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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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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.

Dr Philip Jennings

Principal Plant Pathologist

Fera Science Limited

Signature ..... Date 22/11/17

Dr Tom Wood

Senior Molecular Plant Pathologist

National Institute of Agricultural Botany

Signature ..... Date...23rd November 2017.....

Mr James Townsend

Project Manager - Plant Pathology

Stockbridge Technology Centre

Signature ..... Date .....23/11/2017.....

Report authorised by

Dr Shaun White

Head of Plant Protection

Fera Science Limited

Signature ..... Date 23/11/2017.....

Dr Jane Thomas

Head of Plant Pathology

National Institute of Agricultural Botany

Signature ..... Date ...23rd November 2017.....

Dr David George

Director of Science

Stockbridge Technology Centre

Signature ..... Date .....23/11/2017.....

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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.

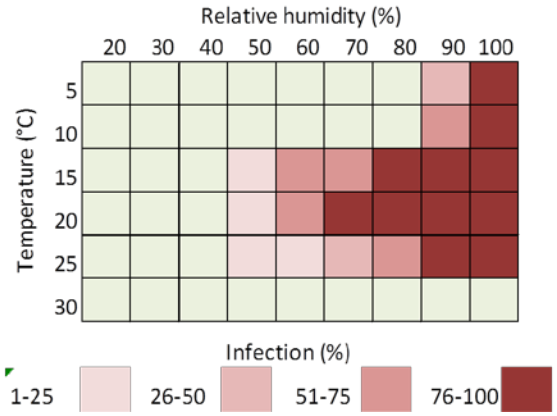
No oospores were detected in any of the seed washings, which suggested that *P. belbahrii* was not present in basil seed lots as an oospore surface contamination. This leads to the conclusion that DNA was present either as an internal contaminant of the seed or as mycelia on the seed surface.

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.

SCIENCE SECTION

Introduction

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. Much 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* (Belbahrii *et al.*, 2005; Thines *et al*., 2009), was first reported in sweet basil in the UK during the summer of 2010 on protected plants grown in the south-east of England. 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.

To date there has been no research carried out on basil downy mildew in the UK. A project funded by HDC (FV 390) was commissioned to look at the epidemiology and control of downy mildew in sage, parsley, mint and basil; however, the lack of basil downy mildew across the industry for the period of the project meant that the basil element of the project was not completed.

The research undertaken on basil downy mildew has focused on epidemiology and control of the pathogen. Work by Garibaldi *et al.* (2004) showed that *P. belbahrii* was seed-borne, with levels of infection as low as 0.02% leading to visible infection of crops. However, it is unclear from the literature whether the pathogen is truly seed-borne (systemic) or simply a contaminant (spores surviving on the outside of seed). It is probable that the disease was introduced into the UK through infested/infected seed; however, it is also possible that it came in on infected plants. This plant material could include infections on alternate hosts. To date two alternate hosts for *P. belbahrii* have been identified, these are agastache (Henricot *et al*., 2009) and coleus (Denton *et al*., 2015). A *Peronospora* species on sage has been shown to have a similar sequence homology to *P. belbahrii* (Thines *et al*., 2009). However, in this case no morphological data were available and, so it was not possible to conclude if the two *Peronospora* species were the same. As the hosts described so far are in the Lamiaceae family, work is required to establish whether other members of the Lamiaceae could also act

as alternate hosts for *P. belbahrii*. The Lamiaceae includes a number of herbs and the common deadnettle weed which are known to be susceptible to downy mildew caused by *Peronospora lamii*.

Studies on the epidemiology of *P. belbahrii* showed that downy mildew infections were most severe where plants had been kept wet for a period of at least 6 h (Garibaldi *et al*., 2007). The highest levels of disease occurred at 20ºC, with no infection occurring below 12ºC or above 27ºC. Following infection, a period of 8 to 10 days was required before production of the conidia was seen on the underside of leaves. For other downy mildew infections this period is related to temperature, with an additive average daily temperature (degree day) of 160 and 170 required for infections on impatiens and pansies, respectively (Jennings *et al*., 2009; Jennings *et al*., 2011). *P. belbahrii* required a period of 7.5 h dark in a moist atmosphere at temperatures between 10 and 27ºC for sporulation to occur (Cohen *et al*., 2013a). Exposure to light supressed the formation of conidia but allowed conidiophores (spore bearing structures) to emerge from the stomata. It was suggested that the inhibition of sporulation in *P. belbahrii*, unlike other oomycetes, operated via a red-light photoreceptor. In plants blue light controls stomatal opening (Ogawa, 1980) and therefore *P. belbahrii* sporulation could be controlled by varying the amount of blue light to which basil plants are exposed. It is not known whether infection requires a period of dark or whether it can occur in the light. However, work on *Peronospora violae* indicated that infection only occurred in the dark (Jennings *et al*., 2009). If this is also the case for infections caused by *P. belbahrii* then the manipulation of the light wavelength that protected crops are grown under could result in reduced infection levels.

To fully appreciate the infection process and provide guidance on risk management for growers a greater understanding of the parameters (humidity, temperature and leaf wetness), time to sporulation and spore survival are required. There are currently no published data on the effect of environmental conditions on the survival of spores produced by *P. belbahrii*. Information concerning the ability of *P. belbahrii* to persist in infested soils and the role of soil-borne inoculum in initiating new disease outbreaks also remains unclear.

Currently there are no resistant varieties, with all commercially popular varieties highly susceptible to disease. Lower disease levels have been observed in red leaf and lemon flavoured basil varieties. Only varieties of *O. americanum* (medicinal herb) have shown no symptoms or sporulation (Djalali Farahani-Kofoet *et al*., 2014). As a result, cultural and chemical control methods will be required to help manage the disease. In terms of cultural control, work with other downy mildews has shown that limiting leaf wetness through adequate ventilation and spacing of plants, and avoiding overhead irrigation and watering late in the evening, is effective. Avoiding the use of fleece/mesh over plants has also been shown

to reduce downy mildew infections. There are a number of approved fungicides which can be used for the control of downy mildew, but there is little published work to indicate how effective these products are against *P. belbahrii*. There has, however, been a report of resistance to mefenoxam (metalaxyl-M) (Cohen *et al*., 2013b), the active ingredient generally most effective against oomycete pathogens. Work on impatiens and pansy downy mildew (Jennings *et al*., 2009; Jennings *et al*., 2011) has indicated that preventative fungicide applications were more effective than curative applications. Studies have also shown that the use of systemic acquired inducers could provide effective disease control depending on the method, rate and timing of application (Mersha *et al*., 2012).

Although a great deal of work has been published on basil downy mildew there are still some knowledge gaps, particularly relating to sources of inoculum, role of alternate hosts, epidemiology and control. These gaps were addressed in this project, with the collaboration between Fera, STCRF and NIAB providing the required skill sets to carry out this work, having already successfully completed similar work on other downy mildew pathogens. This Final report details research carried out in project year 2; Results from project year 1 are described in the Annual report for PE 024.

Materials and methods

Epidemiology

Determine whether *Peronospora belbahrii* is present in UK seed lots and whether infected seed acts as a primary source of infection

Seed samples were sourced in two ways, firstly through acquiring lots most commonly used by growers from suppliers of basil seed (to give an indication of how widespread any problem might be across the industry) and secondly by contacting growers who had reported basil downy mildew to try and obtain a sample of the seed from the lot they had sown.

Determination of the presence of *Peronospora belbahrii* DNA in basil seed samples

DNA was extracted from seed using a CTAB extraction method and Kingfisher™ mL magnetic particle processor. For each seed sample five replicates of 100 seed were counted into a labelled 5 ml capped transport tube containing a single 7/16-inch ball bearing and 2 ml of CTAB soil extraction lysis buffer with 2% antifoam B added. Two additional tubes were prepared, the first minus seed and the second with seed and basil downy mildew sporangia. These tubes acted as negative and positive controls respectively. All seed samples and

controls were ground for 2 minutes using a Kleco 96 grinding machine; if required a further 2 ml of lysis buffer (with antifoam) was added and the sample ground for an extra 30 seconds. The ground sample and negative control were transferred from the 5 ml tubes to 2 ml centrifuge tubes containing 250 µl Buffer B (Wizard Magnetic DNA purification system for food) and 750 µl of precipitation solution (Wizard kit). The tubes were briefly vortex mixed, centrifuged at 13,000 rpm for 10 minutes and 750 µl of the supernatant transferred to a clean 2 ml centrifuge tube. To this, 50 µl of Magensil beads (Wizard kit) and 600 µl of isopropanol were added, the samples vortex mixed and incubated for 5 minutes at room temperature with occasional mixing by tube inversion. DNA was retrieved via the 'gDNA' program on the Kingfisher using an incubation period of 5 minutes at 65ºC. The extracted DNA was stored at -25ºC until required.

The extracted DNA was analysed using an Applied Biosystems 7900HT Fast Real-Time PCR machine in combination with the primers and probes described by Belbahri *et.al.* (2005). The temperature cycle consisted of denaturation (50ºC) for 2 min, annealing (95ºC) for 10 min and 40 amplification cycles with cycles alternating between 95ºC for 15 sec and 60 for 1 min.

Infection of basil plants from contaminated seed

Two seed lots were chosen for testing based on the level of *P. belbahrii* DNA in the seed and the amount of seed available. For each seed sample, 100 seed were sown on five occasions (between Jan and March 2017) and grown until plants were at the fourth true leaf stage. Plants were regularly assessed for the appearance of downy mildew symptoms. If no symptoms were observed 25 plants per sowing were removed and tested for the presence of *P. belbahrii* DNA using a method similar to the one described in the previous section.

Determination of the presence of oospores of *Peronospora belbahrii* in basil seed samples

For each seed sample, 100 seed were washed in 200 µl 70% industrial methylated spirit (IMS) and the number of oospores present counted. The use of 70% IMS did not affect the integrity of oospores, but helped reduced the level of the mucilaginous coating produced by the basil seed when they encountered water. The test was replicated five times and samples ranked according to the average number of oospores recorded.

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

Soil sampling and DNA extraction

Soil was sampled from land where *P. belbahrii* symptoms had been identified in the previous crop. Fifty, 20 g sub-samples, were collected in a 'W' pattern across growing areas (<1Ha) from the top 10-20 cm of the soil profile using a corer/trowel, to form a 1 kg bulk sample. Soil samples were left to desiccate at room temperature for a minimum of 48 hours before DNA was extracted. A total of four 1 kg soil samples were tested for the presence of *P. belbahrii*; these comprised a single sample from the 2016 outdoor production site (Sept 2016, Cambridge, U.K.) and three grower samples collected from approximately 1 ha area of beds (April 2017, Cambs, UK). Soil bulks were dried and homogenised in a pestle and mortar. DNA was extracted from 3 x 10 g sub-samples using a DNeasy Powersoil Maxi kit (Qiagen UK, formerly MO Bio Powersoil kit) according to the standard protocol. Extracts were left in a fridge at 4°C over-night.

Quantification of *P. belbahrii* DNA in soil samples:

Each DNA extract was screened in triplicate for *P. belbahrii* contamination by quantitative PCR with specific forward and reverse primers (Thines *et al*., 2009) using SYBR green chemistry in a 10 µl reaction (5 µl mastermix 1 µl of DNA, 1 forward primer (5 µM), 1 µl reverse primer (5 µM), 1 µl ddH20. PCR cycle: 95°C/5 mins; 40 x 95°C/60 s, 63°C/30 s, 72°C/30 s. Results are presented as a cycle threshold (Ct 0-40) with a lower Ct value denoting higher concentration of DNA. A positive control for bacterial DNA (16s;341F CCTACGGGAGGCAGCAG, 534R ATTACCGCGGCTGCTGGCA)) was also included to ensure the quality of the DNA extract in the absence of *P. belbahrii*.

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

The effect of humidity on leaf wetness

In the first year of the project testing was carried out on basil leaves grown under glass (PE 024 Year 1 Annual Report). As it is likely that the surface of leaves grown outdoors will differ from those grown under protected conditions, and this difference may affect how water is distributed over the leaf surface, the test carried out in the first year was repeated on leaves taken from plants grown outdoors.

Basil seed (cv. Sweet Genovese) was sown in pots (05/06/2017) and grown outdoors until plants had at least 6 true leaves. Five replicate basil leaves were removed and placed in humidity chambers maintained at 19, 49, 81, 97 and 100 % using concentrated salt solutions

(**Table 1**). Leaves were sprayed with water to run-off and leaf wetness recorded every half hour until leaves were dry, or for 48 hours (which ever was the longest).

Table 1. Solutions used to achieve different moisture chamber relative humidity

Solution	Recorded humidity (%)
Lithium chloride	19.9
Potassium carbonate	49.0
Sodium chloride	81.4
Potassium chloride	97.5
Water	100

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

Experiments to determine the effect of temperature on the survival of *P. belbahrii* conidia were carried out on three occasions using the temperature range outlined below.

1) 10, 15, 20 and 25ºC
2) 5, 20 and 30ºC
3) 5, 10, 15, 20 and 25ºC

For each experiment sufficient six-week-old basil plants (cv. Sweet Genovese) were inoculated so that three, freshly sporulating, replicate basil plants could be placed at each temperature. From each plant two sporulating leaves were removed at time 0 and then after 24, 48 and 72 hours incubation. The conidia were removed from each set of two leaves by washing in 2 ml of SDW. From this conidial suspension a 40µl aliquot was incubated for 24 h at 15ºC and a germination count carried out on 100 conidia. The remaining conidial suspension was sprayed onto a four-week-old basil plant which was placed at 100% humidity overnight, to encourage infection, and then grown on in the glasshouse. Each plant was assessed for symptoms of basil downy mildew after 12 days.

Oospore production under protected and outdoor conditions

Protected conditions

Basil seeds (cv. Sweet Genovese) were sown into a seed tray (38x23 cm) and germinated under 18 hours light (22°C)/6 hours dark (18°C). At the first true leaf, batches of 10 seedlings were transplanted into 36, 12 cm pots. Seedlings were grown on until the 3-4 leaf stage. 9 individual pots were placed into four large clear plastic bags. These were inoculated with 20 ml of spores ($1x10^5$) from a single *P. belbahrii* isolate (CREA, FERA), co-inoculated with both isolates (CREA + FERA), or 20 ml water respectively. The plants were kept covered for 48

hours and then grown on until they flowered, at which point watering was reduced. After senescence, 1-2 g dry leaf material was selected randomly from the plants in each pot and ground into a fine powder in a pestle and mortar to release any oospores from the tissue. The ground tissue was re-suspended in 20 ml water and filtered through a 75 µm and 20 µm sieves. The residue on the 20 µm sieve was then re-suspended in 5 ml water. 50 µl aliquots of the suspension were transferred onto a microscope slide with a pipette, covered and viewed at 100 x. This was repeated five times for each plant sample.

Outdoor conditions

Plant material was sampled randomly from four inoculated control plots and discard rows from the outdoor production trial (Cambridgeshire, Oct 2016). Samples were processed in an identical manner to the material grown under protected conditions.

Oospore testing from soil

In addition to testing for *P. belbahrii* DNA, soil samples with a positive qPCR result were screened for the presence of oospores using wet-sieving extraction (Van der Gaag & Frinking, 1997, with modifications). Three 10 g sub-samples were taken from each 1 kg bulk and suspended in 70 ml water in a beaker, stirring with a motorised flea for 10-15 minutes. The samples were then filtered through 75 µm and 20 µm sieves. The residue on the 20 µm screen was re-suspended in 7.5 ml water, with 50 µl of 1% tween 80 added and then stirred. The suspension was poured onto 20 ml of 70% sucrose solution, in a 50 ml corning tube. Tubes were centrifuged for 3 mins at 2,800 rpm. This was passed through a 20 µm sieve and the residue was re-suspended into 10 ml water in a corning tube. The tubes were centrifuged at 6,000 rpm for 3 minutes to concentrate any oospores. The top 8ml of supernatant was carefully removed leaving 2 ml of concentrate. 50 µl aliquots of the suspension were transferred onto a microscope slide with a pipette, covered and viewed at 100 x. This was repeated five times for each soil sample.

Control

Evaluate fungicide programmes in large-scale 'commercial' trials (protected and outdoor production) for efficacy against *P. belbahrii*.

Fungicide longevity trial

Five fungicides (chosen based on results from the outdoor trial reported in the Year 1 annual report) were tested for longevity of efficacy against the basil downy mildew pathogen *P. belbahrii* (**Table 2**). All products were applied according to the manufacturer's recommended rate as foliar sprays. Sprays were applied at three timings: 7, 10 and 14 days pre-inoculation

in a water volume equivalent to 1000L/ha. Control plants were sprayed with an equivalent volume of sterile distilled water (SDW).

Table 2. Fungicides used in fungicide longevity testing against *Peronospora belbahrii*.

Product	Active ingredient	Application rate/ha	UK approval
Fubol Gold	metalaxyl-M (38.8 g/kg) + mancozeb (640 g/kg)	1.9 kg	Off label for outdoor and protected basil
Fenomenal	fenamidone (60 g/kg) + fosetyl-aluminium (600 g/kg)	4.5 kg	Off label for outdoor basil
Revus	mandipropamid (250g/L)	0.6 L	On label for outdoor and protected basil
Paraat	dimethomorph (500 g/kg)	3 kg	Off label for outdoor and protected basil
HDC F225	-	1 L	-

Inoculum production for the tests was started two weeks prior to the proposed inoculation date (8/12/2016). Leaves bearing freshly produced conidia of *P. belbahrii* were removed from infected plants. The conidia were washed from the leaves in SDW and the concentration of conidia established using a counting chamber. The conidial concentration was adjusted to 10^4 conidia ml^{-1} and six-week-old basil plants inoculated to run-off with the conidial suspension. The inoculated plants were incubated for 18 hours in the dark at room temperature; plants were incubated in propagator tops to prevent drying out of inoculum. Plants were then transferred to a glasshouse and grown at 20°C for 10-14 days. During this period plants were watered from the base to ensure conidia were not produced prematurely. Conidial production was initiated by wetting the upper surface of leaves the evening before they were required and incubating overnight in a propagator top.

For each fungicide and control treatment, three replicate basil plants (6-week-old) were inoculated to run-off with a spore suspension containing $2.5x10^4$ conidia ml^{-1}. Plants were transferred to a propagator top and incubated in the dark at room temperature for 18 hours before being placed in a glasshouse maintained at 20°C. After 12 days the percent leaves per plant showing downy mildew symptoms was assessed.

Protected production

Basil seed (cv 'Marian') were sown (15/2/2017) in plug trays and raised under controlled environment conditions of 16 hours light period and 8 hours dark period. Once at the two-true leaf stage plants were potted on into 10 cm pots. The trial consisted of 11 treatments and an untreated control (**Table 3**). These included eight individual products applied as foliar sprays and three experimental spray programmes. Products were applied using an Oxford

Precision Sprayer fitted with Lurmark 30HCX2 nozzles. The plots for the single product treatments were split in two; half were treated with two spray applications applied fortnightly and the other half with four spray applications applied weekly (**Table 4**). The experimental spray programmes included two, three or four treatment applications. The trial was replicated four times and laid out in a fully randomized block design.

Table 3. Product list and application rate used in the protected downy mildew trial.

Product	Active ingredient	Application rate/ha	UK approval
Untreated	-		-
Fubol Gold	metalaxyl-M + mancozeb	1.9 kg	Off label for outdoor and protected basil
Revus	mandipropamid	0.6 L	On label for outdoor and protected basil
HDC F237	-	2.5 L	
Paraat	dimethomorph	0.36 L	Off label for outdoor and protected basil
Previcur Energy; Amistar	fosetyl-Al + propamocarb HCl; azoxystrobin	2.5 L 1 L	Off label for outdoor and protected basil
Infinito	fluopicolide + propamocarb HCl	1.6 L	Off label for outdoor basil
HDC F226	-	0.7 L	-
HDC F225	-	1 L	-
Programme 1	Fubol Gold; Paraat	1.9 kg; 0.36L	See above
Programme 2	Previcur Energy; Paraat; Revus	2.5 L; 0.36 L; 0.6 L	See above
Programme 3	Infinito; HDC F225; HDC F226; HDC F237	1.6 L; 1 L; 0.7 L; 2.5 L	See above

The first treatment applications were applied three days after potting. Plants sporulating with downy mildew were introduced into the plots three days after the first treatment applications. The trial was watered thoroughly and covered with polythene sheeting for 24 hours to create a humid environment for infection to occur. This created optimum conditions for downy mildew development and provided a stern test for the plant protection products.

The trial was assessed for disease incidence and severity 14, 21 and 28 days after the introduction of the sporulating plants. The trial was then covered with polythene sheeting to create a humid environment for 48 hours and a final assessment carried out.

Outdoor production

An outdoor site comprising 76 mini-plots (1 m x 0.4 m) was established at NIAB (Cambridge, UK) for conducting the fungicide efficacy trial. Thirty seed trays (375 mm x 250 mm) were filled with M2 compost and sown with 250 mg (~125) basil cv. Sweet Genovese seed (CN Seeds, Pymoor, Ely) (1/8/17). The seedlings were germinated outdoors and grown on to second true-leaf (16/8/17). Twenty-five plants were then transplanted to each of the mini-plots, irrigated with a sprinkler and allowed to grow until four true leaves before starting the

Table 4. Treatment list and application timings used in the protected basil downy mildew trial.

No.		Treatment	active ingredient (a.i.)	T1 (1000 L/ha water rate) 2-3 days after potting	T2 (600 L/ha water rate) 7 days after T1	T3 (600 L/ha water rate) 7 days after T2	T4 (600 L/ha water rate) 7 days after T3
1		Untreated	Water control	+	+	+	+
2	A	Fubol Gold	mancozeb + metalaxyl-M	+	-	+	-
	B			+	+	+	+
3	A	Revus	mandipropamid	+	-	+	-
	B			+	+	+	+
4	A	HDC F237	-	+	-	+	-
	B			+	+	+	+
5	A	Paraat	dimethomorph	+	-	+	-
	B			+	+	+	+
6	A	Previcur Energy (T1 only)	fosetyl-aluminium + propamocarb HCl	+	-	+	-
	B			+	+	+	+
6	A	Amistar(T2-T4)	Azoxystrobin	+	-	+	-
	B			+	+	+	+
7	A	Infinito	fluopicolide + propamocarb HCl	+	-	+	-
	B			+	+	+	+
8	A	HDC F226	-	+	-	+	-
	B			+	+	+	+
9	A	HDC F225	-	+	-	+	-
	B			+	+	+	+
10		Experimental Programme 1 (2-spray)		Fubol Gold	-	Paraat	-
11		Experimental Programme 2 (3-spray)		Previcur Energy	Paraat	Revus	-
12		Experimental Programme 3(4-spray)		Infinito	HDC F225	HDC F226	HDC F237

Plots were split (A/B) when the same treatment was applied at each application timing so that a 2-spray programme could be compared with a 4-spray programme (+ indicates spray, - indicates no spray)

treatments (24/8/17). The trial was arranged in a fully-replicated block design with 30 cm spaces between plots to limit the effects from spray drift.

The trial assessed 15 individual treatments, three programmes and a control (**Table 5**). Treatments were diluted appropriately to a final application rate of 1500 litres/ha and applied preventatively, in the evening, using a small hand sprayer. Care was taken to ensure products were applied evenly to both upper and lower surfaces of the leaves. Sporulating (*P. belbahrii*) spreader plants were positioned at the end of each plot, throughout the trial area the following morning (two pots per plot, five plants per pot). Plots were irrigated at dusk/dawn and covered with plastic sheeting for three consecutive nights to ensure optimal infection conditions. Two additional foliar applications of aqueous spore suspension ($1x10^5$ conidia ml^{-1}) were applied evenly across the trial area until run-off, during the first and second evenings after treating, to encourage infection.

Table 5. Product list and application rate used in the outdoor downy mildew trial established during August 2017.

Product	Active ingredient	Application rate/ha
Control	N/A	N/A
Amistar	Azoxystrobin	1 L
HDC F238		1 L
HDC F223		10 g
HDC F239		500 ml
HDC F240		500 ml
HDC F241		2.5 g
HDC F242		2.5 g
HDC F243		5 L
Fenomenal	fenamidone/fosetyl-aluminium	2.5 kg
Fubol Gold	metalaxyl-M + mancozeb	1.9 kg
Infinito	fluopicolide + propamocarb HCl	1.6 L
Invader	dimethomrph + mancozeb	2 kg
HDC F244		4 L
Paraat	dimethomorph	3 kg
Revus	mandipropamid	0.6 L
Programme 1	Amistar, Paraat, Revus	1 L; 3kg; 0.6 L
Programme 2	Fenomenal, Revus, Fubol Gold	2.5 kg; 0.6 L; 1.9 kg; 0.6 L; 1.9 kg
Programme 3	Invader, Infinito, Revus	2 kg; 0.6 L; 1.6 L; 0.6 L; 1.6 L

Symptoms were assessed as the leaf area affected (%) and the severity of sporulation (0 – no sporulation and 5 – profuse sporulation), every 2-3 days over a six-week period. Treatment re-application was conducted according to guidance on product labels (listed in **Table 6**). Trial data was analysed using a one-way analysis of variance.

Table 6. Treatment list and application timings used in the outdoor basil downy mildew trial established during August 2017.

Product	Active ingredient	Day 1 (24/8/17)	Day 7 (31/8/17)	Day 14 (6/9/17)	Day 21 (13/9/17)	Day 28 (19/9/17)	Day 35 (26/9/17)	PHI (days)
Control	N/A	x	x	x	x	x	x	N/A
Amistar	Azoxystrobin	x						14
HDC F238		x		x		x		14
HDC F223		x	x	x	x	x	x	7
HDC F239		x		x		x		14
HDC F240		x		x		x		14
HDC F241		x	x	x	x	x	x	7
HDC F242		x	x	x	x	x	x	7
HDC F243		x	x	x	x	x	x	7
Fenomenal	fenamidone + fosetyl-Al	x		x				35
Fubol Gold	metalaxyl-M + mancozeb	x		x		x		14
Infinito	fluopicolide + propamocarb HCl	x						14
Invader	dimethomrph + mancozeb	x	x	x				21
HDC F244		x	x	x	x	x	x	7
Paraat	dimethomorph	x	x	x	x	x	x	7
Revus	mandipropamid	x	x	x	x	x	x	7
Programme 1	Amistar, Paraat, Revus	Amistar	Paraat	Revus	Paraat	Revus	Paraat	7
Programme 2	Fenomenal, Revus, Fubol	Fenomenal	Revus	Fubol Gold	Revus	Fubol Gold		14
Programme 3	Invader, Revus, Infinito	Inv	Revus	Inf	Revus	Inf		14

Safety of fungicides and programmes for use on basil seedlings

Seeds were sown (10/07/2017) in pots containing Levington F2 + sand commercial substrate on benches in a glasshouse. There were 12x10 cm pots in a carry tray per plot and four replicates per treatment arranged in a randomised complete block design. The trial consisted of eight different products and an untreated control (**Table 7**). When two true leaves were fully expanded each plot was split into two with half sprayed at the standard recommended rate and the other half sprayed at double the standard rate for that treatment. The trial was assessed for symptoms of phytotoxicity seven days and 14 days after application.

Table 7. Product list and application rate used during the crop safety trial

Product	Active ingredient	Application rate/ha
Untreated	-	-
Fenomenal*	fenamidone/fosetyl-aluminium	2.5 (1N) and 5 (2N) kg
Fubol Gold	metalaxyl-M + mancozeb	1.9 (1N) and 3.8 (2N) kg
Infinito*	fluopicolide + propamocarb HCl	1.6 (1N) and 3.2 (2N) L
HDC F245	-	2 (1N) and 4 (2N) kg
HDC F226	-	0.7 (1N) and 1.4 (2N) L
HDC F238	-	0.5 (1N) and 1 (2N) L
HDC F239	-	0.5 (1N) and 1 (2N) L
HDC F240	-	0.5 (1N) and 1 (2N) L

*Products not currently approved for use on protected herbs.

The effect of illumination during the night on infection and sporulation by *P. belbahrii.*

Seeds were sown (7/07/2017) in pots containing Levington F2 + sand commercial substrate on benches in a glasshouse. Nine light regimes were trialled (**Table 8**) with twelve 10 cm pots per plot and four replicate plots per lighting regime. Plots were arranged in a randomised block design. The benches were covered with white polythene and there was a piece of capillary matting under each carry tray. Four weeks after sowing each plot was spray inoculated with 20 ml of a *P. belbahrii* spore suspension containing $2.3x10^4$ conidia/ml. Following inoculation, the plots were split, and a polythene bag placed over half the plot to create the humid environment required for spore germination, infection and subsequent sporulation. Plants were maintained in near optimum high humidity conditions for sporulation, but the length of exposure to light during the dark period was varied according to treatment. During the dark period, plants were illuminated with light at an intensity of approximately 10 µmol m^2 s^{-1} for different periods of time, ranging from 0 to 8 hours depending on treatment. Plots were shielded from each other by black screens covered with white polythene (**Figure 1**) to prevent light contamination between plots. The other half of the plots were left uncovered for the duration of the trial, but were exposed to the same lighting regime. Plants

were watered regularly and fed with appropriate nutrient solution as required. Two weeks after inoculation, the plants were assessed for downy mildew sporulation. At the end of the trial the plants in the parts of the plots which had been left uncovered were covered with a polythene bag and placed under conditions of the control treatment (8 hours dark period) for 48 hours and then reassessed after 24 hours. Plot assessments were made for crop height, vigour and chlorosis.

The trial was carried out in mid-August. Sunset ranged from 20:45 at the start of the trial to 20:15 at the end of the trial and sunrise ranged from 05:30 at the start of the trial to 06:00 at the end of the trial. There were periods of approximately one hour of diminished light levels at dusk (the beginning of the night period) and dawn (the end of the night period). The period of darkness ranged from 7:45 hours at the start of the trial to 8:45 hours at the end of the trial.

Table 8. Lighting treatments trialled to determine the effect of light on infection of basil by *Peronospora belbahrii*.

Period of night lighting (hrs)	Timer switch		Sunset		Time														Sunrise	
	On	Off	20:00	20:30	21:00	21:30	22:00	22:30	23:00	23:30	00:00	00:30	01:00	01:30	02:00	02:30	03:00	03:30	04:00	04:30	05:00	05:30
0	-	-	Twilight		Period of darkness																	Twilight
1	00:30	01:30																				
2	00:00	02:00																				
3	23:30	02:30																				
4	23:00	03:00								period of time plants lit during the night												
5	22:30	03:30																				
6	22:00	04:00																				
7	21:30	04:30																				
8	21:00	05:00																				
			Dusk																		Dawn	

Figure 1. Shielding used in trial to test the effect of overnight lighting on infection of basil by *Peronospora belbahrii*.

Results

Epidemiology

Determine whether *P. belbahrii* is present in UK seed lots and whether infected seed acts as a primary source of infection

Determination of the presence of *Peronospora belbahrii* DNA in basil seed samples

20 seed lots were sourced (**Table 9**) and tested for the presence of *P. belbahrii* DNA. Results are presented as cycle threshold values or Ct values which represent the number of amplification cycles after which fluorescence, and therefore DNA, can be detected above a background level. Each amplification cycle breaks down the DNA strands and then rebuilds them, thus doubling the amount of target DNA each cycle. In each test 40 amplification cycles were carried out. The Ct value is inversely proportional to the amount of target DNA, so the lower the Ct value the more target DNA is present in the sample. A value of 40 (the maximum number of amplification cycles) indicates a negative result (either no DNA present in the sample, or the DNA present was not detected by the primer set). 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.

Of the seed samples tested, all but one contained *P. belbahrii* DNA. Of the remaining 19 samples, 15 contained *P. belbahrii* DNA at similar levels across all five replicates, suggesting an evenly distributed contamination of the sample. Of these 15 samples, nine contained high levels of *P. belbahrii* DNA, with six samples containing abundant 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. The remaining four samples all contained low levels of *P. belbahrii* with between one and four replicates containing no *P. belbahrii* DNA.

Infection of basil plants from contaminated seed

Two seed lots, samples 2 and 7, were chosen for this study due to the presence of large amounts of *P. belbahrii* DNA and a large enough quantity of seed on which to carry out the test. Despite regular wetting of plants, to encourage disease development and sporulation, no basil downy mildew symptoms were observed on any basil plants. PCR analysis of 25 plants selected at random (**Table 10**) indicated that low levels of *P. belbahrii* DNA were detected (at noticeably lower levels than found on seed), with plants from sample 2 having

on average slightly higher Ct values than those from sample 7; this mirrors the result observed in the seed.

Table 9. Average cycle threshold value for *Peronospora belbahrii* DNA and oospore count for 20 basil seed samples.

Sample	Supplier	Ave. Ct value (range)	Oospore count
1	1	37.3 (32.1 to 40)	0
2	2	29.9 (29.3 to 30.9)	0
3	3	30.4 (29.9 to 30.7)	0
4	4	33.6 (33.4 to 34.2)	0
5	4	24.7 (24.4 to 25.4)	0
6	5	37.3 (36.1 to 38.7)	0
7	5	30.2 (29.7 to 31)	0
8	-	39.7 (38.5 to 40)	0
9	-	38.5 (37.6 to 40)	-
10	-	37.8 (35.5 to 40)	-
11	6	40	-
12	7	38.2 (37.5 to 39.6)	
13	8	31.8 (31.6 to 32.2)	-
14	8	37.7 (36.7 to 38.5)	-
15	8	32.5 (32.2 to 32.9)	-
16	8	31.0 (30.6 to 31.6)	-
17	8	30.4 (29.9 to 31.3)	0
18	8	33.8 (33.4 to 34.2)	
19	8	29.3 (29.2 to 29.5)	0
20	8	38.4 (36.7 to 39.6)	-
+ve control	n/a	22.6 (22.5 to 22.6)	n/a
-ve control	n/a	40	n/a

Table 10. *Peronospora belbahrii* DNA levels (expressed as cycle threshold (Ct) value) in basil plants grown from seed samples 2 and 7.

Sowing	Ave. Ct value (replicate range)	
	Sample 2	Sample 7
1	38.8 (37.6 - 40)	40
2	40	38 (36 - 40)
3	39.4 (38.7 - 40)	37.7 (35.3 - 40)
4	37.9 (35.7 - 40)	40
5	36.2 (36 - 36.3)	40
Average Ct	38.4	39.1
Original seed Ct	29.7	30.24

Determination of the presence of oospores of *Peronospora belbahrii* in basil seed samples

Spore washings were carried out on ten of the seed samples with no oospores (or conidia) detected in any of the washings.

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

Four soil samples from diseased crops have been screened for *P. belbahrii* contamination; one sample from an inoculated field-grown trial and three from a naturally infested crop with high disease incidence. The results for the soil DNA tests are presented as cycle threshold (Ct) values representing the number of amplification cycles, analogous to the seed lot testing (**Table 11**). Ct values are inversely proportional to the amount of target DNA present in a sample, with a lower Ct indicating a greater quantity of DNA. A Ct value 40 indicates no target DNA is present.

Table 11. Average cycle threshold (Ct) values for DNA extracted from 1 kg bulk soil samples from the inoculated outdoor production site (Trial A/B) and naturally infested crop (Grower 1-3, A/B). Positive control = *Peronospora belbahrii* DNA, negative control = uncontaminated soil.

Soil sample	Average Ct (0-40)
Trial (A)	40
Trial (B)	40
Grower 1 (A)	36.4
Grower 1 (B)	37
Grower 2 (A)	34.9
Grower 2 (B)	35.2
Grower 3 (A)	35.2
Grower 3 (B)	34.9
Positive control	12
Negative control	40

Soil sampled from the artificially inoculated trial tested negative for *P. belbahrii* in both replicates from the 1 kg bulks tested. In contrast, both replicates from all three 1 kg samples from the naturally infested site tested positive for *P. belbahrii*; Ct values ranged from 34.9 to 37 indicating *P. belbahrii* DNA was present at low but relatively uniform levels in each of the respective samples.

The absence of *P. belbahrii* DNA observed in soil from the artificially infested crop could be due to the lower levels of disease symptoms observed in the 2016 trial (Year 1 Annual report) compared to the naturally infested crop where disease symptoms were severe and hard to

control (D. Shailes, personal communication). This would result in a reduced amount of infected crop-debris at the artificially inoculated trial site compared to those naturally infected.

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

The effect of humidity on leaf wetness

In the first year of PE 024 the effect of humidity on the length of time basil leaves grown under protected conditions remained wet was determined. This experiment was repeated in year 2 to establish whether growing basil plants outdoors produced a significantly different result. Whether plants were grown under protection or outdoors did not affect the length of time leaves remained wet at the different humidity regimes tested.

Combining the data produced on the length of time leaves remain wet at the different relative humidity regimes, with data on infection at different temperatures allows the production of a basil downy mildew risk matrix (**Figure 2**). The grid highlights that downy mildew infections are most likely to occur at temperature between 15 and 20ºC when the relative humidity is 70 % or greater.

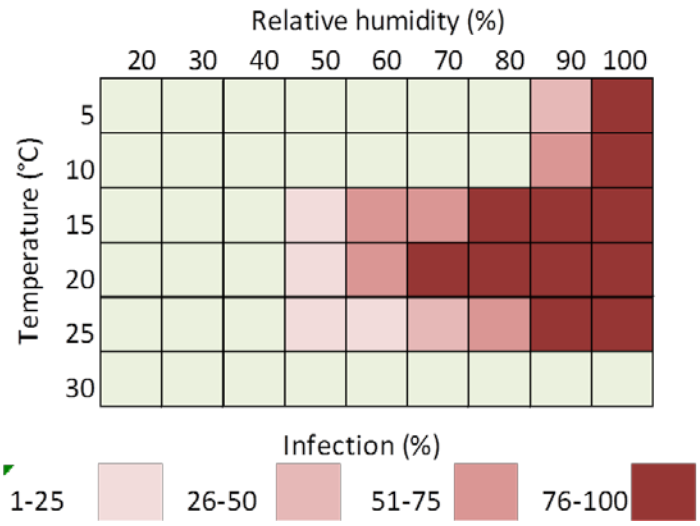
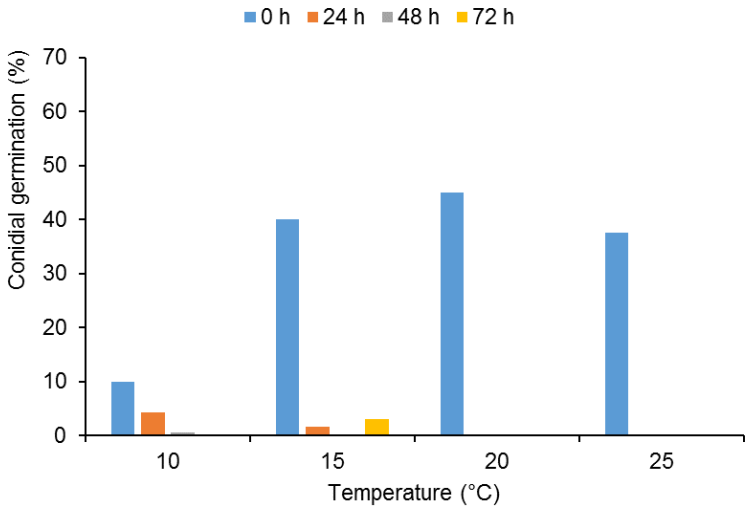


Figure 2. Basil downy mildew infection risk grid for protected and outdoor basil crops

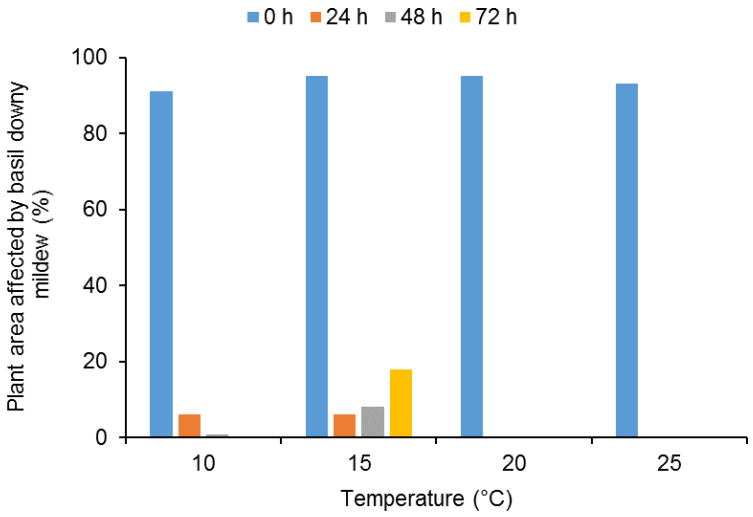
The effect of temperature on the survival of *Peronospora belbahrii* conidia

Three sets of experiments were carried out to establish how conidia of *P. belbahrii* survive at different temperatures. In the first experiment conidia were exposed to temperatures of 10, 15, 20 and 25ºC for 24, 48 and 72 hours. An initial (0 h) assessment of conidial germination gave an average of 33%, with a range between 10 and 45% (**Figure 3a**). Even though there was a wide range in conidial germination across temperature treatments, the severity of infection produced by these spores was similar with an average of 94%, ranging between 91

and 95% (**Figure 3b**). This may suggest that conidial germination is not necessarily a good indication of infectivity. Over the 72-hour exposure period germination and plant infection reduced at 10 and 15ºC, with no conidial germination or plant infection observed after 72 hours exposure at 10ºC. Exposure of conidia to temperatures of 20 and 25ºC resulted in no germination of conidia or plant infection, even after 24 h exposure.



a)

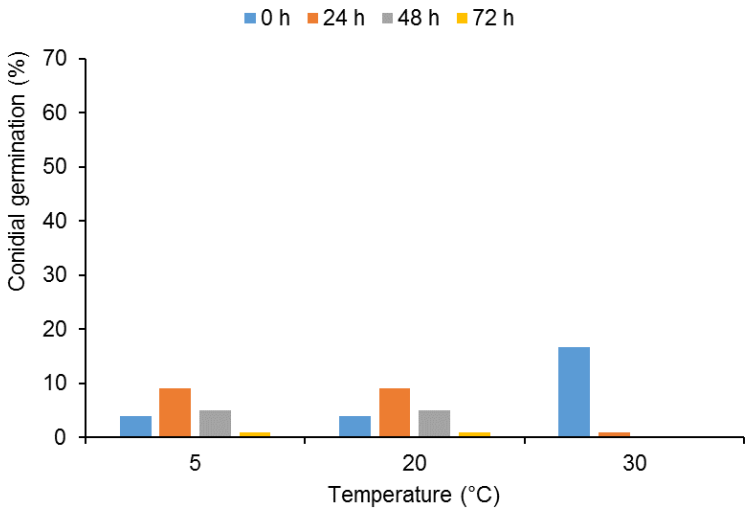


b)

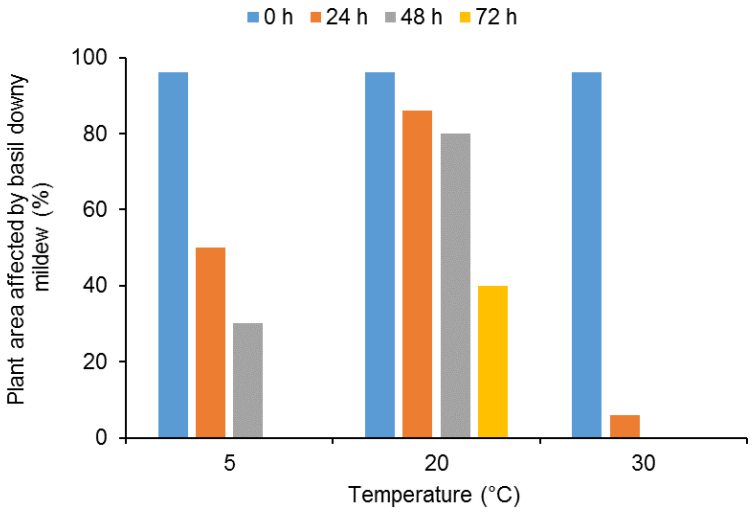
Figure 3. Experiment 1 – the effect of temperature and exposure period on survival of *Peronospora belbahrii* conidia through assessment of, a) conidial germination and b) severity of plant infection.

The second experiment examined conidial survival following exposure to temperatures of 5, 20 and 30ºC; this provided data on conidial survival at more 'extreme' temperatures, plus a repeat to confirm data from the first experiment. The initial (0 h) assessment of conidial germination was lower than the first experiment with an average of 8%, ranging between 4 and 17% (**Figure 4a**). Even though conidial germination was lower than in the first experiment the severity of plant infection was similar at 96% (**Figure 4b**). Exposure of conidia to 5 and

20ºC for 24 h resulted in a higher rate of germination (9%) than assessed at 0 h (4%). In contrast, the severity of plant infection caused by these conidia was lower than the infection observed at 0 h. Infection of plants by conidia exposed to 5ºC for 24 h was lower than that seen by conidia exposed to 20ºC (50% compared to 86%, **Figure 4b**).



a)



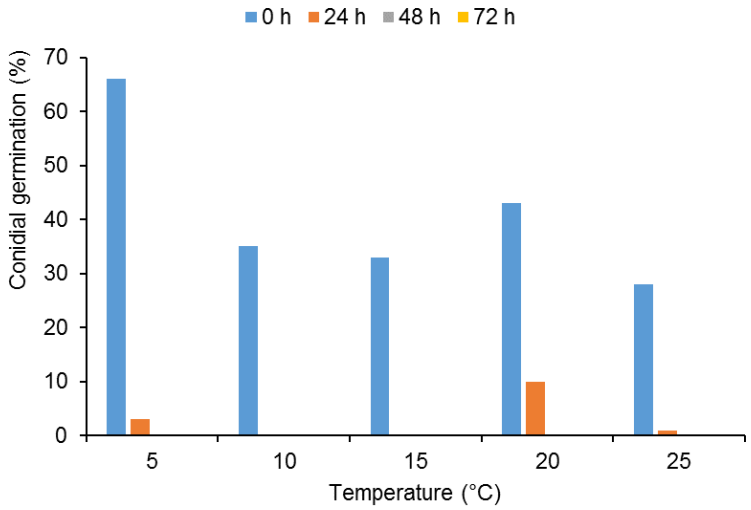
b)

Figure 4. Experiment 2 – the effect of temperature and exposure period on survival of *Peronospora belbahrii* conidia through assessment of, a) conidial germination and b) severity of plant infection.

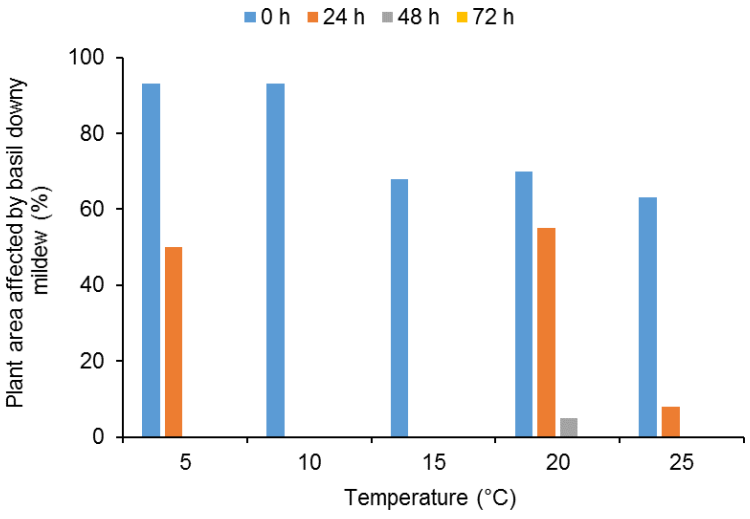
Infection of plants occurred following inoculation using conidia exposed to 30ºC for 24 h (**Figure 4b**), the level of infection observed was lower than that following exposure at 5 and 20ºC. At all temperatures increasing the exposure from time 24 h resulted in a decrease in conidial survival (**Figure 4a**), with no infection following an exposure of 48 h at 30º C and 72 h at 5ºC (**Figure 4b**). Conidia exposed to 20ºC survived the full 72 h exposure although the severity of plant infection reduced from 96% at 0 h to 40%. This result contradicted the first

experiment where no plant infection occurred (suggesting conidia had not survived) following exposure to 20ºC.

Inconsistency of data between experiments 1 and 2 resulted in a third experiment where conidial survival was examined at 5, 10, 15, 20 and 25ºC. The initial (0 h) assessment of conidial germination was as observed in the first experiment with an average of 41%, ranging between 28 and 66% (**Figure 5a**). The severity of plant infection observed following plant inoculation with these spores was high (77%) but more variable than the first two experiments ranging between 63 and 93% (**Figure 5b**). Exposure of conidia for 24 h only resulted in



a)



b)

Figure 5. Experiment 3 – the effect of temperature and exposure period on survival of *Peronospora belbahrii* conidia through assessment of, a) conidial germination and b) severity of plant infection.

conidial germination or plant infection at 5, 20 and 25ºC. After 48 h, only conidia exposed to 20ºC produced any infection when inoculated onto basil plants. No conidia appeared to survive 72 h exposure at any temperature.

Oospore production under protected and outdoor conditions

No oospores were observed in downy-mildew infected plant tissue grown under protected or outdoor conditions, or in soil samples collected from diseased crops. This indicates, at least in the isolates tested in this series of experiments, that the formation of oospores in *P. belbahrii* is unlikely.

Control

Evaluate fungicide programmes in large-scale 'commercial' trials (protected and outdoor production) for efficacy against *P. belbahrii*.

Fungicide longevity trial

Five fungicides were trialled to determine the maximum timing between applications to achieve control of *P. belbahrii* on basil. Three timings were used 7, 10 and 14 days pre-inoculation. All five products trialled significantly (p = 0.05) reduced symptoms of basil downy mildew compared to the water control when applied 7 days pre-inoculation (**Figure 6**). At this timing there was no significant (p = 0.05) difference in the reduction achieved between Paraat, Fenomenal or Revus. The control achieved by Fubol Gold was significantly less than that of Paraat, Fenomenal or Revus, but not HDC F225. It should be noted that Fenomenal is only approved for outdoor use.

Extending the application timing from 7 days pre-inoculation to 10 days pre-inoculation only resulted in a significant (p = 0.05) increase in disease following the application of HDC F225 (**Figure 6**); there was no significant increase following treatment with Paraat, Fenomenal, Revus or Fubol Gold. As with the 7-day pre-inoculation treatments, disease reduction following the application of Paraat, Fenomenal or Revus 10 days pre-inoculation was significantly (p = 0.05) greater than that of Fubol Gold.

Further extending the application time to 14 days pre-inoculation resulted in a significant (p = 0.05) increase in disease for all treatments compared to the 7 and 10-day pre-inoculation timings (**Figure 6**). The application of Paraat, Revus or Fenomenal gave a significant (p = 0.05) reduction in disease compared to the control; however, any reduction achieved following treatment with Fubol Gold or HDC F225 was not significant (p = 0.05). Overall the treatment of basil with Paraat resulted in the greatest reduction in downy mildew.

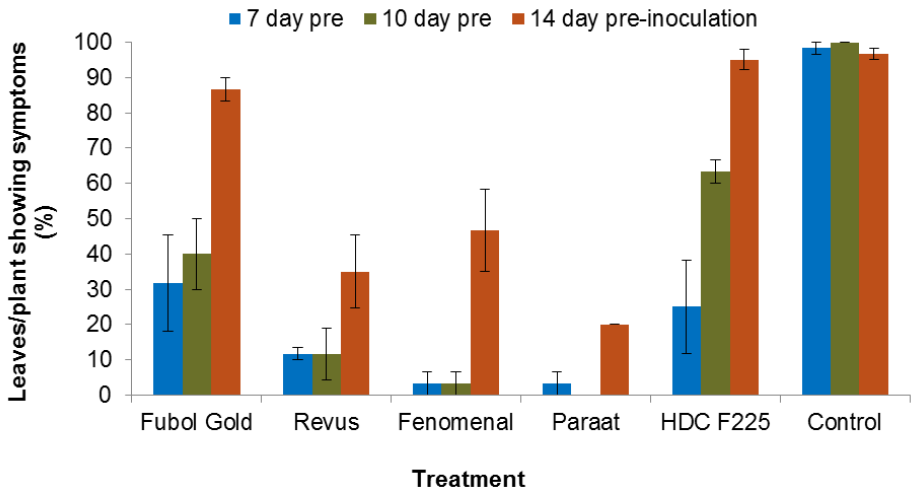


Figure 6. The effect of pre-inoculation fungicide application timings on basil downy mildew symptoms caused by *Peronospora belbahrii.* Error bars represent standard error of the mean, LSD (0.05) = 19.9

The lack of downy mildew control by Fubol Gold was unusual and so a soil drench of Subdue (metalaxyl-M) (full rate) was applied to basil plants two days prior to inoculation with *P. belbahrii* to try and establish whether the isolate used in the trial was resistant to metalaxyl-M. Downy mildew symptoms were observed on both the Subdue treated and control plants (**Figure 7**) which suggested that the isolate was resistant to metalaxyl-M. Some disease control was achieved following the application of Fubol Gold, which suggested that either the mancozeb element of Fubol Gold was providing some control or the culture being used contained a mixture of both metalaxyl-M resistant and sensitive isolates, with the Fubol Gold controlling the sensitive isolate within the mix.

Figure 7. Comparison of downy mildew symptoms on a control plant (right) and a full rate Subdue treated plant (left) following inoculation with the *Peronospora belbahrii* isolate used in the longevity trial. Inserts show sporulation on the underside of symptomatic leaves.

Protected production

Significant differences in levels of downy mildew incidence (**Table 12**) and severity (**Table 13**) were observed between treatments 14 and 21 days after introduction of infector plants. No individual product completely controlled disease, however the lowest levels of disease were observed following treatment with Revus, HDC F237 and HDC F226. For all treatments increasing the number of applications resulted in a decrease in disease incidence and severity. Where Previcur Energy was used first, in a programme or as an individual product, no disease control was observed.

By 28 days after the introduction of infector plants, only plants which had received four applications of Revus had significantly lower disease incidence (78.35 %, **Table 12**) versus the untreated control (100 %). No individual product or spray programme gave 100 % control of disease; however, there were significant reductions in disease severity following four applications of Revus, Paraat or HDC F226 and the four-spray programme (**Table 13**). The disease control observed following treatment with Fubol Gold was lower than anticipated. The protected production trial used the same *P. belbahrii* isolate as the longevity trial, thus these data back up the observations on reduced control by Fubol Gold made in the longevity trial.

A phytotoxic effect was observed in plants which had received multiple applications of Fubol Gold. Symptoms included stunted plants with deformed, crinkled and distorted leaves. The effect was more pronounced in plants which had received four applications compared to those that had received two.

Table 12. Basil downy mildew incidence following treatment with individual products and spray programmes for the fungicide trial carried out under protected conditions.

No.	Treatment	Active Ingredients	No. of applications	Disease Incidence (Days after introduction of infector plants)			
				14[$]	21[$]	28[$]	31*
1A	Untreated		2	100 a	100 a	100 a	100 -
1B			4	100 a	100 a	100 a	100 -
2A	Fubol Gold	mancozeb + metalaxyl-M	2	58.33 b	59 b-e	98.9 a	91.67 -
2B			4	25 cde	31 def	97.65 a	100 -
3A	Revus	mandipropamid	2	12.5 e	37 c-f	98.9 a	100 -
3B			4	8.33 e	4 g	**78.35 b**	91.67 -
4A	HDC F237	-	2	20.83 de	50 b-f	100 a	100 -
4B			4	4.17 e	16 fg	98.9 a	95.83 -
5A	Paraat	dimethomorph	2	29.17 cde	68 bcd	100 a	100 -
5B			4	16.67 e	23 efg	98.9 a	100 -
6A	Previcur Energy[#]	fosetyl-aluminium + propamocarb HCl	2	100 a	100 a	100 a	100 -
6B	Amistar	azoxystrobin	4	100 a	100 a	100 a	95.83 -
7A	Infinito^	fluopicolide + propamocarb HCl	2	50 bc	68 bcd	100 a	100 -
7B			4	29.17 cde	44 b-f	100 a	100 -
8A	HDC F226	-	2	12.5 e	19 fg	100 a	95.83 -
8B			4	20.83 de	19 fg	98.9 a	91.67 -
9A	HDC F225	-	2	70.83 b	72 bc	98.9 a	100 -
9B			4	50 bc	70 bc	100 a	100 -
10A	2 Spray	Fubol Gold, Paraat	2	20.83 de	46 b-f	100 a	100 -
10B			4	29.17 cde	36 c-f	100 a	100 -
11A	3 Spray	Previcur Energy, Paraat, Revus	2	100 a	100 a	100 a	100 -
11B			4	100 a	100 a	100 a	100 -
12A	4 Spray	Infinito, HDC F225, HDC F226, HDC F237	2	58.33 b	77 b	100 a	100 -
12B			4	45.83 bcd	63 bcd	100 a	95.83 -
	P value			0.0001	0.0001	0.0442	0.5305

* after overnight humidity. [#] Previcur Energy was applied as the first application and Amistar used for applications 2, 3 and 4. [$]Values followed by the same letter are not significantly different. ^Infinito is not currently approved for use on protected herbs.

Table 13. Basil downy mildew severity following treatment with individual products and spray programmes for the fungicide trial carried out under protected conditions.

No.	Treatment	Active Ingredients	No. of applications	Disease Severity (Days after introduction of infector plants)							
				14[$]		21[$]		28[$]		31*[$]	
1A	Untreated		2	52.79	a	50.82	a	69.02	a	67.67	a
1B			4	47.04	ab	46	ab	64.33	a	65.28	a
2A	Fubol Gold	mancozeb + metalaxyl-M	2	1.76	efg	1.23	def	3.83	e-i	4.74	g-j
2B			4	0.66	fg	0.71	e-h	3.12	f-i	3.34	j
3A	Revus	mandipropamid	2	0.11	g	0.35	fgh	4.61	d-h	5.79	f-j
3B			4	0.04	g	0.06	h	1.71	i	2.73	j
4A	HDC F237	-	2	0.18	fg	0.44	fgh	5.67	c-h	8.82	e-i
4B			4	0.02	g	0.12	h	3.72	e-i	3.48	j
5A	Paraat	dimethomorph	2	0.63	fg	1	efg	6.25	c-g	9.24	e-i
5B			4	0.42	fg	0.44	fgh	2.68	hi	4.1	ij
6A	Previcur Energy[#]	fosetyl-aluminium + propamocarb HCl	2	37.42	bc	41.9	ab	36.61	ab	46.41	ab
6B	Amistar	azoxystrobin	4	21.16	d	27.46	b	24.66	b	17.22	cde
7A	Infinito	fluopicolide + propamocarb HCl	2	1.84	efg	1.19	def	8.71	cd	20.08	cd
7B			4	0.96	fg	0.76	e-h	4.75	c-h	9.37	e-h
8A	HDC F226	-	2	0.07	g	0.21	gh	4.85	c-h	5.67	f-j
8B			4	0.09	g	0.09	h	2.97	ghi	2.73	j
9A	HDC F225	-	2	4.75	e	3.24	c	9.62	c	9.94	d-h
9B			4	2.86	ef	1.74	cde	7.19	cde	9.99	d-g
10A	2 Spray	Fubol Gold, Paraat	2	0.65	fg	0.57	e-h	6.58	c-f	10.96	def
10B			4	1.4	efg	0.74	e-h	6.98	cde	9.29	e-i
11A	3 Spray	Previcur Energy, Paraat, Revus	2	44.27	ab	41.36	ab	36.13	ab	33.55	abc
11B			4	26.3	cd	33.4	ab	30.25	b	24.74	bc
12A	4 Spray	Infinito, HDC F225, HDC F226, HDC F237	2	2.03	efg	2.55	cd	6.04	c-g	4.41	hij
12B			4	1.15	efg	1.35	def	2.98	ghi	2.87	j
	P value			0.0001		0.0001		0.0001		0.0001	

* after overnight humidity. [#] Previcur Energy was applied as the first application and Amistar used for applications 2, 3 and 4. [$]Values followed by the same letter are not significantly different. ^Infinito is not currently approved for use on protected herbs.

Outdoor production

Results from the outdoor production trial are shown in **Table 14**. Disease symptoms were first observed in control plots 14 days after the initial inoculation, with an average of 63% of the plot area affected, with moderately severe levels of sporulation. Significant differences were observed between treatments for both severity (*p*=0.001, LSD 5%: 0.44) and the percentage leaf area affected (p = 0.001, LSD 5%: 8.9). No disease symptoms were observed in any of the plots treated with F239 and Programme 3. Plots treated with HDC F240, Fubol Gold, Revus, Paraat and Programme 3 exhibited low levels of sporulation (severity <1) and less than 1% of the plot area was affected, demonstrating an effective level control could be achieved with these products. Although not as effective as the previous products, symptoms were also significantly reduced in plots treated with Programme 1, Invader and Fenomenal (severity <2, <7% area affected) compared to the control.

Table 14. Basil downy mildew disease levels following treatment with individual products and spray programmes for the outdoor basil trial established during August 2017.

Product	Active ingredient	Severity (0-5*) (LSD 5% = 0.44)	Plot affected (%) (LSD 5%: 8.9)
Control	N/A	3.30	62.94
Amistar	azoxystrobin	1.44	22.17
HDC F223		2.34	25.5
HDC F238		3.03	56.81
HDC F239		0	0
HDC F240		0.02	0.08
HDC F241		2.72	49.44
HDC F242		2.81	55.0
HDC F243		2.60	35.67
Fenomenal	fenamidone/fosetyl-aluminium	1.36	6.36
Fubol Gold	metalaxyl-M + mancozeb	0.05	0.08
Infinito	fluopicolide + propamocarb HCl	1.54	29.42
Invader	dimethomorph + mancozeb	0.33	2.17
HDC F244		1.47	20.94
Paraat	Dimethomorph	0.36	0.89
Revus	Mandipropamid	0.11	0.33
Programme 1	Amistar, Paraat, Revus	0.28	1.39
Programme 2	Fenomenal, Fubol Gold, Revus	0.28	0.97
Programme 3	Invader, Infinito, Revus	0	0

*Downy mildew disease symptoms (sporulation) scored as severity (0-5); 0: no sporulation, 5: heavy, profuse sporulation).

Safety of fungicides & programmes for use on basil seedlings

Seven days after the application of Fubol Gold, at both standard and double rate, a residue was observed on the leaves of the plants. Plants treated with HDC F245 were noticeably larger, greener and more vigorous than the untreated control plants. This effect was accentuated by double the application rate. Some of the plots treated with HDC F240, at double the standard rate, were slightly smaller than the untreated control, although this was not consistent across all replicates.

Fourteen days after product application the residue on leaves of plants treated with Fubol Gold was no longer visible. Plants treated with HDC F245 remained noticeably larger, greener and more vigorous compared to the untreated control. Plants treated with HDC F240, at double the standard rate, were smaller than the untreated control, although the size reduction was less obvious than at the seven-day assessment.

The effect of illumination during the night on infection and sporulation by *P. belbahrii.*

Eight different overnight lighting regimes (1 to 8 h) were used to determine how lighting during the night affects *P. belbahrii* infection and sporulation on basil plants. Symptoms of downy mildew were observed on all plots 14 days after inoculation. The introduction of illumination during the night resulted in a reduction in disease severity compared to the control (**Table 15**); however, the differences seen were not significant and were only observed on plots which had been left uncovered for the duration of the trial. Although there was a reduction in disease severity between the 1 and 8-hour lighting regimes, the reduction was not consistent for the regimes trialled between these extremes. The lowest disease levels were observed under the 4, 2 and 5-hour night light treatments.

Table 15. Effect of night time illumination on infection of basil by *Peronospora belbahrii*.

Treatment	Disease Severity (% leaf area affected)			Height (cm)	Vigour	Chlorosis
	Uncovered	Covered	Uncovered then covered for 24 hrs		(-5 to +5 scale compared to 0 hours night lighting)	
None	10.38	17.59	15.08	28.75	0	0
1 hour	9.63	10.51	14.5	29	-0.25	0.06
2 hours	6.25	13.57	14.29	29.5	0	0.4
3 hours	8.67	14.25	14.58	29.5	0	0.2
4 hours	5.42	12.4	11.17	28.5	-0.5	0.9
5 hours	6.54	14.56	10.58	28.75	0.25	0.92
6 hours	6.75	12.09	12.29	29.25	-0.25	0.27
7 hours	7.5	9.69	12.79	30.25	0.25	0.81
8 hours	7.71	10.95	9.83	28	-0.25	1.05

Discussion

Work by Garibaldi *et al*. (2004) showed that downy mildew of basil, caused by *P. belbahrii* was seed-borne, with levels of infection as low as 0.02% leading to visible infection of crops. However, from their work it was unclear whether the pathogen was truly seed-borne (systemic) or simply a contaminant (spores surviving on the outside of seed). Determining the type of contamination will help guide the industry to establish the optimum method for producing and treating seed to reduce levels of downy mildew caused by contaminated seed. The seed testing carried out in this project was generally of seed lots where basil downy mildew had proven to be a problem in the growing crop. Most of the seed lots tested contained high levels of *P. belbahrii* DNA; as no oospores (or conidia) were detected in seed washings it seems likely that the DNA detected resulted either from mycelia growing on the seed surface and/or internal seed infection by *P. belbahrii*. Growing on tests of two seed lots, containing high levels of *P. belbahrii* DNA, did not result in any downy mildew infection so the seed-borne nature of the disease could not be confirmed in this project. There are several possible explanations for this result, firstly too few seed were tested to detect a low level of infection or secondly, the DNA detected, whether from mycelia on the seed surface or as an internal contamination, was wholly or partly non-viable. Mycelia on the seed surface would desiccate rapidly and so would not remain infectious for long, however internal seed contamination has the potential to remain viable for much longer. The use of heat, or other seed treatments, could potentially reduce the viability of the *P. belbahrii* DNA detected as an internal seed contaminant. Such treatments would reduce the level of infective seed within a seed sample and hence reduce the chance of detecting a viable infection. Further work, using a larger number of seed and seed collected from an infected crop where it is known that no seed treatment has been applied, would be required to provide confirmation of the seed-borne nature of basil downy mildew.

Epidemiological studies in the first year of the project focused on the how light, temperature and period of leaf wetness affected infection of basil by *P. belbahrii*. In the second year, the focus switched to how temperature affected the survival of conidia produced on the underside of leaves. The data obtained were highly variable, but suggested that the optimum temperature for conidial survival was between 15 and 20ºC. Even at these temperatures it appeared that conditions suitable for plant infection were required within 72 hours of their production on the leaf if subsequent infection was to occur. A metalaxyl-M resistant isolate of *P. belbahrii* was used during the survival study. It is possible that the variability in germination observed for fresh conidia was a ‘fitness-cost’ associated with the metalaxyl-M

resistance and would not be observed with a metalaxyl-M sensitive isolate. However, similar studies carried out with other *Peronospora* species have also shown that conidial germination can be highly variable. The short-term survival of conidia combined with the apparent lack of oospore production by *P. belbahrii,* suggests that the introduction of infection onto production sites is likely to either come from seed or infected plants brought onto site.

A range of biological and chemical products have previously been demonstrated to be suitable for treating downy mildew symptoms in basil (Gullino *et al,* 2009; Gilardi *et al.,* 2013). Thus, over the two years of this project the aim was to identify products suitable for use in the UK under both protected and outdoor conditions. Across the various trials carried out, Revus (mandipropamid), Paraat (dimethomorph) and Fenomenal (fenamidone + fosetyl-aluminium) (outdoor use only) proved to be consistently the most effective for controlling *P. belbahrii,* provided they were applied prior to the onset of infection and re-application was within 10 days. Other products which also performed well were Invader (dimethomorph + mancozeb) and Infinito (fluopicolide + propamocarb HCl) (both approved only for use on outdoor herbs). This information will provide producers and growers with improved knowledge to guide product selection and aid them to implement effective control measures in both protected and outdoor production areas. There was also useful activity against downy mildew with coded products HDC F 237 and 226 under protection and HDC F 239 and 240 outdoors.

Fubol Gold was shown to be effective in the outdoor trials but less effective in the trials carried out under glass. Further examination revealed that the isolate used for the trials carried out under glass was resistant to metalaxyl-M, whereas the isolate used outdoors was sensitive. There was some control of the resistant isolate following the application Fubol Gold, this resulting from the presence of mancozeb in the formulation. As products containing metalaxyl-M are used both as seed and foliar treatments there is the potential for disease control failures to occur unless the prevalence of metalaxyl-M resistance is established within the UK.

Experiments carried out in the first year of the project showed that infection by *P. belbahrii* only occurred when inoculated plants were incubated in the dark, while incubation in the light totally inhibited the infection process. This finding was consistent with those for other *Peronospora* species, e.g. *P. violae* responsible for pansy downy mildew, and suggests that infection only occurs during the night. This was also consistent with the work undertaken by Cohen *et al*. (2013a) who demonstrated that exposure of *P. belbahrii* infections to light suppressed the formation of conidia. Both these findings suggest that the manipulation of the light wavelength protected crops are grown under could result in reduced infection levels, and subsequent disease development and spread. In the second year of PE 024 this hypothesis was examined. Results from the trial did not show any significant differences in

disease levels between the different lighting treatments, though disease severity was generally and consistently reduced with night time lighting treatments versus the control. There are a number of potential explanations for why increased levels of control and/or statistically significant differences were not uncovered:

1. As each plot was surrounded by white sheeting the leaves of basil plants were not exposed directly to sunlight. Despite the white sheeting, during overcast periods in the daytime and at twilight, light intensity dropped below 3 $\mu mol.m^{-2}s^{-1}$ which may have enabled infection by *P. belbahrii* to occur. Previous work by Cohen *et al* (2013a) reported that incandescent light was almost fully inhibitory [of sporulation] at 3.5 $\mu mol.m^{2}s^{-1}$, but only partially inhibitory at 2 $\mu mol.m^{2}s^{-1}$.
2. During the dark period, light from each of the individual plots reflected off the roof of the glasshouse and illuminated unlit plots. Light intensity in unlit plots was measured at between 0.03 and 0.18 $\mu mol.m^{-2}s^{-1}$ so there was never complete darkness during the dark period.

Further, more detailed, work would be required to fully establish the effect of overnight lighting to control infection and sporulation of *P. belbahrii* on basil plants though results support that such work could be worthwhile in terms of developing light management as a component of integrated disease management (the same conclusion having been reached for pest management in AHDB project CP 125).

Conclusions

Source of infection

- *Peronospora belbahrii* DNA was detected in a high proportion of seed samples tested.
- No oospores were detected in any of the seed washings suggesting that *P. belbahrii* was not present in basil seed lots as a surface contaminant, but that it is present as an internal contaminant of the seed.
- Symptoms of basil downy mildew were not observed on plants grown from seed heavily contaminated with *P. belbahrii* DNA. It is unclear whether the seed samples tested had been pre-treated in some way which rendered the DNA detected non-viable, or whether insufficient seeds were tested or if the disease is not seed-borne.
- Oospores were not detected on seed, in plants or in soil suggesting that they are not produced by *Peronospora belbahrii*. The non-production of oospores has wider implications as this would indicate that the pathogen is not likely to overwinter.

Infection of basil by *P. belbahrii*

- Dark was required for infection of basil by *P. belbahrii* to occur.
- Infection of basil by *P. belbahrii* occurred over a wide temperature range (between 5 and 25ºC), with infection requiring a minimum of 4 hours of leaf wetness at temperatures between 15 and 25ºC.
- Germination of freshly produced conidia is highly variable, though, those that germinated were highly infectious. Conidia did not appear to survive longer than 72 hours at temperatures between 15 and 20ºC. Conidial survival was reduced to 48 hours at temperatures between 5 and 10ºC and 24 hours between 25 and 30ºC.

Alternate hosts

- Agastache, lavender, sage and catnip were susceptible to infection by *P. belbahrii*.
- None of the weed species tested were susceptible to infection by *P. belbahrii*.

Control

- To achieve effective protection of basil crops the gap between fungicide sprays should not exceed 10 days.
- Resistance to metalaxyl-M has been highlighted within the UK population of *P. belbahrii*.
- Under field and protected conditions spray programmes and specific individual products demonstrated comparable performance in controlling *P. belbahrii* symptoms, provided the gap between applications did not exceed 10 days.
- Crop safety trials showed that HDC F245 had beneficial growth effects. Multiple applications of Fubol Gold resulted in significant phytotoxic effects.
- Illumination of basil plants overnight reduced disease severity compared to unlit controls. The reduction seen was not consistent over the lighting regimes and further work is required to confirm whether the introduction of night lighting would reliably reduce levels of basil downy mildew.

Knowledge and Technology Transfer

- Presentation to the British Herbs Trade Association Technical meeting held on 1st February 2017 at Nailcote Hall Hotel, near Kenilworth, Warwickshire.
- Article in July/August 2017 edition of AHDB Grower
- Presentation to the SCEPTREplus meeting held on 19th October 2017, at Stockbridge Technology Centre.
- Presentation to the British Herbs Trade Association Technical meeting held on 30th November 2017 at Cranfield University.
- Project featured in AHDB Field Vegetable Review publication for 2017
- Factsheet update in preparation.

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