Project title	Outdoor herbs: epidemiology and control of downy mildew in outdoor sage, parsley, mint and in basil under protection
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Project leader:	Kirsty Wright, Stockbridge Technology Centre
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Location of project:	Stockbridge Technology Centre ADAS Boxworth Grower sites, Norfolk and N. Yorks
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Date project commenced:	1 April 2011
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***** Left STC in Dec 2011

** Maternity leave cover for Kirsty Wright

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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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CONTENTS

GROWER SUMMARY

Headlines

- Sage downy mildew may persist in woody plant material between growth seasons.
- Parsley downy mildew infection occurred only in autumn, despite the occurrence of leaf wetness periods of greater than 24 hours and conditions of high relative humidity earlier in the growing season.
- Basil downy mildew, whilst no longer a notifiable disease in the UK, is a particularly aggressive pathogen requiring effective control measures.

Background and scope

Downy mildew has become an increasing problem over recent years on a range of outdoor grown herbs, particularly sage, mint, and parsley. Downy mildew has also emerged as a problem on UK protected basil. Some growers whose crops were severely affected by downy mildew on sage in 2009 reported up to 80% of the crop being lost to infection.

Downy mildew diseases are caused by a number of different oomycete fungus-like organisms that frequently have narrow host ranges. The first record of *Peronospora lamii* on sage and rosemary in the UK was in May 2004. The same downy mildew species also affects mint. Downy mildews in parsley and basil are caused by different organisms, namely *Plasmopara umbelliferarum* (syn. *Plasmopora petroselini*) and *Peronospora belbahrii*, respectively.

The cool, wet seasons experienced during 2007–2010 were highly conducive to downy mildew infections on a wide range of crops. These high-risk seasons may have given rise to increased inoculum levels in soil and crop debris, and to long-term systemic infections in perennial herb crops like mint and sage.

Given the appropriate environmental conditions, downy mildew can infect herbs in propagation and those grown under protection (tunnels) as well as field-grown perennials or seed-raised crops. Downy mildews of some crops are seed-borne, and, while seed transmission has not been demonstrated for all the herb downy mildews under discussion here, a seed-borne route cannot be discounted, and there is circumstantial evidence to suggest a seed-borne route for dispersal. For example, *Peronospora belbahrii* has been found in association with basil seed, but the significance of this with respect to infection and disease spread is unclear.

A range of fungicide active ingredients such as metalaxyl-M and dimethomorph are available for use on both outdoor and protected herbs. These should provide good activity against downy mildews and other oomycete pathogens, assuming that reduced sensitivity and/or resistance have not developed in the pathogen population. However, the industry is keenly aware of pesticide residue issues and the need to reduce the use of pesticides in line with client (retailer) requirements and this currently presents a particular challenge, especially as there are no effective biopesticide products currently available that have activity against oomycetes. Fungicide applications need to be targeted appropriately, preferably when environmental conditions are favourable for disease development on unprotected crops.

The aims of this project were to determine the environmental conditions posing the greatest risk for downy mildew development through field monitoring, controlled environment work, and literature mining (Objective 1), collate data from Objective 1 to develop a forecasting model for environmental periods of high infection risk (Objective 2), determine whether disinfestation of crop debris and soil could mitigate against infection (Objective 3), and investigate fungicide programmes (Objective 4).

The project was hampered at all stages by lack of reliable infection during the project period (2011–2014). Downy mildews are obligate pathogens, meaning that they cannot be cultured artificially for use as inoculum. Problems were experienced in obtaining suitable inoculum, and, when inoculum was sourced successfully, achieving effective artificial infection proved difficult.

Summary of the project and main conclusions

Downy mildew and environmental conditions were monitored in field crops of parsley (*Petroselinum crispum*) and sage (*Salvia officinalis*) in Norfolk and in sage and mint (*Mentha* spp.) in North Yorkshire in the 2011 and 2012 growing seasons. Environmental parameters were recorded at each site. Disease monitoring was carried out in crops managed under commercial conditions with standard products applied for pests, disease and weeds as necessary. No downy mildew developed in either crop in North Yorkshire in the 2011 season, and only a small amount of disease was seen in 2012, in sage.

Downy mildew was first observed in the Norfolk parsley crop in late September in both 2011 and 2012. In 2011, this subsequently spread rapidly, affecting 80% of assessed plots by the middle of October. The similar emergence date in both years suggests that cooler autumn temperatures are likely to be particularly conducive to disease development and spread. This is supported by the controlled environment work (2011). Artificial infection levels were minimal under controlled environment conditions; nevertheless, parsley downy mildew

infection appeared to occur more readily at lower temperatures (5–15°C) than other downy mildews, and infection occurred with leaf wetness periods as short as 1 hour. Summer 2012 was particularly wet, yet parsley downy mildew still did not develop in commercial crops until early autumn. This suggests that temperature may be of more importance than high relative humidity for disease development, though this may also be indicative of waning plant strength and persistence/effectiveness of chemical control as the crop moved into autumn. By contrast, parsley downy mildew was sustained throughout periods of higher temperatures (early to late spring, 2014) in a small number of test plants at STC when high-humidity was maintained through fleecing. In summary, both lower temperatures and prolonged periods of high humidity were conducive to disease development, but early autumn appears to be a period of particularly high risk.

Downy mildew was present at the majority of assessments in the sage crop (Norfolk) in both 2011 and 2012. In 2011, downy mildew symptoms increased with time on new growth and after each cut but then declined towards the end of the season. No clear correlation between disease development and environmental conditions was apparent, but disease severity increased in early autumn 2012 coinciding with high rainfall levels. Controlled environment work indicated that the optimal conditions for infection were 10–20°C with leaf wetness periods of 6–24 h. However, pesticide treatment of sage plants and crop debris between the 2011 and 2012 seasons had only minimal effect (in spring) and no effect over the whole 2012 season, suggesting that infection in sage may derive from pathogen persistence in woody plant material, despite pesticide application, or that infection arrives each year as airborne spores (sporangia). The products used were SL567A, Previcur Energy, Invader, Signum, and a tank mix of SL567A and Invader, and these were applied in winter 2011 and/or early spring 2012. Sprays were applied over the top of plants to the point of run-off (1000 l/ha) so that the plants and soil were well treated, simulating a drench spray. At the time the work was conducted, the fungicides used were authorised for use on sage under the Extension of Authorisations for Minor Use (EAMU) system (formerly SOLA). Some efficacy was seen with Invader and Signum at the spring assessment (prior to the second spray), but disease severity at later assessments did not differ between treatments. This suggests that disinfestation may be of some value when clearing ground for new crops, but is not likely to minimize disease development in an existing crop.

Financial benefits

- This project has provided additional information regarding the development and persistence of herb downy mildews, particularly in parsley and sage, and the conditions under which the diseases are most problematic. Unfortunately, due to low disease levels in commercial crops during the project, less progress was made than hoped and further work is necessary towards effective control of the pathogen

Action points for growers

- Winter weather severity and spring/summer rainfall are factors that might be used to assess seasonal risk and hence the prospects for early downy mildew infections. Parsley downy mildew is more likely to occur during cooler autumn periods.
- Herb irrigation should be completed early in the day, wherever possible, so that the crop dries and does not remain wet overnight. Long periods of leaf wetness and cool temperatures will favour downy mildew infection and spread, and are best avoided where possible.
- Open planting and increased spacing within the crop aids airflow around plants and is likely to increase drying of the crop after surface wetness periods. Where possible, reducing leaf wetness periods to less than 24 hours may also help reduce infection periods.
- Downy mildew infection in sage may persist in woody material between seasons or may arrive seasonally as airborne spores. Pesticide treatment of sage material between seasons had minimal effect on disease development. However, growers may wish to consider this type of disinfestation when replacing plants or starting a new crop.
- When this project commenced, basil downy mildew was under statutory control and this made it extremely difficult to source isolates of *P. belbahrii* for study. Statutory control has now been lifted, allowing easier access to isolates for research purposes. The HDC has put out a call for further work on Basil Downy Mildew. Growers will be kept updated on progress.
- Growers should source seed from reputable suppliers. Where possible, seed should be from a disease-free crop that has been treated for downy-mildew.

- This project demonstrated that downy mildew is difficult to control once established and fungicides applied at this point have limited efficacy. Treatment for downy mildew should therefore be applied at or before the first sign of disease.

SCIENCE SECTION

General introduction

Downy mildew has become an increasing problem on a range of outdoor grown herbs, particularly sage, mint and parsley, over the last 4–5 years. Furthermore, outbreaks of downy mildew on protected basil were reported at several sites in the UK in 2010. Some growers whose sage crops were severely affected by downy mildew in 2009 reported that up to 80% of the crop was lost as a result of infection. Basil downy mildew was, until recently, notifiable and of quarantine significance but, since 2013, this is no longer the case.

The aim of this study was to provide information on the epidemiology of the three oomycete pathogens causing downy mildew on sage and mint (*Peronospora lamii),* parsley (*Plasmopara umbelliferarum*) and basil (*Peronospora belbahrii*) as a basis for improved disease control.

Downy mildew pathogens are notoriously difficult to research due to four main factors. Firstly, downy mildews are obligate pathogens and as such cannot be cultured and maintained on artificial growth media in the laboratory. Downy mildews survive only on living plant material or on crop debris, which can lead to situations where research cannot be performed until a natural infection occurs to provide inoculum. Secondly, downy mildew species are often highly host-specific and pathogen inoculum for research trials must to be carefully matched to the correct host genera. Thirdly, some downy mildew pathogens comprise different strains with a high level of specificity to particular cultivars and in some crops (e.g. lettuce) it is necessary to match a particular downy mildew strain with a specific variety for successful infection to occur. This host-pathogen interaction is well understood for lettuce downy mildew, but the situation for herb downy mildews is much less clear at present. Lastly, the pathogen requires the presence of a particular set of environmental conditions for successful infection of the host crop. In general this equates to cool, moist conditions that are typically experienced in spring and autumn in the UK. More specifically, plants need to undergo an extended period of leaf-wetness to ensure occurrence of infection, and temperatures of below 20°C are frequently needed to allow the production of spore bearing structures for disease spread.

Four main objectives were included in this study: epidemiology (Objective 1), forecasting (Objective 2), disinfestation of crop soil and debris (Objective 3), and examination of fungicide programmes (Objective 4)

Objective 1: Epidemiology

The aims of Objective 1 were to conduct a review of recent downy mildew literature and gather data regarding the environmental conditions conducive to disease development through field and controlled environment work. In addition, pathogen survival within soil and crop debris was to be examined.

Objective 1.1: Literature review

A review of the recent literature pertaining to downy mildew pathogens in general, and those that infect herbs in particular, was carried out in 2011, and is available in the 2012 annual report. A summary is provided below.

Summary of literature review

Downy mildew (DM) is the common name of a number of oomycete diseases closely related to *Phytophthora* and *Pythium* spp. DMs are obligate biotrophic plant pathogens, meaning that they can only infect and survive on/in living host material and cannot be cultivated on artificial media (e.g., agar plates) in the laboratory. DMs reproduce primarily asexually, producing sporangia on the outside of the infected leaves. Spores are dispersed by the wind and water splash and, when leaf surface moisture is high, by swimming zoospores. Sexual reproduction can also occur via the production of resting spores or oospores within plant tissues, which leads to greater genetic diversity and an increased likelihood of developing novel pathogenic or fungicide-resistance traits. Oospores have a thick wall and can survive in plant debris in the soil, providing a potential source of inoculum for subsequent host crops. Some downy mildew species can also infect plants systemically within perennial crops (e.g. mint), which can provide an additional means by which the pathogen persists.

DMs in temperate climates generally require cool, moist conditions for survival and dispersal. Temperatures of 10–17°C with high relative humidity are most conducive to growth within the host and production sporangia. Spores landing on new host material require a period of leaf surface wetness to allow the infection process to be completed. However, once plants are infected the fungus can survive higher temperatures, only reproducing (sporulating) once environmental conditions become favourable.

At later stages of DM infection, affected leaves develop angular chlorotic lesions bound by leaf veins. The angular leaf chlorosis is usually accompanied by the production of a felty fungal growth on the underside of the leaf that may be white, brown, grey, or purplish depending on the host and DM species. Several DM species affect herbs, and those of relevance to this study are shown in Table 1.1.

Table 1.1. Herb hosts and downy mildew pathogens examined in this study

Host family	Host genus (and most common species)	Common names	Downy mildew species confirmed on this host
Lamiaceae	*Salvia officinalis*	Sage	*Peronospora lamii, P. swinglei* and others
	Mentha spicata	Mint	*Peronospora lamii*
	Ocimum basilicum	Basil	*Peronospora belbahrii*
Apiaceae	*Petroselinum crispum*	Parsley	*Plasmopara umbelliferarum*

Each of the herb downy mildews under investigation in this study now seem to be established in UK commercial crops. Both protected and outdoor crops can be at risk and infection is highly dependent on environmental conditions such as cool, damp weather. Plants display symptoms 6–15 days after infection, depending on temperatures, and sporulation can occur very rapidly under the correct conditions.

Where conditions permit, DM occurs on herbs worldwide, and it is possible that this spread may have been exacerbated by the dispersal of infected seed, plants and foliar material across global markets. Crop epidemics can start from a single infected seed locus and spread through crops once sporulation occurs, and therefore seed and plant provenance is of importance. Growers should also consider using cultivars that exhibit disease tolerance or reduced susceptibility, if available. Protectant pesticides may also be of value as eradication once infection has established can be extremely difficult. The environmental conditions favourable to infection should also be avoided where possible to limit incidence and spread of disease. For example, open planting or spacing within a crop, use of trickle or drip irrigation to reduce leaf wetness, and scheduling of overhead irrigation to reduce leaf wetness duration may be of benefit.

A variety of protection measures have been examined for the control of downy mildews in other crops such as lettuce, grapevines, and cucumber, and also some herb crops, such as chives. Approaches included the use of forecasts to assess times of greater risks and guide pesticide application, and the use of specific light wavelengths to reduce sporulation. These approaches met with varied success in terms of disease control and crop yield.

Little scientific work has been previously performed on the epidemiology and control of the herb downy mildews examined in this study. The gathering of baseline data on the occurrence of herb DMs will be of value in developing future control strategies.

Objective 1.2: Monitoring disease development in commercial outdoor herb crops in relation to weather patterns

Introduction

The aim of monitoring was to assess field sites for disease development while simultaneously monitoring environmental conditions to help determine if weather conditions could be accurately linked to disease occurrence. This knowledge would allow growers to better identify 'high disease risk' events and assist decision-making with respect to instigating appropriate control strategies. Monitoring performed in 2011 is described in the 2012 Annual Report and is summarised below. Monitoring performed in 2012 is described in this report.

Summary of previous work (2011)

Parsley and sage crops were established in 2011 in Norfolk (monitored by ADAS) and sage and mint crops were established in North Yorkshire (monitored by STC). Crops were managed as per commercial practice. No DM symptoms were observed in the crops at the North Yorkshire site.

At the Norfolk site, a disease epidemic commenced in the parsley crop around 30/09/2011 and spread rapidly, affecting ~80% of the crop by 20/10/2011. This corresponded with an extended period in September when leaf wetness periods frequently lasted 30–40 hours and temperatures were mild (5–15°C).

DM was present in all plots at all assessments in the Norfolk sage crop, except when plots had been recently harvested. This suggested the possibility of a systemic infection within the woody parts of the sage plants that remained after harvest. DM symptoms increased with time after harvest and infection was more prevalent on younger leaves. The mode of infection (systemic or from air-borne inoculum) was unclear. Harvesting may have masked any effect of weather on pathogen development due to the removal of susceptible leaf material. However, even prior to harvesting, infection decreased in June and this decrease corresponded with an increase in temperatures and thus there may be a number of factors influencing infection and symptom expression.

Monitoring disease development in commercial outdoor herb crops in relation to weather patterns (2012)

Materials and methods

Site and crop details

The Norfolk and Yorkshire crop sites were as described in the 2012 annual report.

Disease assessments

Disease assessments were conducted as described in the 2012 annual report.

Results

Environmental monitoring / weather records

General weather conditions are provided for the Norfolk and North Yorkshire sites in this section (Tables 1.2 & 1.3). Detailed leaf wetness data are provided in Objective 2, which discusses environmental factors with regard to infection.

Environmental data for the Norfolk site are shown in Table 1.2. In general, 2012 was cooler than normal due to cloud cover and rain was recorded on almost a third of the days. Although some hot days occurred in late summer, rainy days were still frequent, and therefore conditions conducive to DM development were present on at least some occasions. For example, although the maximum temperature on 18th August was 31°C, rain still fell on this day.

Records for the North Yorkshire site are shown in Table 1.3. Rainfall was not determined every day; in these cases, rainfall was estimated from the next available measurement.

As in 2011, air temperatures were similar at the two sites but had a tendency to be lower at the North Yorkshire site in autumn. However, spring temperatures were generally warmer at the North Yorkshire site than the Norfolk site. There was a striking difference in rainfall recorded at the two sites, with the North Yorkshire site receiving substantially more rain. This may be due to the different ways in which the measurements were taken: the Norfolk site data were obtained using a Delta-T logger and the data from the North Yorkshire site were based on farm site records. However, higher relative humidities at the North Yorkshire site support the higher rainfall values.

Table 1.2. Overview of weather conditions at the Norfolk field monitoring site, 2012

	Average Max. Air Temp. (°C)	Average Min. Air Temp. (°C)	Total rain (mm)	Average rel. humidity (%)	Rainy days (number)	Rainy days (%)
March (from 21st)	19.5	6.0	2.0	59.2	2	20.0
April	11.7	4.1	7.2	76.0	9	30.0
May	16.6	8.4	7.2	76.2	10	32.3
June	18.1	10.2	9.2	77.2	13	43.3
July	20.8	12.0	12.8	80.5	11	35.5
August	22.9	12.7	8.6	79.9	10	32.3
September	19.1	8.1	5.6	80.0	6	20.0
October (to 12th)	15.8	5.8	1.8	85.6	2	16.7

Table 1.3. Overview of weather conditions at the N. Yorks. field monitoring site, 2012

	Average Max. Air Temp. (°C)	Average Min. Air Temp. (°C)	Total rain (mm)	Average rel. humidity (%)	Rainy days* (number)	Rainy days* (%)
May (from 22nd)	23.7	9.9	0	79.4	0	0
June	17.6	9.4	114	86.5	23	76.7
July	19.5	10.8	74	87.0	19	61.3
August	21.4	10.9	101	87.6	19	61.3
September	17.3	7.5	123	87.9	14	46.7
October (to 25th)	12.7	5.0	71.5	94.7	11	44.0

*where data are missing, rainy days are estimated from the next available measurement.

Disease occurrence and development (Norfolk)

Parsley

Outbreaks of parsley downy mildew were expected in July and August due to the frequent occurrence of Smith periods (Fig. 2.1). However, no disease was noted until the final crop visit on 26/09/12, and symptoms were minimal. This lack of observed disease may have been due to harvesting of crops after infection occurred but before symptoms could develop. Lack of disease may also have been due to inhibition of the pathogen by the higher temperatures experienced in the summer months. In addition, spores may have been dislodged from leaves during strong rainfall events (> 1mm in 30 min) that occurred on 25/05/12, 11/07/12, 27/07/12, and 18/08/12, and this may have limited the potential for infection.

Sage

With the exception of the assessment on 09/08/2012, downy mildew was found in the majority of plots at each assessment (See Appendix 1 for assessment data)

Disease occurrence and development (North Yorkshire)

Mint

No downy mildew was observed upon any assessment in the mint crop.

Sage

The sage crop was assessed on 20/06/12 and 07/08/12 and downy mildew was observed on both occasions (Table 4). Disease appeared to be more severe at the June assessment. However, the crop had recently been cut and the assessment was performed largely on older, unharvested, leaves. Rainfall was heavier at the Yorkshire site than in the Norfolk site, and it is therefore surprising that infection levels were higher in the Norfolk sage crop. It is reasonable to speculate that this difference may have been due to infection status in the previous year. Early infection of the Norfolk crop in 2011 may have carried over into the 2012 crop. This is supported by the limited efficacy of fungicide disinfestation of sage crop debris between seasons (see Objective 3). Conversely, no sage downy mildew was seen in the North Yorkshire crop in 2011 and this may have limited infection in 2012 to that caused by airborne sporangia.

Table 4. Incidence and severity of sage downy mildew, North Yorkshire - 2012

Assessment date	Number of plots affected (n = 20)	Average severity in affected plots (% leaf area)	Severity range in affected plots (% leaf area)
20/06/12	14	23 %	1 – 50 %
07/08/12	7	4 %	1 – 5 %

Objective 1.3: Assessment of environmental parameters on incidence and severity of downy mildew on parsley and sage

Introduction

The objective of this work was to provide information on conditions conducive to infection of sage and parsley with *Peronospora lamii* and *Plasmopara umbelliferarum*, respectively. The effects of temperature, leaf wetness and relative humidity on infection by *Peronospora lamii* and *Plasmopara umbelliferarum* were examined under controlled environment (CE) conditions.

Summary of previous controlled environment work (2011–2012)

Replicate parsley and sage crops were inoculated with *Plasmopora umbelliferarum* and *Peronospora lamii*, respectively, and were incubated under a variety of controlled temperature and leaf wetness conditions at ADAS (Boxworth). Disease incidence was low overall, and, although insufficient data were obtained for statistical analysis, several trends were noted. Sage infection occurred most readily at average temperatures of 15°C (range; 10–20°C) and prolonged leaf wetness periods of 24 h (range; 6–24 h). By contrast, parsley infection occurred most readily at 5°C (range; 5–15°C) and leaf wetness duration appeared less important, with infection occurring with wetness periods as low as 1 h. No parsley infection occurred at 20°C.

These data must be viewed with caution due to the low incidence of disease and lack of statistical significance.

Environmental parameters (2012–2014)

The controlled environment experiments were due to be repeated in 2014 but were hampered by the absence of suitable inoculum for the obligate pathogen *P. lamii*. Growers were repeatedly approached for samples, but inoculum was either unavailable or was non-viable. Researchers at ADAS used two main strategies in an attempt to produce inoculum for controlled environment experiments. First, parsley seedlings were placed outside on a sheltered hard standing area in case of natural infection from airborne sporangia. Second, seedlings were grown and obtained from growers for artificial inoculation with infected material sourced from STC[1] or growers. The parsley variety Giant Italian Oscar was used as this had previously proved susceptible to downy mildew sourced from STC. Plants were maintained under conditions of high relative humidity and leaf wetness (e.g., glasshouse, polytunnel, outside with fleece). For inoculation, plants were sprayed late in the afternoon with spore suspensions of ≥ 1×10^4 spores/ml. In addition, infected leaves were placed around the plants intended to be infected. Inoculation attempts were unsuccessful, and it was therefore not possible to determine the precise mechanisms and parameters underlying parsley downy mildew infection.

Objective 1.4: Assessment of downy mildew survival in soil / crop debris

Introduction

The aim of this objective was to determine whether downy mildew pathogens could survive

[1] In an attempt to produce inoculum for the controlled environment work in 2014, a small number of parsley plants infected with downy mildew were removed from an STC field study in February 2014 and incubated under fleece with frequent watering and agitation. These plants became heavily infected and were beginning to senesce by April, yet no oospores were found in plant tissues when examined microscopically. In June, fresh curly-leaf and flat-leaf parsley plants were incubated with the infected plants and these also became infected. As these plants were raised to provide inoculum for the controlled environment study, the plants were not monitored and this information is therefore of limited value. However, it is worth noting that infection was active throughout periods of higher daytime temperatures up to 22°C. This contrasts with the controlled environment work from 2011 in which infection was absent above 15°C. This suggests that parsley downy mildew infection may be able to persist at higher daytime temperatures when plants are kept at a constantly high humidity, particularly when a moderate-high level of inoculum is present. Thus, advice to reduce leaf wetness duration and limit overhead watering may be appropriate at higher temperatures as well as lower temperatures. However, this may be difficult to achieve in practice.

in soil and crop debris and re-infect subsequent crops. This information would be of value in determining the necessity for soil disinfestation (see Objective 3).

Outcomes

This part of the study was not performed on parsley and sage at the ADAS site due to a lack of available inoculum.

For basil downy mildew, soil was obtained in autumn 2013 from a North Yorkshire polytunnel in which a substantial basil downy mildew infection had occurred. The soil was overwintered in a protected dry frost-free environment (0–10°C ±2°C) and basil was sown into the soil in spring 2014. No infection occurred; however, it cannot be concluded that this was due to lack of pathogen survival as no oospores were observed in the soil or plant debris upon microscopic examination at collection, and therefore we cannot be sure that pathogen was originally present. It should be noted that it is not clear whether basil downy mildew routinely forms oospores in infected leaf and stem tissues, and further monitoring is advisable[2].

Objective 2: Forecasting environmental periods of high risk for downy mildew infection

The aim of Objective 2 was to collate data gathered in Objective 1 and develop a system for forecasting periods with high risk for downy mildew infection.

Summary of previous work (2011-2012)

The literature review and experimental work highlighted conditions conducive to disease development. The full details can be found in the previous annual report, but the results are summarised in Table 2.1.

[2] STC are currently working with basil downy mildew in a separate project: sporadic microscopic examinations of heavily-infected plant material have not found any evidence of oospore development to date.

Table 2.1: Details of the conditions required for the various herb downy mildew species to infect their hosts and potential high-disease risk periods.

Downy mildew	Host(s)	From the literature		From our laboratory tests	
		Infection temp.	Leaf wetness period required	Infection temp.	Leaf wetness period required
Peronospora lamii	Sage Mint Rosemary	20-25°C	Not recorded	10-20°C	6-24 hrs
Plasmopara umbelliferarum	Parsley	18-20°C	24 hours	5-15°C	1-24 hrs
Peronospora belbahrii	Basil	20°C	Minimum 24 hours	Not tested	

Disease and environmental monitoring

Further disease and environmental monitoring was carried out during the 2012 growing season, but additional controlled environment work was not possible due to lack of infection (see Objective 1).

Sage and Parsley Downy Mildew (Norfolk)

Summer 2012 was characterized by generally low temperatures and high rainfall (Figure 2.1). Consequently, as shown in Figure 2.2, leaf wetness levels were high, and several Smith periods were noted (Smith periods are defined as at least two consecutive days where the minimum temperature on both days is ≥10ºC and 11 hours of relative humidity greater than 90% occur). However, these climatic conditions did not fully correlate with occurrence of downy mildew in sage or parsley: downy mildew emerged in parsley only on 26/09/2012, and sage downy mildew was present in the crop throughout the season. Anecdotal evidence does suggest that parsley downy mildew infections may correlate to a degree with the occurrence of Smith periods, which would be as expected given the humidity and temperature requirements of the pathogen. The results of the sage disinfestation trial (Objective 3) indicate that sage downy mildew may be systemic within woody plant material. This suggests that temperature and humidity might be less significant for initial infection than initially thought, but environmental conditions would still contribute to disease development and symptom expression. In the case of potential systemic infection, the pathogen may have the potential for continued development within the plant irrespective of environmental conditions; however, the external environment would still be important for sporulation and spore release, pathogen dispersal, and any subsequent reinfection of new crops and fresh shoots of the same crop. This is supported by the apparently more severe disease

symptoms observed on sage later in the growing season and after periods of high leaf wetness and humidity (Appendix 1).

Sage and Mint Downy Mildew (Yorkshire)

No downy mildew was seen at the Yorkshire site in the mint crop. Sage downy mildew was apparent during both crop assessments, but the level of infection found was insufficient to allow conclusions to be drawn regarding the impact of climate conditions on disease emergence.

Figure 2.1: Climate conditions and downy mildew presence on Parsley and Sage, Norfolk 2012

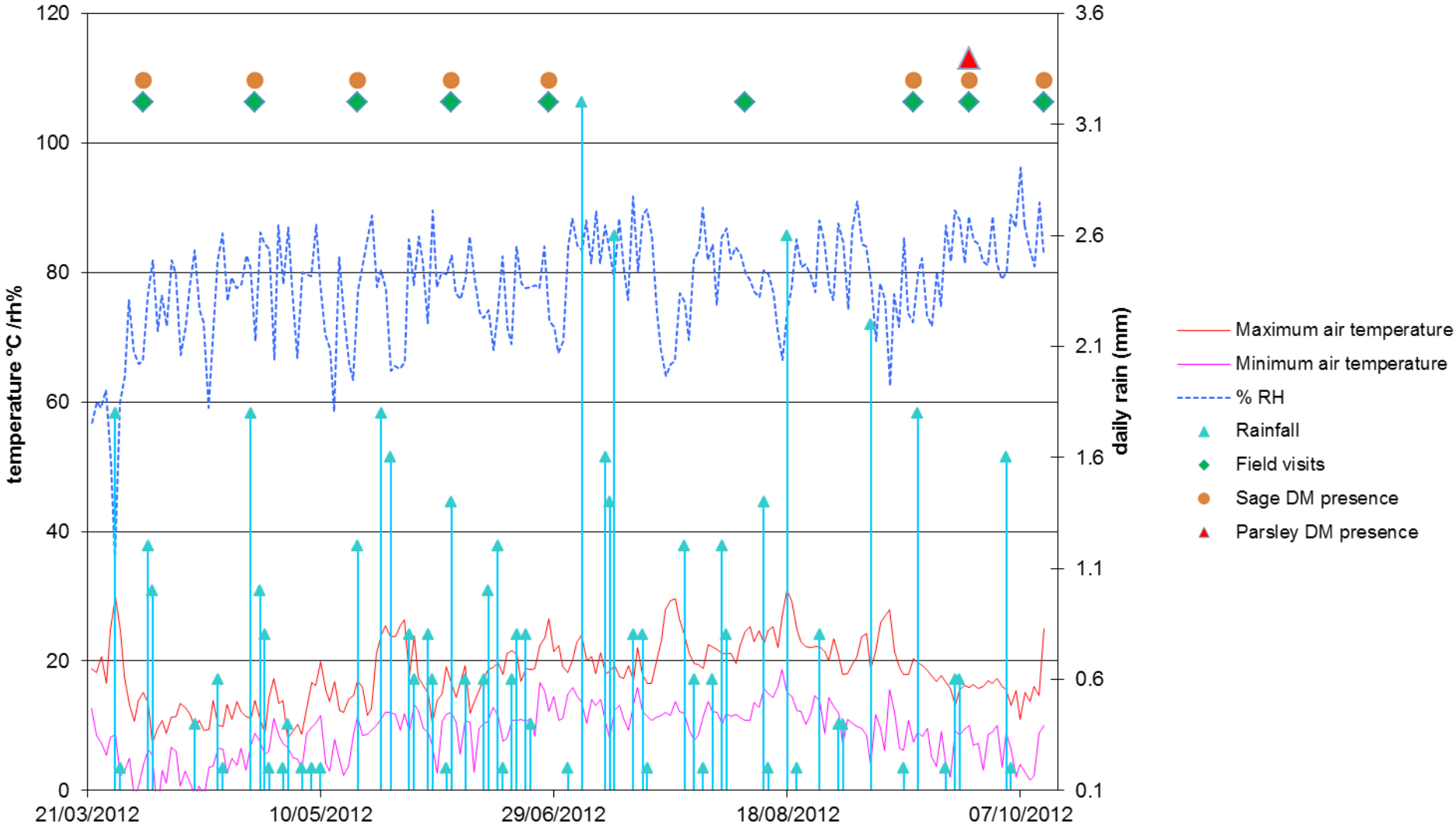
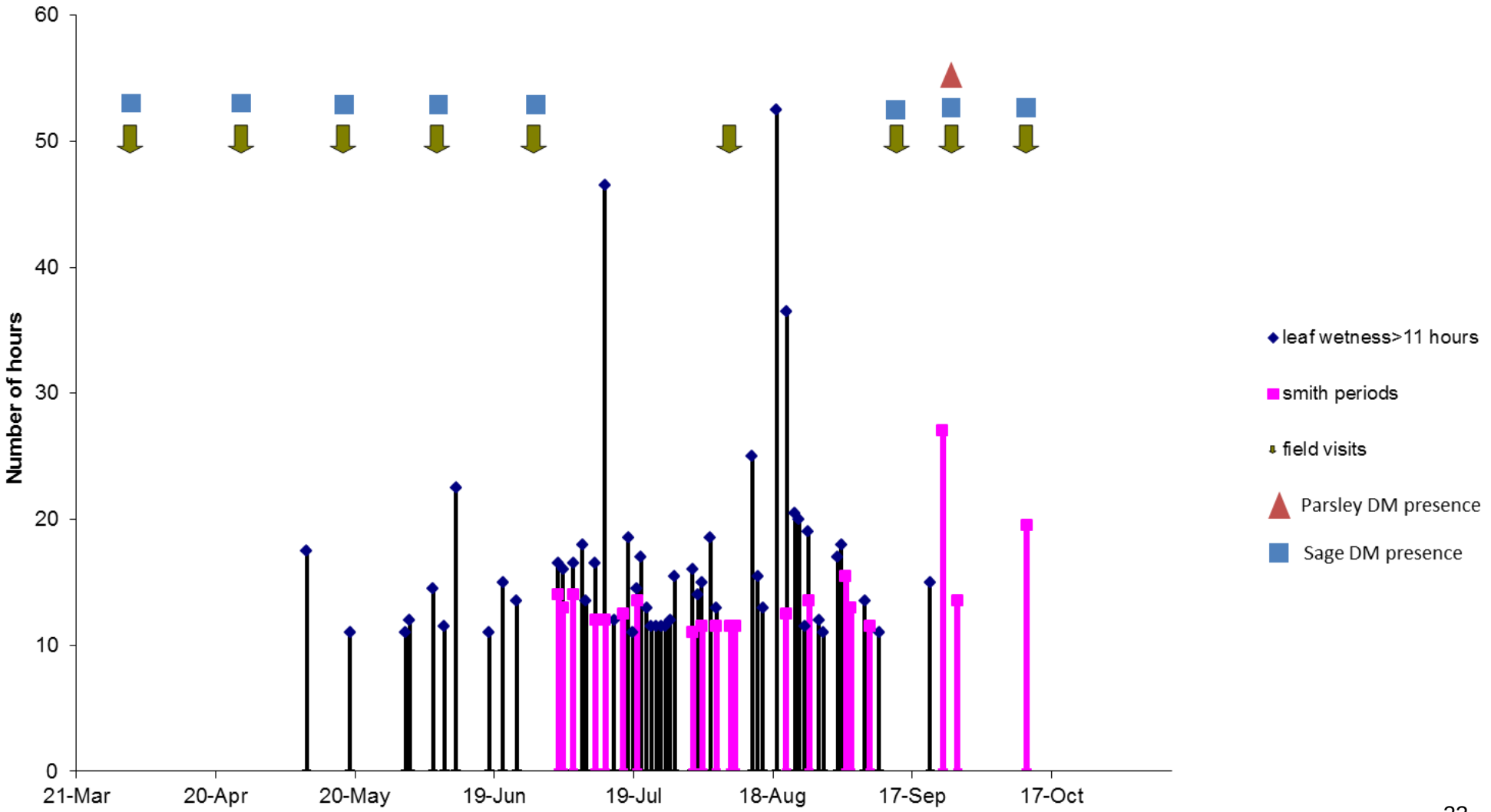


Figure 2.1: Leaf wetness, Smith periods, and downy mildew presence on Parsley and Sage, Norfolk 2012



Objective 3: Disinfestation of soil and crop debris

The aim of this objective was to investigate soil disinfestation by UV light treatment (Objective 3.1), burning (Objective 3.2), and pesticide application (Objective 3.3). Objective 3.1 was not performed due to the lack of available soil with confirmed infective material. Soil sourced from a polytunnel containing infected basil contained no oospores (see Objective 1.4). Objective 3.2 was to assess the effect of propane burning on a perennial mint crop; however, this was not performed due to an absence of infection within the crop during the project period.

Objective 3.3: Effect of overwinter pesticide drenches to soil and crop debris for control of downy mildew in sage

Introduction

The objectives of this work were to test the effectiveness of high volume pesticide sprays against downy mildew (*Peronospora lamii*) when applied as overwinter and early spring disinfestation treatments to dormant sage and to debris between rows. The site and crop details are provided in the 2011 annual report, but are also included here for clarity.

Materials and methods

Site and crop details

The trial was carried out on a commercial crop of sage in Norfolk with a history of downy mildew infection in the previous season. The trial was located in an area sheltered from the prevailing winds to avoid spread of debris between plots.

Treatments

Pesticides were applied in winter (06/12/2011) and/or spring (28/03/2012) as detailed in Table 3.1. Treatments were applied as HV sprays in 1,000 litres water/ha at a pressure of 200–300 kPa using flat fan nozzles and an Oxford precision sprayer. Sprays were applied over the top of plants to the point of runoff so that the plants and soil were well covered. At the time of application, the products described in Table 3.1 were authorised for use on outdoor sage via the Extension of Authorisation for Minor Uses (EAMU) scheme (formerly SOLA). Current EAMU advisory information for Signum relates to use against *Sclerotinia* on leaf herbs but, whilst downy mildew is not mentioned, the target is not a statutory condition of approval.

Experimental design and statistical analysis

The experiment was arranged in a randomised block design with five treatments each applied at three timings: winter only, spring only, and winter and spring. There were four replicate blocks. Each plot consisted of a 1 m length of a 1.5 m wide bed. Blocks were arranged so that all plots in a block received the same irrigation. A spray guard was used between plots when spraying.

At the time of spray application a baseline assessment was performed for % leaf retention and background disease levels. In subsequent assessments, downy mildew development was examined in the central $1m^2$ area of each plot. Downy mildew severity as % leaf area affected was assessed at each spray timing and again on 26/04/12 and 28/06/12 to establish the effect of the treatments on symptom development. Results were analysed by ANOVA.

Results

Plots were assessed for severity of sage downy mildew (Table 3.2; Appendix 2) and examined for significant differences between products, between blocks, and between treatment timings.

Assessment at the time of the second treatment (28/03/2012) revealed that disease levels were lower in the treated (winter and winter + spring plots) than in the not-yet treated plots (spring-only). Significant differences were observed between pesticides only at the time of the second application, with lower infection levels seen in plots treated with Signum and Invader. No products were effective at either eradicating or sustainably reducing downy mildew infection, as indicated by the assessments 24 and 56 days after the final application. Therefore, while treatment was initially effective, no sustained control was achieved over time. It is known from DM work on other crops (including other HDC-funded projects) that such products are most effective when applied as protectants in advance of infection. This cropping scenario, in which there is a chance of overwintered and/or systemic infection, makes the challenge of pathogen control much more difficult. This further emphasises the importance of early action to prevent infection establishing in such perennial crops.

Significant differences were noted in disease severity between replicate blocks throughout the trial, and this was thought to be due to differential irrigation across the field.

These results suggest that downy mildew in sage may not be heavily dependent on inoculum from crop debris, but may instead be due to systemic infection or arrival of new airborne infection on a yearly basis. Both these would explain the lack of efficacy achieved

by reducing inoculum from crop debris between seasons. The host grower reported a general lack of infection at the start of the season and anecdotal evidence from growers suggests that downy mildew may arrive on asymptomatic transplants but not on seed.

Table 3.1. Details of overwinter/early spring pesticide treatments applied to sage for control of downy mildew in 2011/12

Treatment number	Product	Rate of product per ha	Active ingredient	Timing
1	Untreated	-	-	-
2	SL567A	0.24 L	metalaxyl-M	Winter
3	SL567A	0.24 L	metalaxyl-M	Spring
4	SL567A	0.12 L	metalaxyl-M	Winter and Spring
5	Previcur Energy	5 L	fosetyl-aluminium + propamocarb HCl	Winter
6	Previcur Energy	5 L	fosetyl-aluminium + propamocarb HCl	Spring
7	Previcur Energy	2.5 L	fosetyl-aluminium + propamocarb HCl	Winter and Spring
8	Invader	4 kg	dimethomorph + mancozeb	Winter
9	Invader	4 kg	dimethomorph + mancozeb	Spring
10	Invader	2 kg	dimethomorph + mancozeb	Winter and Spring
11	SL567A + Invader (tank mix)	0.24 L + 4 kg	metalaxyl-M and dimethomorph + mancozeb (tank mix)	Winter
12	SL567A + Invader (tank mix)	0.24 L + 4 kg	metalaxyl-M and dimethomorph + mancozeb (tank mix)	Spring
13	SL567A + Invader (tank mix)	0.12 L + 2 kg	metalaxyl-M and dimethomorph + mancozeb (tank mix)	Winter and Spring
14	Signum	3 kg	boscalid + pyraclostrobin	Winter
15	Signum	3 kg	boscalid + pyraclostrobin	Spring
16	Signum	1.5 kg	boscalid + pyraclostrobin	Winter and Spring

Applications were performed on 06.12.11 and 28.03.12.

Table 3.2. Downy mildew severity in overwintered sage treated with pesticides

	Downy mildew severity (% leaf area affected)		
Treatment	**28.03.12**	**26.04.12**	**28.06.12**
Untreated	3.9	0.1	2.0
SL567A	3.7	0.1	2.3
Previcur	3.8	0.1	4.2
Invader	*2.3	0.1	3.5
SL567A+Invader	2.9	0.1	2.6
Signum	*1.5	0.1	2.4
p value	**0.001**	NS	NS
LSD	**1.074**	-	-

* Downy mildew severity significantly reduced compared to untreated; NS, not significant.

Objective 4: Efficacy of control measures against parsley and basil downy mildew.

The aim of this objective was to assess control measures against parsley and basil downy mildew in small pot trials, then extend promising treatments to larger scale trials. A programme of treatments was devised (see 2011 annual report). However, this objective could not be completed either due to a lack of inoculum, or, when inoculum was obtained, due to difficulties encountered in maintaining consistent infection on a sufficient scale. For example, a parsley field crop sown at STC in autumn 2013 became infected with downy mildew, but only a small number of scattered plants were affected and this was very late in the season. This prevented meaningful trials from being performed at this stage.

Project conclusions

- *Overall, despite the complexity of dealing with several herb and obligate pathogen species and fluctuations in environmental conditions, progress has been made to unravel the influential environmental factors that differentially affect various herb downy mildews.*
- *The project demonstrated the that fungicide applications have limited efficacy once infection is established.*
- *Work in this project suggests that lower temperatures may be as important as humidity/wetness for the development of parsley downy mildew symptoms, although, in reality, a combination of both parameters would be required for epidemic disease development. Controlled environment work indicated that infection occurred at 5–10°C and leaf wetness periods as short as 1 hour were sufficient to allow infection.*
- *Sage downy mildew was present at the Norfolk site at the majority of assessments. Correlations with environmental conditions were, however, unclear. Controlled environment work indicated that sage infection required leaf wetness periods longer than 12 hours and temperatures of 10–20°C. Fungicide work to explore pathogen persistence in/on crop debris demonstrated continued pathogen survival after treatment. Growers should be mindful that inoculum could simply arrive as airborne sporangia from other local or distant sources, i.e., other infected host crops/plants.*
- *No downy mildew was observed in the mint crops during the time course of the project and therefore no conclusions can be drawn regarding the environmental conditions conducive to infection in this crop.*

- *Unfortunately, during the majority of the time course of this project, basil downy mildew was under quarantine status in the UK. Whilst arrangements were in place to work on the pathogen at Fera, we were unable to source inoculum from commercial sites for a variety of reasons.*

  Further research priorities

- *This project and other studies have demonstrated the limited efficacy of fungicide applications once infection is established. For perennial crops it is therefore of paramount importance to determine whether reinfection in spring is due primarily to systemic or new infections. The use of molecular or immunological techniques to examine overwintering material for pathogen presence and the monitoring of airborne sporangia populations would go some way towards answering this question, and the information gleaned would assist grower decision-making.*
- *Monitoring of airborne sporangia populations, alongside environmental monitoring, would also be valuable in the context of predicting infection events. For example, parsley downy mildew emerged only in early autumn in the two monitored years in this study; however, it is not known whether this was due to a lack of inoculum earlier in the season or whether inoculum was present but environmental conditions were unsuitable for infection Work elsewhere in other crops (e.g., brassicas and cucumber) has clearly demonstrated the potential of using a serology-based spore-trap technique (Kennedy & Wakeham, University of Worcester) for early and routine pathogen detection and disease prediction.*
- *It is recommended that further work requiring artificial infection only be carried out if robust protocols are already established.*

Technology transfer

Presentation to the British Herb Trade Association on 13 March 2012 (A Huckle & K Wright).

Presentation to the British Herb Trade Association on 28 December 2012 (K Wright).

Presentation to the British Herb Trade Association on 26 February 2013 (K Wright).

Appendix 1: Incidence and severity assessment data for sage downy mildew (Norfolk 2012)

Assessment 1			
Date	Plot	incidence	%severity
02/04/2012	1	100	3
02/04/2012	2	100	4
02/04/2012	3	100	5
02/04/2012	4	100	4.5
02/04/2012	5	100	4
02/04/2012	6	100	2
02/04/2012	7	100	6
02/04/2012	8	100	2
02/04/2012	9	100	1.5
02/04/2012	10	100	10
02/04/2012	11	100	10
02/04/2012	12	100	6
02/04/2012	13	100	4
02/04/2012	14	100	3
02/04/2012	15	100	5
02/04/2012	16	100	8
02/04/2012	17	100	6
02/04/2012	18	100	8
02/04/2012	19	100	10
02/04/2012	20	100	10

Assessment 3			
18/05/2012	Plot	incidence	%severity
18/05/2012	1	1	0.1
18/05/2012	2	1	0.01
18/05/2012	3	1	0.05
18/05/2012	4	1	0.01
18/05/2012	5	1	0.05
18/05/2012	6	1	0.1
18/05/2012	7	1	0.01
18/05/2012	8	1	0.01
18/05/2012	9	1	0.05
18/05/2012	10	1	0.01
18/05/2012	11	0	0
18/05/2012	12	1	0.01
18/05/2012	13	1	0.01
18/05/2012	14	1	0.01
18/05/2012	15	1	0.01
18/05/2012	16	0	0
18/05/2012	17	0	0
18/05/2012	18	1	0.01
18/05/2012	19	1	0.01
18/05/2012	20	1	0.01

Assessment 2			
26/04/2012	Plot	incidence	%severity
26/04/2012	1	1	9.1
26/04/2012	2	1	0.5
26/04/2012	3	1	7
26/04/2012	4	1	5
26/04/2012	5	1	2
26/04/2012	6	1	1
26/04/2012	7	1	3
26/04/2012	8	1	5
26/04/2012	9	1	3
26/04/2012	10	1	4
26/04/2012	11	1	4
26/04/2012	12	1	7
26/04/2012	13	1	4
26/04/2012	14	1	1
26/04/2012	15	1	4
26/04/2012	16	1	2
26/04/2012	17	1	2
26/04/2012	18	1	3
26/04/2012	19	1	6
26/04/2012	20	1	5

Assessment 4			
07/06/2012	Plot	incidence	%severity
07/06/2012	1	1	0.5
07/06/2012	2	1	0.01
07/06/2012	3	1	0.02
07/06/2012	4	1	0.02
07/06/2012	5	1	0.05
07/06/2012	6	1	0.02
07/06/2012	7	1	0.01
07/06/2012	8	1	0.01
07/06/2012	9	1	0.01
07/06/2012	10	1	0.01
07/06/2012	11	0	0.01
07/06/2012	12	1	0.1
07/06/2012	13	1	0.1
07/06/2012	14	1	0.01
07/06/2012	15	1	0.01
07/06/2012	16	0	0
07/06/2012	17	0	0
07/06/2012	18	1	0.01
07/06/2012	19	1	0.01
07/06/2012	20	1	0.01

Assessment 5								
28/06/2012	Plot	incidence	%severity		upper	middle	lower	
28/06/2012	1	1	10			15	25	
28/06/2012	2	1	7			10	15	
28/06/2012	3	1	3			5	10	
28/06/2012	4	1	2			2	5	
28/06/2012	5	1	1			1	5	
28/06/2012	6	1	1			1	5	
28/06/2012	7	1	1			1	5	
28/06/2012	8	1	1			1	5	
28/06/2012	9	1	0.5			0.5	3	
28/06/2012	10	1	1			1	4	
28/06/2012	11	15	0.01		2	20	20	
28/06/2012	12	1	6		0.1	10	2	
28/06/2012	13	1	5			5	5	
28/06/2012	14	1	5		1	5	3	
28/06/2012	15	5	2		0.2	7	3	
28/06/2012	16	0	0		0.1	1	5	
28/06/2012	17	0	5		0.1	5	5	
28/06/2012	18	1	0.5			0.5	3	
28/06/2012	19	1	0.5			0.5	2	
28/06/2012	20	1	1			1	3	

Assessment 6			
09/08/2012	Plot	incidence	
09/08/2012	1	0	
09/08/2012	2	0	
09/08/2012	3	0	
09/08/2012	4	0	
09/08/2012	5	0	
09/08/2012	6	0	
09/08/2012	7	0	
09/08/2012	8	0	
09/08/2012	9	0	
09/08/2012	10	0	
09/08/2012	11	0	
09/08/2012	12	0	
09/08/2012	13	0	
09/08/2012	14	0	
09/08/2012	15	0	
09/08/2012	16	0	
09/08/2012	17	0	
09/08/2012	18	0	
09/08/2012	19	0	
09/08/2012	20	0	

Assessment 7			
14/09/2012	Plot	incidence	
14/09/2012	1	5	
14/09/2012	2	5	
14/09/2012	3	5	
14/09/2012	4	5	
14/09/2012	5	0 no plant	
14/09/2012	6	3	
14/09/2012	7	5	
14/09/2012	8	5	
14/09/2012	9	7	
14/09/2012	10	8	
14/09/2012	11	0 no plant	
14/09/2012	12	0 no plant	
14/09/2012	13	0 no plant	
14/09/2012	14	0 no plant	
14/09/2012	15	0 no plant	
14/09/2012	16	0 no plant	
14/09/2012	17	0 no plant	
14/09/2012	18	5	
14/09/2012	19	0 no plant	
14/09/2012	20	8	

Assessment 8							
26/09/2012	Plot	incidence	%severity		upper	middle	lower
26/09/2012	1	15				15	
26/09/2012	2	5			2	5	7
26/09/2012	3	5					5
26/09/2012	4	no plant					
26/09/2012	5	10			2	10	10
26/09/2012	6	5				5	7
26/09/2012	7	no plant					
26/09/2012	8	20				20	
26/09/2012	9	no plant					
26/09/2012	10	20				10	30
26/09/2012	11	5				5	10
26/09/2012	12	0 no plant					
26/09/2012	13	0 no plant					
26/09/2012	14	0 no plant					
26/09/2012	15	0 no plant					
26/09/2012	16	0 no plant					
26/09/2012	17	0 no plant					
26/09/2012	18	5				5	10
26/09/2012	19	2				5	
26/09/2012	20	5			10		

Assessment 9							
	Plot	incidence	%severity		upper	middle	lower
12/10/2012	1	1	5		0	8	
12/10/2012	2	1	10		5	15	10
12/10/2012	3	1	5		2	20	5
12/10/2012	4	0	no plant				
12/10/2012	5	1	5		1	10	5
12/10/2012	6	1	5		5	5	10
12/10/2012	7	0	no plant				
12/10/2012	8	0	no plant				
12/10/2012	9	0	no plant				
12/10/2012	10	1	10		1	20	2
12/10/2012	11	1	3		0	5	5
12/10/2012	12	0	no plant				
12/10/2012	13	0	no plant				
12/10/2012	14	0	no plant				
12/10/2012	15	0	no plant				
12/10/2012	16	0	no plant				
12/10/2012	17	1	5		2	10	5
12/10/2012	18	1	5		0	10	5
12/10/2012	19	1	5		0	10	5
12/10/2012	20	0	no plant				

Appendix 2: Sage disinfestation trial 2012: Statistical assessment

Table A2.1. Results of factorial analysis - spring assessment, 28 March, 2012

Downy mildew severity (% leaf area affected) Spring assessment (28 March)							
Treatment		**Timing**			**Timing x Treatment**		
		Autumn	Spring	Aut + Spr	Autumn	Spring	Aut + Spr
Untreated	3.87	2.65	3.50	2.40			
SL567A	3.71				3.87	4.12	3.12
Previcur	3.79				3.00	4.62	3.75
Invader	2.33				2.12	3.00	1.87
SL567A+Invader	2.92				2.87	4.00	1.87
Signum	1.52				1.40	1.75	1.40
p value	**0.001**		**0.047**			NS	
LSD	1.074		1.302			1.193	

Table A2.2. Results of factorial analysis - disease assessment, 26 April, 2012

Downy mildew severity (% leaf area affected) Disease assessment (26 April)							
Treatment		**Timing**			**Timing x Treatment**		
		Autumn	Spring	Aut + Spr	Autumn	Spring	Aut + Spr
Untreated	0.03	0.13	0.09	0.05			
SL567A	0.11				0.28	0.02	0.02
Previcur	0.04				0.02	0.07	0.04
Invader	0.07				0.03	0.03	0.14
SL567A+Invader	0.12				0.26	0.05	0.04
Signum	0.11				0.07	0.27	0.01
p value	NS		NS			NS	

Table A2.3. Results of factorial analysis - disease assessment, 28 June, 2012

Downy mildew severity (% leaf area affected) Disease assessment (28 June)							
Treatment		**Timing**			**Timing x Treatment**		
		Autumn	Spring	Aut + Spr	Autumn	Spring	Aut + Spr
Untreated	2.00	3.06	2.75	3.14			
SL567A	2.25				1.62	2.62	2.50
Previcur	4.22				5.28	3.75	3.62
Invader	3.50				3.50	2.75	4.25
SL567A+Invader	2.57				2.75	2.38	2.58
Signum	2.38				2.12	2.25	2.75
p value	NS		NS			NS	