in seed-lots represent live or dead pathogen and a viability qPCR using propidium monoazide to block amplification of non-viable DNA was successfully developed in CP184. Unfortunately, there is a need for complex internal controls for this teat including the need for live mildew spores and this makes the test as it stands unwieldy and uneconomic for routine screening
- 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

Downy Mildew of Lettuce (*Bremia lactucae*)

O'Neill, T (2019) Diseases of lettuce crops. AHDB, pp.6-7 (LettuceDiseases1846_190109_WEB.pdf (windows.net)) provides excellent basic background information and descriptions of this important disease.

- Fungicide resistance tests were carried out at JHI on *B. lactucae* isolates obtained from the industry 2019-2021. These tests 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 used 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.

- The work in CP184 has established a foundation for the development of more informed pathogen marker-based race testing which will need further development.
    - 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 are ongoing, whilst race testing results for all of the isolates tested from 2019 to 2021 were included in the IBEB EU Groslist 2021.
- These findings are strongly linked with parallel AHDB-funded PhD project: Integrated management of lettuce downy mildew (CP 186 https://archive.ahdb.org.uk/integrated-management-of-lettuce-downy-mildew-cp-186), where the identification of pathogen races using genetic markers, which would obviate the need for time-consuming seedling tests, is being investigated.

Decision Support Systems/Tools (DSS/DST) for managing downy mildews

Background and application

Decision-support systems in integrated pest and disease management (IPM) are tools designed to help decide which pest/disease management options to employ when and to make (spray) decisions. For effective IPM some form of decision support is essential. DSS are concerned with wider pest and disease management situations whereas currently most decision supports for downy mildew diseases currently act as 'stand-alones' focussed solely on downy mildew and so would be more appropriately referred to as 'tools' or DST. Effective DST developed for downy mildews range in complexity from simple risk matrices (AHDB Reports HNS 196a & PE 024) which, whilst not giving spray advisories, do provide realistic estimates of when disease risks are low giving the basis for safer protected environment management protocols, to models that contain sub-routines predicting sporulation, inoculum concentration, infection, and latent periods in relation to meteorological data and using meteorological forecasts to predict disease risks and even provide spray advisories. Regardless of this intricacy, it is always important to remember that DSS/DST are risk predictors and should never be used in a prescriptive way but as Xiangming Xu (AHDB Report HNS 165 & HNS 173) puts it; '[they should be used] more in the way of a weather forecast – when rain is forecast it is up to you – are you going to take an umbrella or coat with you? Or go out as normal?'

In the general area of downy mildew management in horticultural systems most advances have been made with *Plasmopara viticola* in grape vines where there is a substantial level of epidemiological modelling and understanding that has led to wide experience of field testing of DST as well as the incorporation of downy mildew DST within full DSS designs and addressing the many issues that hinder/help the implementation of DSS, e.g. vite.net® and AusVit. The more recent general DSS, vite.net®, is composed of two parts the first is concerned with real-time monitoring of vineyard parameters concerned with the air, soil, plants, pests, and diseases, whilst the second is the 'front end' – a web-based tool that uses models to analyse the data from this monitoring and provides the user with bespoke updates, alerts and advisory decision supports. Rossi *et al*. (2014) (Addressing the implementation problem in agricultural decision support systems: the example of vite.net® - ScienceDirect) felt that by addressing a number of key factors, most importantly :-

1) the holistic treatment crop issues (P&D, fertiliser use, agronomy and irrigation)

2) breaking complex decisions into straightforward 'decision supports'

3) easy and rapid access *via* internet connection (no frustratingly-lengthy loading up procedures)

4) strong emphasis on the decision support and NOT decision-making role of DSS/DST

5) two-way communication between users and providers to make it possible to consider and resolve site specific issues and information,

- the likelihood of potential users adopting the vite.net® DSS was greatly increased.

This process requires ongoing active support of the DSS/DST and is a far cry from the majority of downy mildew DST which are currently at an earlier phase of development and are generally not presented to potential users as parts of holistic systems and often have quite difficult-to-handle 'front ends' as indicated by the preference of Australian lettuce growers in comparison trials for DOWNCAST (designed for onion DM) over the less straightforward BREMCAST (designed for lettuce DM) (Minchinton *et al*., 2010 Managing Downy and Powdery Mildew, Anthracnose and White Blister | AUSVEG).

What Inputs are needed for effective Downy Mildew DST?

Environmental data: Whether they are simple 'rules of thumb' like the '10:10:24' (**10**mm of rainfall when air temperatures are ≥**10**°C and the soil surface remains wet for ≥**24**h) developed to determine conditions conducive to downy mildew of the vines (Magarey, 2010 201003-Managing-downy-mildew (wineaustralia.com)) or relatively complex models, the basic data inputs required remain fairly generic. All models require air temperature and the majority use relative humidity (RH%), the only exception being FSP for *P. sparsa* in boysenberry (Kim *et al*., 2014 PHYTO-02-13-0058-R (apsnet.org)) which uses rainfall (Table S2). Rainfall measurements are used by several other DST, whilst the second most frequently used moisture parameter is plant (leaf) surface wetness (LW, Table S2). The majority of DST models utilise hourly/half hourly meteorological data and the quality and relevance of this data to the crops being managed is of key importance to the veracity of the warnings/advisories provided by the DST/DSS.

Table S2: Summary of decision support tools and models that have been worked up for aerial oomycetes (predominantly downy mildews) considered in CP184 (see yr1 report CP184).

Pathogen	Crop	DST/DSS (Decision Support Systems/Tools)	Inputs
Bremia lactucae	Lettuce	BREMCAST (Kushalappa, 2001)	Hourly: Air temp., RH%, Leaf Wetness Disease presence +/- (scouting)
		California 1 (Wu *et al*., 2002 adaptation of Scherm *et al*., 1995)	Hourly: Air temp., RH%, Leaf Wetness, Solar Radiation *(model sets SR threshold of* 8Wm^{-2} *for start of 3h morning {'infection'} Leaf Wetness Duration)*
		California 2 (Kunjeti *et al*., 2016; Klosterman *et al*., 2016) Spore trapping qPCR – Risk thresholds	-
		Inagro/PCG/PSKW Glasshouse DST (van Hese, 2015) Basically, grower aims to keep RH% <90% - DST issues optimised spray advisories	Plant and harvest dates of crops Meteorological data is collected by 'climate box' installed in crop and sent to DST provider by internet
Hyaloperonspora parasitica	Matthiola/ Stocks	Rapeseed model (Neog *et al*., 2013) (very basic – not a DST as such)	Hourly: Air temp, Twice daily RH%, (daily rainfall)
Peronospora aquilegiicola	Aquilegia	Risk matrix (Jennings & Thorp, 2016)	Air temp., RH%
Peronospora belbahri	Basil	Risk matrix (Jennings *et al*., 2017)	Air temp., RH%
Peronospora destructor	Onions	MILIONCAST (Gilles *et al*., 2004)	Hourly: Air temp., RH%
		DOWNCAST modified (de Visser, 1998)	Hourly: Air temp., RH%, Leaf Wetness
		ONIMIL (Battilani *et al*., 1996)	Hourly: Air temp, RH%, Hourly/daily rainfall
		ZWIPERO (Friedrich *et al*., 2003)	Hourly: Air temp, RH%, Leaf Wetness, rainfall
Peronospora effusa	Spinach	Spore trapping qPCR – Risk thresholds (Choudhury *et al*., 2016; 2017) Possible development of recombinase-polymerase assays which will greatly improve this approach (Klosterman *et al*., 2017; 2019)	-
Peronospora sparsa	Rose	Aegerter *et al*. (2003)	Hourly: Air temp., Leaf Wetness
		Rose DST version 3.0 (Xu, 2012; Xu & Robinson, 2011)	≤ ½ hourly Air temp. RH%, Leaf Wetness, rainfall
		Fuzzy Peronospora Sparsa (FPS) Model (Kim *et al*., 2014)	Hourly Air temp., Daily rainfall
Phytophthora infestans	Tomatoes	BlightCast (www.syngenta.co.uk/blightcast) Used to access regional forecasts using Smith and Hutton criteria as a guide for extra vigilance with Greenhouse environmental controls (RH% and LW)	-
		EuroBlight – compares range of *P.infestans* DSTs for potato crops (Hansen et al., 2010 & ongoing)	Hourly: Air temp., RH%, rainfall

Detection and quantitation of downy mildew inoculum: With improvements in detection technologies allowing the rapid and precise identification and quantitation of pathogen propagules, there has been an increasing move towards direct monitoring of airborne inoculum to determine the disease risks. This is very good since spray decisions based solely on meteorologically-based DST predictions without considering the level of inoculum or even the presence/absence of inoculum are likely to lead to some unnecessary spray applications. Another important consideration regarding the real-time *in situ* measurement of downy mildew inoculum in current UK horticultural crops is that, with the almost entire absence of reliable curative treatments in the fungicides available, there is a strong need to guide the timing of early applications of protectant treatments in relation to the presence and concentration of inoculum, to prevent early infection and disease establishment. The best disease risk forecasts are likely to result from simulations using a combination of meteorological and real-time inoculum measurements (AHDB Final report CP 099c). A key problem with inoculum measurements is setting 'disease risk thresholds' as these will vary with inoculum potential or infection efficiency (IE) which is influenced by a range of factors such as crop variety/resistance, agronomic treatments such as fertiliser use, fungicide treatment history (usually accommodated within DST according to simple spray interval timing rules), plus other factors such as the crop plant microbiome composition/activity. Currently consideration of these factors is reliant on the local ('gut') experience and knowledge of individual growers and consultants. The important question of IE feeds directly into the area of work in project CP184 concerned with pathogen fungicide resistance/host disease resistance in *Bremia* populations in lettuce crops (see above).

Concluding remarks

It is important not to lose track of the fact that as decision tools DST/DSS need not be overly intricate and that some very effective/useful assistance with crop management decisions can come from relatively simple systems. A good example of this is the risk tables drawn up from controlled environment observations for infection risks in sweet basil and aquilegia crops (AHDB Reports HNS 196a & PE 024), which, whilst not giving spray advisories, do provide a realistic estimate of when disease risks are low and the basis for the development effective protected environment management protocols. Also, it may be possible to adapt already-developed DST for other crops as seen in the Australian work with the DOWNCAST onion DST on lettuce and poppy crops (Scott *et al*., 2008 Adaptation of the Forecasting Model DOWNCAST for Determination of Downy Mildew Epidemics of Oilseed Poppy in Tasmania, Australia - Scott - 2008 - Journal of Phytopathology - Wiley Online Library; Minchinton *et al*., 2010 Managing Downy and Powdery Mildew, Anthracnose and White Blister | AUSVEG). Review work in CP184 (Year 1 report) collated the optimal environmental parameters for 14 downy mildews in UK horticulture Figure S1 shows the latent period and optimal infection temperature ranges for these pathogens (where available) to give an illustration of potential overlaps and therefore potential for adaptation of DST between pathogens (see also Table S3 for key environmental parameters for Spinach and Column Stock downy mildews).

Figure S1: Latent period and infection temperature ranges of a selection of downy mildew diseases important in UK horticulture, plotted to illustrate overlaps of ranges between diseases.

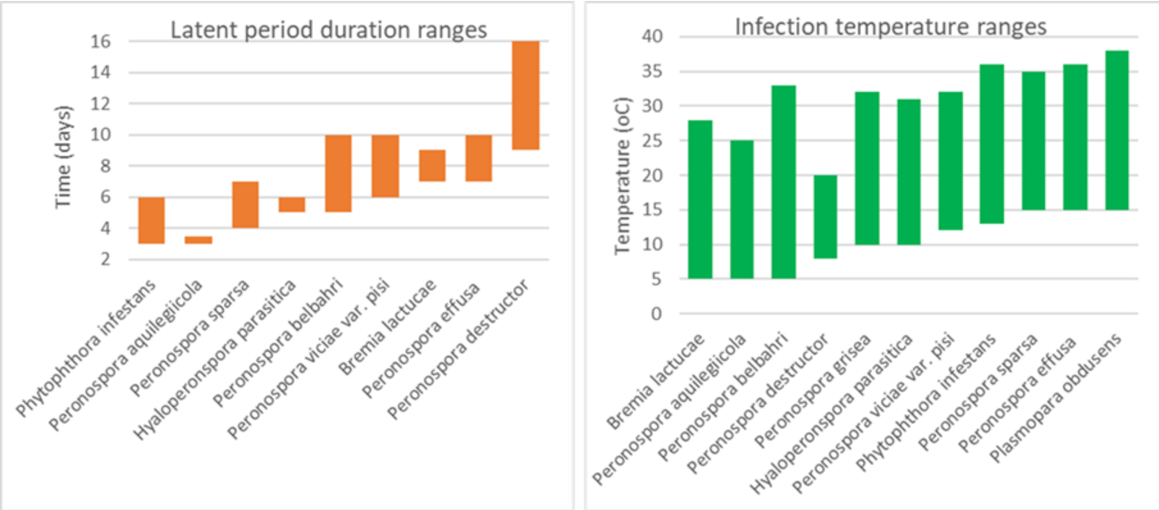

Table S3: Comparison between Spinach, Brassica general and Matthiola Downy Mildews of effects of environmental factors on infection and downy mildew disease

	Survival of spores (conidia)	Latent period	Infection		
			Leaf wetness duration	Optimum Temperature	Minimum RH%
Spinach DM (*P. effusa*)	Several days	7-10 days	>3h	15-21°C	90%
Brassica DM (*Hyaloperonspora parasitica*)	>3days	5-6 days	>4h	10-21°C	90%
Matthiola, Column stocks DM (*P. matthiolae*)	>3days	5-6 days	>3h	12-22 °C	90%