

Project title	Improving the value of downy mildew resistance information for UK spinach growers
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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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# Grower Summary

## Headline

- Understanding the 'R' gene content of a spinach variety helps growers to expect disease and act accordingly.
- Knowledge on the Race distribution of downy mildew isolates identified in the UK assists in instigating appropriate control methods.

## Background and expected deliverables

Spinach downy mildew (*Peronospora farinosa*) causes considerable losses to UK spinach growers. Crops with low levels of infection may be completely unsaleable, resulting in substantial loss of income. Controlling the disease requires the use of expensive fungicides. Growers need high levels of disease resistance in varieties in order to be able to reduce fungicide use. There are a number of resistance ('R') genes available, which can confer immunity to the disease. However, several of these have been "overcome" by changes in the pathogen, rendering the variety susceptible.

Knowledge of the 'R' gene content of a variety will tell growers whether or not to expect disease, and thus what approaches for disease control would be most appropriate. The validity of this information for the UK is critical. Growers receive information on the "R gene" content of varieties from breeders, but there have been instances when a variety expected to be resistant has been susceptible. This may be due to the emergence of new Races, or to errors in the claimed resistance profile.

The project carried out independent testing of varieties nominated by growers with the most relevant typed isolate set from the Netherlands, and also with local UK collections. This report provides growers with an independent assessment of resistance gene content, and a prediction of the risk of attack on different varieties in the main UK production areas.

## Summary of the project and main conclusions

Typed Races and seed of the differential set of varieties of spinach were obtained from Natkuinbouw in The Netherlands. Standard tests on cotyledons were successfully carried out in controlled environment rooms to establish expertise in the UK for Race monitoring and variety testing. Race 5 and Race 7 were both found in the first year of the project (2006). In addition, an unknown Race which infected all the differential lines available was found. This was later confirmed as Race 10 by its reaction on a further differential obtained from Natkuinbouw. Only a few isolates were obtained during 2007. These proved to be Race 8, the first time this was identified in the UK but no further Race 10 isolates were found. In 2008, despite favourable disease conditions, again only a few isolates were received. These were Race 8, and again there was no evidence of Race 10.

Confirmation of "new" pathotypes in a country usually requires recording the new Race over at least two seasons and in a number of different growing areas. By this definition, Race 10 cannot be confirmed as established in the UK, as it is in the US and some European countries. However, Race 8 should be considered as established in this country. Despite the fact that Race 10 was seen in only one year of this project, the initial record emphasises the continuing ability of *P. farinosa* to produce new variants and use of Race 10 resistant varieties would be an effective preventative strategy.

Race 8 should be considered as an established threat, and deployment of resistant varieties should offer an effective means of control. However, several observations from growers have suggested that varieties with claimed Race 8 resistance have shown significant infection in the field. Samples were received from some Race 8 resistant varieties, but all proved to be Race 8 when tested on differentials, and not a new pathotype. During the final year of the project, several varieties with claimed resistance to Race 8 were tested with a UK Race 8 isolate to investigate the incidence of susceptible reactions under controlled conditions. For these tests, 125 plants were used, and for some varieties, up to 17 plants in one variety showed fully susceptible reactions on the cotyledons (see table). Infection also appeared on true leaves of some of the same plants.

Variety	Number of plants (out of 125) with cotyledon infection	
	7 days after infection	17 days after infection
Emilia	5	5
Amazon	0	5
Monza	17	17
Lazio	2	2
Indurain	0	5
Fiorano	4	4
Swan	0	4
Ottawa	0	0
Apollo	0	0

There may be several reasons for this observation, though the most likely is the existence of susceptible “sib” or off type lines within the resistant seed stock. On a field scale, the appearance of susceptible plants at up to around 10% of the population would cause significant quality problems, though the great majority of resistant plants would prevent any major field epidemic.

The appearance of susceptible plants in the field may thus not always be due to a new Race, or to erroneous information on resistance profiles. However, if resistant seed stocks are contaminated, growers will need to monitor crops carefully, and be prepared to use fungicides rather than rely completely on resistance for disease control.

Some key points:

- Races 5, 7, 8 and 10 of spinach downy mildew have all been identified in UK growing areas during a three year monitoring period (2006, 2007 and 2008),
- Race 10 was only found in one year (2006) and location (Kent), and at present cannot be confirmed as established in the country,
- Race 8 has been found in two seasons (2007 and 2008), from different areas, and should be considered as an established pathotype,
- Tests with typed isolates on a panel of varieties selected by growers confirmed resistance profile claims from breeders,
- There was no consistent evidence of resistance on juvenile leaves being different from the resistance expressed in cotyledon tests,
- Some varieties with overall resistance to Race 8 showed significant numbers of plants with susceptible reactions, corresponding to grower field observations.

Financial benefits

Financial benefits from the project will accrue indirectly from the awareness that while resistance claims have been found to be accurate, low level contamination with susceptible types in some seed stocks may be present. Crops should be monitored carefully and preventative sprays applied, even where resistance is present. However, the emergence of new Races is also likely to occur, which may lead to the loss of effectiveness of some resistances.

#### Action points for growers

- Monitor crops with no claims for Race 10 resistance carefully, as the Race may emerge again
- Race 8 is established in the UK, and varieties without resistance to it may succumb quickly to widespread infection in the field
- Several varieties with Race 8 resistance were found to contain low levels of susceptible material, and continued vigilance will be necessary to control the disease with fungicides when scattered infected plants begin to be seen

## Science Section

### Introduction

Spinach downy mildew is caused by the oomycete pathogen *Peronospora farinosa* f.sp. *spinaciae*. It is found readily in European and American production areas. Low levels of infection in a crop will result in major quality problems and loss of crop. While effective fungicides exist currently, there is always a risk that actives may be lost in the future, or that the pathogen may develop insensitivity. Growers therefore rely on variety resistance as an important tool in the management of the disease. Resistance to spinach downy mildew is governed by a series of genetic factors which are probably single genes of major effect ("R genes") and which give complete control of the pathogen. Some varieties are also claimed to have background or field resistance, which, though not usually at a high level, can offer protection when disease pressure is low.

It is well established that *P. farinosa* is a highly variable pathogen, capable of giving rise to new pathotypes or Races which overcome previously effective R genes. This has led to a series of breakdowns of resistant varieties in the field, which often occur very rapidly, and continual breeding effort is needed to produce new varieties with resistance to the newly emerged Races.

In the UK, growers became concerned that varieties in commercial production with claimed resistance to the known existing Races were becoming infected and leading to significant losses. There are several possible reasons for these observations. The emergence of new Races is an obvious explanation, but other causes may be present. Firstly, the existence of erroneous claims for resistance may be possible. Secondly, there is a possibility that adult plant reactions may be different from those expressed on cotyledons which are usually used to profile resistance factors, and finally the existence of "off-types" in a variety which lack the claimed resistances may lead to significant field infection.

This work was undertaken to investigate the identity of Races occurring in UK production areas; to validate claims for resistance in selected varieties; and to investigate the uniformity of the reaction of further varieties to typed Races.

### Materials and methods

A set of typed Races (Pfs 1-7) was obtained from Natkuinbouw, The Netherlands, at the start of the project in 2006 together with seed of the European spinach differential set. An additional differential with Race 10 resistance was obtained at the end of 2006. R gene content of this differential set is summarised below. Races were maintained on cotyledons of cv Giant Winter in a growth room at 15° C with a 16h day and frozen at -20° C for longer term storage. Samples of downy mildew from outbreaks of disease in the UK were received from growers, incubated in a damp chamber at between 5 and 10° C to induce further sporulation if necessary and transferred onto Giant Winter before testing on the differential set and other varieties. Inoculations were carried out by spraying a spore suspension at 10<sup>5</sup> spores/ml on cotyledons, and covering the plants with either small plastic domes or larger polythene bags for 48h. Plants were then allowed to grow for a further 7 to 10 days, before covering again for 48h to induce sporulation. Cotyledons were scored for the presence or absence of infection. All tests were carried out in growth room at a constant 15° C, with a 16h day. A total of 20 plants were used for differential tests. For investigation of off-type frequency, a total of 125 plants per variety were used.



R gene content of European differential set supplied from Natkuinbouw

Differential	Resistance to Pfs Races
Nores	1,2
Califlay	1,3,5,8,9
Polka	1,2,3,5,8,9
Rushmore	1,3,4,5,8,9
Bolero	1,2,3,4
Spinnaker	1,2,3,4,5,8,9
Spicer	1,2,3,4
San Felix	1,2,3,4,5,8,9
Clermont	1,2,3,4
Lion	1,2,3,4,5,6,7,8,9
Eagle	1,2,3,4,5,6,7
Boeing	1,2,3,4,5,6,7
069727	(+) 10

Results

During 2008, four samples were received, all from east and south east of England growing areas. Source variety and results of the differential test are shown in Table 1. All the isolates proved to be Race 8.

Table 1: Reaction of 2008 downy mildew samples on differential series

Differential	Source variety			
	Columbia	Rhino	Parrot	Lazio
Nores	+	+	+	+
Califlay	-	-	-	-
Polka	-	-	-	-
Rushmore	-	-	-	-
Bolero	+	+	+	+
Spinnaker	-	-	-	-
Spicer	+	+	+	+
San Felix	-	-	-	-
Clermont	+	+	+	+
Lion	-	-	-	-
Eagle	+	+	+	+
Boeing	+	+	+	+
069727 (Race 10 resistor)	-	-	-	-

+ = fully susceptible reaction on cotyledons of all 20 plants, - = no sporulation seen

Two Race 8 isolates obtained from Toscane and Divina during 2007 was used to test the reaction of a panel of commercial cultivars. Reactions to both the isolates were the same and generally uniform within a variety, though in one or two cases "off-type" reactions were observed (Table 2). Only 40 plants were included in this test..

Table 2: Reaction of commercial spinach varieties to two Race 8 isolates

Variety	Race 8 from Toscane	Race 8 from Divina
Giraffe	+	+
Grizzly	+	+
Apollo	-	-
Crocodillo	-	- (2 plants +)
Swan	-	-
Toscane	+	+
Campania	+	+
PV 0385	+	+
PV 501	- (5 plants +)	- (7 plants +)
Tarpy	+	+
SP 890	-	-
SP 896	-	-
Verdi	-	-
Bach	-	-
Bizet	-	-
RX 2084	-	-
Allegro	-	-
Ventis	-	- (1 plant +)
RX 1340	-	-
Whale	-	-

Following discussion with growers, seed of a further nine varieties were obtained, and tested with Race 8. Seed of Yabi, Bonbini, Sardinia, Toucan, Pelican, Silverwhale, Marabu and Squirrel was also requested but could not be sourced. For this test, and increased number of plants (125) were used. There was clear evidence for non-uniformity of reaction to Race 8 (Table 3). Monza appeared particularly variable.

Table 3: Number of susceptible plants out of 125 in Race 8 resistant varieties inoculated with a Race 8 isolate

Variety	Number of susceptible plants	
	7 days after infection	17 days after infection
Emilia	5	5
Amazon	0	5
Monza	17	17
Lazio	2	2
Indurain	0	5
Fiorano	4	4
Swan	0	4
Ottawa	0	0
Apollo	0	0

## Discussion

During the course of this project, two “new” Races have appeared in the UK, corresponding to Race 8 and Race 10 profiles on the standard differential series. This mirrors experiences elsewhere. Race 8 and Race 10 have been recorded elsewhere in Europe, and Race 8, 9 and 10 have all emerged in the US (Irish *et al.*, 2007), only a short time after the emergence of Races 5, 6 and 7 (Irish *et al.*, 2003).

Despite favourable conditions for disease development during 2007 and 2008, only a few samples were received compared to 2006, and growers reported relatively little disease. This may reflect effective fungicide application and the deployment of varieties with Race 1-10 resistance on a comparatively large area. This would tend to drive down overall inoculum levels, and reduce opportunities for new populations to develop and spread.

Nevertheless, the fact that two new Races were detected in a three year testing period confirms that continuing vigilance is necessary. The existence of Race 10 in the UK was notified to the International Working Group on Peronospora, though at present, its status as an established Race in this country cannot be confirmed. It was only detected in one year, and at one growing site. In contrast, Race 8 should be considered as established, since more than one sample, from different growing sites, was received in each of two years.

A putative Race 11 (see [www.westernfarmpress.com](http://www.westernfarmpress.com) January 2009) has recently been identified in the Californian growing area, which is infecting varieties with Race 10 resistance such as Lazio. While the identity of this Race remains to be confirmed, it may well emerge in Europe, and growers should inspect varieties with Race 1 to 10 resistance regularly for signs of disease.

It was clear that some varieties with claimed resistance to Race 8 were becoming significantly infected in the field. When samples from these varieties (eg Lazio and Columbia) were tested, a clear Race 8 profile was obtained on the differentials, showing that the varieties were not breaking down to a new Race. Growth room tests with a number of varieties with claimed Race 8 resistance, including Lazio, showed that the varieties were predominantly resistant, but that a variable number of plants showed susceptible reactions, and should be considered as off-types. In the field, this may have significant consequences, especially at high planting densities. Only one seed stock per variety was tested in these cases, from 2008, and it is possible that future seed stocks will be uniformly resistant.

Comparison of variety resistance “claims” with the controlled environment tests carried out with typed isolates did not reveal any discrepancies. Not all the typed isolates obtained from Natkuinbouw were successfully retrieved from the frozen material received, and no tests were carried out with Races 2 and 3, though these appear comparatively unimportant currently. Tests with juvenile leaves compared to cotyledons carried out in previous years of the project showed that reactions were very similar, and there was no indication of significant changes in resistance depending on plant age.

## Conclusions

Results obtained during the course of this project show that resistance claims for spinach varieties are reliable, and growers should expect these resistances to persist on mature material in the field. However, new Pfs Races have emerged during the course of the project and caused previously resistant varieties to become susceptible. New Races now appear to occur relatively frequently, as with other downy mildew pathogens. Close cropping, high planting densities, and possibly organic production may all allow new populations to develop and increase.

A new Race (Pfs 11) which occurred in the US in 2008 may well appear in Europe, but any new Race must occur in at least two seasons, before it can be considered as established. This should ensure that growers and the breeders react to definitive problems, rather than transient threats which do not persist. Race 8 should however be considered as established in UK growing areas though not Race 10 at present.

Particular problems have arisen with the existence of off-types in some Race 8 resistant varieties, which leads to significant infection in the field. This may occur with other resistant material, to Race 8 and other Races, in the future. It is critical that such infections are Race tested so that off-type problems can be separated from the emergence of new and potentially threatening Races.

#### Technology transfer

A summary of results on UK Race testing was included in the proceedings booklet of the British Leafy Salads Conference, November 2006.

An article on the project and related work on fungicide control appeared in May 2007 HDC News

A summary of the project findings was presented to the Leafy Salads Crop group meeting in October 2008

The International Working Group Peronospora was made aware of this project, and the finding of Race 10 during 2006

#### References

B M Irish, J C Correll, S T Koike, J Schafer, T E Morelock, 2003. Identification and cultivar reaction to three new Races of the spinach downy mildew pathogen from the United States and Europe. *Plant Disease*, 87, pp 562-72

B M Irish, J C Correll, S T Koike, T E Morelock, 2007. Three new Races of the spinach downy mildew pathogen identified by a modified set of spinach differentials. *Plant Disease*, 91, pp 1392-1396

## Appendix

### Variety sources and geographic origin of samples received

Source variety (where known)	Area of collection
Monza	Hants
Unknown	Kent
Cherokee	Kent
RX 22059	Kent
Silverwhale	Kent
Whale	Kent
PU0772	Kent
Toscane	Essex
Divina	Surrey
Unknown	Sussex
Unknown	Sussex
Rhino	Cambs (site 1)
Columbia	Cambs (site 2)
Parrot	Cambs (site 3)
Lazio	Lincs