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Project number:	PE 024
Project leader:	Philip Jennings, Fera Science Limited
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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.

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GROWER SUMMARY

Headline

- Basil downy mildew infections can occur at temperatures between 5 and 25ºC.
- Agastache, lavender, sage and catnip can all act as alternate hosts for *Peronospora belbahrii*, the pathogen responsible for basil downy mildew.

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. The majority 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 will be addressed in this project.

Summary

In the first year of the project the main focus of work has been to,

1) Determine whether *P. belbahrii* is present in UK seed lots and whether infected seed can act as a primary source of infection.
2) Establish risk factors for infection of basil by *P. belbahrii*
3) Establish alternate hosts for *P. belbahrii*.

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

To date 11 seed lots have been sourced, the majority of these have come from seed where there had been a problem with downy mildew in the ensuing crop. Of the 11 seed samples examined all but one contained *P. belbahrii* DNA. Six of the samples contained *P. belbahrii* DNA at similar levels across all five replicates suggesting an evenly distributed contamination of the sample. Of the six samples all but one contained high levels of *P. belbahrii* DNA, with two samples containing extremely high levels, with average cycle threshold (Ct) values of 24.7 and 29.9 respectively. The remaining four samples all contained low levels of *P. belbahrii* with between 1 and 4 replicates containing no *P. belbahrii* DNA.

Generally, Ct values of 29 or less are considered strong positive reactions and are indicative of abundant target DNA in the sample. Ct values of 30-37 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.

Seed washings and oospore counts have been carried out on five samples. No oospores were detected in any of the seed washings suggesting that the *P. belbahrii* DNA detected in the seed was from an internal contamination.

Seed will continue to be sourced and analysed in the second year of the project.

Determine the conditions required for infection of basil by *P. belbahrii*

a) The effect of light on infection of basil by *P. belbahrii*.

Infection of basil by *P. belbahrii* was significantly affected by light. Incubation of basil plants in the light for the first 18 h after inoculation resulted in no plants developing downy mildew symptoms. In contrast, over 50% of the plants incubated in the dark developed the yellow discoloured areas on the upper leaf surface and brown downy sporulation on the underside of the leaves associated with basil downy mildew. This suggests that basil downy mildew infections are likely to occur overnight rather than during daylight hours.

b) The effect of humidity on leaf wetness

Downy mildew relies on the presence of water on the leaf surface for infections to occur. Environmental conditions such as humidity have a significant effect on the time taken for leaves to dry. Experiments carried out to determine the length of time it took for wet basil leaves to dry showed that increasing the humidity increased the length of time it took for water to dry from the surface of basil leaves. Leaves dried after 1 hour when exposed to a humidity

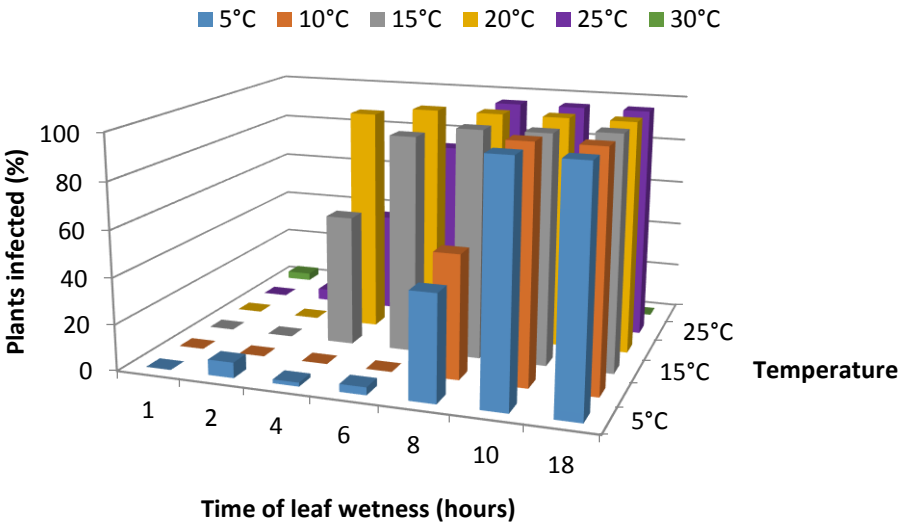
of 20%, 2.5 hours at 50% humidity and 6 hours at 80% humidity. Leaves subjected to 100% humidity remained wet for the duration of the test.

This testing was carried out on basil grown under protection. As leaves grown outdoors may have different physiological characteristics which, in turn, affect how water is distributed on the leaf these tests will be repeated on outdoor grown basil.

c) The effect of temperature and leaf wetness on the infection of basil by *P. belbahrii*

Infection of basil by *P. belbahrii* was examined at six temperatures (5, 10, 15, 20, 25 and 30°C) and six leaf wetness periods (1, 2, 4, 6, 10 and 18 hours). Downy mildew infection occurred when plants were incubated at temperatures between 5 and 25ºC with the infection highly dependent on the length of time a leaf was wet (see Figure below). Limited or no infection of basil plants occurred at 30ºC irrespective of leaf wetness duration. Limited infection also occurred, irrespective of temperature, when leaves were wet for 2 hours or less. Increasing leaf wetness to 4 hours resulted in 97% of basil plants becoming infected by downy mildew when they were incubated at 20ºC. Four hours of leaf wetness also resulted in infection when plants were incubated at 15 or 25ºC, however the level of infection was reduced compared to plants incubated at 20ºC, with 56 and 43% of plants infected at 15 and 25ºC respectively. Increasing leaf wetness to 6 hours increased infection at 15ºC and 25ºC to 93% and 78% of plants respectively; 100% of plants were infected at these temperatures when leaf wetness was increased to 8 hours. A minimum of 8 hours of leaf wetness was required for downy mildew infection of basil to occur at 5 or 10ºC, with 45 and 53% of plants infected respectively. Increasing leaf wetness to 10 hours resulted in 100% of plants infected at both 5 and 10ºC.

Combining all these data suggest that infection of basil is only likely to occur overnight when humidity is greater than 70%. A risk grid based on these data will be produced to help growers determine when the basil plants are most at risk of infection by downy mildew.



Effect of temperature and leaf wetness on infection of basil by *Peronospora belbahrii*.

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

Fourteen plant species from across the Lamiaceae genus 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.

Fungicide control

Basil downy mildew symptoms in an outdoor fungicide efficacy trial were low due to the warm/dry conditions, with symptoms first observed in the 4th week of the trial. Treatment programmes proved most effective in reducing downy mildew symptoms with a range of products showing suitability for use in fungicide programmes. The most effective programme consisted of treatments with Revus (mandipropamid) / a coded product HDC F226, closely

followed by programmes with treatments of Fubol gold (metalaxyl-M + mancozeb) / Revus, and Fenomenal (fenamidone + fosetyl-aluminium) / Revus.

These will be further evaluated in project year 2. No oospores were observed in the diseased material.

Financial Benefits

It is too early to predict the likely financial benefits from this project. However, the pathogens responsible for downy mildew are aggressive and, under favourable environmental conditions, can cause significant economic 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 from 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. The majority 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 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. There is little published work to indicate how effective these products are against *P. belbahrii*, however there has 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. 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 will be addressed in this project, the collaboration between Fera, STCRF and NIAB provide the required skill sets to carry out this work having already successfully completed similar work on other downy mildew pathogens.

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.

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 come into contact with water. The test was replicated five times and samples ranked according to the average number of oospores recorded.

Infection of basil plants from contaminated seed

A trial to establish whether seed contamination could lead to the expression of basil downy mildew infected plants was established using the variety Italiano Clasico. The trial was established in 3 L pots with 100 seed per pot and five replicates per treatment. Treatments consisted of three watering regimes 1) from the bottom, 2) from above during the morning and 3) from above during the evening. Two sets of replicate pots were established per treatment with one set covered with a polythene sheet each evening to increase the humidity and the second set left uncovered. Plants were checked daily for signs of disease.

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

Work in this period has focussed on optimisation of DNA extraction. Soil DNA extraction procedures have been standardised using MO Bio powersoil kits. DNA will be detected by PCR using primers from Thines *et al.,* (2009) and a newly designed isothermal assay. Assays

will be used to screen for *P. belbahrii* DNA in soil collected from the outdoor trial site at Cambridge and other infested sites.

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

The effect of light on infection

Ten basil seeds were sown into each of 12 pots and grown in the glasshouse until seedlings were at the first true leaf stage. Six pots were inoculated with a spore suspension containing 10^4 conidia/ml of *P. belbahrii* and the remaining six sprayed with water as a control. All pots were placed in a moisture chamber, to prevent leaves from drying out, and incubated at 20°C for 18 h. For the duration of the incubation period three inoculated and three control pots were kept under conditions of constant day light, while the remaining pots were kept in the dark. After incubation the plants were removed from the moisture chambers and leaves left to dry naturally. Once dry the plants were transferred to the glasshouse and grown at 20ºC for 10 days. The level of infection was assessed based on leaf yellowing and sporulation.

The effect of humidity on leaf wetness

Five replicate basil leaves were placed in humidity chambers maintained at 19, 49, 81, 97 and 100% using concentrated salt solutions (**Table 1**). Leaves were misted with water to run-off and leaf wetness recorded every half hour until leaves were dry or 48 h 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 and leaf wetness on time taken for *P. belbahrii* to infect basil

Infection of basil by *P. belbahrii* was examined at six temperatures (5, 10, 15, 20, 25 and 30°C) and six leaf wetness periods (1, 2, 4, 6, 10 and 18 hours). At each temperature and timing three replicate pots containing 20 basil plants at the first true leaf stage were inoculated to run off with a spore suspension containing 10^4 *P. belbahrii* conidia ml^{-1} water and incubated at 100% humidity. At the end of each wetness period pots were removed and leaves rapidly air dried to prevent further infection. Plants were transferred to the glasshouse and grown at

18°C for 10 days after which plant infection was assessed based on the percentage of plants showing leaf yellowing and sporulation typical of infection by *P. belbahrii*.

Oospore production under protected and outdoor conditions

Testing for oospore production under protected conditions has been conducted using *P. belbahrii* isolates from CREA in Italy and another provided by FERA where oospore production had been observed previously. Potted basil plants were inoculated with pure and mixed cultures of each isolate (1 x 10^4/ml) and grown on until the plants were senesced. The plant tissue was then assessed for the presence of oospores by weighing, grinding and suspension in water for microscopic analysis.

Outdoor plots of basil have been inoculated/co-inoculated with sporangial suspensions and subsequently covered/irrigated for three consecutive nights to encourage sporulation. Sporulation is being recorded as a percentage leaf area affected and severity (0-5). As a contingency, pots of basil grown under field conditions that have been treated in the same fashion as the field plots have been established.

The existence and importance of alternate hosts for P. belbahrii

Fourteen plant species, including two reported alternate hosts, from a range of genera within the Lamiaceae (**Table 2**) have been sourced for testing for susceptibility to *P. belbahrii*. Each species was sown under conditions appropriate for optimal germination and grown to at least four true leaf stage. At this point plants were inoculated to run off with a spore suspension containing 10^4 *P. belbahrii* conidia ml^{-1} water and incubated overnight in a moisture chamber at 20ºC. Plants were transferred to the glasshouse and grown at 18°C for 10 days after which plant infection was assessed based on leaf yellowing and sporulation.

Where infection was suspected DNA was extracted from symptomatic leaf material and the presence of *P. belbahrii* determined using the primers described earlier. In addition any spores formed on the underside of leaves were removed and inoculated onto clean basil plants.

Table 2. Plant species from the Lamiaceae used in susceptibility test to basil downy mildew *(Peronospora belbahrii).*

Plant species	Common name	Plant type
Mentha piperita	Pepper mint	Crop
Salvia officinalis	Common sage	Crop
Majorana hortensis	Sweet marjoram	Crop
Thymus vulgaris	Common thyme	Crop
Lavandula angustifolia	Lavender	Crop
Nepeta cataria	Catnip/cat mint	Crop
Lamium purpureum	Red dead nettle	Weed
Clinopodium vulgare	Wild basil	Weed
Mentha aquatica	Water mint	Weed
Origanum vulgare	Oregano or wild marjoram	Crop
Teucrium scorodonia	Wood sage	Weed
Thymus polytrichus	Wild thyme	Weed
Solenostemon scutellaroides	Coleus Warpaint	Crop
Agastache	Apricot Spirite	Crop

Control

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

Indoor production

These studies have started and will be reported on in the final report.

Outdoor production

An outdoor trial site was established at NIAB in Cambridge for conducting the fungicide efficacy trial. The trial site was prepared with two rounds of glyphosate treatment with subsequent flailing and cultivation. Nitrogen was applied at a rate of 160 kg/ha (Yara prilled 34.5%, 464 kg/ha). The trial area of 12 m x 6 m (72 m^2) was sown with basil cv Sweet Genovese (CN Seeds, Pymoor, Ely,seed rate 5 g/m^2) on 15/7/16. The trial was arranged in a fully replicated block design with plots 0.6 m x 0.6 m, with 0.3 m buffers surrounding them. The original aim was to use 1 m plots. As the weed pressure was high, even with additional control, the area was reduced to enable a more concentrated effort to remove the weeds in the smaller plots, ensuring reliable data could still be obtained. Plots were irrigated and cultivated until the plants had reached the 4-6 true-leaf stage, at which point treatment

applications were initiated (31/8/2016). Additional hand weeding was performed and the trial was treated with insecticide (deltamethrin, Decis, Bayer Crop Science) at 2 and 6 true-leaf stage to maintain the condition of the plots.

The trial assessed 10 individual treatments, three programmes and a control. Treatments/programmes are listed in **Table 3**. Treatments were diluted appropriately to a final application rate of 1500 L/ha and applied preventatively in the evening using a small hand sprayer or as a drench in the case of HDC F227. Care was taken to ensure products were applied evenly to both surfaces of the leaves. Sporulating (*P. belbahrii*) spreader plants (four pots per plot, five plants per pot) were distributed evenly throughout the trial area the following morning (1/9/2016). Plots were irrigated at dusk/dawn using a sprinkler and covered with plastic sheeting for three consecutive nights to ensure optimal infection conditions. Two additional foliar applications of aqueous spore suspension (1×10^5 spores / ml) were applied evenly across the trial area until run-off, during the first and second evenings after treating to encourage infection.

Symptoms were scored visually as the percentage leaf area affected and the severity of sporulation (0 – no sporulation and 5 – profuse sporulation), in the morning every 2-3 days over a four week period. Treatment re-application was conducted according to guidance on product labels (listed in **Table 4**).

Table 3. Product list for outdoor basil trial

Product	Active ingredient	Application rate/ha
HDC F223		70.07 g
Amistar	Azoxystrobin	1 L
HDC F224		2.5 kg
Control	N/A	N/A
HDC F225		1 L
Fenomenal	Fenamidone/fosetyl-aluminium	4.5 kg
HDC F226		1 L
Paraat	Dimethomorph	3 kg
HDC F227		5 ml/2litres
Revus	Mandipropamid	0.6 L
Fubol Gold	Metalaxyl-M + mancozeb	1.9 kg
Programme 1	Metalaxyl-M + mancozeb/Azoxystrobin/ Metalaxyl-M + mancozeb	1.9 kg/1 L/1.9 kg
Programme 2	Mandipropamid / HDC F226/ Mandipropamid	0.6 L/1 L/0.6 L
Programme 3	Fenomenal/ Mandipropamid/ Mandipropamid/	4.5 Kg/0.6 L/ 0.6L

Table 4. Re-application dates for treatments

Product	Active ingredient	Day 1 (31/8/16)	Day 7 (7/9/16)	Day 14 (14/9/16)	Day 21 (21/9/16)	PHI (days)
Control		x	x	x	x	
HDC F223		x	x	x	x	7
Amistar (Am)	Azoxystrobin	x		x		14
HDC F224		x		x		
Fenomenal	Fenamidone/fosetyl-aluminium	x				35
Fubol Gold (Fg)	Metalaxyl-M + mancozeb	x		x		14
HDC F226		x		x		14
Paraat	Dimethomorph	x				35
HDC F225		x				35
HDC F227		x				35
Revus (Re)	Mandipropamid	x	x			7
Programme 1	Mandipropamid/ azoxstrobin	Fg	Am	Fg		14
Programme 2	Mandipropamid/ HDC F226	Re	Cy		Re	7
Programme 3	Fenamidone/fosetyl-aluminium/ mandipropamid	Fe		Re	Re	7

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

To date 11 seed lots have been sourced (**Table 5**) with all tested for the presence of *P. belbahrii* DNA. The majority of these samples were from lots where basil downy mildew had been an issue in the resulting crop.

Table 5. Details of basil seed samples used for seed testing.

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)	-
6	5	37.3 (36.1 to 38.7)	0
7	5	30.2 (29.7 to 31)	-
8	-	39.7 38.5 to 40)	-
9	-	38.5 (37.6 to 40)	-
10	-	37.8 (35.5 to 40)	-
11	6	40	-
+ve control	n/a	22.6 (22.5 to 22.6)	n/a
-ve control	n/a	40	n/a

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 are 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 29

or less are considered strong positive reactions and are indicative of abundant target DNA in the sample. Ct values of 30-37 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. Six of the samples contained *P. belbahrii* DNA at similar levels across all five replicates suggesting an evenly distributed contamination of the sample. Of the six samples all but one contained high levels of *P. belbahrii* DNA, with two samples containing extremely high levels with average Ct values of 24.7 and 29.9 respectively; as the test undertaken cannot distinguish between viable and non-viable DNA it is not clear whether the DNA detected would be infective. The remaining four samples all contained low levels of *P. belbahrii* with between one and four replicates containing no *P. belbahrii* DNA.

Seed will continue to be sourced and analysed in the second year of the project.

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

Spore washings have been carried out on five of the samples with no oospores detected in any. This work will continue in the second year of the project.

Infection of basil plants from contaminated seed

No plants under any of the watering regimes tested became visibly infected during the course of the trial. A further trial in the second year of the project will be set up (using a protocol developed in a related project) where plants only be grown under ideal conditions for disease development to try and establish whether the presence of high levels of *P. belbahrii* DNA in seed lots translates to downy mildew infected plants.

Detection of P. belbahrii in soil from outdoor production sites

Soil detection work using the methods developed will commence once scoring of disease symptoms in control field assessment plots at Cambridge has ceased. Submission of contaminated soil from growers will also be requested.

Conditions required for infection of basil by P. belbahrii

The effect of light on infection of basil by P. belbahrii

Infection of basil plants by *P. belbahrii* was significantly affected by light (**Figure 1**). Incubation of basil plants in the light for the first 18 h after inoculation resulted in no plants developing downy mildew symptoms. In contrast over 50% of the plants incubated in the dark

developed the yellow discoloured areas on the upper leaf surface and brown downy sporulation on the underside of the leaves associated with basil downy mildew.

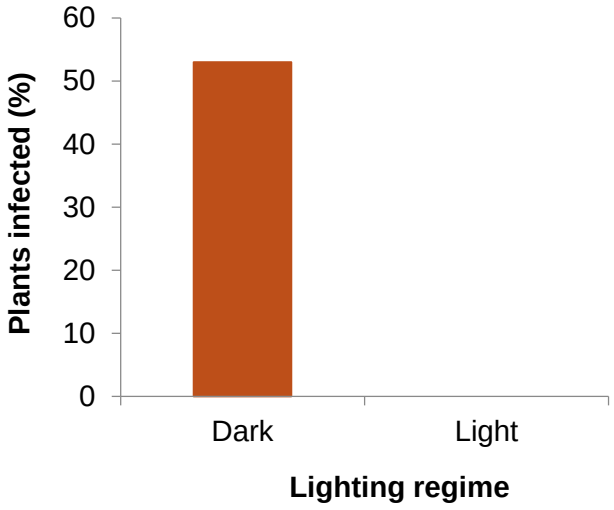


Figure 1. Effect of light on infection of basil by *Peronospora belbahrii*

The effect of humidity on leaf wetness

Increasing humidity increased the length of time it took for water to dry from the surface of basil leaves (**Figure 2**). Leaves dried in 1 hour at a humidity of 20%, 2.5 hours at 50% humidity and 6 hours at 80% humidity. Leaves subjected to 100% humidity remained wet for the duration of the test.

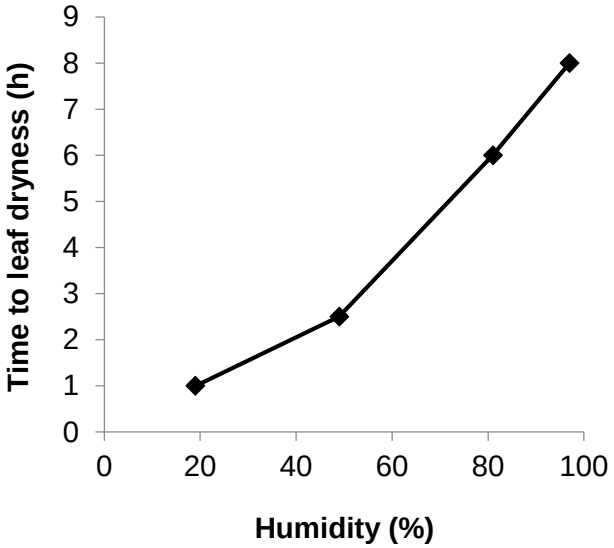


Figure 2. The effect of different humidity regimes on the leaf drying time of basil grown under protection.

The effect of humidity on the length of time basil leaves remained wet was examined using basil grown under protection. As leaves grown outdoors are likely to have slightly different physiological characteristics which could affect water distribution over the leaf surface the test will be repeated in year 2 on basil leaves taken from plants grown outdoors.

The effect of temperature and leaf wetness on incidence of basil downy mildew

Infection of basil by *P. belbahrii* (as determined by symptom development, 10 days after inoculation) occurred when plants were incubated at temperatures between 5 and 25ºC with infection highly dependent on the length of time a leaf was wet (**Figure 3**). Limited or no infection of basil plants occurred at 30ºC irrespective of leaf wetness duration. Limited infection also occurred, irrespective of temperature, when leaves were wet for 2 hours or less. Increasing leaf wetness to 4 hours resulted in 97% of basil plants becoming infected by downy mildew when incubated at 20ºC. Four hours of leaf wetness also resulted in infection when plants were incubated at 15 or 25ºC, however the level of infection was reduced compared to plants incubated at 20ºC, with 56 and 43% of plants infected respectively. Increasing leaf wetness to 6 hours increased infection at 15ºC and 25ºC to 93% and 78% of plants respectively; 100% of plants were infected at these temperatures when leaf wetness was increased to 8 hours. A minimum of 8 hours of leaf wetness was required for downy mildew infection of basil to occur at 5 or 10ºC, with 45 and 53% of plants infected respectively. Increasing leaf wetness to 10 hours resulted in 100% of plants infected at both 5 and 10ºC.

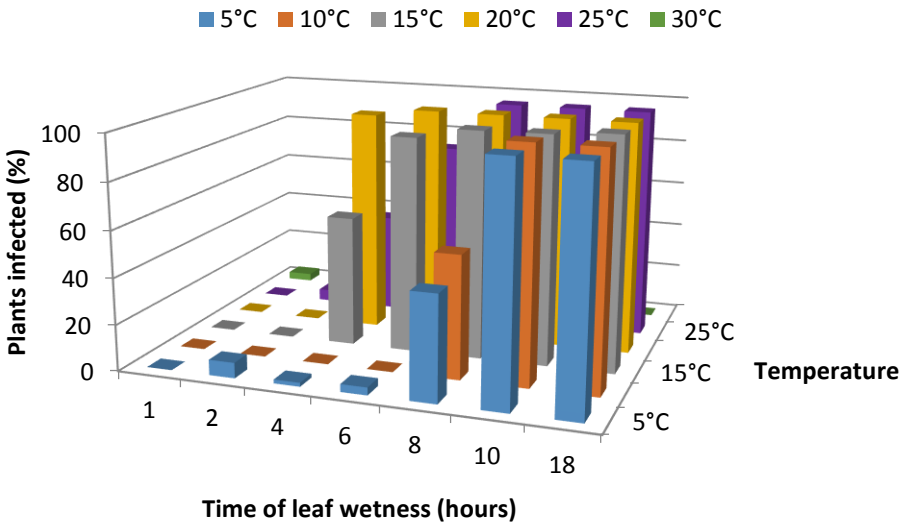


Figure 3. Effect of temperature and leaf wetness on infection of basil by *Peronospora belbahrii*.

The level of oospore production under protected and outdoor conditions

Under protected conditions no oospore production has been recorded in any of the isolates tested (FERA, CREA, FERA + CREA). A secondary infection in inoculated basil plants was identified and isolated late-on in the experiment. Colonisation of the abaxial surface of the leaf/old sporangia by *Lecanicillium lecanii* was observed and this could have affected oospore production. Therefore the experiment is being repeated using fresh seed stocks and cultures, in a clean growth area. The study on the outdoor plots is ongoing.

The existence and importance of alternate hosts for P. belbahrii

Fourteen plant species from across the genus Lamiaceae were tested for susceptibility to *P. belbahrii* (**Table 5**); two of these species, agastache and coleus had previously been reported as alternate hosts for *P. belbahrii*. Of the 14 species tested only agastache, lavender, common sage and catnip showed symptoms associated with *P. belbahrii* (**Figures 4b, 5b, 6b** and **7**). Profuse sporulation was observed following infection of agastache and lavender (**Figures 4c** and **5c** respectively), however sporulation was sparse following infection of common sage and no sporulation was observed on catnip. *P. belbahrii* DNA was present at high levels in leaf material collected from all plants expressing downy mildew symptoms. Basil plants inoculated with spores obtained from infected lavender, sage and agastache all produced symptoms and sporulation associated with *P. belbahrii*.

Table 6. Plant species used, leaf symptoms and level of sporulation obtained during *Peronospora belbahrii* alternate host testing.

Plant species	Common name	Leaf symptoms	Sporulation
Mentha piperita	Pepper mint	No	-
Salvia officinalis	Common sage	**Yes**	Sparse
Majorana hortensis	Sweet marjoram	No	-
Thymus vulgaris	Common thyme	No	-
Lavandula angustifolia	Lavender	**Yes**	Profuse
Nepeta cataria	Catnip/cat mint	**Yes**	No
Lamium purpureum	Red dead nettle	No	-
Clinopodium vulgare	Wild basil	No	-
Mentha aquatica	Water mint	No	-
Origanum vulgare	Oregano	No	-
Teucrium scorodonia	Wood sage	No	-
Thymus polytrichus	Wild thyme	No	-
Solenostemon scutellaroides	Coleus Warpaint	No	-
Agastache	Apricot Spirite	**Yes**	Profuse
Ocimum basilicum	Basil	**Yes**	Profuse

a)  b)  c) 

Figure 4. Symptoms and sporulation observed following inoculation of Agastache var. Apricot Spirite with conidia of *Peronospora belbahrii* a) uninoculated control, b) inoculated and symptomatic plants, c) downy sporulation on the underside of the leaf.

a)  b)  c) 

Figure 5. Symptoms and sporulation observed following inoculation of *Lavandula angustifolia* (lavender) with conidia of *Peronospora belbahrii* a) uninoculated control, b) inoculated and symptomatic plants, c) downy sporulation on the underside of the leaf.

a)  b) 

Figure 6. Symptoms and sporulation observed following inoculation of *Salvia officinalis* (common sage) with conidia of *Peronospora belbahrii* a) uninoculated control, b) inoculated and symptomatic plants.

Figure 7. Symptoms observed following inoculation of *Nepeta cataria* (catnip) with conidia of *Peronospora belbahrii*.

Control

Preliminary results from the outdoor production fungicide trial are shown in **Table 7**. Disease levels in the trial were low due to the warm/dry conditions. Symptoms were first observed in the 4th week of the trial. Significant different differences were observed for treatment programmes 1-3 in both symptom severity (Table 7; *p*=<0.05, LSD 5%=1.52) and the percentage plot affected (*p*=<*0.05*, LSD 5%= 12.81%) compared with the untreated control. In contrast, individual treatments of Fenomenal and Paraat were the only products observed to significantly reduce downy mildew symptom severity. Effects on the percentage plot area affected where however non-significant for the two products.

Treatment programmes therefore proved most effective in reducing downy mildew symptoms. A range of products were shown to be suitable for use in fungicide programmes including Paraat, Revus, Fenomenal, Fubol Gold, and HDC F226. No oospores were observed in the diseased material. Results of this trial are being used as the basis of fungicide selection for use in the product longevity trial.

Table 7. Control of basil downy mildew by individual fungicide products and fungicide programmes.

Treatment	Active Ingredient	Severity (0-5*, (LSD = 1.52)	Plot affected (%) (LSD = 12.8)
Control		2.66	16
Fubol Gold	Metalaxyl-M + mancozeb	1.33	5
HDC F227		1.66	11.66
Programme 1	Metalaxyl-M + mancozeb/Azoxystrobin	1	3.33
Programme 2	Mandipropamid / HDC F226	1	1.66
Programme 3	Fenamidone/fosetyl-aluminium/ Mandipropamid	1	3.33
HDC F223		1.33	6.66
Amistar	Azoxystrobin	2	14.33
HDC F224		2.66	12.33
HDC F225		2	15.66
Fenomenal	Fenamidone/fosetyl-aluminium	1	6.66
HDC F226		2.66	11.66
Paraat	Dimethomorph	1	7
Revus	Mandipropamid	1.33	5.33

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

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 establish the optimum method for producing and treating seed in order to reduce levels of downy mildew caused by contaminated seed. To date the seed testing carried out within this project has generally been limited to seed lots where basil downy mildew has proven to be a problem, with the majority of seed lots containing high levels of *P. belbahrii* DNA. To date, washing of these seed has not yielded any oospores from the seed surface suggesting that the DNA present was a result of internal seed infection by *P. belbahrii*.

Epidemiological studies have so far focused on the effect of light, temperature and period of leaf wetness on infection of basil by *P. belbahrii*. The testing carried out within the first year of the project has shown that infection only occurred when inoculated plants were incubated in the dark, while incubation in the light totally inhibited the infection process. This finding is consistent with those for other *Peronospora* species e.g. *P. violae* responsible for pansy downy mildew and suggests that infection will only occur during the night. This is 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.

Experiments to determine the effect of temperature and leaf wetness on symptom development following inoculation by *P. belbahrii,* showed that symptoms were seen resulting from incubation at temperatures between 5 and 25ºC, with no infection of basil plants occurring at 30ºC. This is a wider infection window than reported by Garibaldi *et al*. (2007) who indicated that no infection occurred below 12ºC or above 27ºC. Within this project the optimum temperature for infection was 20ºC, consistent with the results of Garibaldi *et al*. (2007), however infection occurred with a shorter duration of leaf wetness in this study than in the Garibaldi study (4 hours compared to 6 hours). Four hours leaf wetness also resulted in high levels of infection at 15 and 25ºC. Combining the data on leaf wetness durations required for infection, with the data obtained on the effect of humidity on leaf wetness will allow a risk map for infection to be produced.

To date two alternate hosts for *P. belbahrii* have been identified, these are agastache (Henricot *et al*., 2009) and coleus (Denton *et al*., 2015). Work in this project looked at a range

of plant from the Lamiaceae, both commercially grown and weed species, to determine whether other alternate hosts exist and what their potential threat is to the industry. A total of 14 plant species were tested and agastache, lavender, common sage and catnip were shown to be alternate hosts; of these agastache has previously been described as an alternate host. This is the first report of lavender and catnip being possible alternate hosts for *P. belbahrii*. A *Peronospora* species on sage has been shown to have a similar sequence homology to *P. belbahrii* (Thines *et al*., 2009) however no morphological data were available and so it was not possible to conclude if the two *Peronospora* species were the same. This work does not show that the *Peronospora* species described by Thines *et al*. was *P. belbahrii* however it does indicate that sage can act as an alternate host to *P. belbahrii*. Although coleus has previously been described as an alternate host the cultivar used in our testing proved not to be susceptible. All the alternate host identified were herb crops so growers should be aware of this and take care if growing the different susceptible crops at the same time. The lack of weed crops in the list of alternate hosts should make disease management easier as there appears to be no route for spread of *P. belbahrii* via these plants, however it cannot be discounted that symptomless infections may occur in some of these species.

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). This study aimed to identify products suitable for use under outdoor conditions in the UK by testing ten individual treatments and three programmes. Experiments to assess the efficacy of fungicidal products for controlling *P. belbahrii* under outdoor conditions identified that treatment programmes were more effective in reducing downy mildew symptoms than individual products. However a level of disease control was achieved with two of the individual products tested. This information will provide growers and producers with greater insight into product selection in order to enable them to implement effective control measures in outdoor basil production areas.

Conclusions

- Initial findings suggest that oospores are not present as surface contamination in basil seed lots but that *P. belbahrii* is found internally within seed.
- Dark is required for infection of basil by *P. belbahrii* to occur.
- Infection of basil by *P. belbahrii* occurred over a wide temperature range (5-25ºC), with infection requiring 4 hours of leaf wetness at temperatures between 15 and 25ºC.
- Agastache, lavender, sage and catnip are susceptible to infection by *P. belbahrii*. No weed species tested were susceptible to infection by *P. belbahrii*.

- Spray programmes were more effective than individual treatments in controlling *P. belbahrii* symptoms under field conditions

Knowledge and Technology Transfer

Presentation to the British Herbs Trade Association meeting held at Fera 8th March 2016.

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