Project title:	Salads and baby leaf brassicas: pathogen survival on crop covers and potential disease spread
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Project leader:	Dr Kim Green, ADAS UK Ltd
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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.

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GROWER SUMMARY

Headline

A series of laboratory experiments demonstrated that three pathogens (*Colletotrichum dematium* and *Cladosporium variabile* on spinach, and *Botrytis cinerea* on lettuce) could survive on crop mesh for at least 6 months but that viability was affected by environmental conditions. Results from a single experiment showed that spores of downy mildew (*Hyaloperonospora parasitica*) could be transferred via crop mesh to subsequently cause disease on wild rocket plants.

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 project involves 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 objective 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 on rocket was considered the most problematic, with two growers abandoning use of mesh on this crop because of associated disease risk.

In addition to salad crop / pathogen combinations highlighted in the questionnaire, discussions with growers in 2008 highlighted further crops that might be at risk of pathogen transfer via meshes and fleeces. These included parsley (e.g. downy mildew and septoria) and watercress (fungal and bacterial leaf spots).

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-walled 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 A.

Table A. 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 series of laboratory experiments was done in project year 2 to determine the duration of survival (up to 6 months) for three plant pathogens on crop mesh under a range of conditions. Mesh pieces (approximately 1 cm^2) were dipped in a spore suspension of the test pathogen. The treated mesh pieces were then stored in sealed Petri dishes under defined conditions for different durations before plating on agar to test for pathogen viability (Figure 1).

The first experiment investigated pathogen survival on mesh under dry or wet conditions. This was an extension of a study done in project year 1 that had looked at pathogen survival over 1 month. 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 6 months on pieces of crop mesh stored either wet or dry, although the viability of *B. cinerea* decreased with time on wet mesh (Table B). Spores of *Colletotrichum dematium* (spinach anthracnose) remained viable on mesh pieces for at least six months when stored wet. However, under dry conditions, percentage viability of *C. dematium* was reduced to 0% after 7 days storage, although a trace of growth was recorded after 3 and 6 months storage.

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

Pathogen	Moisture regime	Mean no. (of 5) mesh pieces with target pathogen after different durations					
		0 days	7 days	14 days	28 days	3 months	6 months
Control	Dry	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	Dry	5.0	5.0	3.0	5.0	4.7	4.0
C. dematium	Dry	5.0	0.0	0.0	0.0	0.7	0.7
C. variabile	Dry	5.0	5.0	5.0	5.0	5.0	5.0
Control	Wet	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	Wet	5.0	4.3	2.3	4.0	1.0	1.3
C. dematium	Wet	4.7	5.0	5.0	5.0	5.0	5.0
C. variabile	Wet	5.0	5.0	4.7	4.3	5.0	4.0

A second experiment investigated the effects of storage under different temperature regimes (3-6°C, 15-20°C and 30-35°C) on pathogen viability. *Botrytis cinerea* was eliminated on mesh stored at 30-35°C for 14 days and was the only one of the three pathogens tested to be controlled by the higher temperature treatment.

A third experiment studied the effects of storage under different light regimes (dark, ambient light and UV-A). *B. cinerea* and *Colletotrichum dematium* were eliminated on mesh following storage under UV-A light for 3 months. *Cladosporium variabile* remained unaffected, due possibly to thicker walled and more darkly pigmented spores.

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

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.

Rocket downy mildew

A series of experiments was done to investigate i) survival of *Hyaloperonospora parasitica* on meshes, and ii) transfer of the pathogen via meshes to infect a new crop. *H. parasitica* is an obligate parasite (surviving only as a parasite on the host) and so cannot be cultured on agar media. For this reason, viability of the pathogen on meshes was tested by placing the mesh onto a healthy crop and checking for symptom development, rather than by plating on to agar as was done for the other three test pathogens.

In one experiment in a polytunnel, typical symptoms of downy mildew developed after 28 days on trays of wild rocket seedlings, when mesh pieces were transferred directly from plants with downy mildew symptoms. No symptoms developed on trays of plants that had been covered with clean previously unused meshes (control treatment). Results from Experiment 1 demonstrated that there is scope for transfer of downy mildew spores via crop mesh and subsequent disease development on a healthy crop. This result occurred when the original crop showed abundant sporulation and when meshes were transferred immediately from the diseased plants to the healthy trays. Two subsequent experiments were done to verify this finding, but the results could not be reproduced. This may be because although plants used as a source of infestation had severe symptoms of downy mildew (leaf chlorosis and black speckling), there were only low levels of spore production.

A laboratory experiment was done to look at the duration for which spores of *H. parasitica* remained viable on crop mesh, but results for disease development were negative throughout, even when mesh pieces were transferred immediately from the spore suspension to test plants. This may have been because spore numbers on the treated mesh pieces were too low, or perhaps environmental conditions for downy mildew infection and symptom development were sub-optimal, despite use of conditions considered favourable for the disease in controlled environment cabinets.

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 6 months under a range of environmental conditions. For these pathogens, the risk of pathogen transfer may be reduced but not eliminated by extending the duration between use of a particular mesh on successive crops.
- There is also evidence to suggest that downy mildew spores can be transferred between crops of wild rocket on mesh, although the longevity of spore survival on the mesh has not been determined.
- 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.
- Pathogen viability may also be reduced by high temperatures and bright sunlight.
- Further work may be warranted to investigate the efficacy and practicality of measures such as disinfection, heat or UV treatment that could be used to reduce the risk of pathogen transfer via crop meshes.

SCIENCE SECTION

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 2 to study the effect of environmental conditions (moisture, temperature and light regimes) on the survival of three key foliar pathogens on crop meshes over time. Experiments were also done to determine whether *Hyaloperonospora parasitica* (cause of rocket downy mildew) could survive on crop mesh. The commercial objective of the project was to enable growers to make more informed decisions about re-use and potential treatment of meshes based on improved knowledge of pathogen survival on meshes and risk to subsequent crops.

Pathogen / crop combinations

Based on a questionnaire, knowledge review and experimental work in project year 1, 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.

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)

Effect of moisture on the longevity of pathogen survival on crop mesh

Introduction

A laboratory experiment was done to determine the duration of survival (up to 6 months) for three plant pathogens on crop mesh when stored under different moisture regimes. This was an extension of a study done in project year 1 that had looked at pathogen survival over 1 month.

Methods

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

Table 2. Moisture treatments applied to crop mesh

No.	Pathogen applied to mesh	Source of pathogen	Moisture 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	-	Wet
6	*Botrytis cinerea*	Lettuce	Wet
7	*Colletotrichum dematium*	Spinach	Wet
8	*Cladosporium variabile*	Spinach	Wet

For each treatment, 15 treated pieces of mesh were plated onto agar (three replicate plates of five pieces each) at each of six intervals: 0 days, 7 days, 14 days, 28 days, 3 months and 6 months 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 air 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^{o}C in the dark, to produce sporulating cultures. *Colletotrichum dematium* from spinach was cultured on PDA+S, and incubated at approximately 18^{o}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, 180 mesh pieces (plus spares) were immersed for 1 hour in the appropriate suspension, agitating periodically. Half of the pieces were air 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 six Petri dishes (15 pieces per dish plus spares) 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 six Petri dishes (15 pieces plus spares 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 day, 7 days, 14 days, 28 days, 3 months or 6 months), 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 (one dish of 15 pieces for each treatment combination) 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 air dry before plating. All of the mesh pieces were then plated on to PDA+S (five pieces per plate), using sterile technique. The dishes were labelled and incubated at 20^{o}C in the dark. For each sampling time, the incidence of each target pathogen on the mesh pieces was assessed after approximately 7 days.

For each treatment, three spare mesh pieces from day 0 that had been dipped in spore suspension were placed in 5 ml SDW containing 0.1% v/v Tween 80. The spore concentration in the liquid was subsequently checked using microscope and haemocytometer, and the number of spores per mesh piece calculated by extrapolation.

Results and discussion

The temperature range for mesh storage (both wet and dry) was 14 to 26^{o}C.

Spore counts on mesh pieces from Day 0, indicated that for *Botrytis cinerea*, *Cladosporium variabile* and *Colletotrichum dematium*, there were approximate mean numbers of 6250, 10,400, and 12,500 spores per cm^2 mesh, respectively.

The proportion of mesh pieces from which different target pathogens grew after different storage regimes and durations is summarised in Table 3. Results up to 28 days reflected findings from a similar experiment done in project year 1.

Control mesh pieces (dipped in SDW) and stored wet or dry remained free of the target pathogens throughout the experiments.

The incidence of viable *Botrytis cinerea* on dry mesh remained high throughout the storage period with the pathogen isolated from at least 60% of mesh pieces, irrespective of storage duration. For dry mesh pieces, there was a trend for pathogen viability to decline over time.

Cladosporium variabile grew consistently from 100% of mesh pieces that had been stored dry, even after 6 months storage. The incidence of recovery was also high from wet mesh pieces (80% or more), indicating that moisture levels have little effect on the survival of this pathogen over time.

Colletotrichum dematium grew consistently from mesh pieces that had been stored wet, and was isolated from 100% of mesh pieces that had been stored for 6 months. In contrast, the viability of *C. dematium* was substantially reduced under dry conditions (in agreement with results from year 1) with nil recovery of the pathogen after 7 days and very low levels of recovery after 6 months storage.

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

Pathogen	Moisture regime	Mean no. (of 5) mesh pieces with target pathogen after different durations					
		0 days	7 days	14 days	28 days	3 months	6 months
Control	Dry	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	Dry	5.0	5.0	3.0	5.0	4.7	4.0
C. dematium	Dry	5.0	0.0	0.0	0.0	0.7	0.7
C. variabile	Dry	5.0	5.0	5.0	5.0	5.0	5.0
Control	Wet	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	Wet	5.0	4.3	2.3	4.0	1.0	1.3
C. dematium	Wet	4.7	5.0	5.0	5.0	5.0	5.0
C. variabile	Wet	5.0	5.0	4.7	4.3	5.0	4.0

Plates with dry mesh pieces of the control treatment remained clean (no microbial growth) even after 6 months storage. For all other treatments, micro-organisms other than the target pathogens developed on agar plates when mesh pieces were plated out, particularly from 14 days storage onwards and on wet mesh pieces. The contaminants were mainly ubiquitous saprophytic fungi such as *Penicillium* species and *Cladosporium* species (distinguished from *C. variabile*) as well as a range of bacterial species.

Effect of temperature on the longevity of pathogen survival on crop mesh

Introduction

A laboratory experiment was done to determine the duration of survival (up to 6 months) for three plant pathogens on crop mesh when stored under different temperature regimes.

Methods

The duration of survival of three pathogens on pieces of crop mesh was tested as listed in Table 4.

Table 4. Temperature treatments applied to crop mesh

No.	Pathogen applied to mesh	Source of pathogen	Temperature regime (°C)
1	Sterile distilled water (SDW) – control	-	3-6
2	*Botrytis cinerea*	Lettuce	3-6
3	*Colletotrichum dematium*	Spinach	3-6
4	*Cladosporium variabile*	Spinach	3-6
5	Sterile distilled water (SDW) – control	-	15-20
6	*Botrytis cinerea*	Lettuce	15-20
7	*Colletotrichum dematium*	Spinach	15-20
8	*Cladosporium variabile*	Spinach	15-20
9	Sterile distilled water (SDW) – control	-	30-35
10	*Botrytis cinerea*	Lettuce	30-35
11	*Colletotrichum dematium*	Spinach	30-35
12	*Cladosporium variabile*	Spinach	30-35

For each treatment, 15 treated pieces of mesh were plated onto agar (three replicate plates of five pieces each) at each of six intervals: 0 days, 7 days, 14 days, 28 days, 3 months and 6 months 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 air dry in a laminar flow cabinet.

Cultures of the three pathogens and spore suspensions of each were prepared as described in Section 2.3.2. Spore suspensions of each pathogen were prepared giving a final volume of 300 ml, 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, 270 mesh pieces (plus spares) were immersed for 1 hour in the appropriate suspension, agitating periodically.

For each spore suspension (or SDW), the mesh pieces were placed moist into 18 Petri dishes (15 pieces per dish plus spares). The dishes were sealed with Parafilm. The dishes were labelled with pathogen name, temperature treatment and sampling duration (0 day, 7 days, 14 days, 28 days, 3 months or 6 months). Plates to be stored at 3-6°C were placed in a refrigerator; plates to be stored at 15-20°C were placed in an incubator; plates to be stored at 30-35°C were placed in a heated propagator unit. All plates were kept in the dark by storing in black plastic bags.

At each sampling time, the appropriate mesh pieces (one dish of 15 pieces for each treatment combination) were selected. When mesh pieces were still moist they were placed in a plastic tray wiped clean with 90% ethanol in a laminar flow cabinet to air 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.

Results and discussion

The proportion of mesh pieces from which different target pathogens grew after different temperature regimes and storage durations is summarised in Table 5.

Control mesh pieces (dipped in SDW) and stored wet or dry remained free of the target pathogens throughout the experiments.

Botrytis cinerea was eliminated on mesh stored at 30-35°C for 14 days and was the only one of the test pathogens to be controlled by the higher temperature treatment. The fungus remained viable at low temperatures for at least 3 months and was recovered from 100% mesh pieces up until this stage. Viability of *B. cinerea* on mesh stored at 15-20°C declined over time, but the pathogen was still present on 46% mesh pieces after 6 months storage.

Cladosporium variabile grew consistently from 100% of mesh pieces that were refrigerated. The pathogen also remained viable at high levels when stored at 15-20°C. When stored at 30-35°C, pathogen viability declined over time but still grew from 26% mesh pieces after 6 months storage.

Colletotrichum dematium remained viable on refrigerated mesh, with the pathogen growing from 100% mesh pieces after 6 months. When stored at 15-20°C, or 30-35°C, viability declined over time, although gradual drying of mesh pieces could have been more of a contributory factor than temperature.

Table 5. Survival of fungal pathogens on crop mesh under three temperature regimes for different storage durations

Pathogen	Temp regime (°C)	Mean no. (of 5) mesh pieces with target pathogen after different durations					
		0 days	7 days	14 days	28 days	3 months	6 months
Control	3-6	-	0.0	0.0	0.0	0.0	0.0
B. cinerea	3-6	-	5.0	5.0	5.0	5.0	0.0
C. dematium	3-6	-	5.0	5.0	5.0	0.3	5.0
C. variabile	3-6	-	5.0	5.0	5.0	5.0	5.0
Control	15-20	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	15-20	5.0	5.0	4.3	5.0	4.3	2.3
C. dematium	15-20	5.0	5.0	5.0	5.0	0.3	0.0
C. variabile	15-20	5.0	5.0	5.0	5.0	5.0	3.0
Control	30-35	-	0.0	0.0	0.0	0.0	0.0
B. cinerea	30-35	-	4.3	0.0	0.0	0.0	0.0
C. dematium	30-35	-	4.7	5.0	5.0	4.0	1.3
C. variabile	30-35	-	5.0	4.0	2.3	3.7	1.3

- mesh not plated out

Mesh pieces stored at 30-35°C remained virtually clean (no microbial growth) apart from target pathogens, even after 6 months storage. For the other treatments, micro-organisms other than the target pathogens developed on agar plates when mesh pieces were plated out. The incidence of contamination was lowest on the control plates. The contaminants were mainly ubiquitous saprophytic fungi such as *Penicillium* species and *Cladosporium* species (distinguished from *C. variabile*) as well as a range of bacterial species.

Effect of light regime on the longevity of pathogen survival on crop mesh

Introduction

A laboratory experiment was done to determine the duration of survival (up to 6 months) for three plant pathogens on crop mesh when stored under different light regimes.

Methods

The duration of survival of three pathogens on pieces of crop mesh was tested as listed in Table 6.

Table 6. Light regime treatments applied to crop mesh

No.	Pathogen applied to mesh	Source of pathogen	Temperature

			regime (°C)
1	Sterile distilled water (SDW) – control	-	Ambient
2	*Botrytis cinerea*	Lettuce	Ambient
3	*Colletotrichum dematium*	Spinach	Ambient
4	*Cladosporium variabile*	Spinach	Ambient
5	Sterile distilled water (SDW) – control	-	UV-A
6	*Botrytis cinerea*	Lettuce	UV-A
7	*Colletotrichum dematium*	Spinach	UV-A
8	*Cladosporium variabile*	Spinach	UV-A
9	Sterile distilled water (SDW) – control	-	Dark
10	*Botrytis cinerea*	Lettuce	Dark
11	*Colletotrichum dematium*	Spinach	Dark
12	*Cladosporium variabile*	Spinach	Dark

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 six intervals: 0 days, 7 days, 14 days, 28 days, 3 months and 6 months 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 air dry in a laminar flow cabinet.

Cultures of the three pathogens and spore suspensions of each were prepared as described in Section 2.3.2. Spore suspensions of each pathogen were prepared giving a final volume of 300 ml, 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, 270 mesh pieces (plus spares) were immersed for 1 hour in the appropriate suspension, agitating periodically.

For each spore suspension (or SDW), the mesh pieces were placed moist into 18 Petri dishes (15 pieces per dish plus spares). The dishes were sealed with Parafilm. The dishes were labelled with pathogen name, light regime and sampling duration (0 day, 7 days, 28 days, 14 days, 3 months or 6 months). Dishes to be stored in the dark were stored in a black plastic bag at ambient temperature on a laboratory bench; dishes to be stored in ambient light were placed on a laboratory bench (ambient temperature); dishes to be stored under UV were placed in a wooden cabinet fitted with black light (UV-A) bulbs at ambient temperature. UV-A (so-called black light) represents the least damaging part of the UV spectrum, but was used in this experiment to give an indication of what could happen if mesh pieces were stored for extended periods in bright sunlight.

At each sampling time, the appropriate mesh pieces (one dish of 15 pieces for each treatment combination) were selected. When mesh pieces were still moist they were placed in a plastic tray wiped clean with 90% ethanol in a laminar flow cabinet to air 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.

Results and discussion

The proportion of mesh pieces from which different target pathogens grew after different light regimes and storage durations is summarised in Table 7.

Control mesh pieces (dipped in SDW) and stored wet or dry remained free of the target pathogens throughout the experiments.

Botrytis cinerea remained viable for longer when stored under ambient conditions (isolated from all mesh pieces after 3 months storage) compared with under dark conditions or UV light (nil isolated after 3 months storage).

The incidence of *Cladosporium variabile* on mesh pieces remained high irrespective of light regime, with growth from 93% mesh pieces stored in ambient or UV light, and from 60% of mesh pieces stored in the dark. This pathogen was less susceptible to the effects of UV light than the other two pathogens and this is likely due to the structure of the spores of *C variabile* which are more thick walled and darkly pigmented than the other two test pathogens.

UV-A light had an apparent effect on *Colletotrichum dematium* viability with none recovered after 3 months storage. In comparison, viability of the pathogen remained high under the other two light regimes.

Table 7. Survival of fungal pathogens on crop mesh under three light regimes for different storage durations

Pathogen	Light regime	Mean no. (of 5) mesh pieces with target pathogen after different durations					
		0 days	7 days	14 days	28 days	3 months	6 months
Control	Ambient	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	Ambient	5.0	5.0	4.0	5.0	5.0	0.3
C. dematium	Ambient	5.0	5.0	5.0	5.0	5.0	4.3
C. variabile	Ambient	5.0	5.0	4.3	5.0	3.7	4.7
Control	UV	-	0.0	0.0	0.0	0.0	0.0
B. cinerea	UV	-	4.3	4.7	5.0	0.0	0.0
C. dematium	UV	-	5.0	5.0	5.0	0.0	0.0
C. variabile	UV	-	5.0	5.0	5.0	5.0	4.7
Control	Dark	-	0.0	0.0	0.0	0.0	0.0
B. cinerea	Dark	-	5.0	1.0	4.7	0.0	0.0
C. dematium	Dark	-	5.0	5.0	5.0	5.0	5.0
C. variabile	Dark	-	5.0	4.3	5.0	4.7	3.0

- mesh not plated out

Mesh pieces stored under UV light remained mainly clean (no microbial growth) apart from target pathogens, even after 6 months storage. For the other treatments, micro-organisms other than the target pathogens developed on agar plates when mesh pieces were plated out. The incidence of contamination was lower on the control plates. The contaminants were mainly ubiquitous saprophytic fungi such as *Penicillium* species and *Cladosporium* species (distinguished from *C. variabile*) as well as a range of bacterial species.

Transmission of *Hyaloperonospora parasitica* (rocket downy mildew) via crop mesh

Introduction

A series of experiments was done to investigate i) survival of *H. parasitica* on meshes, and ii) transfer of the pathogen via meshes to infect a new crop. *H. parasitica* is an obligate parasite (surviving only as a parasite on the host) and so cannot be cultured on agar media. For this reason, viability of the pathogen on meshes was tested by placing the mesh onto a healthy crop and checking for symptom development, rather than by plating on to agar as was done for the other three test pathogens.

Methods

Experiment 1

Clean (previous unused) pieces of crop mesh (Wondermesh, 0.8 mm mesh diameter) were placed over three half-size trays of wild rocket seedlings (1-2 true leaf stage) in a polytunnel, with the mesh in contact with the seedlings, and edges tucked underneath the trays. The

seedlings were naturally infected with downy mildew (*H. parasitica*) and were showing typical symptoms of the disease with chlorosis and black speckling on the upper side of leaves, and white sporulation on the under side of leaves.

After the meshes had been in contact with the downy mildew-infected seedlings for 7 days they were each transferred to cover a half-size seed tray of healthy rocket plants (1-2 true leaf stage). The meshes were again placed in contact with the seedlings with the edges tucked underneath. As a control treatment, three clean unused mesh pieces were placed over three trays of healthy rocket seedlings at the same growth stage. These control trays were placed in a separate area of the polytunnel to avoid cross-contamination. The trays were overhead watered as required. Plants were monitored weekly for symptom development for 4 weeks.

Experiment 2

Experiment 1 was repeated at a later date, using rocket seedlings grown in 9 cm pots instead of half-size seed trays.

Experiment 3

Experiment 1 was subsequently repeated using diseased seedlings in half-size seed trays. For this run, mesh pieces were left in contact with the seedlings for approximately 1 month before transfer. Three infested mesh pieces were placed in contact with healthy seedlings, three infested mesh pieces were suspended over healthy seedlings and three clean mesh pieces (controls) were placed in contact with healthy seedlings. Trays of seedlings were maintained in individual clear polythene bags (to maintain high humidity and leaf wetness) in a controlled environment cabinet (18^{o}C, 12 h light, 12 h dark). The plants were checked weekly over 4 weeks for disease development.

Experiment 4

Half-size seed trays were sown with wild rocket in F2S compost, with three rows per tray. Trays were sown approximately 2 weeks before they were required and were maintained in a polytunnel.

200 mesh pieces (approximately 1 cm^2) were cut and immersed for 1 hour in a spore suspension of *H. parasitica* prepared from wild rocket leaves with typical symptoms of downy mildew. The concentration of the suspension was approximately 10^4 spores/ml (checked using a haemocytometer and microscope). 100 mesh pieces were soaked in SDW for 1 hour (control treatment). Half of the inoculated mesh pieces were air-dried in a plastic tray (wiped clean with 90% ethanol) before placing in six clean labelled Petri dishes, sealed with

Parafilm. The remaining half of the inoculated mesh pieces were placed wet in labelled Petris and sealed. The same was done for the control mesh pieces.

Mesh pieces were transferred to trays of healthy wild rocket plants (2-4 true leaves) at 0, 1, 3, 7, 14 and 21 days after soaking in downy mildew spore suspension. At each inoculation time, for each treatment, five mesh pieces were placed onto each of three rows of healthy rocket seedlings. The plants were misted with distilled water to the point of run-off. Each tray was placed in a sealable polythene bag in a controlled environment cabinet set at 10^{o}C for 2-3 days (12 h day / 12 h night). Subsequently the trays were moved to a separate cabinet at 18^{o}C (12 h day / 12 night). The bags were perforated to allow air movement without the plants drying out. The trays were monitored for symptom development and sporulation at 7 and 14 days after introducing the mesh pieces.

Results and discussion

Experiment 1

Typical symptoms of downy mildew developed after 28 days on trays of wild rocket seedlings, when mesh pieces were transferred directly from plants with abundant downy mildew sporulation. No symptoms developed on trays of plants that had been covered with clean meshes (control treatment).

Experiment 2

No downy mildew developed on pots of wild rocket seedlings following transfer of mesh from mature wild rocket that had symptoms of downy mildew but limited sporulation.

Experiment 3

No downy mildew developed on trays of wild rocket seedlings following transfer of mesh from mature wild rocket that had symptoms of downy mildew but limited sporulation.

Experiment 4

The sowing procedure had to be repeated three times due to pest damage (slugs and stem weevils) in the polytunnels, followed by the development of downy mildew on seedlings grown for use as healthy plant material (due to cross contamination in a polytunnel).

Once experimental set-up was achieved satisfactorily, there was no development of downy mildew symptoms on any of the trays of wild rocket following transfer of infested mesh pieces to the plants at different intervals after treatment.

Results from Experiment 1 demonstrated that there is scope for transfer of downy mildew spores via crop mesh and subsequent disease development on a healthy crop. This result occurred when the original crop showed abundant sporulation and when meshes were transferred immediately from the diseased plants to the healthy trays. Results from Experiments 2 and 3 were not able to verify this finding, possibly because although the source crops had severe symptoms of downy mildew, sporulation was at low levels in these experiments. Experiment 4 was designed to look at the duration for which spores of *H. parasitica* remained viable on crop mesh, but unfortunately results for disease development were negative throughout, even when mesh pieces were transferred immediately from the spore suspension to plants. This may have been because spore numbers on the immersed mesh pieces were too low, or perhaps environmental conditions for downy mildew infection and symptom development were sub-optimal, despite use of conditions considered favourable for the disease in controlled environment cabinets.

Project 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 particularly problematic, with two growers abandoning use of mesh on this crop because of associated disease risk.
- In addition to salad crop / pathogen combinations already considered, discussions with growers in 2008 highlighted further crops that might be at risk of pathogen transfer via meshes and fleeces. These included parsley (e.g. downy mildew and septoria) and watercress (fungal and bacterial leaf spots).
- 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.

- Laboratory experiments in years 1 and 2 demonstrated that in a temperature range of 14-26°C and ambient light, *Botrytis cinerea* (grey mould) and *Cladosporium variabile* (cladosporium leaf spot on spinach) can remain viable for at least 6 months on pieces of crop mesh stored either wet or dry, although the viability of *B. cinerea* declined during this period on wet mesh. Spores of *Colletotrichum dematium* (spinach anthracnose) remained viable on mesh pieces for at least six months when stored wet. However, under dry conditions, percentage viability of *C. dematium* was substantially reduced.
- Further laboratory experiments were done to investigate the effects of storage under different temperature and light regimes on pathogen viability. *Botrytis cinerea* was eliminated on mesh stored at 30-35°C for 14 days and was the only one of the three pathogens tested to be controlled by the higher temperature treatment. *B. cinerea* and *Colletotrichum dematium* were eliminated on mesh following storage under UV-A light for 3 months. *Cladosporium variabile* remained unaffected, due possibly to thicker walled and more darkly pigmented spores.
- 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.
- A series of experiments was done to study the transfer of rocket downy mildew on crop mesh. Results from a single experiment demonstrated that spores of *H. parasitica* from wild rocket could be transferred on mesh to subsequently cause symptoms of downy mildew on a healthy crop. However, it was not possible to verify this result in subsequent experiments.
- Further work may now be warranted to investigate the efficacy and practicality of measures such as disinfection, drying, heat or UV treatment that could be used to reduce the risk of pathogen transfer via crop meshes.

Technology transfer

Year 2:

- Information was provided for a summary article on the project in the May 2008 edition of *HDC News*.
- Project results were presented by K. Green at a meeting of the SPGA at ADAS Boxworth, Cambridgeshire, February 2008.
- Project results will form part of an invited presentation (K. Green) at the Conference of the British Leafy Salads Association in November 2008.