Project title	Outdoor herbs: epidemiology and control of downy mildew in outdoor sage, parsley, mint and in basil under protection
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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

Headline

- Long periods of leaf wetness appear to be required for downy mildew infection; extreme temperatures may hamper development and sporulation of the disease even if leaf wetness period is ideal.

Background and expected deliverables

Downy mildew has become an increasing problem over the last 3-4 years on a range of outdoor grown herbs, but particularly on sage, mint and parsley. Downy mildew has also more recently become a problem on protected basil in the UK. 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.

The first record of *Peronospora lamii* (downy mildew) on sage and rosemary in the UK was made in May 2004. The same species of downy mildew also affects mint. Parsley crops are susceptible to a different host-specific downy mildew pathogen – *Plasmopara umbelliferarum* (syn. *P. petroselini*), whilst the latest research on basil downy mildew has identified the pathogen as *Peronospora belbahrii*.

The cool, wet conditions experienced in each season from 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 in soil and crop debris, and long-term systemic infections in herb crops grown perennially e.g. mint and sage.

Downy mildew can infect herbs in propagation, those grown under protection (tunnels) as well as field grown perennials or seed-raised crops, given conducive environmental conditions. Whilst it has not necessarily been demonstrated in the case of these specific pathogens, some downy mildews are seed-borne and a seed-borne route cannot be discounted.

Fungicide actives 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 been detected 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 biological 'biopesticide' products with activity against oomycetes. Fungicide

applications need to be targeted when environmental conditions are likely to lead to a high risk of disease development on unprotected crops. The use of fungicides, bio-control products and other possible novel control mechanisms will be investigated for the control of the relevant downy mildew species. The potential efficacy of UV lights, burning-off of crop debris and over-wintering fungicide applications to eradicate spores (sporangia and oospores) and latent mycelium of the pathogen will be explored as a possible disinfection methodology for soil and affected plant material.

Summary of the project and main conclusions

Downy mildew literature review

A review of the recent literature on downy mildew pathogens in general and those which infect herb crops in particular was carried out. Background information on the main genera of the downy mildews which cause economic losses to horticulture and agriculture were discussed. Details of the possible sources of inoculum, environmental conditions conducive for disease spread and development and also the range of existing and potential methodologies for disease control and eradication have also been gathered. The full review is available in the Science Section of this report.

Parsley, mint and sage downy mildew – Monitoring environmental conditions and disease development in field crops

Downy mildew and environmental conditions were monitored in field crops of parsley (*Petroselinum hortense*) and sage (*Salvia officinalis*) in Norfolk from 13 April to 28 October 2011 (ADAS) and in sage and mint (*Mentha* spp.) in North Yorkshire from 17 May to 7 November (STC) to gain information on how weather affects the occurrence of these diseases. The environmental parameters recorded at each site were temperature, rainfall, relative humidity and leaf wetness. Disease monitoring was carried out in crops managed under commercial conditions with standard products applied for pests, disease and weeds as necessary.

Parsley (Norfolk): Downy mildew (*Plasmopara umbelliferarum)* was first seen in the parsley crop in Norfolk on 30 September, but there was no evidence in the experimental plots until 13 October when 0.2% leaf area affected was observed in four plots. By the final assessment on the 20 October, the disease had increased to affect 0.5% of total leaf area in the majority of plots.

Criteria on how temperature and leaf wetness duration influence disease were used to examine whether they explained disease development in the parsley field crops in 2011. The

criteria used were taken from work elsewhere on basil downy mildew and from growth cabinet work in this project on parsley. Previous work by other groups has suggested that the period of leaf wetness required for infection is >12 hours, while the optimum temperature range for disease development, based on the growth cabinet work, was taken as 5-15°C. The onset of infection on parsley correlated with a period during September when there were frequently long periods (30-40hrs) of leaf wetness and the temperatures were cool (5-15°C). Subsequent spread occurred during the cooler average temperatures of October (7.7°C).

Sage (Norfolk): In the sage crop monitored in Norfolk, downy mildew (*Peronospora lamii)* was present in each plot from 13 April to 28 October except after harvest cuts when there were no leaves present for assessment. Downy mildew symptoms increased with time on new growth and after each cut but then declined towards the end of the season.

Sage and Mint (North Yorkshire): No downy mildew developed in either of the crops monitored in North Yorkshire during the 2011 season.

Effect of temperature, leaf wetness and relative humidity on incidence and severity of downy mildew on parsley and sage

The effects of environmental conditions on the incidence and severity of parsley and sage downy mildews was investigated in three experiments in controlled environments. Two experiments tested the effects of four temperatures and four leaf wetness durations on parsley and sage inoculated with *Plasmopara umbelliferarum* and *Peronospora lamii* respectively. A third experiment tested the effects of four temperatures and six combinations of leaf wetness duration and relative humidity on parsley seedlings inoculated with *Plasmopara umbelliferarum.*

Disease levels were low in all experiments. Downy mildew on parsley appeared most prevalent at 5-10°C, especially where there was 24hr leaf wetness followed by 24hr high humidity. Sage downy mildew occurred mainly at 15°C.

Effect of overwinter fungicide drenches to soil and crop debris on downy mildew in sage

The objectives of this work were (1) to test the effectiveness of fungicide drenches against sage downy mildew when applied as overwinter and early spring disinfestation treatments to a dormant crop and to debris between rows and (2) to establish that products are safe to use on sage crops when applied as drenches.

Five fungicides (SL567A, Previcur Energy, Invader, Signum and a tank mix of SL567A and Invader) were applied in winter and/or early spring. 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 covered, simulating a drench spray. The fungicides used are currently authorised for use on sage under the Extension of Authorisations for Minor Uses system (formerly SOLA).

A replicated plot experiment was established with the five fungicides each applied at three timings: winter only, spring only, and winter and spring. The trial will be assessed for disease and phytotoxicity during 2012 and results available at the end of the 2012 growing season.

Financial benefits

- Information regarding the epidemiology of the key downy mildew pathogens, along with investigation into their survival, dissemination and control will help herb growers to understand the pathogen and therefore be better equipped to manage outbreaks in crops in the future.
- Project results will contribute to better understanding and management of a new downy mildew disease on basil (*Peronospora belbahrii*), first reported during 2010.
- Additional knowledge regarding the potential use of biological control products, cultural control and risk-based pesticide strategies to disease control may help herb growers reduce the risk of detectable fungicide residues on cut herb crops.

Action points for growers

- There are indications that winter 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 irrigation should be completed early in the day, wherever possible, so that the crop dries and does not remain wet overnight. Data indicate that long periods of leaf wetness and cool temperatures favour parsley downy mildew infection and spread.
- Open planting and increased spacing within the crop aids air flow around plants and is likely to increase drying of the crop after surface wetness periods. This may help reduce infection periods.

SCIENCE SECTION

General introduction

Downy mildew has become an increasing problem over the last 2-3 years on a range of outdoor grown herbs, but especially on sage, mint and parsley and, in 2010, an outbreak of downy mildew on protected basil was reported at several sites in the UK. Some growers whose sage crops were severely affected by downy mildew in 2009 reported up to 80% of the crop being lost to infection. On basil the pathogen is still notifiable and of quarantine significance, although this status is currently under review by FERA.

The aim of the proposed work is to provide information on the epidemiology of the three fungal 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, the pathogen is an obligate fungus and as such cannot be cultured and maintained on artificial growth media in the laboratory. It only survives on living plant material or on crop debris and this can lead to situations where the work cannot be carried out until a natural infection occurs to provide inoculum. Secondly, different downy mildew species are very host-specific and pathogen inoculum for research trials has 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. 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 the cool, moist conditions typically experienced in spring and autumn in the UK, although recent growing seasons have been anything but typical. However, more specifically, plants need to undergo an extended period of leaf-wetness to ensure infection occurs, and temperatures usually <20°C to allow the production of spore bearing structures for disease spread.

Literature review

General downy mildew information

Downy mildew (DM) is the common name for a fungus that belongs to the taxonomic kingdom Chromalveolata (sub-group of Chromista); they are oomycetes or water moulds, closely related to *Phytophthora* and *Pythium* spp. There are 17 genera within the group e.g.

Peronospora, Pseudoperonospora, Plasmopara, Bremia and *Sclerospora* each with a restricted host-range. DMs are obligate biotrophic plant pathogens, that is, they only infect and survive on/in living host material and are not saprophytic, invading dead or decaying hosts. The fungus reproduces principally in an asexual way, producing sporangia on the outside of the infected leaf. Spores are dispersed by the wind and water splash and can re-infect new material via fungal penetration of the leaf surface and via natural leaf openings e.g. stomata under suitable environmental conditions. When leaf surface moisture is high, swimming zoospores can be produced as infectious agents. DMs can also reproduce sexually via the production of oospores in plant tissues and this method of reproduction provides the fungus with the ability to alter its genetic material and this can make the new fungus more difficult to control, particularly if fungicide resistance traits are passed on. Oospores can also provide a method of longer-term survival of the fungus due to the thicker cell wall which allows this type of spore to survive in plant debris in the soil, providing a potential source of inoculum for subsequent host crops. Some downy mildew species also infect plants systemically within perennial crops (e.g. mint) – this can provide an additional method of pathogen survival.

A schematic diagram showing the different phases of one member of the pathogen's life-cycle can be seen overleaf (Figure 1).

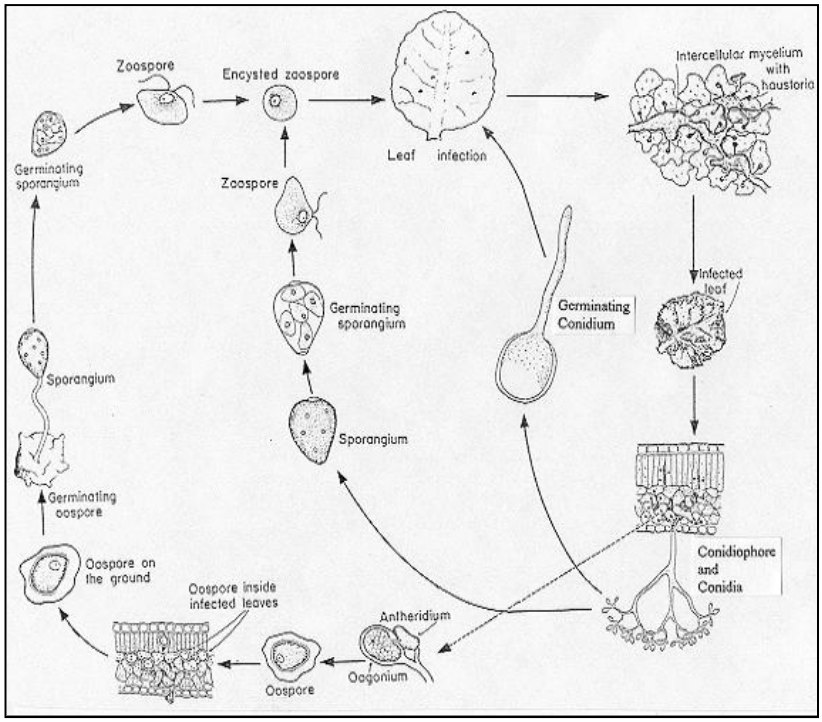

Figure 1. The lifecycle of the lettuce downy mildew fungus Bremia lactucae.

Modified from Agrios 1988

Figure 2. Downy mildew (Peronospora farinosa *f.sp. betae) on red beet*

Figure 3. Downy mildew (Peronospora violae) on pansy

Figure.4. Downy mildew (Peronospora antirrhini) on antirrhinum

Figure 5 Downy mildew (Plasmopara obducens) on impatiens

Several genera of DM infect a number of crops of global importance causing severe economic losses. Host specific downy mildew genera infect members of the *Asteraceae, Brassicae, Poaceae* and *Ranunculaceae* plant families. The DM genera *Peronospora* and *Plasmopora* have the largest host range infecting a number of globally important crops e.g. *Plasmopara viticola* on grapevines, *Peronospora tabacina* on tobacco, *Bremia lactucae* on lettuce, *Plasmopara halstedii* on sunflower*, Scleropora graminicola* on pearl millet and *Pseudosclerospora sorghi* on sorghum.

DMs generally require cool, moist conditions for survival and dispersal. It is widely accepted that temperatures in the 10-17°C range with high relative humidity are most suitable for growth of the fungus within the host and to allow the production of sporangia for reproduction, although some species will tolerate greater extremes of temperature. 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 more conducive. High humidity also plays an important part in the infection and survival processes.

The early stages of plant infection may be recognisable by only a slight lack of gloss or vigour in the affected leaves. This may be followed by the development of angular chlorotic lesions on leaves, bounded by the leaf veins. The angular leaf chlorosis is usually accompanied by the production of a felty fungal growth on the underside of the leaf. Depending on the host and DM genera this may be white, grey, brown or purplish in colour (see Figures 2-8).

Herb downy mildews

There are several species of the *Peronosporales* which affect herbs and these are shown in Table 1. Those relevant to this study are shown in bold.

Table 1. Details of hosts and relevant downy mildew pathogens

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***
	Rosmarinus officinalis	Rosemary	*Peronospora lamii*
Apiaceae	***Petroselinum crispum***	**Parsley**	***Plasmopara umbelliferarum***
Amaryllidaceae	*Allium schoenoprasum*	Chives	*Peronospora destructor*

Holcomb reported the first finding of DM caused by *P. lamii* on *Salvia splendens* and *S. coccinea* in 2000 in the USA. He described the development of angular chlorotic spots which could become necrotic and cause leaf-drop. Scattered patches of white mycelia with brown spores were observed on the underside of the leaves. He identified the fungus as *P. lamii* based on the morphological characteristics of the fungus. In pathogenicity tests he

showed that spores from the fungus infected the above named sage species following incubation in a dew chamber at 20°C for 3 days, followed by further incubation in a glasshouse with temperatures from 18-32°C. Typical lesions developed within 6-8 days. No sporulation was observed at these temperatures; however when leaves were removed and placed in a moist chamber at 25°C sporulation was induced in 2-3 days.

The first report of DM on sage in New Zealand, in 2003, was attributed to *P. lamii* by Hill, Pearson & Gill (2004). The pathogen was considered to be established in NZ. The same fungus was reported for the first time on samples of *Salvia officinalis* in the UK in 2004 by Humphreys-Jones, Barnes & Lane (2008). They describe the early lesions as pale green patches on the upper surfaces of the leaves, that later became yellowish then brown. Dense purple-coloured sporangiophores were observed on the lower leaf surface, in association with the yellow/brown leasions. They also observed the development of thick-walled golden yellow oospores between veins on some dead leaves. They report a later finding in 2005 of the same fungus affecting *Salvia* sp. and *Rosmarinus officinalis* (rosemary) grown under protection.

Liberato, Forsberg, Grice and Shivas report the first occurrence of *P. lamii* on sage in Queensland, Australia in June 2005, although they do report previous occurrences of the fungus on *Salvia, Lamium* and *Mentha*.

Figure 6. Sage infected with Peronospora lamii (courtesy of ADAS)

Previous morphological studies reported the causal agent of sage DM as *P. swinglei* (Kochman & Majewski 1970; Ul'yanishchev *et al* 1985; Vanev *et al* 1993; Stanevicienė 1995; Műller 1999; Shin & Choi 2006), whilst other studies identified it as *P. lamii* (McMillan & Graves 1994; Gamliel & Yarden 1998; Minuto *et al* 1999; Holcomb 2000; Byrne 2001; Plenk 2002; Hill *et al* 2004). More recent work by Belbahri *et al* (2005) and Thines *et al* (2009)

demonstrated that a newly occurring DM on *Salvia officinalis* was distinct from *P. swinglei* and from *P. lamii*. Choi *et al* (2009) carried out further work to resolve the closely related species of *Peronospora* which were parasitic to *Lamiaceae*. They found three groups of morphologically and molecularly differing *Peronospora* spp. on the sage samples examined, two of them differed from both *P. swinglei* and *P. lamii* and they introduced the names *P. salvia-officinalis* and *P. salvia-plebeiae*, based on the host sage species on which they were detected. They hypothesized that the diversity of sage downy mildews reflects host ranges and also geographical origins. Choi *et al* suggest that due to global seed trading the downy mildew of *Salvia officinalis* (common sage) is recorded worldwide, but that previously most findings have been attributed to *P. lamii, P. swinglei* or simply *Perenospora* spp. based on the host range of the specimens. Their findings indicate that at least some of these may well have been other, morphologically and molecularly different species of *Peronospora* and that this is likely to be linked to the broad polyphyletic genus of Salvia species.

Parsley (*Petroselinum crispum*) is a member of the Apiaceae (or Umbelliferae) family of plants. Both flat leaf and curly cultivars can be infected by the DM fungus *Plasmopara umbelliferarum* (previously *P. petroselini*). The disease had been observed in an experimental field crop in Sweden in September 2004 and was confirmed as *P. petroselini* at that time. Local commercial growers reported having observed symptoms consistent with DM every year since 2001 (Amein *et al*. 2006). Soylu *et al* (2009) reported an outbreak on parsley in Turkey where infection levels in crops were between 40 and 100%. Early symptoms of faint leaf chlorosis with off-white mycelium developing on corresponding areas on the under surface of the leaves were reported. As the disease progressed the mycelium turned dark grey and leaves and stalks became shrivelled and eventually died. Similar reports of the same pathogen/host combination were reported in Belgium (Crepel & Inghelbrecht 2003) and the Czech Republic (Muller & Kokes 2008). No formal report of this pathogen in the UK has been found; however, symptoms consistent with DM have been reported by many commercial herb growers and confirmed by diagnostic plant clinics, so there is little doubt that the pathogen is established in the UK.

Figure 7. Parsley infected with P. umbelliferarum

UK herb growers first noted the presence of a new fungal disease on sweet basil growing under protection in July 2009. The fungus was confirmed as downy mildew and diagnosed presumptively as *Peronospora belbahrii* by a number of diagnostic plant clinics.

Figure 8. Peronospora belbahrii sporulating on the underside of a basil leaf

The disease was first reported in Uganda, Africa in 1933 (Hansford). There appear to have been no findings/reports of the pathogen/host combination for almost the next 70 years. The fungus was reported in Switzerland in 2001 (Heller & Baroffio 2003) and then thought to have spread to many other European countries including Italy in 2003 (Garibaldi *et al* 2004),

Northern Iran in 2006 (Khateri *et al* 2007), Argentina in February 2008 (Ronco *et al* 2008), Cameroon in March 2007 (Voglmayr and Piatek 2008), and Cuba in February 2009 (Martínez-de la Part *et al* 2009). The majority of these reports record the presence of DM as either *Peronospora* sp. or *P. lamii* based principally on the morphology of the fungus. The fungus is also pathogenic on coleus (*Solenostemon* spp.) and Agastache (Henricot *et al* 2009). Several researchers noted a significant molecular difference to *P. lamii*. Work carried out by Belbahrii *et al* (2005) and Thines *et al* (2009) proved conclusively that the DM infecting basil and coleus was morphologically and molecularly different to *P. lamii* and proposed the new species name of *Peronospora belbahrii* for the DM infecting basil, but felt that the DM on coleus may show further differences and additional research was required before confirming the taxonomy of coleus DM.

Each of the herb downy mildews under investigation in this study now seem to be established in UK commercial crops. Reports of outbreaks indicate that both protected and outdoor crops can be at risk and that infection is highly dependent on environmental conditions. Cool, wet or damp weather is highly conducive to spread of DM infections as they provide the correct environmental conditions for infection and sporulation to occur on plants. The spores produced on the underside of affected leaves are dispersed by air currents, water splash and by plant-to-plant contact; and will infect fresh material via direct penetration of the leaf epidermis or via natural leaf openings such as stomata with ease providing there is an adequate period of leaf wetness. Once infected, plants can show symptoms between 6 and 15 days later, depending on temperatures, and sporulation can occur immediately under the correct conditions.

It is possible that the pathogens have spread over large geographical areas via the sale of infected seed and through commercial trade in plants and foliar material across global markets. Although not yet proven it is likely that one of the main methods of global spread of some DM pathogens may well be via infected seed, as this is known to occur in some situations. Therefore ensuring the use of non-infected seed is of great importance in controlling disease in susceptible crops. Crop epidemics can start from a single infected seed locus and spread through crops once sporulation occurs. Growers should also consider using cultivars which show tolerance to the infection, or at least are less susceptible. Studies have been carried out in the US by Weynandt *et al* (2010) to investigate the relative susceptibility of 30 basil cultivars to *P. belbahrii*. Where applicable, protectant fungicides should be applied to crops and the use of fungicide treated seed considered. Previous fungicide studies (PC 230, HDC) carried out on the Impatiens DM pathogen *Plasmopara obducens* showed the importance of applying fungicides in a protectant capacity, as once established in the crop it can prove virtually impossible to

eradicate the fungus. Where possible environmental conditions should be manipulated to limit the likelihood and spread of infection e.g. reduce humidity by more open planting or spacing within the crop, minimising periods of leaf wetness e.g. by using trickle or drip irrigation in glasshouses and by carrying out overhead irrigation at times of the day which will result in minimal leaf wetness time.

The development and use of a robust forecasting system which can advise growers about high risk periods for DM development within crops can be a useful tool to help growers take precautionary measures e.g. fungicide application or increased environmental controls. The majority of forecasting systems are based on weather monitoring e.g. rainfall and temperature. The system developed by Hyre (1959) is an example of this and similar forecasting systems are used for blight control in potatoes. Santamaria *et al* (2006) at the University of Delaware investigated the role of leaf wetness and sporangial release in the development of DM (*Phytophthora phaseoli)* in lima beans. They found that a minimum of 4 hours of leaf wetness was required to establish infection and sporulation. Spore trapping over 3 seasons indicated that sporangial release was mostly occurring between 11am and 3pm daily. Therefore a combination of spore release and leaf wetness during the afternoon represented a very high risk of infection.

Work carried out for this study provides further details of the environmental conditions required by two of the downy mildew species responsible for infection of herbs (section on effect of temperature, leaf wetness & relative humidity on incidence and severity).

Downy mildew of lettuce (*Bremia lactucae*) (one of the most studied downy mildews over several decades) is controlled principally in crops by the development of genetic resistance in new cultivars and by the application of protectant fungicides. Wu *et al* (2000) carried out a range of experiments to identify the factors affecting the survival of *Bremia* sporangia after deposition on lettuce leaves. They exposed sporangia to a number of different controlled factors including temperature, leaf wetness, RH and light exposure. They found that *B. lactucae* sporangia on leaves can survive for fairly long periods at more or less adverse temperatures and RH levels, although survival time was longer at cooler temperatures. RH differences were not important and this was deemed to be due to the fact that leaf transpiration was likely to ensure almost complete moisture saturation around sporangia deposited on the leaf surface, and air effects were inconsequential. The studies also showed that spore viability decreased rapidly when exposed to solar radiation and in particular to the shorter wavelengths of the spectrum e.g. UVB or UVC.

Hovius *et al* (2007) carried out studies to evaluate weather-based spray programmes for the control of downy mildew in lettuce in Canada. They developed a forecasting system based

on lesion development following a recorded sporulation-infection period (SIP) with leaf wetness between 3.00 AM and 10.00 AM and temperature between 5 and 20°C. They applied the protectant fungicide mancozeb before lesions were expected, based on 110 accumulated degree-days following a SIP and also post-latent-period sprays (metalaxyl-M and mancozeb) applied after forecasted lesion development based on 135 accumulated degree-days and compared these with weekly applications of mancozeb and metalaxyl-M at a total of 9 sites. They did not observe significant reductions in the level of disease observed, nor in overall yield, although they did find that the forecasted sprays reduced the amount of fungicides being applied to the crops by between 16-60%, and this had no measurable detrimental effect on the crop in terms of increased disease. They concluded that the timing of spore release was crucial when it coincided with leaf wetness periods.

The potential for inducing a systemic acquired resistance (SAR) in sweet basil to *Peronospora belbahrii* was investigated by Mersha and Zhang (2011) in the USA. They stated that basil downy mildew has now been found in 20 US states. Current management strategies, including the use of fungicides, are inadequate to control the pathogen. They investigated the effect of five SAR inducers; Actigard® (ASM), 3-amino-butanoic acid (BABA), isonicotinic acid (INA), salicylic acid (SA) and sodium salicylate (NaSA). Treatments were applied as foliar sprays pre, post and pre + post-inoculation. They found that ASM significantly reduced infection compared to the untreated control, particularly when applied 3 days after inoculation (93% reduction). BABA applied pre + post inoculation reduced infection by 91%. The other products gave somewhat variable results. Such products are not currently approved for use in the UK.

Reuveni & Raviv (1997) discuss the potential merits of reducing downy mildew in greenhouse grown cucumbers using spectrally filtered polythene covers with and without blue pigments. They found that the addition of the blue pigment to films significantly inhibited colonisation and sporangial production of *Pseudoperonospora cubensis*. However, the reduction in light did affect yields and they therefore suggest that further work and development of better claddings may be required before this potential control mechanism could be beneficial in glasshouse raised crops.

Suthaparan & Torre (2010) carried out similar work using LED light treatments on rose powdery mildew (*Podosphaera pannosa*). They used a number of different light arrays and exposure times, but found that a brief exposure (1hr) to red light during the dark period (night) could be as effective as continuous illumination with red light in suppressing PM in greenhouse rose plants.

The literature reviewed indicates that although little scientific work has been carried out on the epidemiology and control of the herb downy mildews relevant to this study, there is a wealth of information on studies of other DM genera which is likely to be highly relevant and useful. The reported work on the development of forecasting systems, the use of spectral filters and LED light regimes, SAR inducers and possible UV effects are all of great interest and relevance for herbs, particularly due to the short harvest intervals in place for some fungicides and the grower's need to reduce and minimise the applications of pesticides on these crops.

Parsley, sage and mint downy mildew – Monitoring environmental conditions and disease development in an outdoor crops

Introduction

The aim of this initial work was to monitor field sites for disease development while simultaneously monitoring environmental conditions to help determine if weather conditions can be more accurately linked to disease occurrence. This knowledge would allow growers to better identify 'high disease risk' events and help them make decisions regarding instigating appropriate control strategies accordingly. In this study "Infection criteria" is used to refer to conditions permitting infection, disease development and sporulation.

Materials and methods

Site and crop details

The experiment was carried out at two commercial sites, one in Norfolk (monitored by ADAS) and the other in North Yorkshire (monitored by STC).

Norfolk: A parsley crop at a farm with a previous history of downy mildew was monitored; although the actual field was new to parsley cropping. A nearby crop of sage was also monitored. The crops were managed as commercial crops; one fungicide (Fubol Gold) was applied to the sage on 1 June 2011. A crop diary is given in Appendix 1.

Loggers (Delta T logger and Spectrum logger) were set up within a farm drilled crop of parsley and sage in April 2011, and arranged so that they did not interfere with commercial farm operations. The leaf wetness sensors for both loggers were positioned at a similar level to the crop canopy.

The Delta-T logger was set up to record air and soil temperature, relative humidity, leaf wetness and rainfall every hour for the duration of the cropping season for parsley (May to October) (Figure 9). A Spectrum temperature and leaf wetness sensor was also set up in

the crop of sage for the duration of the cropping season (May to October). Any extreme weather conditions were recorded throughout the trial.

Figure 9. Parsley experiment environmental trial set-up showing Delta T logger in the foreground – Norfolk, 2011.

Figure 10. Environmental monitoring equipment in sage trial in North Yorkshire

North Yorkshire: A sage crop, growing in a mypex covered bed (Figure 10) was monitored, and also a mint crop in a nearby field. The crops were managed as commercial crops and received the following fungicide treatments during the 2011 growing season:

Sage: Signum (x 2), Previcur Energy, Fubol Gold and Amistar

Mint: Previcur Energy

Delta T loggers were used to monitor rainfall, leaf wetness and air temperature, whilst Hoboware loggers recorded soil temperature and relative humidity.

Disease assessments

Natural infection and disease development was monitored at intervals during the season at both sites and in all crops. Assessments were made at 20 points using 0.5 x 0.5m quadrats marked by ringot pegs in both the sage and parsley crops in Norfolk and by detailed assessment of 20 marked plants/crop in North Yorkshire. These plots were sprayed as per commercial practice and harvested by the grower as normal.

In Norfolk the incidence and severity of downy mildew and any other foliar diseases within the 20 quadrats was recorded for each crop. Eleven disease assessments for sage and parsley were made on 11 and 26 May; 15 June; 1 and 21 July; 18 August; 1, 15 and 30 September; 13 and 20 October. The sage crop had an additional assessment on 28 October; the parsley had already been harvested.

The Yorkshire crops were visited and assessed on 17 and 26 May, 14 June, 7 July, 9 and 19 August, and 7 November. Unfortunately, during this time some mint monitoring data was lost due to technical problems with the loggers. Both crops were cut back or harvested on one occasion during the monitoring period.

At each visit, crop growth stage, crop vigour, % leaf area affected by downy mildew, distribution through crop and incidence and severity of other pests and diseases was recorded. The initial points where disease was found were marked with a ringot peg in order to map spread of infection.

Results

Environment monitoring

Records from the in-field Delta T logger at the Norfolk site are presented in Table 2.. Rainfall measured at Boxworth, Cambs and interpolated for the site using Irriguide are included for comparison with records from the rain gauge set up on the trial site. It is possible that the rain gauge was faulty as actual rainfall recorded at the site was much lower than at Boxworth

or as determined by Irriguide. However, another explanation for these low totals at the trial site is that there is a tendency for rain to be localised during the summer months. Table 3 gives an indication of how many days the leaves would have remained wet and hence have favoured infection, based on actual records and Irriguide. Irriguide interpolates weather data for a defined site and altitude using a least squares distance model and real data from five surrounding Met Office weather stations (Bailey & Spackman, 1996).

The weather over the course of the trial was compared with an average of weather data from Boxworth for the past 30 years. April, which was warmer than normal, was followed by June, July and August that were cooler than average (maximum temperatures 4°C cooler on average). September minimum temperatures were warmer than normal while October minimum and maximum temperatures were higher by 1 to 2.5°C. April and May were very dry with few days of rain. June, July and August had average rainfall and while September was drier than normal, there were more days when rain occurred than in mid-summer. October was a lot drier than had been recorded in previous seasons.

Table 2. Weather records from the field monitoring site, Norfolk - 2011

Month	Average max air temp (°C)	Average min air temp (°C)	Average soil temp (°C)	Average relative humidity (%)	Sum of rain in rain gauge (mm)	Boxworth rain (mm)	Rain levels from Irriguide (mm)
April	18.8	6.4	14.5	71.9	1.8	1.6	3.7
May	18.1	7.2	15.3	69.3	6.8	21.0	15.1
June	19.7	10.1	16.9	78.9	12.8	49.4	60.0
July	20.2	10.6	17.0	81.2	9.6	46.8	56.3
August	20.8	10.8	16.7	82.2	9.0	47.0	47.2
September	20.4	9.9	15.2	83.6	7.4	26.8	20.6
October	17.2	7.0	12.5	84.2	5.2	14.6	29.1

Table 3. Number of days with rain at field monitoring site, Norfolk - 2011

Month	Number of days with rain detected in raingauge	Number of Irriguide rain days
April	2.0	2.0
May	4.0	14.0
June	10.0	17.0
July	6.0	17.0
August	7.0	26.0
September	7.0	21.0
October	4.0	16.0

Table 4. Weather records from the field monitoring site, North Yorkshire - 2011

Month	Average max air temp (°C)	Average min air temp (°C)	Average soil temp (°C)	Average relative humidity (%)	Sum of rain in rain gauge (mm)
May	23.4	6.5	13.9	72.7	-
June	30.6	8.3	17.2	74.1	75.4
July	24.4	10.5	17.4	73.7	61.0
August	26.3	10.0	16.9	82.8	99.8
September	21.9	9.0	14.7	84.8	20.2*
October	16.1	6.3	11.9	92.7	-

* only recorded until 19.9.11

Table 5. Number of days with rain at field monitoring site, North Yorkshire - 2011

Month	Number of days with rain detected in raingauge	Number of Irriguide rain days
June	18	18
July	14	13
August	20	22
September	8*	13**

* only recorded until 19.9.11

**Irriguide data up to and including 19.9.11

A comparison of the weather data for the two sites suggest that minimum air temperatures were similar at the 2 sites, perhaps dropping lower at the Yorkshire site into the autumn months (Table 4). Maximum air temperatures for each month appear higher at the northern site, although we suspect this may be an inaccuracy in the data due to the absence of a Stephenson screen over the temperature sensor at the northern site. This was rectified for the 2012 monitoring season. Soil temperatures and relative humidity levels were similar at both sites. The most notable difference between the sites was in the amount of rainfall recorded. This was on average, approximately 60% higher in Yorkshire than in Norfolk (Table 5).

Disease occurrence and development – parsley (Norfolk)

Downy mildew symptoms in parsley were first observed, by the growers' agronomist, in a crop near to the experimental plots, on 30 September (Fig 9). The disease was not present in the experimental plots at this time, but was observed at a later date by ADAS scientific staff on 13 October. There were four parsley harvest periods: 1 July, 18 August, 15 September and 20 October.

There are various optimal environmental conditions that are specific to different life cycle stages (infection, growth and sporulation) of the pathogen on its specific host. As there is no published information on the infection criteria for parsley and sage, for the monitoring trial a period of leaf wetness greater than 12 hours (Garibaldi *et al*., 2007), and a temperature range of 5-15°C (based on results from the growth cabinet experiment on parsley) have been used as the presumed optimal conditions for infection and symptom development. A period of leaf wetness greater than 12 hrs is based on findings from Garibaldi *et al*, (2007) regarding the effect of leaf wetness duration on infection of downy mildew of basil; this study concluded that the pathogen required a period of leaf wetness of at least 6-12 hours immediately after inoculation and that infection was particularly severe after 12 hours wetness duration. The temperature range was based on the controlled environment experiment that concluded lower temperatures in particular are conducive to disease infection; development and sporulation.

Chart 1 shows the incidences when leaves were wet for various lengths of time when the temperature was in the range 5-15°C, with respect to the harvest dates. The start of this downy mildew epidemic in the experimental plots appears to correlate with a period of extended leaf wetness and a mild temperature. Chart 2.1 shows that after the third harvest, there was a relatively long period, from 15 – 30th September, during which there were many days that incurred 30-40 hrs leaf wetness and a temperature within the 5-15°C range, thereby fulfilling the assumed infection criteria of this study. The parsley plants at this time

may also have been naturally more vulnerable to infection as these conditions occurred soon after harvest, so the plants would have been young and therefore the leaves may have been more susceptible.

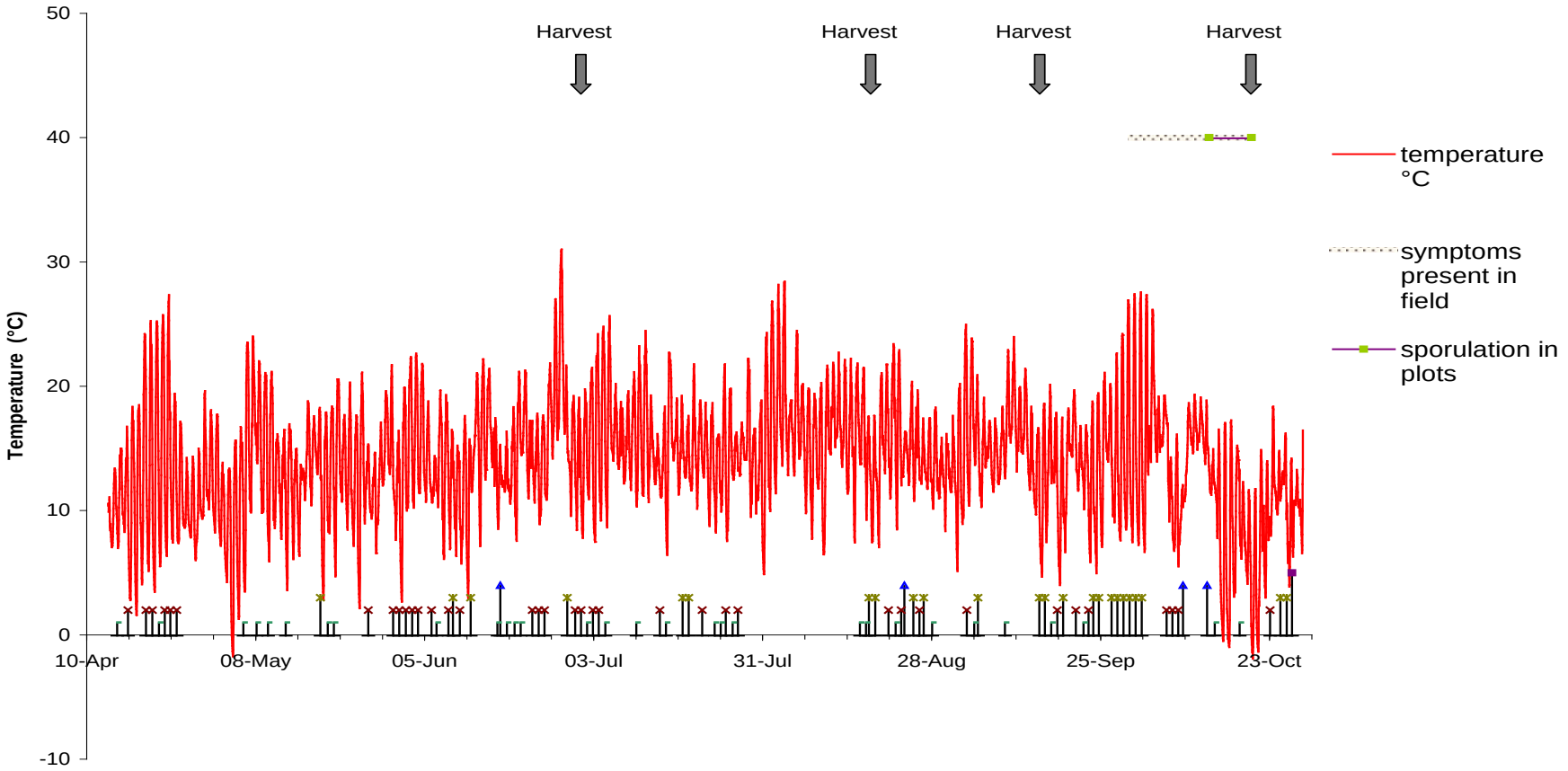

Chart 1. Summary of environmental monitoring in parsley crop showing potential infection conditions and sporulation based on infection criteria with reference to four harvest dates – 2011; (leaf wetness conditions are shown on the x axis as: - < 20h; x – 20-30h; ✳ - 30-40h; △ - 40-50h; ■ - > 50h.

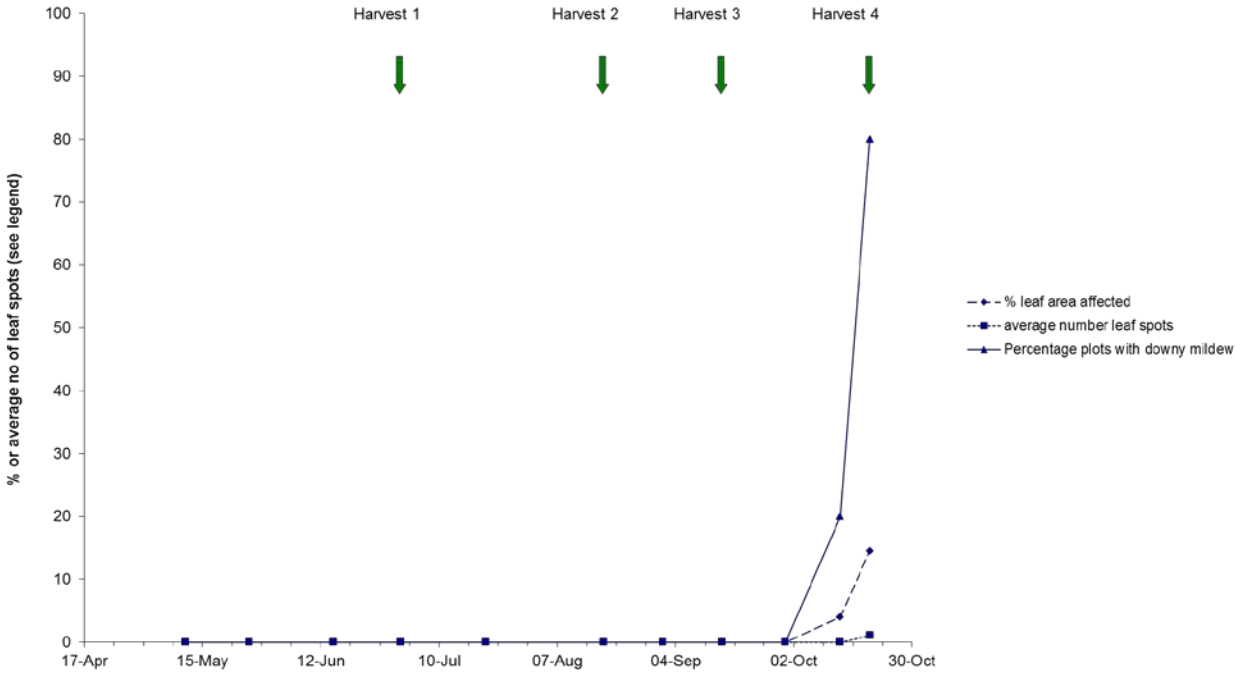

Chart 2. Progress of infection of downy mildew in parsley with reference to four harvests - 2011.

Visits to the site were approximately fortnightly so it was difficult to tell exactly when the infection started. Although downy mildew was present in the field on 30 September there was no evidence in the monitored plots until 13 October. The infection spread rapidly until 80% of the plots had downy mildew by 20 October (Chart 2). It appeared that, on having infected the parsley, the downy mildew spread rapidly in the cooler weather experienced in October. Table 6 shows that from 13 – 20 October the average temperature was 7.7°C, much lower than for the main period of cropping.

Table 6. Average temperatures after four harvests of parsley at field monitoring site, Norfolk - 2011

Period	Start	End	Average temp °C
Harvest 1-2	01-Jul	18-Aug	15.6
Harvest 2-3	19-Aug	15-Sep	15.1
Harvest 3-4	16-Sep	20-Oct	13.3
Infection	13-Oct	20-Oct	7.7

Disease occurrence and development – sage (Norfolk)

Downy mildew was found in every plot at every assessment visit except when there were no leaves present (due to recent harvesting). It is a possibility that the pathogen was systemic in the woody parts of the sage plants (these remain after harvest).

During the monitoring period the trial plots were harvested differently. A partial cut on plots 1 to 6, 13 and 14 was performed before 11 May (visit 1); plots 7-10, 17 and 18 were partially cut before 26 May (visit 2) and all of the crop was cut just before 1 July (visit 4). Some leaves were present after the partial cuts but only the woody stems were present on 1 July. Disease severity of different stages during the season is shown in Chart 3. The disease symptoms (% leaf area affected) increase with time after the cuts (just before assessments 1, 2 and 4). After visit 7 the percentage of leaf with symptoms declines.

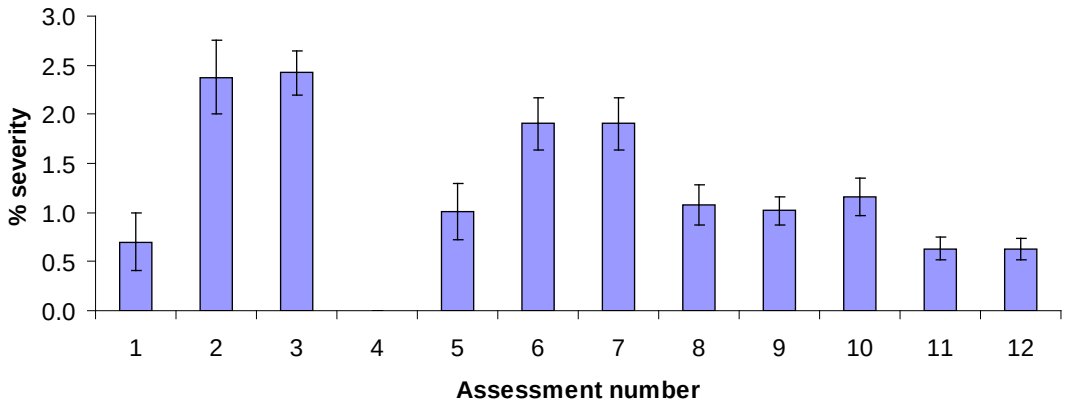

Chart 3. % leaf area affected (shown as % severity) of downy mildew on sage on a particular visit date in 2011. See Appendix 1 for dates of visits 1 to 12 (between 13 April and 28 October). The whisker bars show standard errors.

The plots were compared by cutting groups (Group 1 was cut before 1st visit, Group 2 was cut before 2nd visit, Group 3 was not cut until 1 July at visit 4). At visit 2 the most recently harvested group of plots had the greatest proportion by leaf area of downy mildew. After the final harvest cut on 1 July, a difference was again seen between the cutting groups with group 3 showing the greatest disease severity right up until the end of the trial. This suggests that development of downy mildew is more likely on younger, softer leaves and this may be linked to the development of waxy cuticle and strengthening tissues in older leaves. Group 3 had significantly higher severity than groups 1 and 2 at visits 6, 7, 8 and 9. By visit 10, group 2 was significantly more affected than group 3, and the differences began to disappear after that. The effect of cutting possibly masks any weather related trends, although there would appear to be a clear dip in the levels of infection in each crop group following a distinct rise in temperatures towards the end of June.

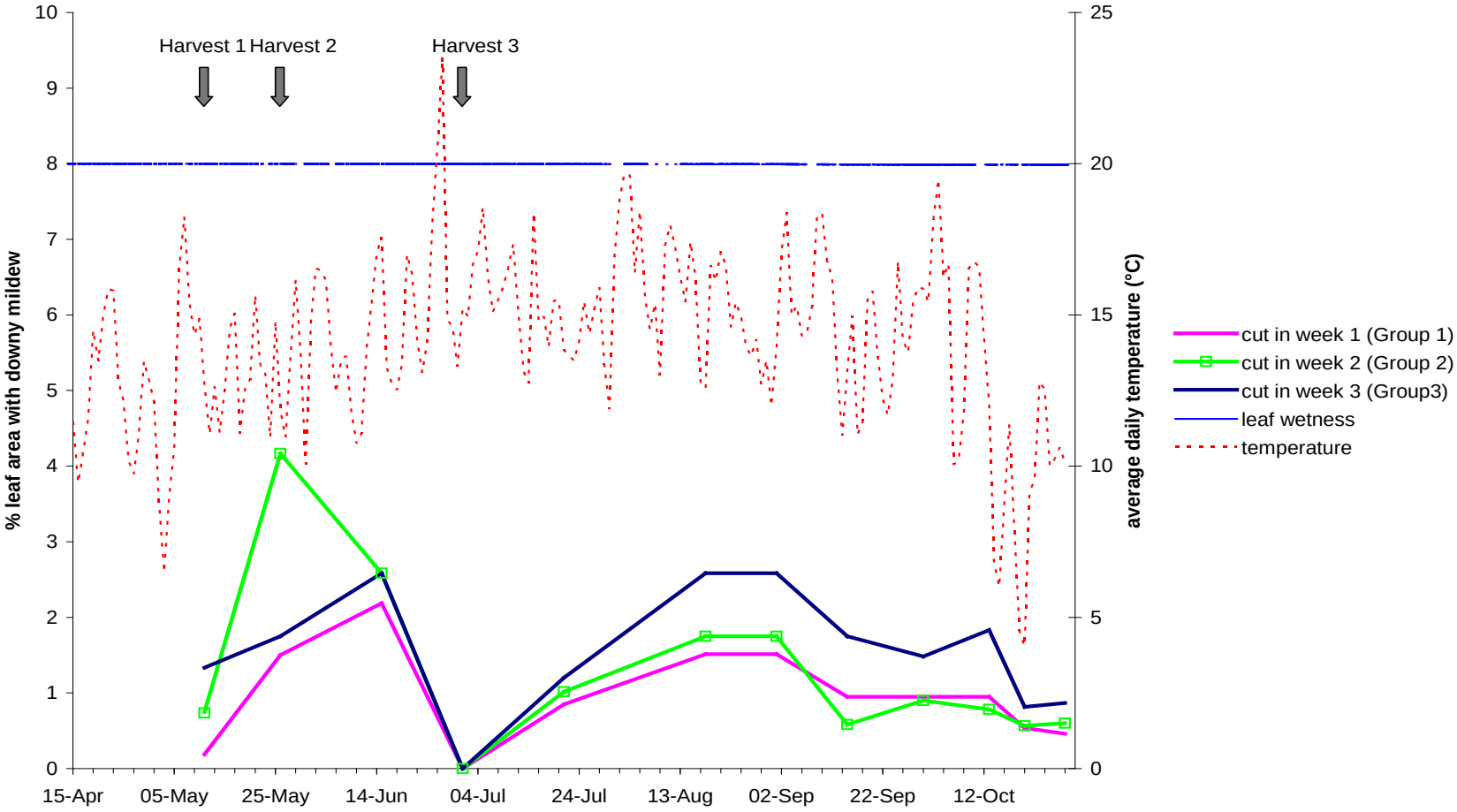

Chart 4. Effect of weather variables and cutting on % leaf area coverage of downy mildew on sage

DM monitoring in mint and sage in North Yorkshire

The crops were monitored on a regular basis between the early summer and late autumn period. No symptoms consistent with downy mildew developed on either crop during this period.

Other temperature effects

On parsley it was shown that severity of the downy mildew increased at low temperatures. This model does not seem to fit sage; as although there is a slight upward trend in all cutting groups after 30 September (Chart 4) the temperature decrease was recorded on 11 October. In controlled environment (CE) cabinet work there was a trend for sage downy mildew to be encouraged by milder temperatures, of approximately 15°C, than parsley, which in our tests appeared to be able to infect and produce spores at much lower temperatures – even as low as 5°C. Mild temperatures occurred in the field through late spring, summer and early September. Coupled with leaf wetness in the crop this would have encouraged the disease to keep progressing at low levels. As the downy mildew was already present in the crop before monitoring commenced it is difficult to pinpoint conditions that led to the first infection.

Discussion

As stated in the introduction, to analyse conditions conducive to infection, development and sporulation of parsley downy mildew (*Plasmopara umbelliferarum*), the infection criteria were assumed to be >12 hours leaf-wetness and a temperature range of 5°C-15°C. From the environmental monitoring of parsley, the start of the disease epidemic around 30 September appears to have correlated with a period in September when leaf wetness periods frequently extended to 30-40hrs and during which the temperature was mild. Having infected the parsley in the experimental plots by 13 October, the pathogen rapidly spread on these plants until approximately 80% of the plots were infected by the 20 October. It was also concluded from controlled environment work (next section) that % incidence and severity of parsley downy mildew was greatest at a low temperature of 5°C and there was a trend for disease reduction as the temperature increased. This may explain the rapid spread of the pathogen in the field of parsley from 13 to 20 October when the temperature averaged 7.7°C (Table 6) which may have promoted further disease development and sporulation.

However, it must be noted that for any conclusions drawn from this monitoring trial, it is impossible to differentiate between conditions required for infection, disease development and sporulation. In further work, it would be interesting to separate these specific parts of the disease cycle in the current analysis as they may all need different conditions.

The environmental monitoring of the sage crop concluded that downy mildew symptoms increased with time on new growth after the cuts, however there is uncertainty whether downy mildew on sage infects systemically or not or whether new leaf material was infected via air-borne inoculum from other adjacent crops. Further fungicide work on downy mildew on perennials such as sage could include timing of fungicide applications post-harvest for optimum control on new growth.

Interestingly, in the study by Garibaldi *et al* (2007), it was found that basil downy mildew appears to prefer higher temperatures than parsley; a temperature of 20°C was most conducive to basil downy mildew development whereas at 12°C and 27°C no disease development was observed.

The study by Garibaldi *et al*, (2007) thus concluded that the causal agent of downy mildew of basil requires mild temperatures to develop and is inhibited by high and low temperatures. Most importantly, it was concluded that a leaf wetness period of at least 24 hours after symptom appearance is required for sporulation. Sporulation of *P. belbahrii* in the presence of favourable environmental conditions is quite intense; this can explain the rapid spread of downy mildew epidemics observed in practice. High relative humidity seems to be the predisposing factor for attacks of *P. belbahrii* on basil, as shown for many other downy mildews (Spencer, 1981).

No downy mildew developed in either of the commercial crops monitored in North Yorkshire. This may have been due to a lack of inoculum in the crops, unfavourable environmental conditions for infection or disease development or effective fungicide control.

Effect of temperature, leaf wetness and relative humidity 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.

Materials and methods

Experiment and crop details

Three separate experiments were carried out: (A) on sage cv. English and experiments (B) and (C) on parsley cv. Bravour. Parsley and sage were inoculated with *Plasmopara umbelliferarum* and *Peronospora lamii*, respectively. In the initial phases of the investigation, two controlled environment cabinets in the pathology laboratory at ADAS Boxworth were used. Following exposure of all inoculated plants to the appropriate temperature and leaf wetness durations in the cabinets, they were moved to a polytunnel at ADAS Boxworth and monitored for symptom development. For all trials the sowing of sage and parsley plants was staggered to ensure that plants of similar age were used for each run of the experiment.

Treatments

For experiments (A) and (B), the inoculated sage and parsley were incubated at four temperatures: (5°C, 10°C, 15°C and 20°C), and four leaf wetness durations (1h, 6h, 24h and 48h) (Table 7). In experiment (C), for each of four temperature treatments, six combinations of leaf wetness duration and relative humidity were tested (Table 8).

Uninoculated control plots were positioned away from the inoculated plants to act as a check for the development of seed-borne disease.

Table 7. Incubation temperature and leaf wetness duration treatments following inoculation of parsley and sage seedlings with downy mildew (Experiments A and B)

Treatment number	Temperature (°C)	Leaf wetness duration (hrs)
1	5	1
2	5	6
3	5	24
4	5	48
5	10	1
6	10	6
7	10	24
8	10	48
9	15	1
10	15	6
11	15	24
12	15	48
13	20	1
14	20	6
15	20	24
16	20	48

Table 8. Incubation temperature, leaf wetness duration and high relative humidity treatments following inoculation of parsley seedlings with downy mildew (Experiment C)

Treatment number	Temp (°C)	Leaf Wetness (hrs)	Relative Humidity (polythene covering regime in glasshouse/ polytunnel)
1	5	1	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
2	5	6	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
3	5	24	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
4	5	48	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
5	5	48	Uncovered post inoculation – Low RH
6	5	48	Continually covered – High RH
7	10	1	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
8	10	6	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
9	10	24	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
10	10	48	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
11	10	48	Uncovered post inoculation – Low RH
12	10	48	Continually covered – High RH
13	15	1	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
14	15	6	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
15	15	24	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
16	15	48	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
17	15	48	Uncovered post inoculation – Low RH
18	15	48	Continually covered – High RH
19	20	1	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
20	20	6	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
21	20	24	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
22	20	48	Covered for 24h when symptoms seen in continually covered plots – 24 h period of RH
23	20	48	Uncovered post inoculation – Low RH
24	20	48	Continually covered – High RH

Experiment design and statistical analysis

Experiments (A) and (B) tested the effects of four temperatures and four leaf wetness durations in a factorial split-plot design, with three replicate blocks (over time) for each treatment combination. Experiment (C) tested the effects of four temperatures and a combination of four leaf wetness durations and, for the 48 hours leaf wetness only, three relative humidity combinations in a fully randomised design, with three replicate blocks (over time) for each treatment combination. For Experiments (A) and (B) a plot comprised 30 sage plants per 1L pot and three half seed trays of parsley. For Experiment (C) a plot comprised two seed trays of 10 parsley plants. For all experiments, once plants were returned to the polytunnel they were placed in a fully randomised block design.

The experiment was run using two controlled environment cabinets each set at a different temperature (Table 9). This resulted in 12 plots in total in each run/cabinet. Results were examined by ANOVA as appropriate for the data.

At each sowing (twice a week for three weeks), an extra plot of 30 plants was sown. These plants remained uninoculated and were transferred directly to the glasshouse at the start of each pair of runs rather than being placed in a CE cabinet. They were positioned away from the inoculated plants and served as a check for development of foliar disease due to seed-borne inoculum rather than due to artificial inoculum.

Table 9. Temperatures of each CE cabinet during various runs – ADAS Boxworth, 2011

Week No.	Run No.	Cabinet 1	Cabinet 2
1	1 & 2 (Mon-Weds)	5	20
	3 & 4 (Weds-Fri)	15	10
2	5 & 6 (Mon-Weds)	15	5
	7 & 8 (Weds-Fri)	10	20
3	9 & 10 (Mon-Weds)	10	5
	11 & 12 (Weds-Fri)	20	15

Production of plants

At each sowing date (twice per week for 3 weeks), 81 x 1L pots and 27 half-size seed trays were filled with Levingtons F2 + S compost and sown with 30 sage and 12 parsley seeds respectively. Each batch of pots and trays was labelled with the sowing date. These were maintained at 20°C in a glasshouse on capillary matting and watered overhead to maintain moist but not waterlogged compost for 4 weeks (until approximately 1-2 true leaf stage).

At each sowing, this provided 36 pots and 12 trays for each of 2 cabinets; there were also pots and trays of uninoculated controls and some spare seedlings to mitigate the risk of poor germination.

Production of sporulating cultures

Plasmopara umbelliferarum was maintained on 12 potted plants of parsley throughout the experiment by potting-on new parsley plants into 1L pots every 2 weeks, and placing them near infected plants in a humid atmosphere. A humid atmosphere was maintained by using a polythene cover over the plants and then misting regularly during the late afternoon.

Peronospora lamii was similarly maintained on 12 potted plants of sage.

Inoculation

At the start of each inoculation treatment, a spore suspension of each pathogen was prepared.

Approximately 20ml sterile distilled water (SDW) was poured into a container and a sterile loop was used to dislodge the spores from the leaves into the water. The spore suspension was poured through muslin and the spore concentration checked using a haemocytometer and high power microscope. For each pair of runs, at least 200ml at a concentration of 1 x 10^5 spores/ml was required. The spore suspension was placed in a clean hand-held mister and the appropriate plants were sprayed to the point of run-off.

If insufficient inoculum was available, as was the case with the sage trial, six plants with sporulating lesions were placed evenly within each cabinet at the start of each temperature run. Also, an 'infector' plant was placed with each of the plots left outside the cabinet for the 1 hour leaf wetness treatment. The 'infectors' were removed after 6 hours.

CE cabinet incubation

Each cabinet was set to the required temperature at least 1 hour before required. Lights were set to 12h day and 12h night, with 0% relative humidity. A mister and tank was set up in each cabinet. When inoculated plants were ready to go in the cabinets, misting commenced at 1.2 L/h for 48h to maintain continuous leaf wetness. In addition to the mister, for experiment (C) the plants were covered in polythene to increase humidity and leaf wetness. In all experiments the plants were misted every hour by hand to maintain leaf wetness.

Leaf wetness durations

Uninoculated plant trays were labelled and maintained on the glasshouse bench.

Trays of plants to receive a 1h leaf wetness treatment were inoculated then left to dry on a laboratory bench or in the incubator room at the required temperature, rather than being placed in the controlled environment cabinet. A small fan heater on low setting was used to dry the leaves if required after 1h. The plants were then returned to the glasshouse bench.

Plants for the remaining leaf wetness treatments were placed in the CE cabinets with misters running immediately after inoculation and left there until the end of the required leaf wetness period (6, 24 or 48h). The plants were removed after the required leaf wetness periods and leaves were dried using a fan heater if necessary and plants returned to the glasshouse bench.

Plant maintenance in polytunnel

Plants were maintained in the polytunnel on capillary matting. The plants were not subjected to overhead watering but kept moist by watering the capillary matting.

For Experiment (C), treatments 6, 12, 18 and 24 were covered immediately post placement into the glasshouse to maintain high relative humidity. Once sporulation occurred on these plants all treatments in the block were covered for 24h except treatments 5, 11, 17 and 23 (low RH) which remained uncovered for the duration of the trial. Figure 11 shows the trial laid out in the glasshouse with plots covered with polythene.

Figure 11. Plants laid out in randomised plots after inoculation and leaf wetness treatment in the CE cabinets – Boxworth, 2012

Meteorological & edaphic records

Temperature and humidity data was recorded hourly using a datalogger (USB logger in CE cabinets, Tinytags on benches). Any extreme weather conditions during the trial were recorded.

Growth stage

The crop growth stage and any variation within the plots was recorded at each visit.

Assessments

For Experiment (A) and (B) at each run, each plot was assessed at 10 and 21 days and again as necessary after inoculation for incidence of downy mildew symptoms (% plants affected per tray) and severity of downy mildew symptoms (% leaf area affected on each plant). Typical lesions were checked under the microscope.

For each run of Experiment (C), each plot was assessed 24 hours after covering when sporulation occurred on treatments 6, 12, 18 and 24 and then again as necessary after inoculation for incidence of downy mildew symptoms (% plants affected per tray) and severity of downy mildew symptoms (% leaf area affected for each plant). Typical lesions were checked under the microscope.

Results and discussion

Disease levels in all three experiments were low and the data was not suitable for statistical analysis. Nevertheless, there were some interesting trends in the data.

For Experiments (A) and (B) there were signs that downy mildew of parsley and sage have different requirements; incidence and severity levels of downy mildew on parsley were greater at lower temperatures and appeared to reduce with the higher temperatures whereas the trend was the reverse for sage. For parsley, incidence was highest (1.7% averaged over all wetness durations) at a temperature of 5°C, while there was no disease occurrence at all for sage at such a low temperature (Table 10). Rather, sage had its greatest incidence of 0.6%, averaged over all wetness durations, at a temperature of 15°C (Table 10). For parsley, it appears that incidence of the disease was favoured by an interaction that included a relatively low temperature and any period of wetness, however the tests did show that infection could take place even when the leaf wetness period was only 1 hour at 5°C. Some degree of infection was observed across a temperature range of 5-15°C in parsley, none was observed in our tests at 20°C, although, due to low levels of infection across the experiment, this result cannot be considered conclusive. In sage, no infection occurred at 5°C in any seedlings, low levels of infection occurred at temperatures between 10-20°C and only in plants which were subjected to 6-24hrs. It appeared that infection occurred more readily with conditions that comprised 24 hours of wetness and a temperature of 15°C that resulted in incidence levels of 2.2% in 2 plots (Table 10). Again, higher levels of disease are required to achieve more confidence in the results obtained.

Table 10. Effect of various temperature and leaf wetness durations on development of parsley and sage downy mildew (Experiments A and B)

Treatment	Temp (°C)	Leaf wetness (h)	Parsley (28 day post inoculation)		Sage (21 day post inoculation)	
			% seedlings affected	% leaf area affected	% seedlings affected	% leaf area affected
1	5	1	4.5	1.3	0.0	0.0
2	5	6	0.0	0.0	0.0	0.0
3	5	24	0.0	0.0	0.0	0.0
4	5	48	2.3	0.3	0.0	0.0
5	10	1	0.0	0.0	0.0	0.0
6	10	6	2.3	0.3	0.7	0.2
7	10	24	0.0	0.0	0.0	0.0
8	10	48	0.0	0.0	0.0	0.0
9	15	1	0.0	0.0	0.0	0.0
10	15	6	2.3	0.7	0.7	0.02
11	15	24	0.0	0.0	1.5	0.3
12	15	48	0.0	0.0	0.0	0.0
13	20	1	0.0	0.0	0.0	0.0
14	20	6	0.0	0.0	0.0	0.0
15	20	24	0.0	0.0	0.7	0.03
16	20	48	0.0	0.0	0.0	0.0

For Experiment (C), we observed a reduction in the incidence and severity of downy mildew in parsley as temperatures increased, with incidence highest (2.8%, averaged across all wetness durations) at 5°C (Table 11). This is the same trend for the previous parsley experiment (A) and suggests that downy mildew on parsley prefers cooler temperatures which may be due to the pathogens' environment of evolutionary adaptation being in a temperate climate. Unfortunately, the pathogen was only detected in plants which had been subjected to a minimum of 24hrs leaf wetness in this experiment, and this did not support the findings of experiment (A) above

In experiment (C), there also appeared to be a trend for the interaction effect of 24hr humidity treatment 7-10 days post-infection combined with inoculation at low temperatures (5 – 10°C) to cause slightly higher incidence and severity of downy mildew. The highest incidence of 13.4% was seen in Trt 3 which combined a temperature of 5°C, 24 hours leaf wetness followed by 24 hours of high humidity. These results are consistent with Garibaldi

et al, (2007) who concluded that a leaf wetness of at least 24 hours after symptom appearance is required for sporulation.

Table 11. Effect of various temperature, leaf wetness and high relative humidity treatments on development of parsley downy mildew (Experiment C)

Treatment	Temp (°C)	Leaf wetness (h)	High RH period on first symptoms	Downy mildew	
				% seedlings affected	% leaf area affected
1	5	1	✓	0.0	0.0
2	5	6	✓	0.0	0.0
3	5	24	✓	13.4	4.8
4	5	48	✓	0.0	0.0
5	5	48	No	0.0	0.0
6	5	48	Continuous	3.4	1.7
7	10	1	✓	0.0	0.0
8	10	6	✓	0.0	0.0
9	10	24	✓	6.7	1.3
10	10	48	✓	0.0	0.0
11	10	48	No	0.0	0.0
12	10	48	Continuous	0.0	0.0
13	15	1	✓	0.0	0.0
14	15	6	✓	0.0	0.0
15	15	24	✓	0.0	0.0
16	15	48	✓	6.7	1.0
17	15	48	No	0.0	0.0
18	15	48	Continuous	0.0	0.0
19	20	1	✓	0.0	0.0
20	20	6	✓	0.0	0.0
21	20	24	✓	0.0	0.0
22	20	48	✓	0.0	0.0
23	20	48	No	0.0	0.0
24	20	48	Continuous	0.0	0.0

It should be emphasized that, as the disease levels were low, no firm conclusions can be drawn. This aspect of the project will be re-examined in 2013.

Development of a risk-based strategy for the control of downy mildew in herbs.

The results of the literature review and the experimental work carried out under controlled environmental conditions has provided a range of information which may help to identify high disease-risk periods in herb crops. The information gathered is presented in Table 12

Table 12. 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 temperature	**Leaf wetness period required**	**Infection temperature**	**Leaf wetness period required**
Peronospora lamii	Sage Mint Rosemary	20-25°C	not recorded	10-20°C	6-24hrs
Plasmopara umbelliferarum	Parsley	18-20°C	24 hours	5-15°C	1-24hrs
Peronospora belbahrii	Basil	20°C	min 24 hours	Not tested	

Interestingly there is some disparity between the environmental parameters required for infection observed in our laboratory experiments and those reported in the relevant literature. In both downy mildew genera tested for this study we observed that infection was possible at much lower temperatures than those reported by other researchers. This is an interesting factor and potentially may suggest that isolates used in our study had evolved in some way to suit the northern European climate where they are prevalent, whilst much of the reported work was carried out either in southern Europe or in the USA. It is important to note that this is the first known study looking specifically at herb downy mildews grown in UK conditions.

Hyre (1959) and Santamaria *et al* (2006) both carried out work looking at the role of leaf wetness, rainfall and temperature effects on downy mildew infection and spread in order to identify specific environmental conditions which might alert growers to potential high disease risk periods and take precautionary actions.

In reality, in outdoor crops the only precautionary actions that are available are the application of preventative fungicide sprays, and this can often be problematic for growers due in part to the fact that their supermarket clients are seeking a virtual zero tolerance to pesticide applications as well as the long harvest intervals for many herb crops, particularly

those with a short, fast growing period such as parsley and basil. For some herb crops the use of fungicide treated seed may prove helpful in providing protection to young emerging seedlings e.g. parsley and basil. Although laboratory and field testing carried out to date in this study still requires some additional work to provide greater confidence in the specific environmental requirements for the downy mildew infections in question, it is fairly clear that a few days with temperatures and leaf-wetness periods in the ranges shown in Table 12 do provide the potential for infection to develop in unprotected crops. The risk may be increased in crops where there has been a previous history of DM infection e.g. from infected crop debris, or from a potentially systemic infection in an over-wintering crop e.g. sage. We would therefore suggest that, where possible, growers should apply preventative fungicide sprays during these high-disease risk periods.

Further details and suggestions should become available as the project is completed in 2013.

Effect of overwinter fungicide drenches to soil and crop debris for control of downy mildew in sage

Introduction

The objectives of this work were (i) to test the effectiveness of high volume fungicide sprays against downy mildew (*Peronospora lamii*) when applied as overwinter and early spring disinfestation treatments to dormant sage and to debris between rows and (ii) to establish that products are safe to use on sage crops when applied as drenches.

Materials and methods

Site and crop details

The trial was carried out on a commercial crop of sage in Norfolk where there was 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

Fungicides were applied in autumn and/or early spring as detailed in Table 4.1. They were applied as HV sprays in 1,000 litres water/ha at a pressure range 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. The fungicides detailed in Table 13 are (at the time of writing) authorised for use on sage via the Extension of Authorisation for Minor Uses (EAMU) scheme (formally SOLA).

Experiment design and statistical analysis

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

Table 13. Detail of overwinter/early spring fungicide treatments applied to sage for control of downy mildew – 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

At the time of spray application a baseline assessment was made for % leaf retention and the background disease levels. As new growth occurs in spring the plots will be monitored for downy mildew development in the central $1m^2$ area of each plot.

After the treatment application in winter the leaves were allowed to dry and then diseased leaves (x 10) and soil were collected from each treated and untreated plot. Soil was collected from the top 1cm of the soil profile and no deeper than 5cm to provide enough material to fill a 1.5 L pot. Sage seeds will be sown into this potted soil/leaf mix once the leaves have decayed and the resulting seedlings monitored for disease development to see if soil/leaf debris is a source of inoculum.

Results

This aspect of the project is still on-going. Full results will be provided in the final report in 2013.

Efficacy trials for the control of parsley and basil downy mildew

A fully randomized parsley downy mildew trial to investigate the efficacy of a range of fungicide, biopesticide and novel UV light treatments will be carried out at STC during late 2012 when environmental conditions are optimal for disease. The treatments (detailed in Table 14) will initially be applied to young parsley plants raised in pots to allow smaller-scale studies and a broader range of treatments. Promising treatments will be taken forward into a larger field-scale study in 2013. Three different cultivars of parsley will be used in the initial tests to ensure there are no varietal susceptibility problems following inoculation.

The results and conclusions of this work will be supplied in the 2013 final report.

Table 14. Details of the proposed treatment list for the parsley downy mildew efficacy testing experiments scheduled for 2012-2013.

Treatment No.	Product	Active Ingredient	Comment
1.	Untreated uninoculated	-	
2.	Untreated inoculated	-	
3.	SL567A	metalaxyl-M	EAMU valid until 30.9.12
4.	Revus	mandipropamid	EAMU valid until 31.1.15
5.	Previcur Energy	fosetyl-aluminium + propamocarb HCl	EAMU valid until 31.10.19
6.	Paraat	dimethomorph	EAMU valid until 25.9.13
7.	Fubol Gold	metalaxyl-M + mancozeb	EAMU valid until 30.7.13
8.	Amistar	azoxystrobin	
9.	Femomenal	fenamidone + fosetyl-aluminium	
10.	Signum	boscalid + pyraclostrobin	
11.	Invader	dimethomorph + mancozeb	
12.	Valbon	benthiovalicarb + mancozeb	
13.	Dithane	mancozeb	
14.	Farm Fos-44	potassium phosphite	
15.	Serenade	*Bacillus subtilis*	
16.	UV Light	UVC lightwaves	To include 3 different exposure timings.

Work to investigate the potential efficacy of a number of fungicide, biopesticide and novel UV light treatments to control basil downy mildew was scheduled for 2011. However, there were no reported outbreaks of downy mildew in this crop in the UK during 2011 and despite, extensive plans being put in place to enable small-scale glasshouse house studies to be carried out in quarantine facilities at Fera (North Yorkshire), the work had to be postponed until inoculum can be gathered. This may prove difficult as commercial herb nurseries are now fore-warned of this potential risk and are likely to be applying preventative fungicides to young seedlings and plants in order to protect their crops from infection. Growers are unlikely to be willing to leave plants untreated to provide inoculum for this trial as the pest is still notifiable under UK plant health legislation, so therefore any report of infection on the nursery could result in an order from Plant Health for crop destruction. This problem will have to be overcome in some way. Late last year STC were made aware of an outbreak of basil downy mildew in northern France. STC have conferred with a Plant Health Inspector and can confirm that it would be possible to import material to the STC Plant Clinic under a special licence to move material, and this may provide a solution to the problem. It is hoped that the work can be carried out in 2013 using either UK or European infected material. Due to space limitations the trial will be carried out on pot raised basil of a susceptible variety. Details of the proposed treatments are shown in Table 15.

Table 15. Details of proposed treatments for the control of downy mildew on basil.

Treatment No.	Product	Active Ingredient
1.	Inoculated, untreated	-
2.	SL567A	metalaxyl-M
3.	Filex	propamocarb HCl
4.	Amistar	azoxystrobin
5.	Experimental	-
6.	Invader	dimethomorph + mancozeb
7.	Experimental	-
8.	Previcur Energy	fosetyl-aluminium + propamocarb HCl
9.	Revus	mandipropamid
10.	Experimental	-
11.	Rose Tonic	potassium phosphite
12.	Serenade	*Bacillus subtilis*
13.	UV light	-

Project conclusions

Monitoring environmental conditions

- The combination of a cold winter followed by a dry spring in 2011 kept downy mildew levels low early in the season but there were some significant attacks by the end of the season in parsley crops.
- There was some evidence that a period of extended leaf wetness and mild temperatures are conducive to infection of downy mildew on parsley.
- Rapid spread of parsley downy mildew was favoured by extended periods of leaf wetness and the cooler average temperature in October (7.7°C).

Controlled environment experiment

- Incidence and severity of downy mildew on parsley appeared to be slightly greater at a low temperature (5°C) and decreased with increasing temperatures.
- Incidence and severity of downy mildew on sage was greatest at 15°C.
- There was a trend for a combination of 24 hours leaf wetness followed by 24 hours of high humidity to give the highest incidence of downy mildew on parsley at low temperatures (5-10°C).

Technology transfer

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

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Appendix 1. Parsley, sage and mint crop diary (monitoring)

Monitoring environmental conditions and disease development in an outdoor crop- Norfolk

Date	Task/Assessment
13 April 2011	Sage & parsley trial marked out. Loggers set up
11 May 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage. Downy mildew found on sage
26 May 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage.
15 June 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage.
1 July 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage. Soil samples taken Both crops recently harvested
21 July 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage.
18 August 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage. Parsley recently harvested.
01 September 2011	Downy mildew assessment completed on parsley & sage
15 September 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage Parsley harvested
30 September 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage Downy mildew now present in parsley field but not found in trial quadrats
13 October 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage Downy mildew found in parsley trial
20 October 2011	Data loggers downloaded Downy mildew assessment completed on parsley & sage
28 October 2011	Data loggers downloaded & removed Downy mildew assessment completed on sage Parsley had been harvested

Monitoring environmental conditions and disease development in an outdoor crop-Yorkshire

Date	Task/Assessment
17 May 2011	HOBO loggers installed in mint & sage crops (measuring RH, soil temperature & air temperature) Downy mildew assessment completed on mint & sage
26 May 2011	Delta-T loggers installed in mint and sage (measuring leaf wetness & rainfall) Downy mildew assessment completed on mint & sage
14 June 2011	Downy mildew assessment completed on mint & sage
07 July 2011	Downy mildew assessment completed on mint & sage Loggers downloaded Mint recently harvested-assessed an adjacent crop
09 August 2011	Downy mildew assessment completed on mint & sage Loggers downloaded
19 September 2011	Downy mildew assessment completed on mint & sage Loggers downloaded
07 November 2011	Downy mildew assessment completed on mint & sage Loggers downloaded and removed from site

Appendix 2. Parsley and sage crop diary (controlled environment)

Herb Downy Mildew – Effect of temperature, leaf wetness and relative humidity on incidence and severity of *Plasmopara umbelliferarum* and *Peronospora lamii*

Experiment A - Parsley

Date	Task/assessment
5 December 2011	Run 1 (10°C) & run 2 (15°C) set up in CE cabinets 1 hour & 6 hour taken to glasshouse
6 December 2011	24 hour taken to glasshouse
7 December 2011	48 hour taken to glasshouse
7 December 2011	Run 3 (20°C) & run 4 (5°C) setup in CE cabinets 1 hour & 6 hour taken to glasshouse
8 December 2011	24 hour taken to glasshouse
9 December 2011	48 hour taken to glasshouse
13 December 2011	Run 5 (10°C) & run 6 (20°C) set up in CE cabinets 1 hour & 6 hour taken to glasshouse
14 December 2011	24 hour taken to glasshouse
15 December 2011	48 hour taken to glasshouse
15 December 2011	Run 7 (5°C) & run 8 (15°C) set up in CE cabinets 1 hour & 6 hour taken to glasshouse
15 December 2011	Runs 1 & 2 assessed
16 December 2011	24 hour taken to glasshouse
16 December 2011	Runs 3 & 4 assessed
17 December 2011	48 hour taken to glasshouse
19 December 2011	Run 9 (15°C) & run 10 (20°C) set up in CE cabinets 1 hour & 6 hour taken to glasshouse
20 December 2011	24 hour taken to glasshouse
21 December 2011	48 hour taken to glasshouse
21 December 2011	Run 11 (10°C) & run 12 (5°C) set up in CE cabinets 1 hour & 6 hour taken to glasshouse
22 December 2011	24 hour taken to glasshouse
23 December 2011	48 hour taken to glasshouse
23 December 2011	Runs 1 – 6 assessed Heating in glasshouse turned off. Parsley covered by plastic.
28 December 2011	Runs 1 – 4 & 7 – 10 assessed. First mildew found.
3 January 2012	Runs 1 – 6 & 11 – 12 assessed.
5 January 2012	Run 7 & 8 assessed
9 January 2012	Run 9 & 10 assessed
11 January 2012	Runs 11 & 12 assessed

Experiment B - Sage

Date	Task/assessment
5 September 2011	Run 1 (10°C) & run 2 (15°C) set up in CE cabinet 1 hour & 6 hour treatments moved to polytunnel
6 September 2011	24 hour treatments moved to polytunnel
7 September 2011	48 hour treatments moved to polytunnel
7 September 2011	Run 3 (20°C) & run 4 (5°C) set up in CE cabinets 1 hour & 6 hour treatments moved to polytunnel
8 September 2011	24 hour treatments moved to polytunnel
9 September 2011	48 hour treatments moved to polytunnel
12 September 2011	Run 5 (10°C) & run 6 (20°C) set up in CE cabinet 1 hour & 6 hour treatments moved to polytunnel
13 September 2011	24 hour treatments moved to polytunnel
14 September 2011	48 hour treatments moved to polytunnel
14 September 2011	Run 7 (5°C) & run 8 (15°C) set up in CE cabinet 1 hour & 6 hour treatments moved to polytunnel
14 September 2011	Runs 1 & 2 assessed
15 September 2011	24 hour treatments moved to polytunnel
16 September 2011	48 hour treatments moved to polytunnel
16 September 2011	Runs 3 & 4 assessed
19 September 2011	Run 9 (15°C) & run 10 (20°C) set up in CE cabinets 1 hour & 6 hour treatments moved to polytunnel
20 September 2011	24 hour treatments moved to polytunnel
21 September 2011	48 hour treatments moved to polytunnel
21 September 2011	Run 11 (10°C) & run 12 (5°C) set up in CE cabinets 1 hour & 6 hour treatments moved to polytunnel
21 September 2011	Runs 1 & 2, 5 & 6 assessed.
22 September 2011	24 hour treatments moved to polytunnel
23 September 2011	48 hour treatments moved to polytunnel
23 September 2011	Runs 3 & 4, 7 & 8 assessed
28 September 2011	Runs 1 & 2,5 & 6, 9 & 10 assessed
30 September 2011	Runs 3 & 4, 7 & 8, 11 & 12 assessed
14 October 2011	All pots assessed
28 October 2011	All pots assessed

Experiment C - Parsley

Date	Task/Assessment
6 February 2012	Run 1 (10°C) & run 2 (15°C) set up in CE cabinets
8 February 2012	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
8 February 2012	Run 3 (20°C) & run 4 (5°) set up in CE cabinets
10 February 2012	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
13 February 2012	Run 5 (10°C) & run 6 (20°) set up in CE cabinets
15 February 2012	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
15 February 2012	Run 7 (5°C) & run 8 (15°) set up in CE cabinets
17 February 2912	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
20 February 2012	Run 9 (15°C) & run 10 (20°) set up in CE cabinets
22 February 2012	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
22 February 2012	Run 11 (10°C) & run 12 (5°C) set up in CE cabinets
24 February 2012	Treatments 5, 11, 17, 23 covered in polythene (48 hours, high RH)
28 February 2012	Downy mildew found in block 2 (5°C, 48 hours & covered). Blocks 1 & 2 covered in polythene except for treatments 4, 10, 16, 22 (48 hours, low RH)
29 February 2012	Polythene removed from all treatments in blocks 1 & 2 except treatments 5, 11, 17, 23 (48 hours, high RH).
1 March 2012	Assessment completed on blocks 1 & 2
7 March 2012	Small amount downy mildew found in block 3 (20°C 48 hours, high RH). Block 3 covered in polythene except treatments 4, 10, 16, 22 (48 hours, low RH).
8 March 2012	Polythene removed from all treatments in blocks 1 & 2 except treatments 5, 11, 17, 23 (48 hours, high RH).
9 March 2012	Assessment completed on blocks 1 & 2 and block 3.
20 March 2012	Assessment completed on blocks 1 & 2 and block 3.

Appendix 3. Sage crop diary (disinfestation trial)

Herb downy mildew – Efficacy testing of overwinter fungicide drenches to soil and crop debris for control of downy mildew in sage – Part A (field trial)

Date	Task/Assessment
6 December 2011	Autumn spray applied. Soil samples collected for part B
9 December 2011	Glasshouse sage trial set up
13 December 2011	Phytotoxicity assessment completed