Contract report for the
Horticultural Development Council

Salads and baby leaf brassicas: pathogen survival on crop covers and potential disease spread

FV 283

August 2007

Project title:	Salads and baby leaf brassicas: pathogen survival on crop covers and potential disease spread
Project number:	FV 283
Project leader:	Dr Kim Green, ADAS UK Ltd
Report:	Year 1 Annual report
Previous reports	-
Key staff:	Steve Wilson, Martin Selby
Location of project:	ADAS Arthur Rickwood
Project coordinator:	Mr Thane Goodrich
Date project commenced:	1 April 2006
Date completion due:	31 March 2008
Key words:	Crop covers, mesh, pathogen survival, foliar disease

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

I declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr K R Green
Research Scientist
ADAS

Signature ..... Date .....

Report authorised by:

Dr W E Parker
Horticulture Sector Manager
ADAS

Signature ..... Date .....

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1 GROWER SUMMARY

Headlines

- Experiments demonstrated that three pathogens (*Colletotrichum dematium* and *Cladosporium variabile* on spinach, and *Botrytis cinerea* on lettuce) survived on crop mesh for at least 1 month, and increased the incidence of disease on subsequent crops.
- Growers can alleviate these risks to future crops by:
    - Increasing the duration between use of meshes on successive crops (ensuring no re-use within 3 days)
    - Allow meshes to dry before rolling for storage

Background and expected deliverables

A range of crop covers, fleeces and meshes, are used in the production of field vegetable crops (e.g. speciality salads and brassicas) to enable early crop production and also to provide protection from insect pests. Meshes in particular are often re-used on later plantings in the same growing season and in subsequent growing seasons, as they are less prone to damage and are relatively expensive to replace. In addition, there is increasing pressure on growers to re-use meshes due to changes in waste regulations. Salads and baby leaf brassicas are affected by a wide range of foliar fungal diseases (e.g. downy mildews and leaf spots) that can develop while crop covers are in place. Little is known of the survival of these fungi on crop covers and growers are concerned that when re-used, meshes could act as a source of fungal inoculum, initiating disease development on subsequent crops. The proposed work is a novel investigation into the survival of key foliar pathogens on crop covers. Increased knowledge of pathogen survival on meshes, risk to subsequent crops and possible control methods if pathogen survival does occur, will enable growers to make more informed decisions about re-use of crop covers.

The overall project aim is to determine the role of crop meshes in the survival and spread of foliar pathogens on salads and baby leaf brassicas. The specific objectives are to:

1. Compile information on the environmental conditions affecting the survival of key foliar pathogens of salad and baby leaf crops on which meshes are used.
2. Select up to four pathogen/crop combinations to use as model systems, based on the findings of 1.
3. Determine the ability of selected pathogens to survive on meshes and infect subsequent crops.

4. Investigate the effect of environmental factors on the longevity of survival of selected pathogens on meshes.

Summary of the project and main conclusions

Use of meshes on UK salad crops

A knowledge review of the specific environmental factors that affect the survival of key foliar pathogens of UK crops on which covers are used was compiled. In addition, a questionnaire was used to collate information from growers on the occurrence of diseases under crop meshes for different crops.

Responses to the grower questionnaire emphasised the diversity of crops for which crop meshes are used. In addition, the repeated re-use of crop mesh in a single growing season was demonstrated. Of a range of diseases observed on crops under meshes, downy mildew and *Colletotrichum dematium* on rocket was considered the most problematic, with two growers abandoning use of mesh on this crop because of associated disease risk.

The knowledge review demonstrated that for many of the key pathogens on salad crops, dispersal spores may survive for at least 12 hours and often longer, under favourable conditions. This fact, together with questionnaire findings that mesh transfer to a new crop can occur within 0-3 days of removal from a previous crop, and observations of diseases on crops under mesh, suggests that there is potential for transfer of viable inoculum of a range of pathogens to subsequent crops via crop meshes.

The ability of different pathogens to survive on mesh for long periods is likely to vary according to spore type. In general, pathogens such as *Alternaria* species, *Stemphylium botryosum* and *Cladosporium variabile* which produce spores that are darkly-pigmented (resistant to damage from solar radiation) and thick-walled (less prone to desiccation), will have a greater chance of survival than colourless, thin-wall spores of e.g. downy mildews and *Colletotrichum dematium*. Spore size may also influence survival duration.

On the basis of the knowledge review and questionnaire, four crop/pathogen systems were selected for further study of potential pathogen transfer on crop meshes: grey mould (*Botrytis cinerea*) on lettuce, Cladosporium leaf spot (*Cladosporium variabile*) on spinach, anthracnose (*Colletotrichum dematium*) on spinach, and downy mildew (*Hyaloperonospora parasitica*) on wild rocket. Pathogen characteristics are summarised in Table 1. This report includes results from experiments on *C. dematium*, *C. variabile* and *B. cinerea.* Development

of a test system for rocket downy mildew is underway and experimental results will be incorporated in the next report.

Table 1. Key characteristics of pathogens used in crop covers survival experiments

Pathogen	Disease	Host	Spore dispersal	Long-term survival
Colletotrichum dematium	Anthracnose	Spinach	Water splash only	Spinach crop debris and volunteers
Cladosporium variabile	Leaf spot	Spinach	Air (& water splash)	Spinach crop debris and seed
Botrytis cinerea	Grey mould	Many E.g. lettuce	Air (& water splash)	Seed, crop debris, other hosts, soil
Hyaloperonospora parasitica	Downy mildew	Brassicas E.g. wild rocket	Air (& water splash)	Other hosts, (survival spores in crop debris, and maybe soil)

Duration of pathogen survival on crop meshes

A laboratory experiment was done to determine the duration of survival (up to 28 days) for three plant pathogens on crop mesh under either dry or moist conditions. Results showed that in a temperature range of 14-26^{o}C and ambient light, *Botrytis cinerea* (grey mould) and *Cladosporium variabile* (Cladosporium leaf spot on spinach) can remain viable for at least 1 month on pieces of crop mesh stored either wet or dry. Similarly, spores of *Colletotrichum dematium* (spinach anthracnose) remained viable on mesh pieces for at least one month when stored wet. However, under dry conditions, percentage viability of *C. dematium* was reduced to 20% after 7 days storage and to 0% by 28 days. See Figures 1-3. Further experiments are now being done to determine the effects of environmental conditions on pathogen survival over longer durations.

Pathogen transmission on crop meshes

Experiments were done to determine whether each of three fungal pathogens could be transferred via crop covers and infect a new crop. *C. dematium* (spinach anthracnose) remained viable on mesh at sufficient levels to infect a subsequent crop of spinach, although there was no significant effect from mesh positioning on the previous diseased crop (in contact or suspended over plants) on the proportion of plants affected after mesh transfer. For *Cladosporium variabile* and *Botrytis cinerea*, there was strong evidence that each of these fungi could remain viable on mesh at sufficient levels to infect a subsequent crop. For each, there was a trend for higher inoculum levels and disease incidence following use of mesh that had been in direct contact with affected plants.

Table 1. Effect of mesh treatments on i) development of symptoms after mesh transfer, and ii) growth of pathogens from mesh pieces plated on agar

Treatment	% plants infected			Colonies of target pathogen from mesh pieces (out of 25)		
	C. dematium	*C. variabile*	*B. cinerea**	*C. dematium*	*C. variabile*	*B. cinerea*
Clean mesh	0.7	25.7	6.3	0	1	0
Mesh previously in contact with Cladosporium-infected plants	19.2	99.4	21.9	3	22	25
Mesh previously suspended over Cladosporium-infected plants	10.6	98.4	5.2	1	4	24

*Symptoms on upper 2/3 of plant

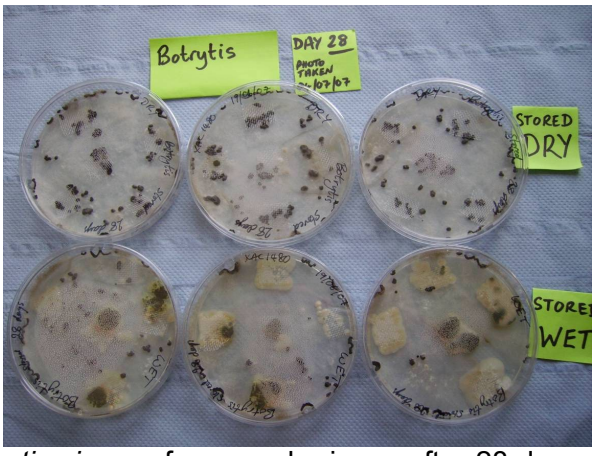

Figure 1. Growth of *Botrytis cinerea* from mesh pieces after 28 days storage under wet and dry storage regimes

Figure 2. Growth of *Cladosporium variabile* from mesh pieces after 28 days storage under wet and dry storage regimes

Figure 3. Growth of *Colletotrichum dematium* from mesh pieces after 7 days storage under wet and dry storage regimes

Financial benefits

- Results will enable salad and baby leaf brassica growers to make informed decisions on re-use of crop meshes based on risk of pathogen carry-over
- Results will be applicable to a wide range of UK vegetable crops on which covers are used
- Findings may also be relevant to the potential survival of foliar pathogens on farm machinery such as harvesting equipment.

Action points for the industry

- Be aware that certain fungal pathogens of lettuce and spinach crops can remain viable on crop meshes for at least 1 month.
- Reduce the risk of pathogen transfer by extending the duration between use of a particular mesh on successive crops for as long as is practical.
- Survival of certain pathogens is favoured by wet conditions (e.g. *Colletotrichum dematium*, cause of spinach anthracnose). Where possible allow meshes to dry before rolling for storage.
- Further work may be warranted to investigate measures such as disinfection that could be used to reduce the risk of pathogen transfer via crop meshes.

2 SCIENCE SECTION

2.1 Introduction

A range of crop covers as fleeces and meshes are used in the production of field vegetables such as salads and baby leaf brassicas, spinach, lettuce, celery, carrot, swede and courgette. In salad and baby leaf production, meshes in particular are regularly re-used on later plantings in the same growing season or in subsequent growing seasons, because they are less prone to damage and are relatively expensive to replace. In addition, there is increasing pressure on growers to re-use crop covers due to changes in waste regulations.

Key foliar diseases (e.g. downy mildews and leaf spots) can and do develop on salad and baby leaf crops while covers are in place. Affected leaves are likely to contact and contaminate the covers with fungal spores. Little is known of pathogen survival on crop covers and growers are concerned that re-used meshes could act as a source of fungal inoculum, thus exacerbating disease development on subsequent crops. Pathogens that cause foliar diseases produce dispersal spores that are either transmitted by air movement or by water splash. In the absence of a growing host crop, these pathogens may produce survival structures in crop debris, but are not generally soil-borne. It is predicted that pathogen survival could occur directly on mesh surfaces or in infected leaf debris, but to a lesser extent in soil.

While there is negligible scientific literature or grower evidence to demonstrate the survival of foliar pathogens on crop covers, previous work has proven the ability of certain root pathogens to survive on inert substrates. There is also a wealth of literature describing the environmental conditions that affect the survival of dispersal spores of certain key pathogens such as lettuce downy mildew (*Bremia lactucae*) and powdery mildews.

This report describes work from project year 1 on the survival of key foliar pathogens on crop meshes. The commercial objective of the project is to enable growers to make more informed decisions about re-use of meshes based on improved knowledge of pathogen survival on meshes and risk to subsequent crops.

2.2 Use of crop meshes on UK salad crops

2.2.1 Introduction

The aim of this knowledge review was to compile information on the specific environmental factors that affect the survival of key foliar pathogens of UK crops on which covers are used. In addition, information from growers has been collated on the occurrence of diseases under crop meshes for different crops. The information will be used to identify pathogens and crop situations for which re-use of meshes could increase the risk of disease spread.

2.2.2 Grower questionnaire

A questionnaire was devised and distributed to members of the Speciality Produce Growers Association (SPGA) in February 2007 (Appendix 1). After a poor initial response, the questionnaire was re-circulated to relevant members by the HDC in May 2007. In total, there were 12 responses to the questionnaire, comprising five growers who used mesh only, three who used mesh and fleece, and four who used fleece only. When interpreting the questionnaire results, the focus was on summarising data from growers who used mesh, as fleece re-use is of less relevance to this project.

Mesh was listed as being used on a wide range of lettuce and salad crops grown both for whole head and baby leaf production including, cos, little gem, butterhead, iceberg, frisee, green and red oak leaf, lolla rosso, red batavia, endive and radicchio. Baby leaf brassica crops on which meshes were used included wild rocket, pak choi, mizuna and curly kale. Other crops where meshes were used were radish (organic), spinach and baby leaf beet. Meshes were reported to be used for early, mid and late season crops, except for speciality lettuce types where meshes were not listed as being used for mid-season crops.

The most frequent reason given for mesh use was to provide protection from pests. Other reasons included advancement of crop growth, protection from hail damage and reduction of contaminants.

Of the eight growers who used mesh, all listed that they re-used mesh in the same growing season and in the following growing season. One grower noted that mesh is continually re-used during a season, and although there is usually a few days gap between crops, mesh may be transferred from a crop to be harvested to a new crop on the same day. One grower out of eight reported that mesh was transferred between farm operations in different countries for re-use. The same respondent said that the mesh was washed annually by machine. None of the other respondents used any washing or disinfection procedures before

mesh re-use. Half of the growers used meshes in contact with the crop while the other half used meshes supported above the crop.

Four of the eight growers listed diseases that had been observed on crops grown under mesh. Disease listed were as follows:

- Lettuce – *Botrytis*, *Sclerotinia* and downy mildew
- Spinach – leaf spots
- Endive – *Sclerotinia* and downy mildew
- Pak choi – Alternaria leaf spot
- Wild rocket – downy mildew

In the case of rocket downy mildew, two growers noted that this crop is no longer grown under mesh due to an increased incidence of downy mildew, which under certain weather conditions is seen as a greater threat to production than flea beetle. One grower noted that there were specific instances where transfer of downy mildew spores on mesh was suspected as the cause of disease on a subsequent wild rocket crop.

2.2.3 Key foliar pathogens of UK salad crops and environmental factors affecting survival

Lettuce (*Lactuca sativa*)

Downy mildew (*Bremia lactucae*) can be severe on lettuce if not avoided through the use of resistant varieties or managed by the use of fungicides. The fungus cannot survive in the absence of a living host, so the main source of inoculum is spores (sporangia) from surrounding plants, weeds and neighbouring lettuce beds. Much research has been done on the conditions required for infection of lettuce by *B. lactucae*, development of downy mildew symptoms, and spore production, release and survival. In general, humid, cool conditions are required for *B. lactucae* to sporulate and to infect lettuce. The spores are produced on leaf lesions at night, and are then released into the air in the early morning. Spore production is restricted to periods of darkness, high relative humidity (>90%), optimum temperatures of 6 to 11^{o}C (UK strains) and low wind speed (Su *et al.*, 2004). Su *et al*. (2000) demonstrated that spore release is triggered by light and a reduction in relative humidity. The spores are dispersed by air currents and splashing water, and once deposited on lettuce leaves, at least 3 hours of leaf wetness and suitable temperatures (10-22^{o}C) are required for spore germination and infection (Powseland, 1954). If conditions required for infection do not occur, then the longevity of spore survival depends mainly on temperature and solar radiation. For example, spores survive much longer at 23^{o}C (>12 h) than at 31^{o}C (2 to 5 h) irrespective of relative humidity (Wu *et al*., 2000). Survival of spores is also reduced after exposure to solar radiation (especially in the UVB spectrum) (Wu *et al*., 2000). Spores may

survive for more than 12 hours but usually only under conditions of cloud or shading, and low temperature.

Grey mould (*Botrytis cinerea*) is common on lettuce and usually of minor importance, but can cause major losses if environmental conditions are favourable for development. Injured or senescing tissues that are wet or that remain in contact with the soil are most susceptible. *B. cinerea* is widespread, surviving as a pathogen on a wide range of crops and weed hosts, as a saprophyte on crop debris and also as sclerotia (survival structures in the soil).

Spores of *B. cinerea* can germinate and infect plant tissue immediately, or may remain dormant on plant surfaces for up to 3 weeks. High relative humidity favours rapid spore germination and infection. Germination can occur over a very wide range of temperatures (5-25ºC) with optimum growth at 18-23ºC and inhibition at 32ºC or above (Koike *et al*., 2007). A complete infection cycle, from spore germination through symptom development to new spore production, can be as short as a few days.

A high humidity level, greater than 95% for a period of more than 3 hours, is the critical threshold for the germination of spores of *B. cinerea* (O'Neill *et al*., 2002). Once this period is exceeded then spore germination will continue even if the humidity is reduced to 80% or less. However, where high humidity periods are kept at 3 hours or less, there is little germination or disease development, even after a period of fluctuating high and low humidity periods.

Once infection has occurred, leaf wetness is important for symptom expression, with disease severity increasing with leaf wetness duration. Symptoms may develop within hours, or the infection may remain dormant and symptomless, not becoming active until days or even weeks later. This is known as latent infection. Due to the potential for latent infection by *B. cinerea,* transplants taken from apparently healthy batches can subsequently develop grey mould symptoms. Similarly, produce that appears healthy at the time of harvesting and packaging can later develop symptoms of grey mould during transit or storage.

Ringspot (*Microdochium panattonianum*) has been particularly problematic on romaine or cos-type lettuce during prolonged periods of wet weather in the last two to three years. Koike *et al*. (2007) describe the following conditions for pathogen survival and spread. The fungus can survive for up to 4 years as survival structures (microsclerotia) in soil. Cool wet conditions are required for infection to take place. Microsclerotia and spores (conidia) are spread by water splash. Optimum temperatures for disease development are approximately 18-20ºC and symptoms can appear 4-8 days after infection. Conidia can survive in soil and

on crop debris, with longevity of survival depending on soil type (Galea & Price, 1988). The pathogen can survive for 14-16 weeks in crop debris at the soil surface, 10-20 weeks when buried at 10 cm depth and 58-70 weeks on debris when suspended in the air (Galea & Price, 1988).

Sclerotinia rot (*Sclerotinia sclerotiorum*) can be devastating on lettuce crops particularly in wet seasons. The life-cycle of *S. sclerotiorum* includes both a soil-borne and an air-borne phase. Sclerotia of *S. sclerotiorum* can survive in the soil for ten years or more. They germinate to produce small funnel-shaped fruiting bodies (apothecia) that are approximately 1 cm in diameter. Apothecia produce air-borne spores, which can cause infection when they land on a susceptible host plant, either via flowers, or in the case of lettuce, by direct germination on leaves. New sclerotia develop in infected plant tissue and when the plant dies these remain on the soil surface or may become incorporated during subsequent soil cultivation. Sclerotinia rot affects a wide range of crops such as celery, carrot, and oilseed rape. Spores or sclerotia produced when any of these crops is affected can be a source of the disease on lettuce.

Conditions favouring the development of Sclerotinia can be summarised as follows (C. Young, ADAS, pers. comm.). After a period of cold conditioning in winter, sclerotia in the top 5 cm of the soil germinate from spring onwards to produce apothecia, when soil temperatures are 10^{o}C or higher and the soil is moist. Sclerotia do not germinate in dry soil or when the soil temperature is above 25^{o}C. Sclerotia buried below 5 cm in the soil are less likely to germinate. Once apothecia are fully formed, spore release can occur in the light or dark but is temperature dependent, so tends to peak around midday. Apothecia can last about 20 days at 15 to 20^{o}C, but shrivel after less than 10 days at 25^{o}C.

On lettuce, infection is mainly by air-borne spores landing directly on leaves. Spores can survive on leaves for several weeks until conditions favourable for leaf infection occur. Spore germination and infection depend on the presence of nutrients on leaves, either from plant wounds or senescing plant material. The optimum spore germination and infection conditions are 15-25^{o}C with continuous leaf wetness and high humidity. Once plant infection has occurred, rapid disease progress is favoured by warm (15-20^{o}C) and moist conditions in dense crops.

Spinach (*Spinacia oleracea*)

Downy mildew caused by *Peronospora farinosa* f. sp. *spinaciae* infects only spinach and a few *Chenopodium* weed species, such as fat hen. The following information is summarised from Koike *et al*., 2007. As for other downy mildews, this pathogen requires cool, wet

conditions for infection and disease development. Densely planted baby-leaf spinach retains much moisture and can create ideal conditions for infection and disease development. Spores may be dispersed between plants and fields by air currents and winds and to a lesser extent by splashing water. Long-lived spore structures (oospores) have been detected on seed and may also develop in leaf tissue; however the role of oospores in disease epidemiology remains unclear.

Anthracnose caused by *Colletotrichum dematium* is a common leaf spot disease on UK spinach with confirmed outbreaks in 2005, 2006 and 2007. Conditions favouring infection by *Colletotrichum* species include high relative humidity and leaf wetness. Once infection has taken place, spores develop in structures (acervuli) on the leaf surface and are readily spread to neighbouring plants or beds by overhead irrigation, rain-splash or wind-driven rain, resulting in rapid disease spread. Moisture is necessary for spore dispersal to occur. Anthracnose epidemics are sporadic but factors such as dense plantings, poor air circulation and low plant fertility can increase the risk of infection (Correll *et al*., 1994). *C. dematium* survives as dormant mycelium in infected plant debris and this is probably the primary source of inoculum for spinach anthracnose. The pathogen is also known to over-winter in the UK on volunteer spinach plants (Green, 2007). *C. dematium* can be seed-borne on spinach at low levels (Hernandez-Perez & du Toit, 2006). However, the relative importance of seed-borne inoculum in outbreaks of anthracnose on spinach has not been documented.

Cladosporium leaf spot (*Cladosporium variabile*) is frequently observed on spinach and has been reported from USA, Europe (including the UK) and Asia. In the UK, it is most severe under cool, wet autumn conditions. The fungus can grow and infect spinach under a wide range of temperatures, but a temperature range of 15-20^{o}C with relative humidity above 80% is most conducive to disease development (Fuentes-Dávila & Gabrielson, 1994). Spores of *C. variabile* can germinate and penetrate leaf stomata within 48 hours of inoculation, in the presence of free moisture and symptoms of the disease generally follow 4-10 days later (Inglis *et al*., 1997; du Toit & Derie, 2002). Once infection is established, the fungus grows in the leaf tissue and spores produced within leaf lesions start new infection cycles. Spores of *C. variabile* can be spread readily by air currents, wind, rain and irrigation splash, or carried on equipment. *C. variabile* is known to be seed-borne on spinach (Hernandez-Perez & du Toit, 2006; Green, 2007). Hernandez-Perez & du Toit (2005) demonstrated seed transmission of the fungus in greenhouse trials but this has not been proven under field conditions. Volunteer spinach can also serve as a ‘reservoir’ for the disease (du Toit & Derie, 2003).

Stemphylium leaf spot (*Stemphylium botryosum*; teleomorph *Pleospora herbarum*) has become increasingly widespread in spinach growing states of the USA, and was reported for the first time in the UK in 2006 (Green, 2006). Infection may take place over a wide range of conditions but is favoured by temperatures in the range 18-24°C and prolonged periods of leaf wetness. Spores may be spread by wind, rain splash, irrigation, and farm implements or workers. *S. botryosum* is seed-borne surviving either as mycelium and conidia, or as survival structures (pseudothecia) of the teleomorph (*Pleospora herbarum*) (Hernandez-Perez & du Toit, 2006). Transmission of *S. botryosum* from infected seeds to seedlings has been demonstrated in glasshouse experiments (Hernandez-Perez & du Toit, 2005). *S. botryosum* can survive on spinach crop debris. For example, *P. herbarum*, the teleomorph of *S. botryosum* was isolated consistently from spinach seed stalk debris and found to be pathogenic on spinach (du Toit & Derie, 2003). Fruiting bodies (pseudothecia) on crop debris discharge airborne ascospores which could be a primary source of inoculum in spring for infection of newly-planted spinach crops (du Toit & Derie, 2004). The same authors found that the viability of fruiting bodies on crop debris could be reduced to 10 weeks or less by debris burial in soil, compared to survival for at least 15 weeks on debris at the soil surface.

Baby leaf brassicas

Downy mildew on wild rocket (*Diplotaxis muralis*) is caused by the fungus *Hyaloperonospora parasitica* (previously *Peronospora parasitica*). It is a major problem in crops of some baby leaf crucifers particularly wild rocket as there has been limited breeding for resistance. Crops are grown at high density and are susceptible to downy mildew from emergence, with cotyledons and first true leaves being particularly susceptible. Ideal conditions were summarised by Koike *et al*., 2007: cool (10-15°C), moist conditions favour downy mildew sporulation, germination of sporangia and infection. Symptom development occurs most rapidly at 20°C and at this temperature the disease cycle may be completed in only 3 or 4 days. Spore dispersal is by air currents and water splash. Infection occurs via the leaf cuticle or stomata. If resting spores (oospores) are produced, these may survive in crop residues and in soil, providing sources of inoculum for subsequent crops if crop hygiene is inadequate (Kluczewski & Lucas, 1982). Some researchers claim that only 30 minutes leaf wetness if required for infection to occur such that many crops may be at risk of disease. Crop covers are used routinely to prevent damage from flea beetle but may contribute to downy mildew problems if air-flow is poor.

Alternaria leaf spot (*Alternaria brassicae*, *A. brassicicola*) affects all the major brassica types but can be particularly important on pak choi and baby leaf brassicas due to reduced leaf quality and appearance. Both *Alternaria* species can be seed-borne but may also survive on crop residues and on a range of crucifer crop (e.g. oilseed rape) and weed

species. Conditions for disease development are summarised by Koike *et al*. (2007) as follows: Twelve hours or more of 90% relative humidity are required for spore production to occur on leaf tissues and the process is also favoured by alternating light and dark periods. Temperature optima for spore production are 18-24°C for *A. brassicae* and 20-30°C for *A. brassicicola*. Spore release occurs as relative humidity decreases (e.g. during the afternoon). Spores are spread by air and water splash, and both species require a minimum leaf wetness period of 6 hours for infection to occur.

2.2.4 Summary

The knowledge review demonstrates that for many of the key pathogens considered on salad and baby leaf crops, dispersal spores may survive for at least 12 hours and often longer, under favourable conditions. This fact, together with the questionnaire finding that mesh re-use on a new crop can occur within 0-3 days of removal from a crop, and observations of diseases on crops under mesh, suggests that there is the potential for transfer of viable fungal inoculum to a subsequent crop via crop meshes.

The ability of different pathogens to survive on mesh for long periods is likely to vary according to spore type. In general, pathogens such as *Alternaria* species, *Stemphylium botryosum* and *Cladosporium variabile*, producing spores that are darkly-pigmented (resistant to damage from solar radiation), and thick-walled (less prone to desiccation), will have a greater chance of survival than colourless, thin-wall spores of e.g. downy mildews and *Colletotrichum dematium*. Spore size may also influence survival duration.

Based on the questionnaire and knowledge review, four pathogen/crop combinations were selected to use as model systems in experimental work (Table 1) to give more insight on survival of pathogens on meshes. This report includes results from experiments on *C. dematium*, *C. variabile* and *B. cinerea.* Development of a test system for rocket downy mildew is underway, in conjunction with HDC project FV 316 (Baby leaf crucifers: improving control of downy mildew), and experimental results will be incorporated in the next report.

Table 1. Key characteristics of pathogens to be used in crop covers survival experiments

Pathogen	Disease	Host	Spore dispersal	Long-term survival
Colletotrichum dematium	Anthracnose	Spinach	Water splash only	Spinach crop debris and volunteers
Cladosporium variabile	Leaf spot	Spinach	Air (& water splash)	Spinach crop debris and seed
Botrytis cinerea	Grey mould	Many E.g. lettuce	Air (& water splash)	Seed, crop debris, other hosts, soil

Hyaloperonospora parasitica	Downy mildew	Brassicas E.g. wild rocket	Air (& water splash)	Other hosts, (survival spores in crop debris, and maybe soil)

2.3 Laboratory study on longevity of pathogen survival on crop mesh

2.3.1 Introduction

A laboratory experiment was done to determine the duration of survival (up to 28 days) for three plant pathogens on crop mesh under either dry or moist conditions.

2.3.2 Methods

The duration of survival of three pathogens on pieces of crop mesh was tested as listed in Table 2.

Table 2. Treatments applied to crop mesh

No.	Pathogen applied to mesh	Source of pathogen	Storage regime
1	Sterile distilled water (SDW) – control	-	Dry
2	*Botrytis cinerea*	Lettuce	Dry
3	*Colletotrichum dematium*	Spinach	Dry
4	*Cladosporium variabile*	Spinach	Dry
5	Sterile distilled water (SDW) – control	-	Moist
6	*Botrytis cinerea*	Lettuce	Moist
7	*Colletotrichum dematium*	Spinach	Moist
8	*Cladosporium variabile*	Spinach	Moist

The experiment comprised a factorial design. For each treatment, 15 treated pieces of mesh were plated onto agar (three replicate plates of five pieces each) at each of seven intervals: 0, 1, 3, 7, 14, 21 and 28 days after treatment.

Crop mesh (Wondermesh, 0.8 mm mesh diameter) was cut into 1 cm^2 pieces and stored in a clean sealable polythene bag. On the day that they were required, the mesh pieces were surface sterilised by dipping in 90% ethanol for 5 minutes, ensuring that all the pieces were completely immersed. Using sterile forceps, the surface sterilised pieces were spread out in a plastic tray (wiped clean with 90% ethanol), to dry in a laminar flow cabinet.

Isolates of *Botrytis cinerea* from lettuce and *Cladosporium variabile* from spinach were cultured on potato dextrose agar amended with streptomycin (PDA+S), and incubated at approximately 20°C in the dark, to produce sporulating cultures. *Colletotrichum dematium* from spinach was cultured on ¼ strength PDA+S, and incubated at approximately 18°C under UV light to produce a sporulating culture. Spore suspensions of the three pathogens were prepared as follows: Approximately 20 ml SDW was poured onto a fungal culture and a

sterile loop used to dislodge spores. The suspension was filtered through muslin and the spore concentration determined using a haemocytometer and microscope. For each pathogen, a final volume of 300 ml was prepared to immerse the mesh pieces at a concentration of 1 x 10^5 spores/ml.

For each spore suspension (or SDW) and using sterile technique, 210 mesh pieces (plus spares) were immersed for 1 hour in the appropriate suspension, agitating periodically. Half of the pieces were dried in a laminar flow cabinet in plastic trays previously wiped with 90% ethanol. The other half of the pieces were left moist. Sterile technique was used to minimise cross-contamination between pieces of mesh treated with different pathogens.

For each spore suspension (or SDW), the wet mesh pieces were placed into seven Petri dishes (15 pieces per dish) each containing one piece of sterile (autoclaved) filter paper moistened with SDW. The dishes were sealed with Parafilm. The dry mesh pieces were placed into seven Petri dishes (15 pieces per dish) without filter paper, and sealed with parafilm. The plates were labelled with pathogen name, storage regime (wet or dry) and sampling duration (0, 1, 3, 7, 14, 21 or 28 days), and were then incubated at ambient temperature and light. A maximum/minimum thermometer was used to record the temperature range for each sampling duration.

At each sampling time, the appropriate mesh pieces (2 dishes of 15 pieces for each pathogen or SDW) were selected. The mesh pieces stored under moist conditions were placed in a plastic tray wiped clean with 90% ethanol in a laminar flow cabinet to dry before plating. All of the mesh pieces were then plated on to PDA+S (five pieces per plate), using sterile technique. The plates were labelled and incubated at 20°C in the dark. For each sampling time, the incidence of each target pathogen on the mesh pieces was assessed after approximately 7 days.

2.3.3 Results and discussion

The temperature range for mesh storage (both wet and dry) was 14 to 26°C.

Both *Botrytis cinerea* and *Cladosporium variabile* grew consistently from 100% of mesh pieces treated with these target pathogens and plated on agar (Table 3; Figures 1 and 2), irrespective of storage regime (wet or dry) or duration (up to 28 days). *Colletotrichum dematium* also grew from all mesh pieces that had been treated with this pathogen and stored in wet conditions, for up to 28 days (Table 2). Dry conditions had a deleterious effect on the viability of *C. dematium*, with the incidence of survival reduced to 20% after 7 days and 0% by 28 days (Figure 3). There was no fungal or bacterial development on the control

mesh pieces that were stored under dry conditions, even after 28 days. There was some fungal growth from mesh pieces stored under wet conditions (Table 3). The fungal growth was identified as *Cladosporium variabile* and it is considered that cross contamination by spore dispersal may have occurred when the control mesh pieces were left to dry in trays.

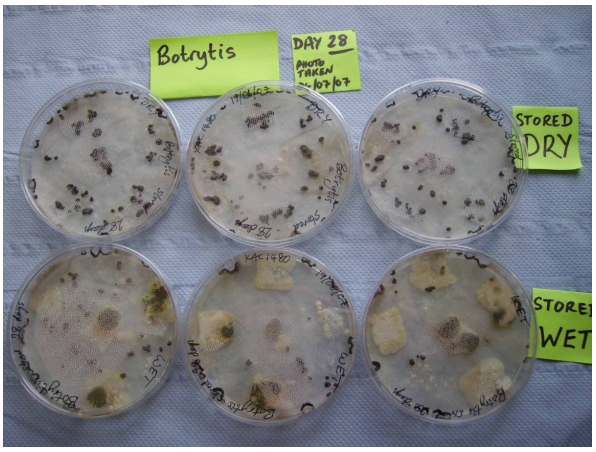

Figure 1. Growth of *Botrytis cinerea* from mesh pieces after 28 days storage under wet and dry storage regimes

Figure 2. Growth of *Cladosporium variabile* from mesh pieces after 28 days storage under wet and dry storage regimes

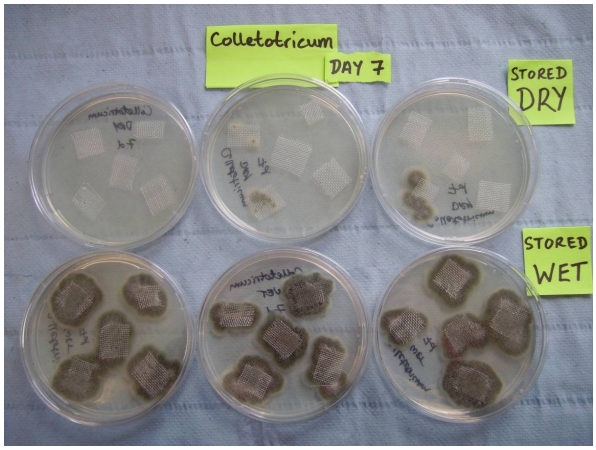

Figure 3. Growth of *Colletotrichum dematium* from mesh pieces after 7 days storage under wet and dry storage regimes

Table 3. Survival of fungal pathogens on crop mesh under wet and dry storage regimes for different storage durations

Pathogen	Mean number (of 5) mesh pieces with target pathogen after different durations													
	0 days		1 day		3 days		7 days		14 days		21 days		28 days	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Control	0	0	0.3	0	1.3	0	0.3	0	1	0	3.3	0	0	0
B. cinerea	5	5	5	5	5	5	5	5	5	5	5	5	5	5
C. dematium	5	5	5	5	5	4	5	1	5	0	5	0.7	5	0
C. variabile	5	5	5	5	5	5	5	5	5	5	5	5	5	5

2.4 Transmission of *Colletotrichum dematium* from spinach via crop mesh

2.4.1 Introduction

An experiment was done to determine whether the fungal pathogen *Colletotrichum dematium* (cause of spinach anthracnose) could be i) transferred via crop covers and ii) infect a new crop.

2.4.2 Methods

Production of diseased plants and infested meshes

Sixty half-size seed trays were sown with spinach (var. Lazio) in F1 compost, with 15 seeds (thinned to ten seedlings) per tray. Six seed trays were placed in each of ten plastic chitting trays and maintained in a glasshouse until the seedlings had reached the two true leaf stage. The spinach plants were inoculated to the point of run-off using a hand mister containing a spore suspension of *Colletotrichum dematium* (*ex* spinach) at a concentration of 7.5 x 10^5 spores/ml. The spore suspension was prepared from sporulating cultures of *C dematium* using a similar method to that described in Section 1.3.2. Trays were covered with polythene for 16-24 h after inoculation, in order to maintain conditions of leaf wetness and high relative humidity conducive for disease development.

Once symptoms of spinach anthracnose (confirmed microscopically) had developed on the inoculated plants, the trays were covered with pieces of crop mesh (Wondermesh, 0.8 mm mesh diameter) measuring approximately 1 x 2 m^2 as follows: for five potato chitting trays (each containing six half-trays spinach), pieces of crop mesh were wrapped over the trays, in contact with the diseased plants. For the other five trays, pieces of crop mesh were wrapped over the trays but suspended above the diseased plants by approximately 30 cm using plastic pegs. The diseased plants covered with mesh were maintained in a glasshouse for approximately 4 weeks with overhead watering through the mesh as required to maintain moist but not waterlogged compost.

Production of test plants

A second set of healthy spinach plants (var. Lazio) was raised on a hard standing area to use as test plants. There were 48 half-size seed trays each sown with 15 seeds in F1 compost, then thinned to ten seedlings. Four trays were placed in each of 12 chitting trays, which were raised up and covered with clean mesh to prevent rabbit and insect damage. The plants were maintained and overhead watered until they reached the 6-8 true leaf stage.

Mesh transfer

The healthy spinach plants were covered with mesh pieces from three different sources with mesh in contact with the plants:

1. Clean crop mesh (control), not previously in contact with plants
2. Crop mesh that had previously been placed over, and in contact with spinach plants with symptoms of anthracnose (*C. dematium*)
3. Crop mesh that had previously been placed over, but suspended above spinach plants with symptoms of anthracnose (*C. dematium*).

For each treatment, a piece of mesh was placed over a chitting tray containing four seed trays each containing ten healthy spinach plants. There were four replicates of each treatment. Trays for treatment 1 were positioned more than 5 m away from treatments 2 and 3, to reduce risk of cross infection. The trays were maintained on a hard standing area.

Assessments

Plants were assessed 28 days after mesh transfer, for the incidence of anthracnose lesions on individual plants. Symptoms were confirmed as being due to *C. dematium* if black setae (spiny structures) and conidia characteristic of the species could be observed microscopically within lesion centres (Sutton, 1992). To aid symptom identification on some plants, individual leaves were placed in a Petri dish sealed with parafilm and incubated in the dark at 20^{o}C, to encourage the development of fungal structures. Data were examined statistically using generalised linear models (GLM) in Genstat.

Laboratory studies

For each of the three treatments, a spare fifth piece of mesh was used for two laboratory studies to check for the presence of *C. dematium* :

i) A 20 x 30 cm piece was cut from the centre of a mesh piece from each treatment, and placed in a plastic tray previously wiped with 90% ethanol. 500 ml of sterilised distilled water was added to each tray and agitated for 45 min. For each treatment, 1 ml of the washing water was pipetted out and used to give a 1:100 and 1:1000 dilution. An aliquot of 100 ul of each dilution was then plated onto each of 3 plates of PDA+S and spread evenly using a sterile plastic loop. The plates (18 in total; three treatments x two dilutions x three replicate plates) were incubated under UV light at approximately 20^{o}C.

ii) Twenty five squares (1 cm^2) were cut from the centre of each mesh treatment, and plated onto five plates of PDA+S (five mesh squares per plate). The plates were incubated at 20^{o}C in the dark for 4 days then transferred to UV light (approximately

20ºC) for 2 days. The proportion of mesh pieces on which colonies of *C. dematium* had developed was determined for each treatment.

2.4.3 Results and discussion

Symptoms of anthracnose were confirmed on the first batch of spinach plants 7 days after inoculation with *C. dematium*. During the period when the trays were under meshes, symptoms became severe such that the leaves were covered with anthracnose lesions and had become desiccated.

Symptoms developed more slowly on the spinach plants to which meshes had been transferred, with lesions first visible 21 days after mesh transfer. For the control treatment, one plant (out of nine) in a single tray was affected with anthracnose lesions, giving an overall incidence of 0.7%. Mesh for this treatment was taken directly from a new roll stored inside, so it is probable that symptom development occurred due to cross-infection from another treatment. Symptom development was more prevalent on the other two treatments with 19.2% of plants affected following transfer of mesh that had been in contact with plants, and 10.6% of plants affected following transfer of mesh that had been suspended over plants. There was no significant effect of mesh positioning from the previous diseased crop (in contact or suspended over plants) on the proportion of plants affected after mesh transfer.

In laboratory studies, there was no development of *C. dematium* (or any other micro-organisms) on agar following serial dilution of washings from mesh pieces. However, when mesh pieces were plated directly on agar, colonies of *C. dematium* developed from 3 out of 25 mesh pieces from treatment 2 (covers in contact with plants) and 1 out of 25 mesh pieces from treatment 3 (covers suspended over plants). Other fungi observed included a *Cladosporium* species (not *C. variabile* that is pathogenic to spinach) on all of the mesh pieces, and occasional development of *Fusarium* sp., *Penicillium* sp. and *Botrytis cinerea*. Nil colonies developed from the control mesh pieces. The mesh pieces had dried out before they were used for the laboratory studies, such that survival of *C. dematium* may have been reduced, in agreement with findings from the laboratory study on longevity of pathogen survival on mesh (Section 1.3.3).

These results strongly suggest that *C. dematium* can remain viable on mesh at sufficient levels to infect a subsequent crop.

2.5 Transmission of *Cladosporium variabile* from spinach via crop mesh

2.5.1 Introduction

An experiment was done to determine whether the fungal pathogen *Cladosporium variabile* (cause of Cladosporium leaf spot on spinach) could be i) transferred via crop covers and ii) infect a new crop.

2.5.2 Methods

Two experiments were done using similar methods to those described in Section 1.4.2., except that the test pathogen was *Cladosporium variabile*.

Production of diseased plants and infested meshes

Methods for raising plants, inoculation, and mesh coverings were as in Section 1.4.2, except for the following:

For Experiment 1, meshes were placed on spinach plants (var. Lazio) that were already showing typical symptoms of Cladosporium leaf spot, following artificial inoculation with *C. variabile* for another HDC project (FV 268a). Plants were raised and maintained in a polytunnel.

For Experiment 2, The spinach plants were inoculated to the point of run-off using a hand mister containing a spore suspension of *Cladosporium variabile* (*ex* spinach) at a concentration of 5 x 10^5 spores/ml. The spore suspension was prepared from sporulating cultures of *C. variabile* using a similar method to that described in Section 1.3.2. Trays were covered with polythene for 16-24 h after inoculation, in order to maintain conditions of leaf wetness and high relative humidity conducive for disease development. Plants were raised on a hard standing area.

Production of test plants

Methods were as in Section 1.4.2., except healthy plants for Experiment 1 were raised in a polytunnel.

Mesh transfer

Methods were mainly as described in Section 1.4.2. For Experiment 1, plant trays were maintained in a polytunnel with overhead watering. Control plants were positioned approximately 5 m from plants for treatments 2 and 3. For Experiment 2, plants were maintained outside on hard standing areas with overhead watering by hand. Control plants were positioned at least 20 m from treatments 2 and 3.

Assessments

For Experiment 1, plants were assessed 21 days after mesh transfer for the incidence of Cladosporium lesions on individual plants and number of lesions per plant. Symptoms were confirmed as being due to *C. variabile* if grey/green sporing structures typical of the species could be observed microscopically within lesion centres (Ellis, 1971). To aid symptom identification on some plants, individual leaves were placed in a Petri dish sealed with parafilm and incubated in the dark at 20°C, to encourage the development of fungal structures.

For Experiment 2, plants were assessed approximately 28 days after mesh transfer for the incidence of Cladosporium lesions on individual plants. Data were examined statistically using generalised linear models (GLM) in Genstat.

Laboratory study

For each of the three treatments, a spare fifth piece of mesh was used for a laboratory studies to check for the presence of *C. variabile*. Twenty five squares (1 cm^2) were cut from the centre of each mesh treatment, and plated onto five plates of PDA+S (five mesh squares per plate). The plates were incubated at 20°C in the dark. The proportion of mesh pieces on which colonies of *C. dematium* had developed was determined for each treatment 9-11 days after plating.

2.5.3 Results and discussion

In both experiments, lesions were first visible on spinach plants to which meshes had been transferred about 10 days after mesh transfer.

In both experiments, lesions of Cladosporium leaf spot developed on plants even when they were placed under clean meshes (Table 4). However, statistical analysis of results from Experiment 2 showed that the incidence of plants affected, was significantly lower than for plants under infested meshes (P<0.05). *Cladosporium variabile* is easily air-borne and may also be spread by water splash, such that cross-infection occurred even when plants were located more than 20 m from infected meshes (Experiment 2). In Experiment 1, there was a trend for lower disease incidence and severity following transfer of crop meshes that had been suspended rather than placed in contact with infected plants. However, in Experiment 2, there was no significant difference in disease incidence between the two treatments.

Table 4. Incidence and severity of Cladosporium leaf spot on spinach plants under different crop mesh treatments

	Mean no. lesions per plant	**Mean % plants affected**	
Treatment	**(Experiment 1)**	Experiment 1	Experiment 2

Clean mesh	Not assessed	25.0 (estimate)	25.7
Mesh previously in contact with Cladosporium-infected plants	6.0	100.0	99.4
Mesh previously suspended over Cladosporium-infected plants	1.8	88.1	98.4

When mesh pieces were plated directly on agar, colonies of *C. variabile* developed from 22 out of 25 mesh pieces from treatment 2 (covers in contact with plants) and 4 out of 25 mesh pieces from treatment 3 (covers suspended over plants). In agreement with results from Experiment 1, there was a reduction in pathogen incidence on mesh pieces that had been suspended rather than placed in contact with infected plants. Other fungi that survived included a *Fusarium* sp. on all mesh pieces from treatment 2, and 22 out of 25 mesh pieces from treatment 3. There was also occasional development of another *Cladosporium* sp., *Penicillium* sp., *Alternaria* sp. and *Botrytis cinerea*. From the control treatment there was colony growth of each of *Cladosporium variabile*, *Cladosporium* sp., *Penicillium* sp. on 1 out of 25 mesh pieces.

The results provide strong evidence that *C. variabile* can remain viable on mesh at sufficient levels to infect a subsequent crop. There was a trend for higher inoculum levels and disease incidence following use of mesh that had been in direct contact with affected plants.

2.6 Transmission of *Botrytis cinerea* from lettuce via crop mesh

2.6.1 Introduction

An experiment was done to determine whether the fungal pathogen *Botrytis cinerea* (cause of grey mould on lettuce) could be i) transferred via crop covers and ii) infect a new crop.

2.6.2 Methods

Production of diseased plants and infested meshes

Healthy lettuces var. Frisco (romaine type) potted in compost (F2 plus sand) in 1 L pots (four per pot) and raised in a glasshouse were used for inoculation. The lettuces were selected for use as they were mature with some senescing tissue that would be susceptible to infection by *B. cinerea*. The plants were placed, 6 pots in each of 10 chitting trays, in a polytunnel with overhead watering. The lettuces were inoculated to the point of run-off using a hand-held

mister containing a spore suspension of *Botrytis cinerea* (*ex* lettuce) at a concentration of 7.75 x 10^5 spores/ml. The spore suspension was prepared from sporulating cultures of *B. cinerea* using a similar method to that described in Section 1.3.2. Trays were covered with polythene for 48 h after inoculation, in order to maintain conditions of leaf wetness and high relative humidity conducive for disease development. Subsequently the trays were overhead watered regularly and side vents of the polytunnel kept closed, also to maintain leaf wetness and high relative humidity.

Once symptoms of grey mould due to *B. cinerea* had developed on the inoculated plants (approximately 14 days), the trays were covered with pieces of crop mesh as described in Section 1.4.2. The diseased plants covered with mesh were maintained in a polytunnel for approximately 3 weeks with overhead watering through the mesh as required to maintain moist but not waterlogged compost, and side vents of the polytunnel closed to maintain high relative humidity.

Production of test plants

A second set of healthy but senescing lettuce plants (var. Frisco) raised in a glasshouse in 1 L pots (four plants per pot) were used as test plants.

Mesh transfer

The healthy lettuce plants were covered with mesh pieces from three different sources with mesh in contact with the plants:

1. Clean crop mesh (control), not previously in contact with plants
2. Crop mesh that had previously been placed over, and in contact with lettuce plants with symptoms of grey mould (*B. cinerea*)
3. Crop mesh that had previously been placed over, but suspended above spinach plants with symptoms of grey mould (*B. cinerea*).

For each treatment, a piece of mesh was placed over a chitting tray containing six pots each containing four healthy lettuce plants. There were four replicates of each treatment. Trays for treatment 1 were positioned more than 5 m away from treatments 2 and 3, to reduce risk of cross infection. The trays were maintained in a polytunnel with overhead watering and side vents closed.

Assessments

Plants were assessed 28 days after mesh transfer, for the incidence of grey mould due to *B. cinerea* on individual plants (confirmed under a low power microscope by the presence of transparent spores on thick brown aerial conidiophores). Symptoms were assessed separately on the lower third of the plant (including the stem) and subsequently on the upper

leaves, after incubation for 72 h in polythene bags. Data were examined statistically using generalised linear models (GLM) in Genstat.

Laboratory studies

For each of the three treatments, a spare fifth piece of mesh was used for two laboratory studies to check for the presence of *B. cinerea* :

i) A 20 x 30 cm piece was cut from the centre of a mesh piece from each treatment, and placed in a plastic tray previously wiped with 90% ethanol. 500 ml of sterilised distilled water was added to each tray and agitated for 45 min. For each treatment, 1 ml of the washing water was pipetted out and used to give a 1:100 and 1:1000 dilution. An aliquot of 100 ul of each dilution was then plated onto each of 3 plates of PDA+S and spread evenly using a sterile plastic loop. The plates (18 in total; three treatments x two dilutions x three replicate plates) were incubated under UV light at approximately 20^{o}C.

ii) Twenty five squares (1 cm^2) were cut from the centre of each mesh treatment, and plated onto five plates of PDA+S (five mesh squares per plate). The plates were incubated at 20^{o}C in the dark for 4 days. The proportion of mesh pieces on which colonies of *B. cinerea* had developed was determined for each treatment.

2.6.3 Results and discussion

Grey mould typical of *B. cinerea* was visible on lettuce plants to which meshes had been transferred about 21 days after mesh transfer.

Symptoms of grey mould developed on lettuce even when they were placed under clean meshes (Table 5). Despite an apparently higher incidence of grey mould on bases of plants from treatment 2, there was no significant treatment effect. The incidence of grey mould on upper leaves was significantly higher for treatment 2 (meshes previously in contact with plants), compared to treatments 1 and 3 (*P*=0.019).

Table 5. Incidence of grey mould (*Botrytis cinerea*) on lettuce plants under different crop mesh treatments

	Mean % plants affected	
Treatment	Lower 1/3 of plant	Upper plant
Clean mesh	21.9	6.3
Mesh previously in contact with Cladosporium-infected plants	38.5	21.9

Mesh previously suspended over Cladosporium-infected plants	24.0	5.2

In laboratory studies, there was no development of *B. cinerea* or other micro-organisms following serial dilution of washings from mesh pieces. However, when mesh pieces were plated directly on agar, colonies of *B. cinerea* developed from 24 out of 25 mesh pieces from treatment 2 (covers in contact with plants) and 25 out of 25 mesh pieces from treatment 3 (covers suspended over plants). Although *B. cinerea* developed from all the treatment 3 mesh pieces, it was observed that colony development was generally slower than from treatment 2 mesh pieces, suggesting lower spore density on mesh pieces that had been suspended rather than placed in contact with infected plants. There was also occasional development of a *Fusarium* sp. (Treatments 2 and 3) and a *Penicillium* sp. (treatment 3 only). There was no fungal growth on mesh pieces from the control treatment.

B. cinerea is a ubiquitous pathogen spread by air currents and water splash. The results confirm that grey mould can develop on senescing lettuce both in the presence and absence of infested meshes. However, results from the plant and laboratory study indicated there was a trend for higher inoculum levels and disease incidence following use of mesh that had been in direct contact with affected plants.

2.7 Conclusions

- Responses to a grower questionnaire emphasised the diversity of crops for which crop meshes are used. In addition, the repeated re-use of crop mesh in a single growing season was demonstrated. Of a range of diseases observed on crops under meshes, downy mildew on rocket was considered the most problematic, with two growers abandoning use of mesh on this crop because of associated disease risk.
- The knowledge review highlighted a range of key pathogens on salad crops that could potentially be transferred via crop meshes. The ability of different pathogens to survive on mesh for long periods is likely to vary according to spore type. In general, pathogens such as *Alternaria* species, *Stemphylium botryosum* and *Cladosporium variabile*, producing spores that are darkly-pigmented (resistant to damage from solar radiation), and thick-walled (less prone to desiccation), will have a greater chance of survival than colourless, thin-wall spores of e.g. downy mildews and *Colletotrichum dematium*. Spore size may also influence survival duration.
- On the basis of the knowledge review and questionnaire, four crop/pathogen systems were selected for further study of potential pathogen transfer on crop meshes: grey mould (*Botrytis cinerea*) on lettuce, Cladosporium leaf spot (*Cladosporium variabile*) on spinach, anthracnose (*Colletotrichum dematium*) on spinach, and downy mildew (*Hyaloperonospora parasitica*) on wild rocket.
- A laboratory experiment demonstrated that in a temperature range of 14-26^{o}C and ambient light, *Botrytis cinerea* (grey mould) and *Cladosporium variabile* (Cladosporium leaf spot on spinach) can remain viable for at least 1 month on pieces of crop mesh stored either wet or dry. Similarly, spores of *Colletotrichum dematium* (spinach anthracnose) remained viable on mesh pieces for at least one month when stored wet. However, under dry conditions, percentage viability of *C. dematium* was reduced to 20% after 7 days storage and to 0% by 28 days.
- Experiments were done to determine whether each of three fungal pathogens could be transferred via crop covers and infect a new crop. *C. dematium* (spinach anthracnose) remained viable on mesh at sufficient levels to infect a subsequent crop of spinach, although there was no significant effect of mesh positioning on the previous diseased crop (in contact or suspended over plants) on the proportion of plants affected after mesh transfer. For *Cladosporium variabile* and *Botrytis cinerea*, there was strong evidence that each of these fungi could remain viable on mesh at sufficient levels to infect a subsequent crop. For each, there was a trend for higher inoculum levels and disease incidence following use of mesh that had been in direct contact with affected plants.

- In further studies on pathogen survival on mesh, methods will be developed to quantify inoculum on mesh pieces at different sampling times, to enable a more accurate prediction of decline in spore numbers over time. This could be done, for example by examination of mesh under high power magnification, or by spore washing from mesh pieces and determination of spore numbers present using a haemocytometer.
- Further work may now be warranted to investigate measures such as disinfection that could be used to reduce the risk of pathogen transfer via crop meshes.

2.8 Technology transfer

A questionnaire was circulated to growers via the SPGA, and subsequently via the HDC.

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2.10 Acknowledgements

The assistance of growers who responded to the project questionnaire is gratefully acknowledged.

3 APPENDIX 1. CROP COVERS QUESTIONNAIRE

HDC FV 283 Salads and baby leaf brassicas: Pathogen survival on crop covers and potential disease spread

The aim of this project funded by the Horticultural Development Council is to determine the role of crop meshes in the survival and spread of foliar pathogens on salads and baby leaf brassicas. Please complete the following form about crop mesh use to enable us to collate information on crop situations for which the re-use of meshes could increase the risk of disease spread.

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1. Name of farm *(this will not be disclosed in reporting)*:

2. County:

3. Please list the salad crops/types on which crop meshes are used at your farm and tick the relevant boxes to show when and what type of meshes are generally used:

Crop/type	When is mesh used:			Type of mesh used
	Early season	Mid season	Late season	
E.g. cos lettuce	✓			

4. Please tick relevant boxes in the table to show the reasons that meshes are used for different crops. Add extra crops or reasons if necessary.

Crop	Reasons for using meshes?				
	Avoid hail damage	Protection from pests	To advance crop growth	Other?	Other?
Lettuce					
Spinach					
Baby leaf brassicas					
Endive					
Others?:					

5. Do you re-use meshes on crops in the same growing season? Yes / No

6. Do you re-use meshes on crops in the following season? Yes / No

7. Do you re-use meshes in one country that have previously been used on crops in another country? Yes / No

8. Are meshes in contact with the crop or supported above the crop?

9. Do you wash or disinfect meshes before re-use? Yes / No

If yes, please give details of prodecures used:

10. Have you seen disease symptoms on crops under meshes? Yes / No

If yes, please list the crop and disease (e.g. lettuce downy mildew)

In 2007:

In previous years:

Thank you for your co-operation. Please return your form to Kim Green by 22 June 2007:

Email kim.green@adas.co.uk

Fax 01354 694488

Or mail
ADAS Arthur Rickwood
Mepal, Ely
Cambs. CB6 2BA