Grower Summary

PE 024

Basil: Improving knowledge and control of downy mildew in protected and outdoor crops

Final 2017

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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.

Project title:	Basil: Improving knowledge and control of downy mildew in protected and outdoor crops
Project number:	PE 024
Project leader:	Philip Jennings, Fera Science Limited
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Key staff:	Gilli Thorp, Fera James Townsend, STC Tom Wood, NIAB
Location of project:	Fera, York STC Research Foundation, Cawood National Institute of Agricultural Botany, Cambridge
Industry Representative:	Simon Budge, Vitacress Ltd, West End Nursery, Roundstone Lane, Angmering, West Sussex, BN16 4AX
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GROWER SUMMARY

Headline

- 95% of the basil seed samples that were screened contained DNA of the basil downy mildew pathogen, *Peronospora belbahrii*.
- No oospores were detected in seed washings, which suggests that the disease was present either as an internal contaminant within the seed or as mycelia on the seed surface.
- Oospores of *Peronospora belbahrii* were not detected in plants or soil, and conidia did not appear to survive longer than 72 hours suggesting that the pathogen is not likely to overwinter.
- Paraat (dimethomorph), Fenomenal (fenamidone + fosetyl-aluminium) (outdoor use only) and Revus (mandipropamid) provided good protective activity when applied up to ten days prior to infection.
- In this trial, Fubol Gold (metalaxyl-M + mancozeb) failed to control the disease which led to the discovery that metalaxyl-M resistant isolates were present within the UK.

Background

A recent BHTA survey showed that approximately 30 ha of sweet basil (*Ocimum basilicum*) is grown in the UK, with about 25% under protected conditions. Most of the crop is grown outdoors in the summer. Several crops can be produced from the same area in the same season, so the total area grown will be considerably larger than this. It has been estimated that the value of the crop is ‘in the order of tens of millions of UK sterling’.

Basil downy mildew, caused by the biotrophic oomycete *Peronospora belbahrii,* was first reported in sweet basil in the UK during the summer of 2010. Initially the disease was given quarantine status, with infected crops subject to statutory action; this status was lifted in 2012. The UK fresh basil industry is highly valuable, and the recurring problem of downy mildew is causing growers major issues.

Although new to the UK, the disease is endemic in many parts of Europe (including Switzerland (2001), Italy (2003), France (2005) and Hungary (2011)), North America, Africa, Asia and South America.

There has been a great deal of work published on basil downy mildew. However, knowledge gaps have been identified, particularly relating to sources of inoculum, role of alternate hosts, epidemiology and control. These gaps were addressed in this project.

Summary

Epidemiology

Presence of *Peronospora belbahrii* in UK seed lots

Twenty seed samples were tested for the presence of *P. belbahrii* DNA. Of these twenty samples, all but one contained *P. belbahrii* DNA; 15 contained *P. belbahrii* DNA at similar levels across all five replicates, suggesting an evenly distributed contamination of the sample. Of these samples, nine contained high levels of *P. belbahrii* DNA, with six samples containing very high levels of target DNA (average Ct values of between 24.7 and 30.4). As the test cannot distinguish between viable and non-viable DNA it is not clear whether the DNA detected in the seed was infective.

Generally, Ct values of 30 or less are considered strong positive reactions and are indicative of abundant target DNA in the sample. Ct values of 31-37 are positive reactions and indicate moderate amounts of DNA, whereas values 38-40 are weak reactions and indicate a minimal amount or no target DNA in the sample.

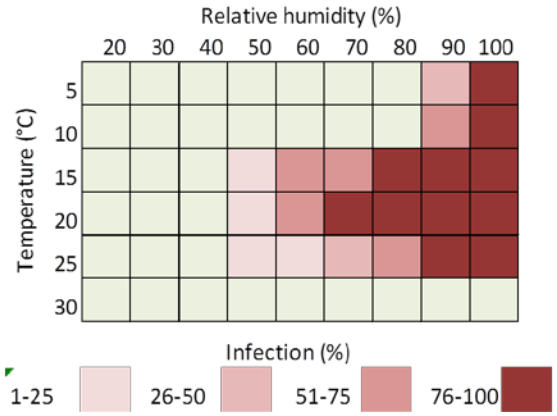
No oospores were detected in any of the seed washings, which suggested that *P. belbahrii* was not present in basil seed lots as an oospore surface contamination. This leads to the conclusion that DNA was present either as an internal contaminant of the seed or as mycelia on the seed surface.

Infection of basil plants from contaminated seed

Two seed samples with a high average *B. belbahrii* DNA content were used to determine whether seed contamination resulted in downy mildew infected plants. Five lots of 100 seed were sown for each of the two samples and plants grown to the fourth true leaf stage. No basil downy mildew symptoms were observed on any basil plants. Analysis of leaf and stem material for the presence of *P. belbahrii* DNA showed that it was present at low levels, with higher Ct values in plant material from the seed batch with the higher Ct value. It is unclear whether the DNA detected was simply transferred from the seed directly onto the growing plant or was present as an internal contamination which came from inside the seed.

Conditions required for infection of basil by *P. belbahrii*

Based on the experimental work carried out in year 1 of the project an infection risk grid was constructed to provide growers with a quick guide to conditions (temperature and humidity) which are most likely to lead to infection of basil plants by downy mildew.



The effect of temperature on the survival of *Peronospora belbahrii* conidia

Three experiments were set up to determine the effect of temperature on the survival of *P. belbahrii* conidia. Assessment of the germination of fresh conidia showed that under the conditions used, conidial germination was highly variable averaging 33, 8 and 41% for experiments 1, 2 and 3, respectively. Inoculating plants with these conidia produced consistently high levels of disease. Exposure of conidia to different temperatures did not produce consistent results over the three experiments, however there were general trends in the data. The optimum temperature for survival of conidia was 20ºC, with conidia capable of surviving for up to 72 hours. Conidia exposed to 15ºC were also capable of surviving 72 hours, but at lower levels than at 20ºC. At temperatures of 10ºC and below conidia survived for up to 48 hours at levels sufficient to cause infection. At temperatures of 25ºC and above conidia did not survive for longer than 24 hours.

These data suggest that conidia of *P. belbahrii* are not capable of surviving for long periods and that conditions suitable for plant infection are required within 72 hours of their production on the leaf.

Oospore production under protected and outdoor conditions

For some downy mildew species, the production of oospores enables longer term survival of the pathogen. For *P. belbahrii*, however, no oospore production was observed for any of the isolates tested (FERA, CREA, FERA + CREA) under protected conditions, across three

independent assessments. Oospores were also absent in leaf material sampled from the inoculated outdoor production trial.

Soil samples testing positive for *P. belbahrii* DNA were screened for the presence of oospores using sieving and centrifugation. No oospores were observed in any of the samples tested. This indicates that *P. belbahrii* does not form oospores, or at least not under the experimental conditions, or in the isolates, tested in this study.

Detection of *P. belbahrii* in soil from outdoor production sites

Soil samples collected from naturally and artificially infested sites were tested for the presence of *P. belbahrii* DNA with quantitative PCR. *P. belbahrii* DNA was found to be present at a low level in the soil sampled from the naturally infested commercial crop. DNA was, however, absent in soil sampled from the artificially inoculated trial crop. As no oospores were found to be present in any of the samples tested it was concluded that the *P. belbahrii* DNA was likely to be from infected plant debris in the soil. Failure to detect *P. belbahrii* DNA in soil from the artificially inoculated site could have been a result of the lower disease incidence and severity compared to the commercial production site.

The existence and importance of alternate hosts for *P. belbahrii*

Fourteen plant species from across the Lamiaceae family were tested for susceptibility to *P. belbahrii*. Of the plant species tested agastache, lavender, common sage and catnip were the only ones which showed symptoms associated with *P. belbahrii*. Profuse sporulation was observed following infection of agastache and lavender, sporulation was sparse following infection of common sage and no sporulation was observed on catnip. Basil plants inoculated with spores obtained from the infections on sage, lavender and agastache all showed symptoms of basil downy mildew.

All the alternate hosts identified were herb crops so growers should take care if growing the alternate host crops at the same time as basil. The lack of weed crops in the list of alternate hosts should make disease management easier as there appears to be no route for overwintering/spread of *P. belbahrii* via these plants.

Disease Control

Fungicide treatment longevity:

Five products identified with potential for the control of basil downy mildew were further examined to determine the most appropriate time between fungicide applications. The results indicated that three products, Paraat (dimethomorph), Fenomenal (fenamidone + fosetyl-

aluminium) (outdoor use only) and Revus (mandipropamid), provided good protective activity when applied up to ten days prior to infection (as a protectant application), and as a result have the potential to be used as part of a weekly fungicide programme for the prevention of downy mildew infection of basil.

In this trial the lack of control by Fubol Gold (metalaxyl-M + mancozeb) led to the discovery that metalaxyl-M resistant isolates were present within the UK. This potentially could lead to control problems as products containing metalaxyl-M are relied on for disease control both as seed and foliar treatments.

Fungicide programme efficacy – Protected

The trial carried out under protected conditions consisted of 11 treatments (eight individual products and three different spray programmes). The individual products were applied either twice (14 days between treatments) or four times (seven days between treatments). No treatment, whether individual product or spray programme, resulted in 100% control of disease. Where individual products were applied weekly, significant control was achieved by application of Revus (mandipropamid), Paraat (dimethomorph) and HDC F226. Significant levels of control were also achieved by the four-spray programme (Infinito (fluopicolide + propamocarb hydrochloride), HDC F225, HDC F226 and HDC F237). Infinito is not currently approved for use on protected herbs.

Phytotoxic effects were observed following multiple applications of Fubol Gold (metalaxyl-M + mancozeb) to the crop.

Fungicide programme efficacy – Outdoor

Efficacy testing for 15 individual products and three spray programmes was conducted under outdoor conditions. Disease symptoms were first observed in untreated control plots 14 days after inoculation with the pathogen. Levels of disease were relatively high, with an average of 63% of leaf surface area showing signs of moderate to heavy sporulation across the duration of the trial (6 weeks). Treatment programme 3 (Invader (dimethomorph + mancozeb), Infinito and Revus) and product HDC F239 performed most effectively, with no disease symptoms observed in any of the replicate plots. Disease symptoms were also observed to be very low (<1% leaf area affected and severity of sporulation ≤1 (0-5 scale)) for plots treated with Fubol Gold (metalaxyl-M + mancozeb), Revus (mandipropamid), Invader, Paraat (dimethomorph), and Programme 2 (Fenomenal, Fubol Gold, Revus).

Crop safety

Eight products were trialled for crop safety. All products were applied at standard and double rate. Seven days after treatment a residue was observed on plants treated with Fubol Gold (metalaxyl-M + mancozeb); this residue was no longer present after 14 days. The phytotoxicity noted in the trial carried out under protection following multiple product applications was not observed in the crop safety trial where a single treatment was applied. Plants treated with HDC F245 were noticeably larger, greener and more vigorous than the controls. Plants treated with HDC F240, at double the standard rate, were smaller than the control plants, although this observation was not consistent across the replicates.

The effect of night time illumination on *P. belbahrii* infection and sporulation

A basil trial inoculated with a spore suspension of *P. belbahrii* was exposed to nine different incandescent night lighting treatments ranging from 0 to 8 hours illumination during the dark period. Half of each plot was covered with polythene to maintain high humidity throughout the trial and half of each plot was left uncovered. After 2 weeks there was downy mildew sporulation in all treatments. Differences in disease severity were observed after 2 weeks in the uncovered plots but these were not significant. Plants which were not illuminated during the dark had the highest disease severity and plants exposed to 4 hours of light during the dark period had the lowest disease severity.

Financial Benefits

It has been estimated that outbreaks of downy mildew caused by *P. belbahrii* can cause over 80% crop loss in field and protected production with associated financial loss and disruption to the supply chain. Outputs from this project have provided information on potential routes of downy mildew infection, conditions under which infections are likely to occur and control strategies. Implementation of these strategies will significantly lower downy mildew infections and hence associated losses.

Action Points

- Check crops regularly and, where practical, if foci of infected plants are found remove them immediately by carefully bagging to avoid dispersing spores to other plants.
- For protected crops ensure there is adequate air circulation around plants to minimise prolonged periods of leaf wetness by better spacing and by increasing the ventilation in the glasshouse. If possible, avoid overhead watering as this is likely to aggravate

the disease. If it is necessary to water from overhead then do this early, on days when solar radiation levels will ensure the leaves have a chance to dry out quickly.

- Remove leaf and other plant debris at the end of the season to minimise the risk of carry-over of the disease and maintain effective weed control in and around the growing areas.
- Consider growing host crops independently to each other.

