| | |
|---|---|
| **Project title:** | Basil: Improving knowledge and control of downy mildew in protected and outdoor crops |
| **Project number:** | PE 024a |
| **Project leader:** | Philip Jennings, Fera Science Limited |
| **Report:** | Final report, March 2019 |
| **Previous report:** | Annual and Final report for PE 024 |
| **Key staff:** | Gilli Thorp, Fera<br>Tom Wood, NIAB |
| **Location of project:** | Fera, York<br>National Institute of Agricultural Botany, Cambridge |
| **Industry Representative:** | Simon Budge, Vitacress Ltd, West End Nursery, Roundstone Lane, Angmering, West Sussex, BN16 4AX |
| **Date project commenced:** | 01 May 2018 |

## DISCLAIMER

*While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.*



*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 3/4/2019

Dr Tom Wood
Senior Molecular Plant Pathologist
National Institute of Agricultural Botany

Signature .. Date .....3rd April 2019

**Report authorised by:**

Dr Shaun White
Head of Plant Protection
Fera Science Limited

Signature ..... Date 3/4/19

Dr Jane Thomas
Head of Plant Pathology
National Institute of Agricultural Botany

Signature ..... Date ..... 4th April 2019

# CONTENTS

**GROWER SUMMARY** ..... 1

Headline ..... 1

Background ..... 1

Summary ..... 2

Action Points ..... 3

**SCIENCE SECTION** ..... 4

Introduction ..... 4

Materials and methods ..... 6

Results ..... 8

Conclusions ..... 11

Knowledge and Technology Transfer ..... 11

References ..... 12

Appendix I ..... 14

# GROWER SUMMARY

## Headlines

- Transmission of *Peronospora belbahrii* from basil seed to seedlings was demonstrated for two naturally infected seed lots, with 0.4% and 1.3% seedlings showing symptoms of downy mildew.
- Metalaxyl-M resistance was found in three out of the four *Peronospora belbahrii* isolates collected from the UK.

## Background

A British Herbs 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 primarily addressed in AHDB Project PE 024 however, on completion of the project, there were two areas where further work was required. These areas were covered in this extension project as follows:

1) Determine whether P. *belbahrii* infections can be transmitted to plants from *P. belbahrii* contaminated seed

2) Determine the level of metalaxyl-M resistance in *P. belbahrii*

**Summary**

Transmission of *Peronospora belbahrii* from contaminated seed to plants.

Eight seed samples were tested in seed transmission studies using two different protocols, a conventional grow-on test and a box test. Six samples were obtained from AHDB project PE 024 and two by NIAB during this project (one from an inoculated field trial conducted in 2018 and the other from Germany). No basil downy mildew infections were reported in the UK during 2018 so fresh seed samples could not be obtained via this route.

Four seed samples were screened using the grow-on screen with between 2500 and 6000 seed analysed per sample. No downy mildew symptoms were observed in any of the seedlings screened, despite all four seed samples testing positive for the presence of pathogen DNA, and the maintenance of suitable environmental conditions for the expression of disease symptoms.

Seven seed samples were screened using the box test protocol with between 100 and 775 plants screened per seed sample. Downy mildew symptoms were observed in seedlings from seed samples coded 17 (0.4 %) and 21 (1.3%), with a higher level of symptom expression in sample 21, which also contained the higher level of *P. belbahrii* DNA. This demonstrates that *P. belbahrii* can be transmitted from basil seed to seedlings. With transmission rates as high as 1.3% it suggests that seed-borne inoculum can play an important role in outbreaks of basil downy mildew.

Despite very high levels of *P. belbahrii* DNA being present in the two seed samples obtained during this project extension, no downy mildew symptoms were expressed. Reasons for this are unclear, however, the germination level in seed from the inoculated NIAB field trial was very low at 6%, likely due to poor pollination during the trial, but it cannot be ruled out that this was due to disease expression in a different form.

The level of metalaxyl-M resistance in *Peronospora belbahrii.*

Four samples of basil downy mildew from the UK were screened for metalaxyl-M resistance; the isolates from STC and NIAB used in PE 024 and two samples sent to Fera by the industry. The samples sent by the industry to Fera were received in August and September 2017, no samples were received during 2018.

The isolates screened from STC and NIAB were shown to be metalaxyl-M resistant and sensitive respectively. These data are consistent with the results obtained from the fungicide trials carried out in PE 024. The two industry isolates were both metalaxyl-M resistant.

This result highlights the need to use a planned fungicide resistance management strategy alternating different modes of action to reduce the chances of further resistance developing.

## Financial Benefits

The UK fresh basil industry is highly valuable, and the recurring problem of downy mildew is causing growers major issues; particularly as an infection results in damaged leaves and thus unmarketable plants. The lack of any tolerance from retailers to blemishes and the rapid spread of downy mildew under favourable conditions have led to complete loss of crops grown under glass and up to 80% losses in the field. Outputs from this PE 024 and the extension project (PE 024a) 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 with your seed supplier as to the health status of seed batches and any treatment methods used.
- Consider using the simple box-test protocol to screen incoming seed batches for basil downy mildew (see Appendix I in Science Section). Note, a negative result does not necessarily imply that seed is free of *P. belbahrii*.
- 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.
- Use a planned fungicide resistance management strategy alternating different modes of action) to reduce the chances of further fungicide resistance developing. Do not rely on metalaxyl-M alone for seed treatment or disease control in the crop.
- 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 survey carried out by British Herbs reported 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 relatively 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.

Prior to the AHDB project PE 024, there had 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). Results from AHDB-funded project PE 024 suggested that the pathogen was systemic in seed with a high proportion of seed-lots tested contaminated with DNA of *P. belbahrii*. However, growing of plants from contaminated seed did not result in disease development. It is unclear whether the DNA detected in seed was rendered non-viable due to seed treatments, such as heat treatment, or whether the contaminated seed were present at a rate lower than could be detected in the screen set up. The significance of the presence of *P. belbahrii* DNA in seed lots remains unclear and was investigated as part of this follow-on project.

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. Alternate host studies carried out in project PE 024 looked at 14 plant species from across the Lamiaceae family and showed that lavender, common sage and catnip were also susceptible to infection by *P. belbahrii*. Profuse sporulation was observed on lavender, with little or no sporulation noted on common sage and catnip. All the alternate hosts identified were crops of one type or another, so growers should take care if growing these along with basil. The lack of weed crops as alternate hosts should make disease management easier as there appears to be no route for overwintering/spread of *P. belbahrii* via weed species.

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). Experiments in PE 024 showed that infection of basil by *P. belbahrii* occurred over a wide range of temperatures (between 5 and 25°C), with the optimum temperature for infection being between 15 and 25°C. High humidity and prolonged moisture on the leaf surface (greater than 4 hours) were also required for infection.

Exposure to light suppressed 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. Infection experiments in the AHDB project PE 024 showed that spore production and infection of basil plants by *P. belbahrii* did not occur during daylight, both requiring a period of darkness to occur. This suggests that, where practicable, it would be advantageous to minimise the period of overnight darkness as much as possible. Work on *Peronospora violae* also showed that sporulation and infection only occurred in the dark (Jennings *et al*., 2009).

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 several 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*. Several active ingredients, screened in Project PE 024, were shown to provide good disease control in protected and outdoor crops. A number of these products offered good protective activity when applied up to 10 days before infection and so could be used in a weekly fungicide programme to prevent the disease. 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). 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. Resistance to metalaxyl-M was reported in *P. belbahrii* isolates collected in the UK. The significance of metalaxyl-M resistance in the UK basil downy mildew population was investigated in the current project (PE 024a).

## Materials and methods

Transmission of *Peronospora belbahrii* from contaminated seed to plants

Two methods were used to screen seed, a conventional growing test and the Box test. Seed samples tested were chosen from those screened in PE 024 based on the presence of *P. belbahrii* DNA. Seed were also planned to be collected from any basil downy mildew outbreaks that occurred during the lifetime of this project. Such seed were analysed for the presence of DNA (using a method based on that reported in PE 024) prior to use in the transmission screening.

Conventional grow-on test

A minimum of 2500 seed were screened for each seed sample chosen. Seed were sown in batches of 100 seed grown until plants were at the fourth true leaf stage. Plants were regularly wetted, placed in high humidity conditions overnight and assessed for the appearance of downy mildew symptoms.

Box test

Basil seed were sown in 7 x 7 cm seed trays (15 seed/pot). The compost was wetted thoroughly, covered with a propagator and placed in a growth chamber at 19-24°C (16 h light) until seedlings reached the 1st true leaf stage (13-15 days, Figure 1a). Seedling were cut at the stem base and placed inverted onto wet filter paper in Perspex boxes (Figure 1b). Lids were placed on the boxes and the seedlings incubated for 24 h in a dark growth chamber and then maintained at 19-24°C with a 16:8 h light/dark for a further 8 days.

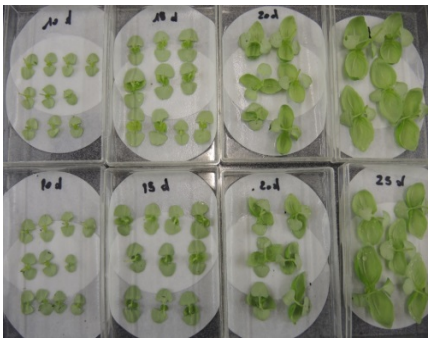
a)  b) 

Figure 1. Box test screening of basil seed a) seedlings at first true leaf stage and b) seedlings transferred to Perspex boxes.

Seedlings were assessed for disease incidence (% seedlings affected) and severity using a 0-5 scale (0 = no sporulation, 1 = 1% to 10% sporulation, 2 = 11% to 25%, 3 = 26% to 50%, and 4 = 51% to 75% 5 = 76% to 100%).

The level of metalaxyl-M resistance in *Peronospora belbahrii*

Basil samples infected with downy mildew were sent to Fera for screening. On arrival at the laboratory, a sample was treated in one of two ways depending on the level of infection.

1) Samples with high levels of sporulation

Infected leaves were removed, and sporangia washed from the leaf surface. The spore suspension was filtered through a double layer of lens tissue to remove any leaf or soil debris and the spore concentration adjusted to give $10^4$ sporangia $mL^{-1}$. The spore suspension was

then used to inoculate three replicate basil plants (6-week-old) treated with a Subdue soil drench containing metalaxyl-M (at 12.5 mL product/100L water @ 10% of pot volume) the day before inoculation. An additional three plants, drenched with an equivalent volume of water, were inoculated as untreated controls.

2) Samples with low level of sporulation

Sporangia were washed from the leaf and filtered through a double layer of lens tissue. The sporangial suspension was then inoculated on to a single untreated basil plant in order to produce additional inoculum for testing.

Plants were kept in a propagator top overnight to prevent drying out of inoculum and then transferred to a glasshouse, maintained at a constant 20°C, with 12 h day period for 14 days. During this period plants were watered from the bottom to ensure sporangia were not produced prematurely. Sporangial production was stimulated by wetting the upper surface of leaves and incubating overnight in a propagator top. The presence of disease was confirmed by assessing the underside of leaves for the downy growth associated with a sporulating downy mildew infection. Sporulation on the control plants only indicated metalaxyl-M sensitivity for the isolate tested, whereas sporulation on both control and metalaxyl-M treated plants indicated metalaxyl-M resistance.

**Results**

Transmission of *Peronospora belbahrii* from contaminated seed to plants

Eight seed samples were tested (Table 1) in seed transmission studies. Six samples were obtained during AHDB project PE 024 and two by NIAB (one from a field trial conducted in 2018 in which basil was inoculated with *Peronospora belbahrii*, and the other from Germany). No basil downy mildew infections were reported in the UK during 2018 so fresh seed samples could not be obtained via this route.

The level of *P. belbahrii* DNA in the seed (Table 1) was expressed 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.

Table 1. Seed samples used for the seed transmission screening.

| Seed sample | Ave Ct value | Test used | Origin |
|---|---|---|---|
| 3 | 30.4 | Grow-on and Box | PE 024 |
| 13 | 31.8 | Box | PE 024 |
| 16 | 31.0 | Box | PE 024 |
| 17 | 30.4 | Grow-on and Box | PE 024 |
| 19 | 29.3 | Grow-on and Box | PE 024 |
| 21 | 29.8 | Grow-on and Box | PE 024 |
| NIAB field trial* | 19.4 | Box | NIAB |
| Lot 21(2018) | 31.1 | Box | NIAB |

*Artificially inoculated with *Peronospora belbahrii*

Four seed samples were screened using the grow-on screen with between 2500 and 6000 seed analysed (Table 2). No downy mildew symptoms were observed in any of the seed screened, despite all four-testing positive for presence of pathogen DNA, and the maintenance of suitable environmental conditions for the expression of disease symptoms.

Table 2. Seed transmission results for four seed samples screened using the Grow-on test

| Seed sample | Number of Seed tested | Symptomatic seedlings | Infected seedlings (%) |
|---|---|---|---|
| 3 | 3500 | 0 | 0 |
| 17 | 2500 | 0 | 0 |
| 19 | 3500 | 0 | 0 |
| 21 | 6000 | 0 | 0 |

Seven seed samples were screened using the Box test with between 100 and 775 plants screened (Table 3). Downy mildew symptoms were observed in seedlings from seed samples 17 and 21, with a higher level of symptoms showing in sample 21 which also contained the higher level of *P. belbahrii* DNA. This demonstrates that *P. belbahrii* can be transmitted from basil seed to seedlings. Transmission rates as high as 1.3% suggests that seed-borne

inoculum plays an important role in outbreaks of basil downy mildew. There have been other reports of seed transmission of *P. belbahrii* (Garibaldi et al., 2004; Farahani-Kofoet *et al*., 2012). The study by Garibaldi *et al*. (2004) showed that seed-borne levels of *P. belbahrii* as low as 0.02% could lead to visible infection in a basil crop. A study in Israel (Falach-Block *et al.*, 2019) seemed to contradict these reports by concluding that *P. belbahrii* was not seed transmitted. Their hypothesis about the apparent contradiction was that the exposure of basil plants, in Europe and other locations, to prolonged periods of wetness during flowering and seed production facilitated seed infection; in contrast the dry summer conditions experienced in Israel inhibited this.

Despite very high levels of *P. belbahrii* DNA being present in both the NIAB field trial sample and 'Lot 21', no downy mildew symptoms were expressed. It is unclear why no symptoms were expressed in these samples, however seed germination in seed from the field trial was very low at 6%; this is likely due to poor pollination during the trial, but it cannot be ruled out that the disease was being expressed in a different form.

Table 3. Seed transmission results for seven seed samples screened using the Box test

| Seed sample | Number of seeds tested | Symptomatic seedlings | Mean severity (0-5) | Infected seedlings (%) |
|---|---|---|---|---|
| 13 | 750 | 0 | 0 | 0 |
| 26 | 750 | 0 | 0 | 0 |
| 17 | 775 | 3 | 1 | 0.4 |
| 19 | 750 | 0 | 0 | 0 |
| 21 | 775 | 10 | 1 | 1.3 |
| NIAB field trial* | 100 | 0 | 0 | 0 |
| Lot 21(2018) | 525 | 0 | 0 | 0 |

*Artificially inoculated with *Peronospora belbahrii*

The level of metalaxyl-M resistance in *Peronospora belbahrii*

Four samples were screened for metalaxyl-M resistance; the isolates from STC and NIAB used in PE 024 and two samples sent to Fera by the industry. The samples sent by the industry to Fera were received in August and September 2017, no samples were received during 2018.

The isolates screened from STC and NIAB were shown to be metalaxyl-M resistant and sensitive respectively. These data are consistent with the results obtained from the trials work carried out in PE 024.

The two industry isolates were both metalaxyl-M resistant.

Resistance to metalaxyl-M (mefenoxam) in *P. belbahrii* has also been reported in Israel (Cohen *et al*., 2013b) and Italy (Pintore *et al*., 2016a). It was also reported by Pinore *et al.* (2016b) that metalaxyl-M resistant strains of *P. belbahrii* could be transmitted through infected seed. Reports from Italy (T. Woods *pers. com*.) suggest that metalaxyl insensitivity in *P. belbahrii* populations has not been seen since metalaxyl was dropped as a control product. However, insensitivity is now being report to the active used to replace it. This highlights the need to use a planned fungicide resistance management strategy alternating different modes of action) to reduce the chances of further resistance developing.

**Conclusions**

- No seed transmission of basil downy mildew was observed using the conventional growing on test.
- Low-level seed transmission in two seed samples was observed in the box test resulting in 0.4 and 1.3% infected seedlings. This demonstrates the potential role of seed-borne inoculum in epidemics of basil downy mildew in both protected and field crops.
- No consistent correlation between level of *P. belbahrii* DNA in the seed and seed transmission was found.
- Metalaxyl-M resistance was shown to be present in three out of the four UK isolates of *P. belbahrii* tested.

**Knowledge and Technology Transfer**

- Presentation to the British Herbs Trade Association Technical meeting held on 22nd November 2018 at Syngenta, Jealotts Hill.
- Article for the Protected Edibles review magazine (published 2019).
- Articles for the Field Vegetable review magazine (published 2018).

**References**

Belbahri L, Calmin G, Pawlowski J, Lefort F (2005). Phylogenetic analysis and real-time PCR detection of a previously undescribed *Peronospora* species on sweet basil and sage. *Mycological Research*, 109, 1276-1287.

Cohen Y, Vaknin M, Ben-Naim Y, Rubin AE (2013a). Light supresses sporulation and epidemics of *Peronospora belbahrii*. PLOS ONE, 8, 1-12.

Cohen Y, Vaknin M, Ben-Naim Y, Rubin AE, Galperin M (2013b). First report of the occurrence and resistance to mefenoxam of *Peronospora belbahrii*, causal agent of downy mildew of basil (Ocimum basilicum) in Israel. *Plant Disease*, 97, 692.

Denton GJ, Beal E, Denton JO, Clover D (2015). First record of downy mildew, caused by *Peronospora belbahrii*, on *Solenostemon scut*ellarioides in the UK. *New Disease Reports*, 31, 14.

Djalali Farahani-Kofoet, R, Romer P, Grosch R (2014). Selecting basil genotypes with resistance against downy mildew. *Scientia Horticulturae*, 179, 248-255.

Falach-Block L, Ben-Naim Y, Cohen Y (2019). Investigation of seed transmission in Peronospora belbahrii the causal agent of basil downy mildew. *Agronomy*, 9, 205.

Farahani-Kofoet RD, Roemer P, Grosch R (2012). Systemic spread of downy mildew in basil plants and the detection of the pathogen in seed and plant samples. *Mycological Progress*, 11, 961-966.

Garibaldi A, Minuto A, Minutto G, Gullino ML (2004). Seed transmission of *Peronospora* sp. of basil. *Zeitschift fur pflanzenkrankheiten und pflanzenschutz*, 111, 465-469.

Garibaldi A, Bertetti, D, Gullino ML (2007). Effect of leaf wetness duration and temperature on infection of downy mildew (*Peronospora* sp.) of basil. *Journal of Plant Disease and Protection*, 114, 6-8.

Gilardi G, Demarchi S, Garibaldi A, Gullino M (2013). Management of downy mildew of sweet basil (*Ocimum basilicum*) caused by *Peronospora belbahrii* by means of resistance inducers, fungicides. biocontrol agents and natural products. *Phytoparasitica,* 41, 59-72

Gullino M, Gilardi G, Garibaldi A (2009). Chemical control of downy mildew on lettuce and basil under greenhouse. *Communications in Agricultural and Applied Biological Science*, 74, 933-40.

Henricot B, Denton J, Scrace J, Barnes AV, Lane CR (2009). *Peronospora belbahrii* causing downy mildew disease on Agastache in the UK: a new host and location for the pathogen. *New Disease Reports*, 20, 26.

Jennings P, Turner JA, Thorp G, McPherson M, Lambourne C, Liddell D, Burdon I (2009). Detection and control of downy mildews on ornamentals. AHDB Horticulture Final Report for project PC 230.

Jennings P, Thorp G, McPherson M, Lambourne C (2011). Control of downy mildew (*Plasmopara obducens*) an economically important foliar disease on impatiens. AHDB Horticulture Final Report for project PC 230a

Mersha Z, Zhang S, Raid RN (2012). Evaluation of systemic acquired resistance inducers for control of downy mildew on basil. *Crop Protection*, 40, 83-90.

Ogawa T (1980). Synergistic Action of Red and Blue Light on Stomatal Opening of Vicia faba Leaves. In The Blue Light Syndrome, Proceedings in Life Sciences 1980, pp 622-628, Ed. H Senger.

Pintore I, Gilardi G, Gullino ML and Garibaldi A (2016a). Risposta di popolazioni di *Peronospora belbahrii* a concentrazioni crescenti di Metalaxyl-M. *Protezione delle colture*, 9(2), 59-60.

Pintore I, Gilardi G, Gullino ML and Garibaldi A (2016b). Detection of mefenoxam-resistant strains of *Peronospora belbahrii*, the causal agent of basil downy mildew, transmitted through seed infection. *Phytoparasitica*, 44, 563-569.

Thines M, Telle S, Ploch S, Runge F (2009). Identity of the downy mildew pathogens of basil, coleus and sage with implications for quarantine measures. *Mycological Research*, 113, 532-540.

Van der Gaag D, Frinking H 1997. Extraction of oospores of Peronospora viciae from soil. *Plant Pathology,* 46, 675-679.

**Appendix I**

**Basil downy mildew box-test**

**Please note when using this test a negative result doesn't necessarily indicate nil pathogen on seed.**

1. Sow >500 basil seed (*Ocimum basilicum* L., cv. Sweet Genovese) in 150-mL plastic pots (7x7cm, 15 plants/pot, image 1) containing Levington M2 compost. Wet thoroughly, cover in a propagator and place in a growth chamber at 19-24°C (16 h light) for 13-15 days (1st true leaves, Figure 1).

Figure 1: 13-15 day old seedlings.

2. Prepare Perspex boxes (125 mm, 82 mm x 22 mm, with lids) by placing two paper filter discs inside and adding 5-7 ml sterile water
3. Sample seedlings (1st true leaves) in a laminar flow hood using a sterile scalpel; hold the base of each stem with forceps, cut directly above and invert on filter paper. Arrange 15 seedlings in each box and cover (Figure 2).

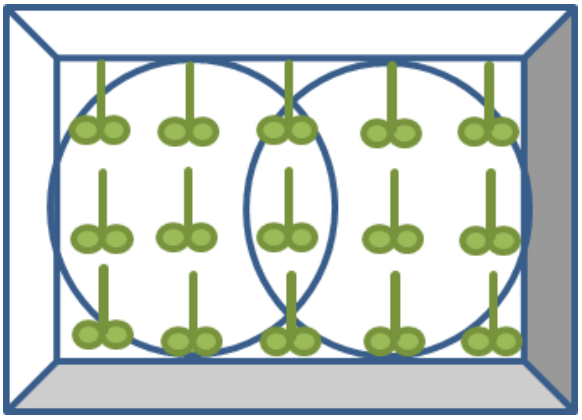


Figure 2: Inverted seedlings arranged in boxes

4. Incubate the seedlings for 24 h in a dark growth chamber to allow development of disease, and then maintain the boxes at 19-24°C with a 16:8 h light/dark for a further 8 days in a growth cabinet to allow for expression of symptoms.
5. Screen for disease symptoms after 7-10 days incubation, scoring disease incidence (% seedlings affected) and disease severity (0-5 scale) visually, with the aid of a stereomicroscope; basil downy mildew is grey/dark grey with a fluffy appearance (Figure 3.)

Figure 3: *P. belbahrii* sporulation.

6. Disease severity should be scored according to the percent sporulation present on each seedling; a five-point categorical scale (0–5) was used in which 0 = no sporulation, 1 = 1% to 10% sporulation, 2 = 11% to 25%, 3 = 26% to 50%, and 4 = 51% to 75% 5 = 76% to 100%. Ideally the test should be repeated in triplicate for each batch of seed.
7. Where no differences in disease severity are observed results are presented as % incidence alone.