Project title	Baby leaf crucifers: Improving control of downy mildew
Project number:	FV 316
Project leader:	Dr Peter Gladders, ADAS UK Ltd
Report:	Annual report, year 1
Previous report	-
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Location of project:	ADAS Boxworth
Project coordinator:	Shaun Clarkson, Langmead Farms
Date project commenced:	1 July 2007
Date project completed (or expected completion date):	31 December 2009
Key words:	Rocket downy mildew, resistance, seed treatments, crop covers

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

AUTHENTICATION

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

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

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GROWER SUMMARY

Headline

- Rocket downy mildew can be reduced through choice of variety and use of seed treatments.
- Evaluation of varieties of wild and salad rocket for resistance to downy mildew identified significance differences between wild rocket varieties, but there were no differences between the salad rocket varieties tested. Good control of downy mildew was achieved with various seed treatments for up to three weeks after sowing. The addition of an insecticidal seed treatment decreased damage by stem weevils.

Background and expected deliverables

Downy mildew is caused by the fungus *Hyaloperonospora parasitica* (previously *Peronospora parasitica*) and is a major problem in crops of some baby leaf crucifers, particularly in rocket. The cotyledons and first true leaves are particularly susceptible. Symptoms range from large yellow blotches to small black speckles. Downy mildew is limiting production on some farms. Crops are grown at high density and are susceptible to downy mildew from emergence. Almost all crops are at risk as downy mildew requires only short periods of surface wetness to achieve infection.

Crops are harvested 20-50 days after sowing depending on the cropping season. The use of foliar fungicides is problematic because of the short harvest intervals and the requirement to use them prior to disease onset. Few products are available at present and the risk of selecting fungicide resistant strains is of concern. Seed treatments would be of interest if effective fungicides can be identified.

The extent to which downy mildew is seed-borne in rocket is not known. The project will develop a PCR test for detecting seed-borne downy mildew in wild and salad rocket seed samples. A sensitive and accurate diagnostic test will provide new information on seed-borne inoculum and be valuable for eliminating problem seed stocks.

Crop covers are used routinely to prevent damage from flea beetles and may also contribute to downy mildew problems if air-flow is poor. The interaction between crop covers and downy mildew has not been quantified on rocket crops. Crops are grown in succession and the most suitable land is used for cropping each year. Although hygiene measures such as

removal of old leaf material and flaming over crop resides are practised, soil-borne inoculum could become important.

There has been limited breeding of salad rocket and little progress has been made on improving disease resistance to downy mildew. Field resistance to downy mildew is highly desirable as single major gene resistance is likely to be overcome by new races of the pathogen (as occurs with downy mildews on lettuce and spinach for example). There is no independent comparative data on downy mildew susceptibility of current cultivars.

Overall aim of the project

To improve the management of downy mildew in rocket leading to enhanced crop quality and more profitable production.

Specific objectives

1. To evaluate cultivars and selections of the major types (wild and salad) of rocket for susceptibility to downy mildew.
2. To evaluate the efficacy of downy mildew seed treatments
3. To investigate the influence of crop covers on downy mildew development in rocket.
4. To develop a molecular diagnostic test for seed-borne downy mildew in rocket and determine the occurrence of downy mildew in seed stocks.

Summary of the project and main conclusions

Varietal resistance

Experiments have been carried out under protected conditions to compare resistance to downy mildew in wild and salad rocket varieties. The varieties tested were provided by several breeders (Table 1). The aim is to identify promising varieties under controlled conditions and then evaluate them in the field where variation in pathogen populations and weather may influence their effectiveness.

Clear differences in resistance to downy mildew were found between the 26 wild rocket varieties tested. The relative resistance levels of different varieties were consistent between two experiments carried out 3-4 weeks apart (Figure 1). The most resistant variety SSC2501 from breeder Shamrock was almost completely free from downy mildew, despite

severe disease pressure. The most susceptible varieties showed symptoms on over 90% of plants, with symptoms covering over 50% of the area of some leaf layers.

These results suggested that varietal resistance provide good control of downy mildew control in wild rocket. The upcoming field experiments are necessary to confirm the robustness of resistance in field conditions, after which the results can be used to help guide variety choice for growers and choice of lines for breeders.

Table 1. Varieties of wild and salad rocket tested for downy mildew resistance

Type of rocket	Variety name	Supplier
Wild	Atlantis	Tozer Seeds Ltd
Wild	Columbia	Tozer Seeds Ltd
Wild	Discovery	Tozer Seeds Ltd
Wild	Voyager	Tozer Seeds Ltd
Wild	SSC2402	Shamrock
Wild	SSC2501	Shamrock
Wild	Wild rocket No.1	CN Seeds
Wild	Wild rocket No.2	CN Seeds
Wild	Wild rocket No.3	CN Seeds
Wild	Wild rocket No.4	CN Seeds
Wild	Wild rocket No.5	CN Seeds
Wild	Wild rocket No.6	CN Seeds
Wild	Wild rocket No.7	CN Seeds
Wild	Wild rocket No.8	CN Seeds
Wild	CN Wild rocket 24549	CN Seeds
Wild	Rucola 1	Enza Zaden
Wild	Rucola 2	Enza Zaden
Wild	Rucola 3	Enza Zaden
Wild	Rucola 4	Enza Zaden
Wild	Rucola 5	Enza Zaden
Wild	Rucola 6	Enza Zaden
Wild	TZ6054	Tozer Seeds Ltd
Wild	TZ5010	Tozer Seeds Ltd
Wild	TZ0309	Tozer Seeds Ltd
Wild	TZ7002	Tozer Seeds Ltd
Wild	TZ0317	Tozer Seeds Ltd
Salad	Astra	Tozer Seeds Ltd
Salad	Challenger	Tozer Seeds Ltd
Salad	Sky Rocket	Tozer Seeds Ltd
Salad	Dentellata 24380	CN Seeds
Salad	Dentellata 24531	CN Seeds
Salad	CN Vistoria 23303	CN Seeds
Salad	CN 2503	CN Seeds
Salad	Rucola 7	Enza Zaden
Salad	Rucola 8	Enza Zaden

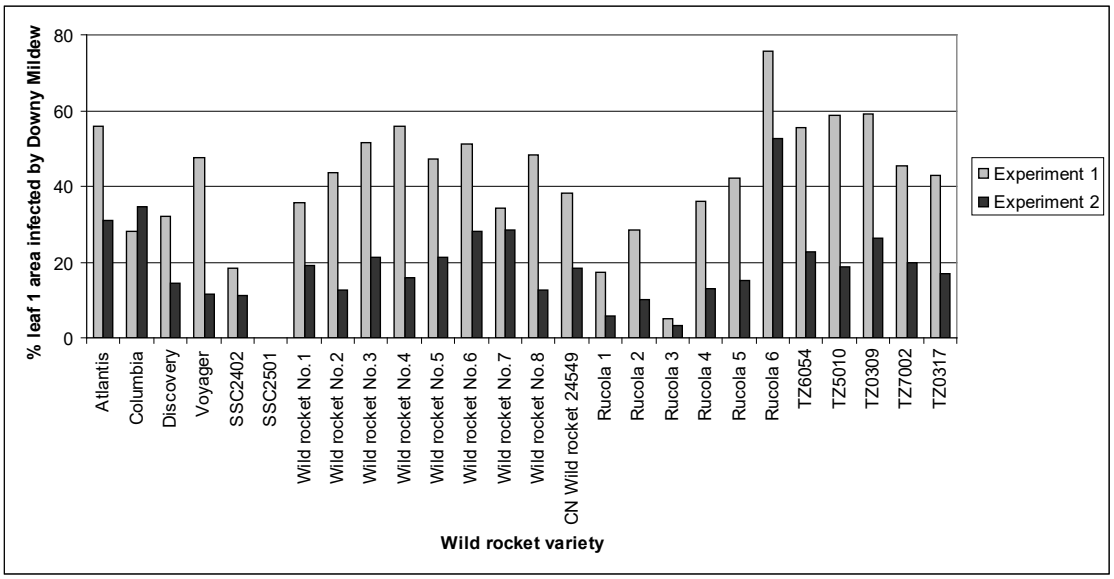


Figure 1. Percentage area of leaf 1 affected by downy mildew in the final assessments of two replicated experiments on wild rocket varieties. Experiment 1 assessed 32 days after sowing; Experiment 2 assessed 30 days after sowing

Nine varieties of salad rocket were tested, but there were no significant differences in downy mildew severity between varieties in either of the two experiments (data not shown). Downy mildew was less severe in salad rocket than in wild rocket and symptoms were mainly restricted to small spots.

Seed treatments

Five fungicide seed treatments were tested in relation to untreated seed on wild and salad rocket for downy mildew control. Seed treatments were provided by Syngenta Crop Protection Ltd. There were three rates of metalaxyl (as Apron XL), Apron XL + insecticide (A9700) and Wakil XL. These seed treatments provided a high level of downy mildew control in wild rocket, with the duration of control being longer at higher treatment doses. Apron XL at the highest rate gave over 85% control for the full 28 days of the trials, relative to untreated seed. The lowest dose gave similar control up to 17 days, but little control after 21 days from sowing (Figure 2).

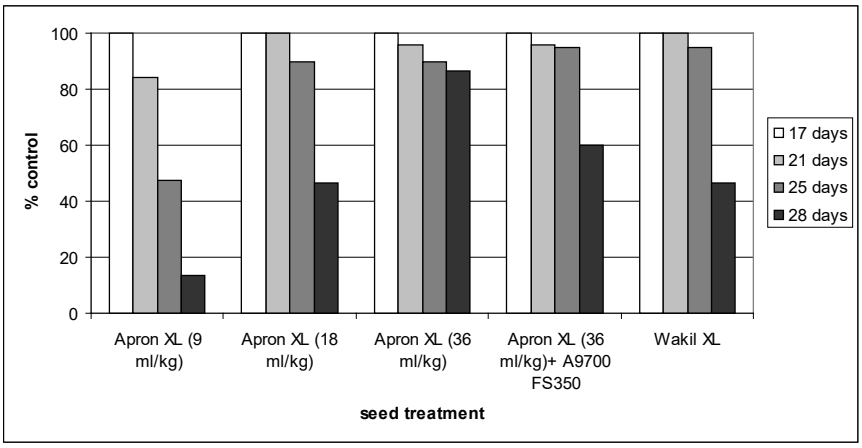


Figure 2. Percentage control of downy mildew incidence, relative to untreated seed, by five seed treatments on wild rocket

In salad rocket, a similar range of seed treatments all gave almost complete control of downy mildew for the duration of the trials. The only downy mildew seen on any treated plants was on the lowest rate of Apron XL, at the final assessment in one of two replicate experiments.

The Apron XL + A9700 treatment also provided a high level of pest control. In both wild and salad rocket experiments, leaf holing by stem weevils was reduced by over 80%, and in some cases 100%, relative to untreated plots (Figure 3).

Figure 3. Reduction in stem weevil damage on wild rocket with Apron XL + A9700 seed treatment (left) four weeks after sowing relative to untreated seed (right).

Phytotoxic effects were caused by seed treatments in both wild and salad rocket experiments. The higher doses of Apron XL caused yellowing of the cotyledon edges, while the Wakil XL treatment delayed emergence and reduced plant vigour.

These experiments have shown that seed treatments have strong potential for effective downy mildew control in both wild and salad rocket. Duration of protection was largely determined by treatment dose, but high doses carry risks of phytotoxicity, so experiments in the field will be important to establish suitable dose rates.

Crop covers

Some types of crop covers caused significant increases in downy mildew incidence and in plant vigour, relative to uncovered crops (Figure 4). A perforated plastic cover was the most conducive to downy mildew spread, but an Enviromesh cover, commonly used for flea beetle control, also raised downy mildew incidence significantly. Cover height did not have a significant effect on downy mildew.

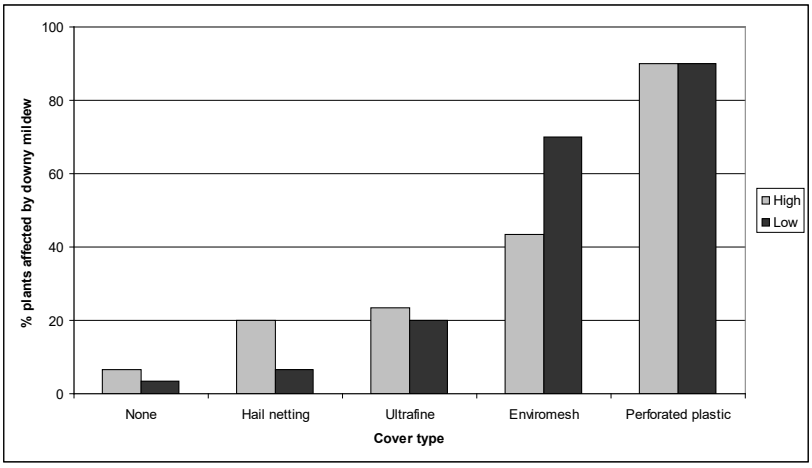


Figure 4. Effects of different crop covers on downy mildew incidence in a commercial wild rocket crop. Crop covers were held at two different heights: high (approx. 15 cm above crop) and low (approx. 10 cm above crop). Disease assessed on 4 September 2008, 26 days after sowing.

PCR test for seed-borne infection

Work has also been begun to develop a PCR test for detecting seed-borne downy mildew in wild and salad rocket seed samples. A range of methods were tested for extracting DNA from rocket seed. Different primers pairs and PCR methods were then tried, in order to develop a sensitive and accurate diagnostic test. A potentially successful technique has been developed from this work, but further work is required to confirm the results obtained and to quantify the detection limits of the technique.

A molecular (PCR) diagnostic test for downy mildew has been developed. Extracting DNA from rocket seed proved to be difficult, due to the oiliness of the seed. Consequently, the

standard method of primary PCR was unsuccessful in detecting downy mildew DNA in the preparations, even in positive controls. Nested PCR, a more sensitive technique, was tried with greater success. Downy mildew was detected with this method, even at very low dilutions. Several batches of rocket seed, obtained for the product from breeders, tested positive for downy mildew using this more sensitive technique. The PCR test does not establish the DNA is viable. Further work may include growing rocket from the seed batches tested, in isolation from any potential inoculum, to see if downy mildew develops in the crop as predicted by the test.

Financial benefits

Rocket growers in England continue to suffer losses of individual crops from downy mildew. Crops are grown in other parts of Europe to ensure continuity of supply. An estimated national loss in production of 20% is worth £200,000 /annum. The industry will benefit through reduced losses due to downy mildew achieved through:

- Information on varieties and their susceptibility to downy mildew
- Increased knowledge of fungicide seed treatments with activity against downy mildew
- Improved understanding of downy mildew in relation to crop covers
- Additional benefits from pest control

Project results will be of relevance to both home and overseas production. When linked to fungicide activity against downy mildew in other crops (e.g. Brassica seedlings, spinach), more general benefits may be forthcoming.

Action points for growers

- Downy mildew is capable of developing very rapidly throughout the cropping period given suitable weather. It continues to cause loss of crops. Management strategies combining resistant varieties and seed treatments appear to be promising from the first stage of this project.
- Downy mildew is able to overcome host resistance and fungicides and therefore a range of varieties with different resistance genes and fungicides with different modes of action will be required to sustain control strategies.

SCIENCE SECTION

Introduction

Baby leaf crucifers are an expanding sector of the salad crop market and comprise a range of species and varieties. The world market value has been estimated to exceed $4 billion/annum. The larger UK growers have production in the UK and in other countries to ensure continuity of supplies.

Baby leaf salads comprise a wide range of species (e.g. lettuce, spinach, beet and crucifers). The cruciferous crops include:

1. Rocket (Wild) (*Diplotaxis muralis*)
2. Salad rocket or arugula (*Eruca sativa*)
3. Mibuna and Mizuna (*Brassica rapa* var *nipposinica*)
4. Mustard (Chinese) (*Brassica juncea*)
5. Mustard spinach (*Brassica rapa* var *pervidis*)
6. Oriental types: Chinese cabbage (*Brassica rapa* var *pekinensis*) (Li-Quan, 1990), Pak choi/bok choi (*B. rapa* var *chinensis*), Choi sum (*B. rapa* var *parachinesis*), Tatsoi (*B. rapa* var *rosularis*).

Related crops include land cress (*Barbarea verna*); cress (*Lepidium sativum*); kale (*Brassica oleracea*); lambs lettuce (*Valerianella locusta*); rapeseed (*Brassica napus*) (Kluczewski and Lucas, 1983); radish (*Rhaphanus sativus*) (Dickinson and Greenhalgh, 1977); turnip (*Brassica rapa*) and watercress (*Rorippa nasturtium-aquatkum*).

The problem

Downy mildew is caused by the fungus *Hyaloperonospora parasitica* (previously *Peronospora parasitica*) and is a major problem in crops of some baby leaf crucifers, particularly in rocket (Garibaldi *et al.*, 2004; Koike *et al.*, 2007). Priority has been given to rocket in this proposal as downy mildew is limiting production on some farms, but the management principles established will be of generic interest to other crop species.

Crops are grown at high density (Padulosi and Pignone, 1997) and are susceptible to downy mildew from emergence. The cotyledons and first true leaves are particularly susceptible. Almost all crops are at risk as downy mildew requires only short periods of surface wetness to achieve infection. Crop covers are used routinely to prevent damage from flea beetles and may also contribute to downy mildew problems if air-flow is poor. Crops are grown in succession and the most suitable land is used for cropping each year. Although hygiene

measures such as removal of old leaf material and flaming over crop resides are practised, soil-borne inoculum could become important (Kluczewski and Lucas, 1982). Downy mildew affects the seed pods and seed infection may occur in crucifers (Vishunavat and Kolte 1993; Acharp, 1995;). The importance of seed-borne infection in rocket is not known.

Crops are harvested 20-50 days after sowing depending on the cropping season. The use of foliar fungicides is problematic because of the short harvest intervals and the requirement to use them prior to disease onset. Few products are available at present and the risk of selecting fungicide resistant strains is of concern.

There has been limited breeding of wild and salad rocket and little progress has been made on improving disease resistance to downy mildew. Wild rocket types are thought to be mainly selections from the wild. There is UK interest in breeding and benefits from this expertise will be gained by the collaboration with Tozer Seeds Ltd and CN Seeds. Field resistance to downy mildew is highly desirable as single major gene resistance is likely to be overcome by new races of the pathogen (as occurs with downy mildews on lettuce and spinach for example) (Anon., 2003). The molecular basis of downy mildew resistance is an active area of current research using *Arabidopsis* as a model plant system (McDowell *et al*., 2005; Slusarenko and Scharich, 2003). There is no independent comparative data on downy mildew susceptibility of current cultivars or selections of rocket.

Fundamental studies of the epidemiology of downy mildew and its control have been undertaken at Warwick–HRI in conventional and organic crops on *Brassica oleracea* (cauliflower). Chemical control of downy mildews continues to be an area of applied research on a wide range crops. Recent work on chemical control in the UK even on major crops such as cauliflower is limited and the proposed seed treatment evaluation is an advance over previous work.

The opportunity

Downy mildew is an obligate pathogen, showing some specialisation to individual host species (Dickinson and Greenhalgh, 1977; Kluczewski and Lucas, 1982; Anon., 2003). The downy mildew strains affecting rocket may be specialised and show limited ability to infect other cruciferous species. The use of species or variety mixtures with a proportion of downy mildew resistant types should provide the opportunity to reduce spread of downy mildew in crops. Current seed regulations prevent the sale of seed mixtures though they could be amended if substantial benefits could be demonstrated.

There is now an opportunity to exploit seed treatments that could provide protection for a short period post-emergence. Seed treatments combining fungicides and insecticides for flea

beetle control would also be valuable. To further improve the benefits of mixtures, seed treatments for downy mildew control could be included on one or more varieties.

DNA fingerprints are available for the downy mildew pathogen (Tham *et al.*, 1994) and there is opportunity to develop a DNA test for seed-borne infection using polymerase chain reaction (PCR) techniques. Such techniques should be more reliable and sensitive than blotter tests and establish whether seed-borne downy mildew is important.

The need

Downy mildew is a major problem on rocket and it is restricting production in the UK and overseas. Improved varieties and selections of rocket are now being developed by various seed companies and growers will benefit from independent evaluation of disease resistance in varieties and other control measures.

Materials and methods

Experiments under protected conditions

Tests were done in an unheated polytunnel on wild rocket (*Diplotaxis tenuifolia/muralis*) and salad rocket (*Eruca sativa*), grown in half seed trays with a John Innes seed compost and artificially inoculated with downy mildew. Two successive experiments were carried out for each of wild and salad rocket varieties, and wild and salad rocket seed treatments. Variety experiments consisted of three replicates, in a randomised block design. Seed treatment experiments included four replicate randomised blocks. Seed of a range of wild rocket and salad rocket varieties were obtained from commercial breeders and sown by hand in two rows per tray (Tables 1 and 2). The wild rocket varieties coded 2, 3, 5, 6 and 16 showed poorer germination in Experiment 1, so lower plant numbers may have influenced the disease severity scores. Fresh seed stocks of wild rocket varieties 2, 3 and 6 were obtained for Experiment 2, and an additional variety added. Wild and salad rocket seed treated with a range of seed treatments was provided by Syngenta Crop Protection UK Ltd. (Table 3). . There were three rates of metalaxyl (as Apron XL), Apron XL + insecticide (A9700) and Wakil XL (cymoxanil + fludioxonil + metalaxyl-M). A data logger was set to record temperature for the duration of all experiments.

A wild rocket downy mildew isolate was collected from a commercial crop in West Sussex on 3 August 2007. This was bulked up on a susceptible variety prior to the start of the experiments. Downy mildew inoculum was prepared by washing spores from infected leaves with distilled water and diluting the resulting suspension to approximately 5000 spores/ml.

Inoculum was applied with a hand mister, using approx 0.7 ml suspension per half seed tray. Immediately after inoculation, experiments were covered with a 'tent' of polythene sheeting for 48 hours to prolong leaf wetness duration and promote high relative humidity. Salad rocket experiments used similar inoculum from a commercial salad rocket crop in Hampshire obtained in August 2007, applied using the same technique.

Variety experiments were inoculated at the two-leaf stage and watered regularly to encourage downy mildew development. Seed treatment experiments were not artificially inoculated, as high levels of downy mildew in the polytunnel caused natural infection. Trials were assessed for differences in emergence, vigour and pest damage between varieties and seed treatments, and for phytotoxic effects of seed treatments. Downy mildew was assessed by leaf layer on 10 plants per plot, destructively sampled. Dates of sowing, inoculation and assessments are shown in Table 4.

Table 1. Wild rocket varieties tested in polythene tunnel tray tests.

No.	Variety	Company
1	Atlantis	Tozer Seeds Ltd
2	Columbia	Tozer Seeds Ltd
3	Discovery	Tozer Seeds Ltd
4	Voyager	Tozer Seeds Ltd
5	SSC2402	Shamrock
6	SSC2501	Shamrock
7	Wild rocket No.1	CN Seeds
8	Wild rocket No.2	CN Seeds
9	Wild rocket No.3	CN Seeds
10	Wild rocket No.4	CN Seeds
11	Wild rocket No.5	CN Seeds
12	Wild rocket No.6	CN Seeds
13	Wild rocket No.7	CN Seeds
14	Wild rocket No.8	CN Seeds
15	CN Wild rocket 24549	CN Seeds
16	Rucola 1	Enza Zaden
17	Rucola 2	Enza Zaden
18	Rucola 3	Enza Zaden
19	Rucola 4	Enza Zaden
20	Rucola 5	Enza Zaden
21	Rucola 6	Enza Zaden
22	TZ6054	Tozer Seeds Ltd
23	TZ5010	Tozer Seeds Ltd
24	TZ0309	Tozer Seeds Ltd
25	TZ7002	Tozer Seeds Ltd
26	TZ0317	Tozer Seeds Ltd
27	WA-47070-15242 (Expt 2 only)	Shamrock

Table 2. Salad rocket varieties tested in polythene tunnel tray tests.

No.	Variety	Supplier
1	Astra	Tozer Seeds Ltd
2	Challenger	Tozer Seeds Ltd
3	Sky Rocket	Tozer Seeds Ltd
4	Dentellata 24380	CN Seeds
5	Dentellata 24531	CN Seeds
6	CN Victoria 23303	CN Seeds
7	CN 2503	CN Seeds
8	Rucola 7	Enza Zaden
9	Rucola 8	Enza Zaden

Table 3. Seed treatments for both wild and salad rocket.

No.	Seed treatment	Rates for wild rocket (ml product / kg seed)	Rates for salad rocket (ml product / kg seed)
1	Untreated	-	-
2	Apron XL	18	6
3	Apron XL	36	12
4	Apron XL	9	24
5	Wakil XL	15g pr	18g pr
6	Apron XL + A9700 FS350	36 + 60	12 + 20

Table 4. Sowing, inoculation and assessment dates for all experiments

Experiment	Sowing	Inoculation	Downy Mildew assessments		
Wild rocket varieties 1	15/05/08	27/05/08	04/06/08	09/06/08	16/06/08
Wild rocket varieties 2	09/06/08	18/06/08	01/07/08	09/07/08	
Salad rocket varieties 1	27/05/08	04/06/08	16/06/08	26/06/08	
Salad rocket varieties 2	18/06/08	27/06/08	03/07/08	09/07/08	
Wild rocket seed treatments 1	28/06/08	Not inoculated	15/07/08	23/07/08	
Wild rocket seed treatments 2	11/07/08	Not inoculated	24/07/08	01/08/08	08/08/08
Salad rocket seed treatments 1	06/07/08	Not inoculated	23/07/08	01/08/08	
Salad rocket seed treatments 2	24/07/08	Not inoculated	01/08/08	15/08/08	

Field experiment on crop covers

A field experiment was carried out in West Sussex, using a commercial wild rocket variety treated with a Wakil XL seed treatment. The crop was drilled using standard farm equipment on 2 August 2008 then covered on 5 August 2008 using a range of mesh covers, stretched over the beds by wire hoops and secured by burying the edges with soil. A fleece cover was

initially planned, but was unavailable when the trial was set up. Covers were supported at two heights, approximately 10 cm or 15 cm above the crop (Table 5). Plots were 10 m long, and arranged in a randomised block design with three replicate blocks. Data loggers were used to record temperature under different covers.

Table 5. Treatments used in crop covers trial, West Sussex 2008

No.	Cover type	Height above crop*
1	None	-
2	None	-
3	Ultrafine	Low
4	Enviromesh	Low
5	Hail netting	Low
6	Perforated plastic	Low
7	None	-
8	Ultrafine	High
9	Enviromesh	High
10	Hail netting	High
11	Perforated plastic	High
12	None	-

*Low covers held approximately 10 cm above crop; high covers 15 cm above crop

Crop emergence and vigour were assessed on 19 August 2008, 17 days after drilling. On 4 September 2008, 26 days after drilling, covers were removed and 10 plants per plot assessed for downy mildew.

PCR test for seed-borne downy mildew: DNA extraction methods

Six methods, detailed below, were tested for extracting DNA from wild and salad rocket seed. Each of the two extraction buffers in method 1 was tested with two sizes of seed aliquot, 0.5 g and 1 g, while the other five methods were tested using a Nucleospin kit to extract DNA from the preparation described. Each method was tested twice, once using only seed, and once spiked with rocket leaves known to be infected with *H. parasitica* (Table 4).

1. CTAB Extraction of DNA from seed.

CTAB Buffer 1 (amounts per litre)

23 g Sorbitol

10 g N-Lauryl-Sarcosine

8 g CTAB

87.7 g NaCl

10 g PVP

CTAB Buffer 2
2.5% CTAB
1.4 M NaCl
1 % PVP
50 mM EDTA
100 mM Tris-HCl pH 8.0

Rocket seed (variety: Shamrock, Ref: 4707015141) was ground up using a coffee grinder. 0.5 g and 1 g aliquots were weighed out and added to 5 ml CTAB buffer (either 1 or 2) and were incubated overnight at 65^{o}C. 800 µl 5M Potassium acetate was added and samples were vortexed for 1 minute. Samples were put in a -80^{o}C freezer for 30 minutes. Tubes were centrifuged at 3000 rpm for 15 minutes then 0.5 ml supernatant was put into a new 1.5 ml tube. Care was taken to avoid the oily layer at the top of the supernatant. Equal volume of Phenol:Chloroform was added and samples were vortexed for 1 minute. Samples were centrifuged for 5 minutes and the top aqueous layer was removed to a new tube. An equal volume of 100% isopropanol was added and mixed and samples were incubated at room temperature for 10 minutes. Samples were centrifuged for 5 minutes at 13000 rpm to pellet the DNA and the supernatant was thrown away. DNA pellets were washed with 70% ethanol. DNA was resuspended in 100 µl dH_2O.

2. Seed wash

1 g rocket seed (variety: Shamrock, Ref: 4707015141) was placed in a Bioreba filter bag with 10 ml TE Buffer and was incubated at room temperature for 5 minutes. The bags were placed into a stomacher machine and were shaken for 5 minutes. The liquid was transferred to a 15 ml falcon tube and centrifuged for 5 minutes at 5000 rpm. The supernatant was removed and the pellet was resuspended in 600 µl lysis buffer containing RNAseA and ProteinaseK. Contents of the pellet were further disrupted using a Qiagen Tissuelyser. The DNA was then extracted from the samples following the method of the nucleospin II. The DNA was eluted from the columns in 100 µl elution buffer supplied with the kit.

3. Seed 48 h incubation – liquid only

250 mg seeds (variety: Shamrock, Ref: 4707015141) were soaked for 48 hours with 10 ml dH_2O in a Bioreba bag. The remaining liquid was collected and centrifuged. The pellet was ground up in liquid N_2.

4. Seed 48 h incubation – total prep: seeds, plants and liquid

250 mg seeds (variety: Shamrock, Ref: 4707015141) were soaked for 48 hours with 10 ml dH_2O in a Bioreba bag. All liquid, germinated plants and seeds were collected. Samples were centrifuged and the pellet was ground up in liquid N_2.

5. Seed 48 h germination on Petri dishes, wash + disruption

250 mg seeds (variety: Shamrock, Ref: 4707015141) were germinated on moist filter paper in Petri dishes for 48 h. All seeds and plants were washed off with 10 ml dH_2O and stomached for 5 minutes then centrifuged. The sample was ground in liquid N_2.

6. Seed 48 h germination -dry

250 mg seeds (variety: Shamrock, Ref: 4707015141) were germinated on moist filter paper in Petri dishes for 48 h. All seeds and plants were scraped off and were ground in liquid N_2.

Table 6. Summary of DNA extraction methods tested

No.	Method name	DNA number (and date)	DNA preparation method	Date prepared	Spike
	Infected Rocket plant: Shamrock	Positive control	Nucleospin	Sep 2007	Positive control
1	CTAB (1) extraction from Shamrock seed	1 (07/07)	CTAB buffer 1 0.5g seed	Sep 2007	
1	CTAB (1) extraction from seed	2 (07/07)	CTAB buffer 1 1g seed	Sep 2007	
1	CTAB (2) extraction from seed	3 (07/07)	CTAB buffer 2 0.5g seed	Sep 2007	
1	CTAB (2) extraction from seed	4 (07/07)	CTAB buffer 2 1g seed	Sep 2007	
1	CTAB (2) extraction from seed	5 (07/07)	CTAB buffer 2 0.5g seed	Sep 2007	Spiked with 100 mg infected rocket leaves
1	CTAB (2) extraction from seed	6 (07/07)	CTAB buffer 2 1g seed	Sep 2007	Spiked with 100 mg infected rocket leaves
2	Seed wash (stomached 5 min)	1 (22/11/08)	Nucleospin	22/11/07	
2	Seed wash (stomached 5 min)	2 (22/11/08)	Nucleospin	22/11/07	Spiked with 100 mg infected rocket leaves
3	Seed 48 h incubation – liquid only	1 (06/02/08)	Nucleospin	06/02/08	
3	Seed 48 h incubation – liquid only	2 (06/02/08)	Nucleospin	06/02/08	Spiked with 100 mg infected rocket leaves
4	Seed 48 h incubation – total prep- seeds, plants and liquid	3 (06/02/08)	Nucleospin	06/02/08	
4	Seed 48 h incubation – total prep- seeds, plants and liquid	4 (06/02/08)	Nucleospin	06/02/08	Spiked with 100 mg infected rocket leaves
5	Seed 48 h germination on petri dish, wash + disruption	5 (06/02/08)	Nucleospin	06/02/08	
5	Seed 48 h germination on Petri dish, wash + disruption	6 (06/02/08)	Nucleospin	06/02/08	Spiked with 100 mg infected rocket leaves
6	Seed 48 h germination –dry	7 (06/02/08)	Nucleospin	06/02/08	
6	Seed 48 h germination –dry	8 (06/02/08)	Nucleospin	06/02/08	Spiked with 100 mg infected rocket leaves

PCR test for seed-borne downy mildew: primer design

A range of primers were tested on DNA extracted from *H. parasitica* infected rocket (variety: Shamrock – P. Gladders, ADAS) (Table 6). Gradient PCR was performed using the primer pairs over a temperature range of 50–60°C. The conditions used were 5 min 95°C; 40 cycles of: 15 s at 95°C, 15 s at 60°C, 30 s at 72°C followed by 1 cycle of 5 min at 72°C (for further information, contact Anna Gordon, NIAB). In all cases 2 µl of DNA was used as the template in the primary reactions, and 2 µl of primary PCR product was used as the template in the nested PCR reactions.

Table 7. Primers tested on DNA extracts

	Primer sequence	Genbank ref:	Expected product size	Actual product size
Peronospora viciae ATPase F1 & R1	F1: CAGTCCACCCATCCTATC R1: CGTTACTTTCTCCACTTTTC	AF217282	310 bp	310 bp
P.v. ATPase F2 & R2	F2: ATTTCCGGTCATGGTCTTAAAG R2: TTGTACTGTTGGTCAATGGCA	AF217282	160 bp	160 bp
Hyaloperonospora parasitica ATR1 F2 & R2	F2:CCATTATGCGCGTCTGCTACTTC R2: ATGCCACTGCTTCCTCCAATC	AY842884	400 bp	800 bp and 400 bp

PCR test for seed-borne downy mildew: commercial samples

For samples 1-12, 5000 seeds were counted using a “Contador” seed counter. These were then weighed to give rise to the thousand seed weight vales in Table 8. The seeds were germinated and grown for 14 days at 14°C on moist filter paper in a 500 cm^2 square culture dish then ground up in liquid N_2. DNA was extracted from 100 mg ground material using a Nucleospin kit. DNA was eluted in 100 µl. DNA extractions were amplified by both primary and nested PCR methods, then 10 µl of each preparation was run on a 1% agarose gel stained with ethidium bromide.

Table 8. Rocket varieties tested for seed borne downy mildew, with thousand seed weights

DNA number	Rocket variety	g/5000 seed	TSW / mg	Date prepared
1 10/04	Discovery TZ 0316 (wild)	1.209	242	10/04/08
2 10/04	TZ 0309 (wild)	1.868	373	10/04/08
3 10/04	T7 6054 (wild)	1.633	327	10/04/08
4 10/04	Columbia (wild)	1.404	281	10/04/08
5 10/04	Voyager (wild)	1.395	279	10/04/08
6 10/04	TZ 5010 (wild)	1.295	259	10/04/08
7 10/04	TZ 7002 (wild)	1.693	338	10/04/08
8 10/04	T7 0317 (wild)	1.550	310	10/04/08
9 10/04	Atlantis (wild)	1.249	249	10/04/08
10 10/04	Challenger (salad)	10.092		10/04/08
11 10/04	Sky Rocket (salad)	10.975		10/04/08
12 10/04	Astra (salad)	11.317		10/04/08
1 25/07	ADAS wild rocket leaf ref:N21			25/07/08
2 25/07	ADAS salad rocket leaf ref: N82			25/07/08
1 29/07	ADAS wild rocket leaf ref:N21			29/07/08
2 29/07	ADAS salad rocket leaf ref: N82			29/07/08

Results

Experiments under protected conditions

Wild rocket varieties

Severe downy mildew was induced on cotyledons and true leaves in both replicated experiments. Significant differences between varieties were identified in all leaf layers (Tables 9-12), and the relative resistance of different varieties was similar in the two experiments.

The Shamrock coded variety SSC2501 (Variety number 6) stood out from the others as having near total downy mildew resistance. This variety also had a highly distinctive leaf shape, closer in appearance to salad rocket than wild rocket. Rucola 3 from Enza Zaden also had very little downy mildew. Atlantis, Rucola 6 and TZ05010 were amongst the most severely affected varieties (Tables 9-12),

Table 9. Downy mildew incidence and severity, wild rocket experiment 1, 9 June 2008 (2-5 leaves, 25 days after sowing)

No.	Variety	Company	% downy mildew on cotyledons 9 June	% downy mildew on true leaves 9 June	% plants with downy mildew 9 June
1	Atlantis	Tozer Seeds Ltd	97.7	40.2	100.0
2	Columbia	Tozer Seeds Ltd	48.3	10.4	80.0
3	Discovery	Tozer Seeds Ltd	52.3	12.2	86.7
4	Voyager	Tozer Seeds Ltd	74.8	15.3	100.0
5	SSC2402	Shamrock	35.7	7.0	73.3
6	SSC2501	Shamrock	0.2	0.0	3.3
7	Wild rocket No.1	CN Seeds	87.2	28.1	100.0
8	Wild rocket No.2	CN Seeds	77.0	19.8	100.0
9	Wild rocket No.3	CN Seeds	83.0	27.1	100.0
10	Wild rocket No.4	CN Seeds	79.5	22.4	100.0
11	Wild rocket No.5	CN Seeds	83.5	33.2	100.0
12	Wild rocket No.6	CN Seeds	79.3	28.3	96.7
13	Wild rocket No.7	CN Seeds	67.8	11.1	96.7
14	Wild rocket No.8	CN Seeds	86.7	31.0	100.0
15	CN Wild rocket 24549	CN Seeds	77.3	16.0	100.0
16	Rucola 1	Enza Zaden	34.7	5.3	86.7
17	Rucola 2	Enza Zaden	55.7	8.3	83.3
18	Rucola 3	Enza Zaden	30.2	0.4	60.0
19	Rucola 4	Enza Zaden	84.2	27.7	100.0
20	Rucola 5	Enza Zaden	81.7	24.7	100.0
21	Rucola 6	Enza Zaden	92.8	38.1	100.0
22	TZ6054	Tozer Seeds Ltd	86.5	17.2	100.0
23	TZ5010	Tozer Seeds Ltd	82.3	42.2	100.0
24	TZ0309	Tozer Seeds Ltd	84.0	29.9	100.0
25	TZ7002	Tozer Seeds Ltd	78.7	28.7	100.0
26	TZ0317	Tozer Seeds Ltd	92.5	27.8	96.7
		SED (50 df)	10.05	6.399	8.72
		F pr.	<0.001	<0.001	<0.001
		LSD (5%)	20.19	12.853	17.51

Table 10. Downy mildew incidence and severity, wild rocket experiment 1, 16 June 2008 (3-6 leaves, 32 days after sowing)

No.	Variety	Company	% downy mildew on leaf 1 16 June	% downy mildew on leaf 2 16 June	% plants with downy mildew 16 June
1	Atlantis	Tozer Seeds Ltd	55.8	20.7	86.7
2	Columbia	Tozer Seeds Ltd	28.1	7.5	73.3
3	Discovery	Tozer Seeds Ltd	32.2	11.9	80.0
4	Voyager	Tozer Seeds Ltd	47.7	13.9	93.3
5	SSC2402	Shamrock	18.3	7.7	50.0
6	SSC2501	Shamrock	0.0	0.0	0.0
7	Wild rocket No.1	CN Seeds	35.6	11.5	93.3
8	Wild rocket No.2	CN Seeds	43.5	16.1	96.7
9	Wild rocket No.3	CN Seeds	51.6	14.6	93.3
10	Wild rocket No.4	CN Seeds	56.0	17.3	90.0
11	Wild rocket No.5	CN Seeds	47.3	13.0	90.0
12	Wild rocket No.6	CN Seeds	51.3	17.4	96.7
13	Wild rocket No.7	CN Seeds	34.3	8.0	86.7
14	Wild rocket No.8	CN Seeds	48.3	8.5	90.0
15	CN Wild rocket 24549	CN Seeds	38.2	12.7	90.0
16	Rucola 1	Enza Zaden	17.4	1.7	63.3
17	Rucola 2	Enza Zaden	28.6	11.9	73.3
18	Rucola 3	Enza Zaden	4.9	0.0	13.3
19	Rucola 4	Enza Zaden	36.2	10.8	83.3
20	Rucola 5	Enza Zaden	42.1	17.6	83.3
21	Rucola 6	Enza Zaden	75.5	24.8	96.7
22	TZ6054	Tozer Seeds Ltd	55.7	21.2	76.7
23	TZ5010	Tozer Seeds Ltd	58.7	24.0	86.7
24	TZ0309	Tozer Seeds Ltd	59.0	24.2	93.3
25	TZ7002	Tozer Seeds Ltd	45.4	18.2	93.3
26	TZ0317	Tozer Seeds Ltd	42.8	19.3	93.3
		SED (50 df)	11.42	7.046	8.54
		F pr.	<0.001	0.021	<0.001
		LSD (5%)	22.94	14.152	17.14

Table 11. Downy mildew incidence and severity, wild rocket experiment 2, 01 July 2008 (2-5 leaves, 22 days after sowing)

No.	Variety	Supplier	% downy mildew on cotyledons 01 July	% downy mildew on leaf 1 01 July	% downy mildew on other true leaves 01 July	% plants with downy mildew 01 July
1	Atlantis	Tozer Seeds Ltd	80.5	48.7	13.83	100.0
2	Columbia (new stock)	Tozer Seeds Ltd	62.3	24.8	9.55	100.0
3	Discovery (new stock)	Tozer Seeds Ltd	57.0	12.8	2.03	86.7
4	Voyager	Tozer Seeds Ltd	69.2	16.8	5.63	100.0
5	SSC2402	Shamrock	33.5	16.3	5.10	76.7
6	SSC2501 (new stock)	Shamrock	0.0	0.0	0.00	0.0
7	Wild rocket No.1	CN Seeds	65.5	30.3	4.67	100.0
8	Wild rocket No.2	CN Seeds	57.3	11.1	0.73	93.3
9	Wild rocket No.3	CN Seeds	63.5	21.3	6.47	96.7
10	Wild rocket No.4	CN Seeds	49.7	16.0	7.43	93.3
11	Wild rocket No.5	CN Seeds	56.8	13.6	3.00	100.0
12	Wild rocket No.6	CN Seeds	47.2	20.4	5.87	96.7
13	Wild rocket No.7	CN Seeds	60.2	25.2	5.23	100.0
14	Wild rocket No.8	CN Seeds	57.3	28.8	6.07	100.0
15	CN Wild rocket 24549	CN Seeds	63.2	18.7	5.17	93.3
16	Rucola 1	Enza Zaden	10.3	3.3	0.07	56.7
17	Rucola 2	Enza Zaden	46.2	10.0	0.93	80.0
18	Rucola 3	Enza Zaden	5.0	0.8	0.07	20.0
19	Rucola 4	Enza Zaden	76.3	29.0	5.67	100.0
20	Rucola 5	Enza Zaden	71.0	30.0	6.10	93.3
21	Rucola 6	Enza Zaden	95.7	66.7	26.07	100.0
22	TZ6054	Tozer Seeds Ltd	38.8	7.3	2.43	90.0
23	TZ5010	Tozer Seeds Ltd	65.8	40.5	11.70	100.0
24	TZ0309	Tozer Seeds Ltd	74.5	36.5	8.50	100.0
25	TZ7002	Tozer Seeds Ltd	72.5	22.7	6.13	100.0
26	TZ0317	Tozer Seeds Ltd	61.2	30.5	7.47	100.0
27	WA-47070-15242	Shamrock	37.0	27.8	5.80	90.0
		SED (51 df)	12.41	8.37	3.744	8.48
		F pr.	<0.001	<0.001	<0.001	<0.001
		LSD (5%)	24.91	16.8	7.517	17.03

Table 12. Downy mildew incidence and severity, wild rocket experiment 2, 09 July 2008 (5-8 leaves, 30 days after sowing)

No.	Variety	Supplier	% downy mildew on leaf 1 08 July	% downy mildew on leaf 2 08 July	% downy mildew on other true leaves 08 July	% plants with downy mildew 08 July
1	Atlantis	Tozer Seeds Ltd	30.8	14.0	0.30	96.7
2	Columbia (new stock)	Tozer Seeds Ltd	34.8	25.0	4.15	100.0
3	Discovery (new stock)	Tozer Seeds Ltd	14.4	3.9	0.27	100.0
4	Voyager	Tozer Seeds Ltd	11.6	5.0	0.53	96.7
5	SSC2402	Shamrock	11.3	11.8	2.37	93.3
6	SSC2501 (new stock)	Shamrock	0.0	0.0	0.00	0.0
7	Wild rocket No.1	CN Seeds	19.1	10.6	1.37	93.3
8	Wild rocket No.2	CN Seeds	12.5	5.8	0.23	96.7
9	Wild rocket No.3	CN Seeds	21.4	13.3	0.87	96.7
10	Wild rocket No.4	CN Seeds	15.8	4.7	0.27	90.0
11	Wild rocket No.5	CN Seeds	21.4	15.5	0.50	96.7
12	Wild rocket No.6	CN Seeds	28.0	14.9	0.87	96.7
13	Wild rocket No.7	CN Seeds	28.5	9.7	0.40	100.0
14	Wild rocket No.8	CN Seeds	12.6	6.9	0.63	100.0
15	CN Wild rocket 24549	CN Seeds	18.3	5.6	0.47	100.0
16	Rucola 1	Enza Zaden	5.8	5.2	0.33	86.7
17	Rucola 2	Enza Zaden	10.0	3.3	0.07	86.7
18	Rucola 3	Enza Zaden	3.2	0.4	0.00	23.3
19	Rucola 4	Enza Zaden	12.9	4.3	0.30	90.0
20	Rucola 5	Enza Zaden	15.0	7.4	0.70	100.0
21	Rucola 6	Enza Zaden	52.5	22.8	1.00	100.0
22	TZ6054	Tozer Seeds Ltd	22.6	10.9	1.33	93.3
23	TZ5010	Tozer Seeds Ltd	18.9	7.9	0.17	90.0
24	TZ0309	Tozer Seeds Ltd	26.2	16.1	0.30	100.0
25	TZ7002	Tozer Seeds Ltd	19.7	5.5	0.57	100.0
26	TZ0317	Tozer Seeds Ltd	16.9	4.0	0.17	90.0
27	WA-47070-15242	Shamrock	24.7	17.5	0.77	100.0
		SED (51 df)	6.135	4.718	0.5316	4.753
		F pr.	<0.001	<0.001	<0.001	<0.001
		LSD (5%)	12.317	9.472	1.0672	9.543

Salad rocket varieties

Downy mildew proved more difficult to work with on salad rocket than on wild rocket. Many of the symptoms were small dark spots with little sporulation and there were difficulties in multiplying up sufficient inoculum for the two experiments. Downy mildew remained at lower levels than in the wild rocket experiments, and no significant differences were identified between varieties in either of the replicated experiments (Tables 13-16).

Table 13. Downy mildew incidence and severity, salad rocket experiment 1, 16 June 2008 (2-4 leaves, 20 days after sowing)

No.	Variety	Supplier	% downy mildew on cotyledons 16 June	Overall DM incidence % plants 16 June
1	Astra	Tozer Seeds Ltd	0.37	10.0
2	Challenger	Tozer Seeds Ltd	2.77	13.3
3	Sky Rocket	Tozer Seeds Ltd	1.43	23.3
4	Dentellata 24380	CN Seeds	0.43	10.0
5	Dentellata 24531	CN Seeds	0.33	6.7
6	CN Victoria 23303	CN Seeds	0.37	10.0
7	CN 2503	CN Seeds	0.77	6.7
8	Rucola 7	Enza Zaden	2.00	13.3
9	Rucola 8	Enza Zaden	0.00	0.0
		SED (16 df)	0.879	7.43
		F pr.	NS (0.082)	NS (0.249)
		LSD (5%)	1.864	15.76

Table 14. Downy mildew incidence and severity, salad rocket experiment 1, 26 June 2008 (3-6 leaves, 30 days after sowing)

No.	Variety	Supplier	% downy mildew on cotyledons 26 June	% downy mildew on Leaf 1 26 June	% downy mildew on later leaves 26 June	Overall DM incidence % plants 26 June
1	Astra	Tozer Seeds Ltd	2.37	0.03	0.07	46.7
2	Challenger	Tozer Seeds Ltd	2.85	0.93	0.30	43.3
3	Sky Rocket	Tozer Seeds Ltd	0.85	1.73	0.27	40.0
4	Dentellata 24380	CN Seeds	2.70	0.67	0.00	23.3
5	Dentellata 24531	CN Seeds	2.17	0.97	0.00	26.7
6	CN Victoria 23303	CN Seeds	1.53	0.62	0.25	33.3
7	CN 2503	CN Seeds	1.43	0.60	0.06	30.0
8	Rucola 7	Enza Zaden	0.83	0.13	0.00	10.0
9	Rucola 8	Enza Zaden	2.60	0.07	0.10	26.7
		Grand Total	1.90	0.64	0.12	31.1
		SED (16 df)	1.291	0.783	0.153	15.18
		F pr.	NS (0.378)	NS (0.500)	NS (0.314)	NS (0.407)
		LSD (5%)	2.788	1.659	0.3244	32.19

Table 15. Downy mildew incidence and severity, salad rocket experiment 2, 03 July 2008 (2-4 leaves, 14 days after sowing)

No.	Variety	Supplier	% downy mildew on cotyledons 03 July	% downy mildew on true leaves 03 July	% plants with downy mildew 03 July
1	Astra	Tozer Seeds Ltd	22.9	2.90	100.0
2	Challenger	Tozer Seeds Ltd	18.8	2.83	90.0
3	Sky Rocket	Tozer Seeds Ltd	23.3	2.03	96.7
4	Dentellata 24380	CN Seeds	27.0	1.97	83.3
5	Dentellata 24531	CN Seeds	17.5	1.83	76.7
6	CN Victoria 23303	CN Seeds	5.7	0.23	70.0
7	CN 2503	CN Seeds	16.7	0.93	93.3
8	Rucola 7	Enza Zaden	6.1	0.70	56.7
9	Rucola 8	Enza Zaden	12.0	0.43	76.7
		SED (16 df)	7.1	1.099	14.74
		F pr.	NS (0.082)	NS (0.184)	NS (0.149)
		LSD (5%)	15.04	2.33	31.25

Table 16. Downy mildew incidence and severity, salad rocket experiment 2, 09 July 2008 (2-5 leaves, 20 days after sowing)

No.	Variety	Supplier	% downy mildew on leaf 1 09 July	% downy mildew on leaf 2 09 July	% downy mildew on later leaves 09 July	% plants with downy mildew 09 July
1	Astra	Tozer Seeds Ltd	5.30	2.07	0.067	63.3
2	Challenger	Tozer Seeds Ltd	7.17	2.63	0.333	86.7
3	Sky Rocket	Tozer Seeds Ltd	5.30	1.40	0.033	76.7
4	Dentellata 24380	CN Seeds	4.77	1.57	0.033	70.0
5	Dentellata 24531	CN Seeds	5.27	2.33	0.067	73.3
6	CN Victoria 23303	CN Seeds	2.77	0.40	0.000	73.3
7	CN 2503	CN Seeds	6.57	1.90	0.333	76.7
8	Rucola 7	Enza Zaden	1.63	0.27	0.000	53.3
9	Rucola 8	Enza Zaden	4.57	0.57	0.033	66.7
		SED (16 df)	2.694	1.473	0.1394	17.29
		F pr.	NS (0.600)	NS (0.689)	NS (0.139)	NS (0.769)
		LSD (5%)	5.710	3.122	0.2956	36.66

Wild rocket seed treatments

All seed treatments provided a good level of downy mildew control up to leaf 2 (Tables 17 & 18). Cotyledons were retained significantly longer in treated plants than in the untreated (Table 17). The lowest rate of Apron XL had more downy mildew than the other fungicide treatments, while the other treatments were not significantly different from each other. Some reduction in the holes left by pest damage from stem weevil adults was evident in treatment 6 (Apron XL + A9700 FS350) (Table 19). Downy mildew caused crinkling and yellowing of the leaves and this resulted in poor growth and low vigour of untreated plants (Table 19).

Table 17. Downy mildew incidence and severity, seed treatments for wild rocket experiment 1, 23 July 2008 (3-6 leaves, 25 days after sowing)

No.	Seed treatment	Rates for wild rocket (ml product/kg seed)	% downy mildew on cotyledons 23 July	% cotyledons remaining 23 July	% downy mildew on leaf 1 23 July	% plants with downy mildew 23 July
1	Untreated	-	0.00	5	26.500	100.0
2	Apron XL	18	0.25	100	0.300	10.0
3	Apron XL	36	0.00	95	0.125	10.0
4	Apron XL	9	7.57	80	5.108	52.5
5	Wakil XL	15g pr	1.25	85	0.025	5.0
6	Apron XL + A9700 FS350	36 + 60	0.25	100	0.000	5.0
		SED (15 df)	3.224	7.26	2.926	10.95
		F pr	NS (0.211)	<0.001	<0.001	<0.001
		LSD (5%)	6.966	15.48	6.237	23.34

Table 18. Downy mildew incidence and severity, seed treatments for wild rocket experiment 2, 08 August 2008 (4-8 leaves, 28 days after sowing)

No.	Seed treatment	Rates for wild rocket (ml product/kg seed)	% downy mildew on leaf 1 08 August	% downy mildew on leaf 2 08 August	% downy mildew on later leaves 08 August	% plants with downy mildew 08 August
1	Untreated	-	16.69	7.50	0.625	75.0
2	Apron XL	18	1.12	1.18	0.225	40.0
3	Apron XL	36	0.05	0.38	0.000	10.0
4	Apron XL	9	8.82	3.03	0.675	65.0
5	Wakil XL	15g pr	3.68	1.35	0.125	40.0
6	Apron XL + A9700 FS350	36 + 60	2.88	0.43	0.150	30.0
		SED (15 df)	2.510	2.186	0.2980	14.41
		F pr	<0.001	0.041	NS (0.178)	0.005
		LSD (5%)	5.349	4.659	0.6531	30.72

Table 19. Vigour and pest assessments following seed treatments in wild rocket experiment 1, 17 July 2008 (2-3 leaves, 19 days after sowing)

No.	Seed treatment	Rates for wild rocket (ml product/kg seed)	Vigour 0-5	% leaf area with holes
1	Untreated	-	2.95	1.75
2	Apron XL	18	4.63	3.00
3	Apron XL	36	4.60	2.35
4	Apron XL	9	4.83	3.00
5	Wakil XL	15g pr	2.58	2.30
6	Apron XL + A9700 FS350	36 + 60	4.53	0.00
		SED (15 df)	0.412	0.904
		F pr	<0.001	0.042
		LSD (5%)	0.879	1.926

All treatments caused some degree of phytotoxicity, with phytotoxic symptoms including yellowing of cotyledon edges and reduced vigour. Treatments 2, 3 and 6 caused the most cotyledon yellowing, while treatment 5 caused the greatest reduction in early vigour (Table 20).

Table 20. Vigour and cotyledon yellowing following seed treatments in wild rocket experiment 1, 07 July 2008 (cotyledon stage, 9 days after sowing)

No.	Seed treatment	Rates for wild rocket (ml product/kg seed)	Vigour (plant size) 0-10	% cotyledon area with yellowing
1	Untreated	-	9.00	0.00
2	Apron XL	18	9.00	3.25
3	Apron XL	36	8.25	7.00
4	Apron XL	9	9.75	0.50
5	Wakil XL	15g pr	4.00	0.25
6	Apron XL + A9700 FS350	36 + 60	7.63	10.00
		SED (15 df)	0.567	1.098
		F pr	<0.001	<0.001
		LSD (5%)	1.209	2.34

Salad rocket seed treatments

In salad rocket, seed treatments gave almost complete control for the duration of the experiments (Tables 21 & 22); the only incidence of downy mildew in any treated plots was in the lowest rate of Apron XL, in the final assessment of experiment 2 (Table 17). As in wild rocket, the Wakil treatment caused a significant reduction in pest damage, measured by percentage leaf area eaten by flea beetles and stem weevils (Table 23).

Table 21. Downy mildew incidence and severity, following seed treatments in salad rocket experiment 1, 01 August 2008 (3-5 leaves, 26 days after sowing)

No.	Seed treatment	Rates for salad rocket (ml product / kg seed)	% Downy mildew on leaf 1 01 August	% Downy mildew on leaf 2 01 August	% plants with downy mildew 01 August
1	Untreated	-	1.06	0.40	60.00
2	Apron XL	6	0.00	0.00	0.00
3	Apron XL	12	0.00	0.00	0.00
4	Apron XL	24	0.00	0.00	0.00
5	Wakil XL	18g pr	0.00	0.00	0.00
6	Apron XL + A9700 FS350	12 + 20	0.00	0.00	0.00
		SED (15 df)	0.198	0.0577	6.24
		F pr	<0.001	<0.001	<0.001
		LSD (5%)	0.423	0.123	13.29

Table 22. Downy mildew incidence and severity following seed treatments in salad rocket experiment 2, 15 August 2008 (2-4 leaves, 22 days after sowing)

No.	Seed treatment	Rates for salad rocket (ml product / kg seed)	% downy mildew on cotyledons 15 August	% downy mildew on leaf 1 15 August	% plants with downy mildew 15 August
1	Untreated	-	15.4	0.98	57.5
2	Apron XL	6	0.25	0.05	5.00
3	Apron XL	12	0.00	0.00	0.00
4	Apron XL	24	0.00	0.00	0.00
5	Wakil XL	18g pr	0.00	0.00	0.00
6	Apron XL + A9700 FS350	12 + 20	0.00	0.00	0.00
		SED (15 df)	4.91	0.372	11.87
				NS	
		F pr	0.034	(0.103)	<0.001
		LSD (5%)	10.46	0.793	25.31

Table 23. Vigour and pest assessments following seed treatments for salad rocket in experiment 1, 01 August 2008 (3-5 leaves, 26 days after sowing), and experiment 2, 12 August 2008, (2-3 leaves, 19 days after sowing)

No.	Seed treatment	Rates for salad rocket (ml product / kg seed)	% leaf area with holes experiment 1 01 August	% leaf area with holes experiment 2 12 August
1	Untreated	-	13.50	7.25
2	Apron XL	6	12.25	9.25
3	Apron XL	12	9.00	9.25
4	Apron XL	24	10.25	7.25
5	Wakil XL	18g pr	12.25	11.25
6	Apron XL + A9700 FS350	12 + 20	1.50	0.80
		SED (15 df)	1.194	1.294
		F pr	<0.001	<0.001
		LSD (5%)	2.546	2.758

As in wild rocket, most of the seed treatments caused some degree of phytotoxicity. Treatments 4 and 6 caused the most yellowing of cotyledons, while treatment 5 caused a delay in emergence and a significant reduction in plant vigour (Tables 24 & 25).

Table 24. Vigour and cotyledon yellowing following seed treatments in salad rocket experiment 1, 17 July 2008 (cotyledon stage, 11 days after sowing)

No.	Seed treatment	Rates for salad rocket (ml product / kg seed)	Vigour (plant size) 0-5 17 July	% cotyledon area with yellowing 17 July
1	Untreated	-	4.58	0.25
2	Apron XL	6	4.78	0.43
3	Apron XL	12	4.85	1.00
4	Apron XL	24	4.58	5.25
5	Wakil XL	18g pr	3.75	0.10
6	Apron XL + A9700 FS350	12 + 20	4.65	2.00
		SED (15 df)	0.2133	0.482
		F pr	0.002	<0.001
		LSD (5%)	0.4546	1.027

Table 25. Emergence and vigour following seed treatments in salad rocket experiment 2, 28 July 2008 (cotyledon stage, 4 days after sowing)

No.	Seed treatment	Rates for salad rocket (ml product / kg seed)	Emergence / plant population 17 July	Vigour (plant size) 0-5 28 July
1	Untreated	-	51.3	4.35
2	Apron XL	6	65.0	4.55
3	Apron XL	12	65.0	4.48
4	Apron XL	24	57.5	4.30
5	Wakil XL	18g pr	37.5	1.88
6	Apron XL + A9700 FS350	12 + 20	85.0	4.75
		SED (15 df)	12.76	0.239
		F pr	0.04	<0.001
		LSD (5%)	27.19	0.509

Field experiment on crop covers

Crop covers had a significant effect on downy mildew incidence and severity, but cover height did not (Table 26). The intended fleece treatments were excluded from the analysis, due to the unavailability of fleece when the experiment started. The two uncovered treatments were assigned as 'low' and 'high' to give a balanced design for two-way analysis of variance. The most severe downy mildew was seen in plots covered with perforated plastic sheeting, followed by Enviromesh (Table 6). Hail netting was the only cover type which did not significantly increase downy mildew incidence relative to uncovered plots.

Crop covers also had an effect on crop vigour (Table 27), with all cover types significantly increasing vigour relative to uncovered plots. The strongest effects were caused by perforated plastic and Enviromesh.

Table 26. Downy mildew severity and incidence, crop covers trial, 4 September 2008 (26 days after sowing)

No.	Cover type	Cover height	% leaf area with downy mildew 4 September	% plants affected by downy mildew 4 September
1	None	(Low)	0.07	3.3
3	Ultrafine	Low	0.40	20.0
4	Enviromesh	Low	0.89	70.0
5	Hail netting	Low	0.05	6.7
6	Perforated plastic	Low	5.27	90.0
8	Ultrafine	High	0.30	23.3
9	Enviromesh	High	0.75	43.3
10	Hail netting	High	0.30	20.0
11	Perforated plastic	High	3.71	90.0
12	None	(High)	0.06	6.7
	Cover type	SED (18 df)	0.630	8.29
		F pr.	<0.001	<0.001
		LSD (5%)	1.324	17.41
	Cover height	SED (18 df)	0.399	5.24
		F pr.	NS (0.445)	NS (0.802)
		LSD (5%)	0.837	11.01
	Interaction	SED (18 df)	0.891	11.72
		F pr.	NS (0.637)	NS (0.207)
		LSD (5%)	1.872	24.62

Table 27. Crop vigour, relative to untreated plots (assigned a vigour score of 5), crop covers trial, 19 August 2008 (17 days after sowing)

Trt No.	Cover type	Cover height 19 August	Crop vigour (control = 5) 19 August
1	None	(Low)	5.0
3	Ultrafine	Low	7.3
4	Enviromesh	Low	9.0
5	Hail netting	Low	7.0
6	Perforated plastic	Low	8.3
8	Ultrafine	High	7.7
9	Enviromesh	High	8.0
10	Hail netting	High	7.3
11	Perforated plastic	High	9.0
12	None	(High)	5.0
	Cover type	SED (18 df)	0.467
		F pr.	<0.001
		LSD (5%)	0.982
	Cover height	SED (18 df)	0.296
		F pr.	NS (0.824)
		LSD (5%)	0.621
	Interaction	SED (18 df)	0.661
		F pr.	NS (0.463)
		LSD (5%)	1.389

PCR test for seed-borne downy mildew: DNA extraction methods

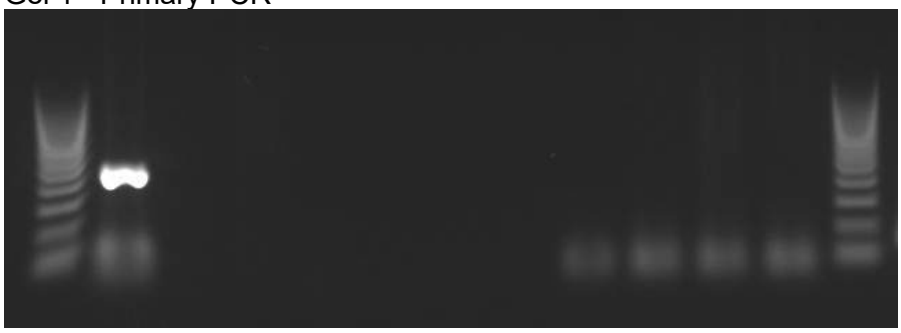
Primary PCR failed on all DNA extractions except the plant material positive control. This is thought to be because the rocket seed extractions were very oily, and there was much carry-over of inhibiting substances. Nested PCR, which uses two sets of primers, was able to overcome the difficulties in amplifying from samples containing inhibitors, and gave a positive result with every DNA extraction (Table 28; compare gel 1 and gel 2). When tested on a dilution series, nested PCR was also able to detect a 1/1000 dilution of the control DNA, compared to a faint band at a 1/100 dilution with the primary PCR reactions.

Table 28. Results from PCR of rocket DNA extractions using primary (gel 1) and nested PCR (gel 2) methods (10 µl loaded onto a 1% agarose gel stained with ethidium bromide)

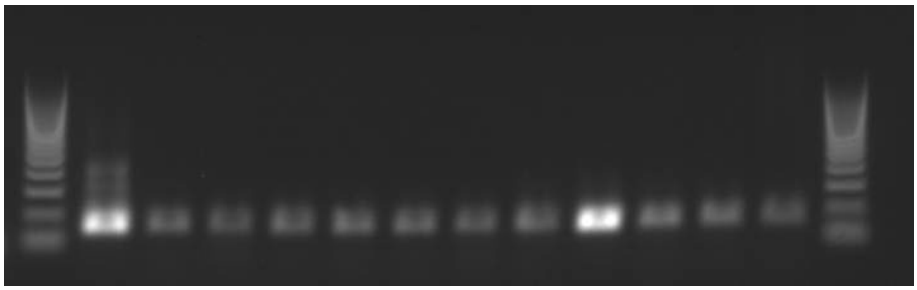
Lane number	Template used	Primary PCR positive?	Nested PCR positive?
1	Positive control	Y	Y*
2	1 07/07 CTAB		Y
3	2 07/07 CTAB		Y
4	3 07/07 CTAB		Y
5	4 07/07 CTAB		Y
6	5 07/07 CTAB + spike		Y
7	6 07/07 CTAB + spike		Y
8	1 22/11/07 Seed wash		Y
9	2 22/11/07 Seed wash + spike		Y
10	1 29/07		Y
11	2 29/07		Y

* Y=positive

Gel 1 Primary PCR



Gel 2 Nested PCR



PCR test for seed-borne downy mildew: primer design

The *Hyaloperonospora parasitica* ATR1 primers produced more than two bands when the annealing temperature was below 58°C suggesting that unspecific binding was taking place. A single band was obtained above the temperature range 50–60°C but it was weak, which was why other primer pairs were used. Consistently robust bands were produced using the ATPase primers designed to an ATPase from *Peronospora viciae*. The ideal annealing temperature for the F1 and R1 primer pair was 60°C. The ideal annealing temperature for the nested primer pair (Pv ATPase F2 + R2) was 58°C.

PCR test for seed-borne downy mildew: commercial samples

Five of the sixteen seed samples tested gave positive results with nested PCR: Columbia (wild), TZ 7002 (wild), T7 0317 (wild), Sky Rocket (salad) and Astra (salad). No positives were identified with the primary PCR. These results are the first indications that downy mildew might be seed-borne in rocket.

Discussion

Variety evaluation

The inoculated screening experiments on wild and salad rocket worked well. Downy mildew was more severe on wild rocket than on salad rocket, reflecting field experience. Named varieties of wild rocket were very susceptible to downy mildew, but some new coded varieties had very high levels of resistance. There appears to be good potential to develop more resistant varieties of wild rocket in future. Salad rocket varieties tested so far showed little variation in downy mildew resistance. New varieties should be routinely tested for downy mildew susceptibility so that only improved types are launched commercially.

Field testing of varieties is an important second stage of the project. High rainfall in August 2008 hampered field experimentation and the scheduled variety trials will be done in spring 2008. These will expose the varieties to different field populations of downy mildew and perhaps identify varieties where the genetic resistance is fragile.

Seed treatments

Seed treatment experiments gave very promising results. There was better control with higher doses of metalaxyl and no indications of poor control because of fungicide resistance. The Wakil seed treatment also gave good control of downy mildew but checked plant growth. This appears to be acceptable if the downy mildew control allows the crop to be harvested. The availability of treatments with different fungicides should reduce the risks of fungicide resistance problems. Pest control options are also promising as control of damage by stem weevil adults was demonstrated

Crop covers

Some crop covers aggravated downy mildew. The mesh covers used to protect crops against insect pests had less effect on downy mildew than a perforated polythene cover. Air flow and higher temperatures within the covered areas are likely to be the main factors involved. Covered crops grew more quickly than uncovered control areas and would have been harvested earlier. A further experiment will be done in 2009 to confirm these results.

Diagnostics

The initial *H. parasitica*-infected plant material was positive in all PCR reactions carried out. Original seed that these plants were grown from (Ref: Shamrock 4707015141) was supplied which was then used to assess different methods of DNA extraction. Rocket seed extractions were very oily and there was much carryover of inhibiting substances as

observed with failure of primary PCR despite the plant/seed material having been spiked with infected rocket leaf tissue. Nested PCR using two sets of specific primers gave much greater sensitivity to the PCR detection method. When tested on a dilution series the nested reactions were able to detect a 1/1000 dilution of the control DNA whereas there was only a faint band present in the 1/100 dilution with the primary PCR reactions. Additionally, the nested PCR was able to overcome the difficulties in amplifying from samples containing inhibitors. Negative controls of potentially contaminating fungi should always be included when trying new primers as the Pv ATPase nested primers appeared to produce a band with these.

Several seed batches of both wild and salad rocket were obtained through the project, which were intended to be tested with the new methods. A detection limit of 1/5000 seeds was desirable so 5000 seeds of each variety were germinated and subsequently ground up for DNA extractions.

The primary PCR did not identify any positives. However there were five seed lots which were positive with the bTubulin nested primers. PCR tests do not indicate whether the DNA is viable or not. Further seed lots are required to establish if downy mildew is seed-borne and capable of initiating field outbreaks.

Conclusions

There were large differences in downy mildew susceptibility in wild rocket, but no significant differences between salad rocket varieties. Field testing is now required to substantiate these results, but growers may wish to undertake their own evaluation of the most promising material.

Seed treatments appear very promising for downy mildew control and for control of some pests. Wakil and a metalaxyl seed treatment were effective and the former is being used by some growers.

There was generally more downy mildew under crop covers than in uncovered areas. A perforated polythene cover produced the greatest effect. Mesh covers for pest control allow air movement and are the most practical option given their role in preventing pest damage.

There are indications that downy mildew may be seed-borne in some seed stocks, but further work is required to demonstrate that this can cause plant infection.

The next stage of the project is field evaluation of varieties to ensure that their performance is robust against different pathogen populations.

Technology transfer

- Presentation by Kim Green at British Leafy Salads Association Conference, Peterborough, 19 November 2008
- Discussion o project at SPGA Meeting, Boxworth, 7 February 2008
- Telephone and email contact with growers, consultants, seed companies and agrochemical manufacturers.

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