Project title:	Pea Downy Mildew diversity in the UK
Project number:	FV 436
Project leader:	Processors and Growers Research Organisation
Report:	Annual report, March 2016
Previous report:	Annual report, March 2015
Key staff:	Dr L Wiesel, K Poulson & R Ward, PGRO Dr C Domoney, C Moreau & M Ambrose, JIC
Location of project:	Processors and Growers Research Organisation, The Research Station, Great North Road, Thornhaugh, Peterborough, PE8 6HJ. John Innes Centre, Norwich Research Park, Colney Lane, Norwich, Norfolk, NR4 7UH.
Industry Representative:	Stephen Francis, Fen Peas Ltd, The Old Farm House, Church End, Old Leake, Boston, PE22 9HR
Date project commenced:	1st April 2014
Date project completed (or expected completion date):	31st March 2018

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

Report authorised by:

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

CONTENTS

GROWER SUMMARY

Headline

A collection of several field-collected pea downy mildew (DM) populations from different locations across the UK has been established at PGRO but the distribution of downy mildew races could not be determined.

Background

Pea DM is a major disease of both vining and combining peas in the UK. Early infection can kill plants, while later infections can reduce yield by up to 55% in the UK. Quality standards for vining peas are high and blemish due to disease infection is not accepted by processors. Downy mildew invades pods, reducing the quality and visual appearance of the produce. Primary infection, caused by soil-borne oospores, can be supressed by the use of the seed treatment Wakil XL (metalaxyl-M, fludioxonil and cymoxanil). Disease tolerance is present in some varieties, although DM race differentiation leads to variable levels of tolerance.

Primary infection of young seedlings can be reduced by growing peas in a rotation of one year in five. Due to the location of processing factories vining peas are grown in intensively cropped areas and, although the rotation in pea crops is maintained, the land may have supported many pea crops for a considerable period, allowing greater build-up of soil-borne inoculum. Wakil XL is used when there is a high risk of DM, either from early sowing into poor soil conditions and when weather is suitable for disease development or where disease pressure is high. Rotation and seed treatment reduce the incidence of primary infection by soil-borne oospores but secondary infection from airborne spores cannot be controlled in this way. Descriptive and recommended lists are produced annually to indicate relative tolerance of current pea varieties to DM (PGRO Vining Pea Growers Guide and PGRO Pulse Agronomy Guide) and growers use the lists to influence their choice of variety and seed treatment.

No single option to reduce the risk of the disease described above gives complete control of DM.

Varieties may be more or less susceptible than expected, in different areas of the country. This is the result of both the varied nature of the DM population and the genetic interaction between the pea variety and the pathogen. The UK DM population is made up of a number of genetically distinct races. A study carried out in the 1980s identified 11 UK races (Taylor, 1986). No studies have been undertaken since then to establish dynamics and geographic

spread of these races. The project will investigate diversity and spread of DM populations across the UK and investigate differences in pea varietal tolerances to the different DM populations.

Summary

During the 2015 growing season, 16 samples of pea DM were collected from different fields by PGRO. The samples were taken across wide region of the UK from Kent to Somerset and Yorkshire. Pea DM can only survive on a living plant (with the exception of the long-lived oospores in the soil) and it is difficult to maintain a living culture under laboratory conditions. A method was developed at PGRO to maintain a DM culture collection and 16 pea DM field samples could successfully be maintained over the whole season. The DM cultures are available at PGRO and can be cultivated to achieve freshly sporulating material for further experiments.

In order to try to determine the race structure of the DM populations, four differential pea host lines with recorded resistances and susceptibilities to UK DM races were used. The pea lines were inoculated with DM and presence or absence of DM infection recorded. This information may then be used to determine the DM race (Table 1). Infection was inconsistent within the same pea line and DM sample but differences were observed between the different DM samples (Table 2). This shows that the DM field samples most likely consist of several DM races. However, differences in susceptibility of the pea differential host lines to the different field samples were observed (see isolate 101 for example, Table 2) which indicates that the DM populations differ in different UK regions. The combined infection success rates of all field samples showed that the pea lines differ greatly in their overall tolerance levels. More than 50% of all seedlings of line JI 1272 were infected by DM whereas lines JI 15 and JI 85 only showed 12.2% and 4.4% infection rates, respectively. These data have implications for pea varietal tolerance in different UK regions.

Table 1. Susceptibility (S) or resistance (R) of four pea differential host lines (JI 411, JI 560, JI 758, JI 1272) to 11 races of downy mildew (UK pathotypes). The differential host lines can be used to determine the race of a DM culture (Taylor, 1986).

DM race	JI 411	JI 560	JI 758	JI 1272
1	S	S	S	S
2	S	S	S	R
3	S	S	R	S
4	S	R	S	S
5	S	R	R	S
6	R	S	S	S
7	R	S	S	R
8	R	S	R	S
9	R	R	S	S
10	R	R	R	S
11	R	R	R	R

Table 2. Downy mildew infection of seedlings of two pea germplasm lines (JI 15 and JI 85) and four pea differential host lines (JI 411, JI 560, JI 758, JI 1272), using six or eight seedlings per test. Number of infected plants out of total of inoculated, growing plants (in brackets).

Sample	Inoculation Date	JI 15	JI 85	JI 411	JI 560	JI 758	JI 1272
I 81	24/11/2015	2 (8)	1 (8)	0 (8)	0 (8)	0 (8)	0 (8)
I 83	02/11/2015	1 (8)	0 (8)	0 (8)	0 (8)	4 (8)	1 (8)
I 84	23/09/2015	1 (8)	0 (8)	2 (8)	6 (8)	2 (8)	8 (8)
I 85	23/09/2015	1 (8)	0 (8)	3 (8)	2 (8)	3 (3)	7 (8)
I 87	11/09/2015	2 (6)	1 (6)	2 (6)	0 (6)	3 (4)	5 (6)
I 90	11/09/2015	2 (6)	1 (6)	2 (6)	2 (6)	2 (6)	5 (6)
I 94	11/09/2015	0 (6)	1 (6)	4 (6)	6 (6)	2 (6)	6 (6)
I 94	30/09/2015	2 (8)	0 (8)	1 (7)	1 (8)	5 (8)	3 (8)
I 96	02/11/2015	0 (8)	0 (8)	4 (8)	1 (8)	0 (8)	0 (8)
I 99	30/09/2015	0 (8)	0 (8)	0 (7)	3 (8)	2 (8)	7 (8)
I 100	19/10/2015	0 (8)	0 (8)	0 (7)	2 (8)	0 (8)	3 (8)
I 101	19/10/2015	0 (8)	0 (8)	0 (7)	7 (8)	0 (8)	2 (8)
Overall % infection		**12.22%**	**4.44%**	**20.93%**	**33.33%**	**27.71%**	**52.22%**

Work at the John Innes Centre has identified a downy mildew resistance locus in the pea genome arising from JI 15 (see Table 2) and genetic markers have been developed for this locus. New crosses using pea lines that carry different sources of resistance have been developed which, in combination with DM screening, will generate further genetic information that can be used for marker-assisted breeding.

Pea leaves carrying a single DM lesion will be collected in 2016 with the assumption that the single lesion was caused by an individual DM race. These DM isolates will be used to inoculate the four differential host lines in order to determine their race. The information will be used to study race distribution in the UK. To determine geographical differences in DM

populations and to assess differences in pea variety tolerance to the different DM populations, field trials will be performed in the 2016 season. The four pea host lines and the two germplasm lines (Table 2) plus a selection of combining and vining pea lines will be grown in fields at six different locations along the East Coast of the UK and DM infection will be assessed.

Financial Benefits

No results on distribution of downy mildew populations across the UK or on pea variety tolerances to different downy mildew populations could yet be obtained and therefore, no recommendations can yet be given.

Action Points

Action points are not applicable at this stage of the project.

SCIENCE SECTION

Introduction

Downy mildew (*Peronospora viciae* f. sp. *pisi*) is a serious disease of pea crops grown in the UK. It was first reported as a serious problem in pea crops in the 1960's with yield losses between 45 and 80% reported (Biddle et al. 1988; Taylor 1986) and, despite the development of more tolerant modern cultivars, it remains a significant source of losses to the profitability of the pea crop firstly by compromising the growth of the plants through lesions of the stem, leaves and stipules and later by spreading into the pods where it directly affects the quality of the developing seeds. Downy mildew is both soil and air-borne, surviving in the soil as oospores. When peas are drilled, root leachates stimulate the germination of the oospores. These move to the seedlings and cause systemic infection which frequently results in plant death. Infected seedlings appear to have a blue velvet texture as a result of the development of sporangiophores on the leaf surface (Fig. 1). These release conidia onto air currents to infect neighbouring and distant plants. This is the secondary infection causing disease on flowering plants and pods. Infected plants have reduced photosynthetic area which can result in substantial yield reduction and poor produce quality.

Some control of primary downy mildew can be achieved through use of cultural practices and fungicidal seed treatments. Growers use crop rotation, growing peas and beans at a minimum of one year in five, to minimise infection. Choice of variety can also reduce the risk of disease. Disease tolerance exists in many combining pea varieties and ratings can be found in the PGRO Pulse Agronomy Guide Recommended List tables. There is less varietal disease tolerance available in vining peas and ratings can be found in the PGRO Vining Pea Guide Descriptive List tables.

The seed treatment Wakil XL (metalaxyl-M, fludioxonil and cymoxanil) is used to control primary infection of seedlings planted in areas where there is a history of disease. However, this does not control secondary or pod infection. There are currently no foliar-applied products to control downy mildew.

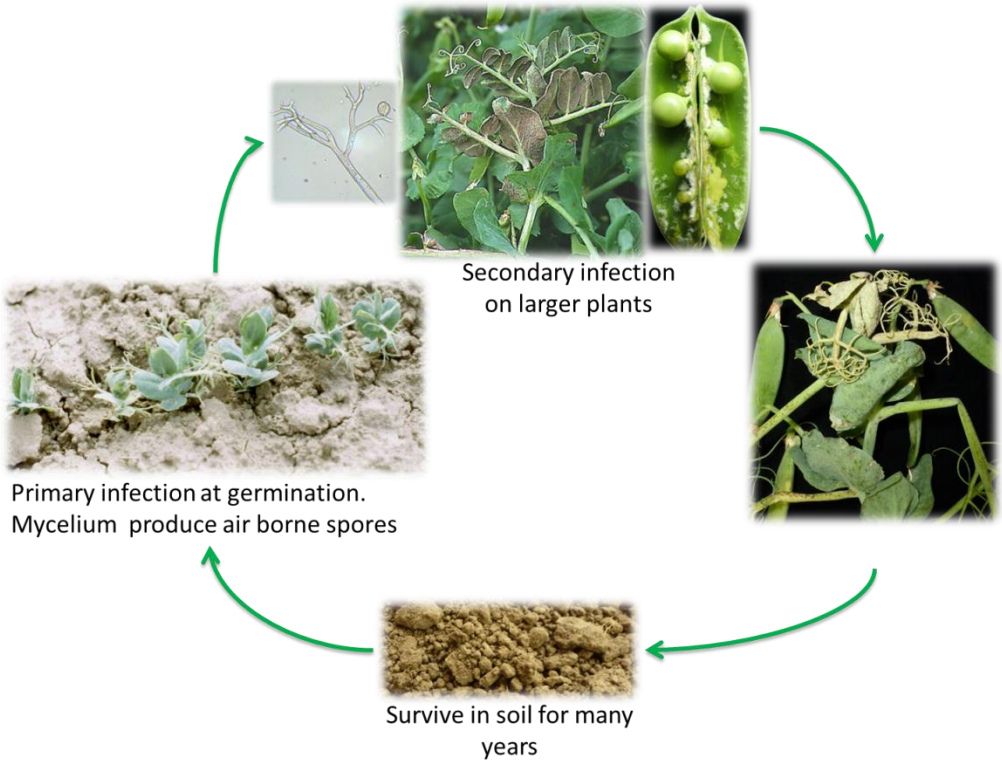

Figure 1. The life cycle of pea downy mildew.

Downy mildew produces large quantities of airborne spores and is able to evolve very quickly (Liu et al. 2013). This results in the development of different populations with subtle genetic differences. The constantly changing population can result in the development of new virulent races that are able to cause severe infections in varieties that were previously only mildly susceptible or moderately tolerant. For example, the marrow fat variety Sakura has scored a 7 for DM tolerance on a 1-9 scale (1 = very susceptible, 9 = resistant) in 2010, a 6 in 2013 and only a 5 in 2016 (PGRO Pulse Agronomy Guides 2010, 2013, 2016). Very little is known about the genetic diversity of downy mildew in the UK. Differences do exist and varieties grown in some areas of the UK appear to be more tolerant to downy mildew than in others, even when disease pressure is high in both areas. More research is needed to assess the impact of differing DM populations on tolerances of pea varieties and to assess whether germplasm resistances hold up to current DM populations. Therefore, this project will carry out field trials in addition to further study distributions of DM populations and races.

Materials and methods

Culture collection (PGRO)

During the 2015 growing season, 16 field samples of pea DM were collected by PGRO (Milestone (M) 6). The samples were either obtained by collection of infected pea plants during field visits by staff from PGRO or were received from pea growers across the UK who sent infected pea plants to PGRO. The samples covered a wide region across the UK from Kent to Somerset and Yorkshire (Table 3, Figure 2).

Table 3. Downy mildew sample ID, collection date, location with postcode of town centre and host variety on which the DM sample has been collected of the DM samples received in 2015.

Sample	Collection date	Location	Centre postcode	Host variety
I 80	04/06/2015	Kilham	YO25 4SH	Celebration
I 81	04/06/2015	Kilham	YO25 4SH	Celebration
I 83	05/06/2015	Kilham	YO25 4SH	Celebration
I 84	19/06/2015	Nocton	LN4 2BN	Span
I 85	19/06/2015	Donington	PE11 4TR	unknown
I 87	19/06/2015	Fosdyke Bridge	PE12 6LH	Avola
I 89	19/06/2015	Fosdyke Bridge	PE12 6LH	Geneva
I 90	23/06/2015	Milverton	TA4 1LN	Legacy
I 91	24/06/2015	Holbeach	PE12 7NQ	Bartesa
I 94	29/06/2015	Romney Marsh	TN28 8TS	Kelvedon Wonder
I 96	02/07/2015	West Ashby	LN9 5PT	Span
I 98	02/07/2015	West Ashby	LN9 5PT	Span
I 99	02/07/2015	Thimbebly	LN9 5RF	Anubis
I 100	02/07/2015	West Ashby	LN9 5PT	Span
I 101	02/07/2015	Baumber	LN9 5NG	Span
I 102	02/07/2015	Ranby	LN8 5LN	Anubis

Figure 2. Origins of pea DM samples received or collected in 2016.

Since pea DM is an obligate pathogen and can only grow on living plant tissue, the first task was to optimise a method to cultivate DM on pea seedlings under laboratory conditions. As reported last year (see Year 1 report for this project, FV 436 Annual Report 2015), the cultivation of DM samples from 2014 was unsuccessful (M2, M3). Several methods have been tested from spraying a weak Tween solution containing DM spores to placing infected pea material next to pea seedlings. The method which resulted in infected pea seedlings most reliably was to place fungal mycelium directly on freshly germinated pea roots and hypocotyls.

In more detail, *Pisum sativum* var. Avola seeds were surface sterilised using a 10% bleach solution and left to germinate on potato dextrose agar for 3-5 days. Freshly germinated seeds with a root of about 1 cm length and a freshly emerged hypocotyl were chosen for inoculation. Using a scalpel, strongly sporulating DM mycelium was scraped off an infected pea plant and carefully placed on the hypocotyl and young root of the seedling (Fig. 3). The seedlings were placed in a tray filled with moist compost, covered with compost and the tray covered with a lid. The trays were kept in a growth room at 15°C with 16 h light for 1-2 weeks before being moved to a glasshouse at 20-22°C. Correct temperature and humidity conditions are crucial for infection to occur and were optimised to achieve strong DM infection (Fig. 4). After about four weeks the infected plants start to die off and therefore sub-culturing onto fresh seedlings has to be performed about every three weeks. Infected plant samples were always kept apart to avoid cross contamination. Sub-culturing was performed in a cabinet enclosed on three sides with as little air movement as possible. The cabinet was thoroughly disinfected after

working with each DM sample. Infected plant material from each DM sample was also dried for long term storage (M6). Future DM samples will also be stored at -80°C.

Figure 3. Demonstration of technique to inoculate pea seedlings with freshly sporulating DM mycelium.

Figure 4. Pea seedlings infected with DM.

Pea differential host lines (PGRO)

In order to determine the race of the DM sample, four differential pea host lines with recorded resistances and susceptibilities to UK DM races were used (Table 4). The pea host lines were infected with the different DM field samples as described above. Six to eight seedlings per pea line were used for each DM isolate and presence or absence of DM infection recorded (M10).

In addition, two pea germplasm lines with potentially strong resistance to DM (JI 15 and JI 85) were also inoculated with the different DM samples to determine their resistance profile (M11). If these two lines show resistance to a wide range of UK DM populations they hold the potential to be used in DM resistance breeding.

Table 4. Susceptibility (S) or resistance (R) of four pea differential host lines (JI 411, JI 560, JI 758, JI 1272) to 11 races of downy mildew (UK pathotypes). The differential host lines can be used to determine the race of a DM culture (Taylor, 1986).

DM race	JI 411	JI 560	JI 758	JI 1272
1	S	S	S	S
2	S	S	S	R
3	S	S	R	S
4	S	R	S	S
5	S	R	R	S
6	R	S	S	S
7	R	S	S	R
8	R	S	R	S
9	R	R	S	S
10	R	R	R	S
11	R	R	R	R

Results

Identification and multiplication of genetic stocks for use in laboratory and field disease trials (JIC)

The pea genetic stocks identified by the desk study (see Year 1 report for this project, FV 436 Annual Report 2015) have undergone two rounds of multiplication in JIC glasshouses. The seeds were supplied initially by the JI Germplasm Resources Unit, which curates over 3000 *Pisum* germplasm accessions, usually as low numbers of seeds of purified stocks, and can provide clean stocks of accessions in limited numbers on demand. Seeds from the first round of multiplication were shared with PGRO to facilitate discrimination in their laboratory of pea DM races in UK soil samples. The genetic stocks were multiplied again to ensure ample seeds would be available for further analysis (Table 5, M5).

Table 5. Lines of pea used for multiplication of stocks; highlighted are lines of particular interest with respect to differential resistance, where a higher number of seeds were re-sown: in pink are the four lines described as host differential variants, in blue are two further lines identified as sources of resistance gene(s). [CRF number = JIC stock identifier and seed storage tracker] (M5).

JI accession	Seeds to PGRO (Aug 15)	Seeds re-sown CRF60059
JI 15	648	**15**
JI 85	185	**15**
JI 411	590	**15**
JI 441	480	**10**
JI 540	717	**10**
JI 560	647	**15**
JI 584	253	**10**
JI 758	541	**15**
JI 952	578	**10**
JI 1215	690	**10**
JI 1272	862	**15**
JI 1273	1120	**10**

The same set of pea lines was sown in the field at JIC in the 2015 season. These stocks were shared with PGRO to enable samples to be sown at a number of UK locations in 2016.

Based on the desk study reported in 2015 (M1), new crosses have been established (M9). The source of resistance (JI 15) identified within PCGIN (Defra-funded Pulse Crop Genetic Improvement Network) is unlikely to offer durable resistance since one major genetic locus is implicated in the resistance derived from this line. The ability of a pathogen to overcome a single genetic locus which determines resistance will be diminished by combining different resistance loci. The desk study revealed that JI 85, a *P. sativum* Afghanistan line, had been reported to be resistant to all downy mildew pathotypes, except for pathotype 7. Neither this line, nor JI 15 (see above), had been included in further research, following that reported in the desk study. Given the differences in their apparent responses to the pathogen and widespread resistance shown by JI 85, we propose that the cross between JI 15 and JI 85 will be of particular interest with respect to developing durable resistance (Table 6). Note that

further crosses have been established between JI 15 and JI 1194 to enlarge the small population (60 recombinant inbred lines) that has been used in the exploitation of the legume genome sequences and PCGIN marker data for DM resistance breeding (see section below).

Table 6. Lines of pea used for establishing crosses (M7, M9) between sources of differential resistance and for extending an existing mapping population between JI 15 and JI 1194, where the latter is a vining type of pea, susceptible to DM.

F1 seeds sown	Number of crossed pods collected
JI 15 x JI 85-Cx-F1-#1	5
JI 85 x JI 15-Cx-F1-#1	3
JI 399 x JI 85-Cx-F1-#1	4
JI 85 x JI 399-Cx-F1-#1	3
JI 1194 x JI 85-Cx-F1-#1	2
JI 15 x JI 411-Cx-F1-#1	2
JI 15 x JI 1194-Cx-F1-#1	10
JI 1194 x JI 15-Cx-F1-#1	2

The crosses will be verified by phenotypic analysis of F1 plants, where possible; for example, crossing pollen from a purple-flowered, tall pea having round seeds with yellow cotyledons (all dominant traits) onto a white-flowered, short pea genotype having wrinkled seeds with green cotyledons allows for F1 plants and seeds to be verified based on four visual dominant traits. Where a cross is performed in the opposite direction, seeds from genuine crosses will have the same phenotype as seeds from selfed flowers. In the latter case and where genotypes do not have distinguishing phenotypes, crosses are verified by genotyping, using amplification of one or more genes to identify sequence-based or amplicon size polymorphism between the parent lines.

Assessment of DM field samples using the pea differential host lines (PGRO)

Five of the 16 DM samples (I80, I89, I91, I98 and I102) grew so poorly over the whole season that the amount of fresh fungal mycelium produced was never high enough to inoculate the host differential pea lines. The remaining DM samples were inoculated using freshly sporulation mycelium onto four pea differential host lines (JI 411, JI 560, JI 758, JI 1272) and onto two germplasm lines (JI 15 and JI 85) and presence and absence of DM infection was

assessed (Table 7). The differential host lines were inoculated in order to determine the race of the DM sample and to get information on DM populations. The germplasm lines were inoculated to assess whether their resistance holds up to current DM populations and to assess the potential of JI 85 to be used in DM resistance breeding. Infection was inconsistent within the same pea line and DM sample which might show that the DM samples were not pure and it is therefore not possible to determine a race. However, differences were observed between the different DM samples which show that DM populations differ in different fields across the UK.

The combined infection success rates of all field isolates showed that the pea lines differ greatly in their overall tolerance levels. More than 50% of all seedlings of line JI 1272 were infected by DM whereas lines JI 15 and JI 85 only showed 12.2% and 4.4% infection rates, respectively. This shows that the resistance based on JI 15 still holds up under current conditions and that JI 85 has great potential for resistance breeding in the future.

Table 7. Downy mildew infection of seedlings of two pea germplasm lines (JI 15 and JI 85) and four pea differential host lines (JI 411, JI 560, JI 758, JI 1272), using six or eight seedlings per test. Number of infected plants out of total of inoculated, growing plants (in brackets). Overall % infection of all DM samples on the six pea lines is given.

Sample	Inoculation Date	JI 15	JI 85	JI 411	JI 560	JI 758	JI 1272
I 81	24/11/2015	2 (8)	1 (8)	0 (8)	0 (8)	0 (8)	0 (8)
I 83	02/11/2015	1 (8)	0 (8)	0 (8)	0 (8)	4 (8)	1 (8)
I 84	23/09/2015	1 (8)	0 (8)	2 (8)	6 (8)	2 (8)	8 (8)
I 85	23/09/2015	1 (8)	0 (8)	3 (8)	2 (8)	3 (3)	7 (8)
I 87	11/09/2015	2 (6)	1 (6)	2 (6)	0 (6)	3 (4)	5 (6)
I 90	11/09/2015	2 (6)	1 (6)	2 (6)	2 (6)	2 (6)	5 (6)
I 94	11/09/2015	0 (6)	1 (6)	4 (6)	6 (6)	2 (6)	6 (6)
I 94	30/09/2015	2 (8)	0 (8)	1 (7)	1 (8)	5 (8)	3 (8)
I 96	02/11/2015	0 (8)	0 (8)	4 (8)	1 (8)	0 (8)	0 (8)
I 99	30/09/2015	0 (8)	0 (8)	0 (7)	3 (8)	2 (8)	7 (8)
I 100	19/10/2015	0 (8)	0 (8)	0 (7)	2 (8)	0 (8)	3 (8)
I 101	19/10/2015	0 (8)	0 (8)	0 (7)	7 (8)	0 (8)	2 (8)
Overall % infection		**12.22%**	**4.44%**	**20.93%**	**33.33%**	**27.71%**	**52.22%**

Exploitation of legume genome sequences and PCGIN marker data for DM resistance to bridge the link between phenotype and precise (perfect) markers for breeding (JIC)

Legume genome sequences and PCGIN marker data for DM resistance have been exploited to provide precise (near-perfect) markers for breeding. Gene-specific markers have been developed for the DM resistance locus identified on Linkage Group I (LG I) in the JI 15 x JI 1194 mapping population (see Table 5), using candidate genes based on synteny with the model genome sequence of *Medicago truncatula* and emerging transcriptome data for pea. Figure 5 shows an example of the assay for one gene linked to DM resistance: a serine/threonine kinase (STK) gene on pea LG I.

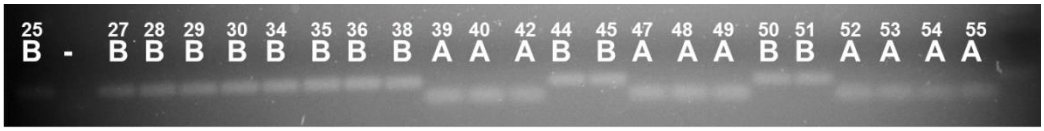

Figure 5. Genetic marker for DM resistance in pea, based on Mt5g035030 (STK gene reference in *Medicago truncatula*), using a dCAPS assay that distinguishes two bands of 70 (JI 1194, B) and 35 (JI 15, A) base pairs in progeny lines (25 – 55).

The genetic map position for a second gene (Function Unknown Protein, FUP) in this region of LG I (Figure 6) supports a position for the disease resistance locus as residing between these two markers. The data suggest that using these two gene-specific markers will allow the LG 1 resistance gene to be followed in crosses (M8).

This will facilitate not only breeding programmes but additionally the distinction of LG I resistance alleles from others that may be characterised later in pea lines showing differential resistance (see above and annual report 2015). The earlier (PCGIN) LG I map position for DM resistance was determined by variation in repetitive DNA around the locus, a consequence of variation in retrotransposon insertion sites across the pea genome. The assays used here generate a multiple and complex array of fluorescently-labelled DNA fragments, which must be analysed using specialised software to align fragments, assign sizes to them and identify variants in progeny lines from crosses. While this complexity benefits genetic mapping by generating many markers, the methodology is not likely to be adopted by breeding programmes, where one or two variant fragments may suffice to follow the linked trait of interest. In contrast, the assays now reported here are based on simplex PCR (two pimers), enzyme digestion and gel electrophoresis, as may be conducted in a basic molecular biology laboratory. The assay results shown in Figures 5 and 6 do not rely on the use of fluorescent primers or specialised software, and hence are much more likely to be adopted by breeders.

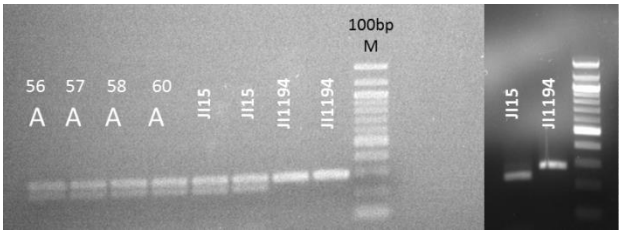

Figure 6: Genetic marker for DM resistance in pea, based on Mt5g036420 (FUP gene reference in *Medicago truncatula*), using a dCAPS assay that distinguishes a single band of ~280 (JI 1194, B) from a doublet of ~200 and ~280 (JI 15, A) base pairs. Progeny lines (56 - 60) are shown alongside the parents, JI 15 and JI 1194. Note that longer digestion times allow for complete digestion of products in JI 15 to generate the ~200 base pair band alone (inset to right of image). A 100 bp size marker is shown alongside for comparison of both gels.

Discussion

In 2015, a method to cultivate pea DM on young pea seedlings was optimised at PGRO. This was crucial to be able to maintain a DM collection in the UK. As a result, 16 DM samples are currently in long-term storage at PGRO. The collection will be increased in the 2016 season.

During the 2015 season, the infection profile of the DM field samples on pea differential host lines was tested. Seedlings of the same pea line were not consistently susceptible or tolerant to the DM samples. Furthermore, the pea differential host lines show different responses to DM samples collected from neighbouring fields (see samples 96 and 100). This most likely reflects that each DM field sample consists of several DM races and represents part of the DM population of the field it was collected from. In order to get a better idea of DM race distribution, single pea leaves carrying a single DM lesion will be collected in 2016 from a few field sites. It is the assumption that each single lesion will be of pure race. The aim is to multiply each single lesion on a susceptible isolate and to use the four differential pea host lines to determine the DM race.

However, differences in susceptibility of the pea differential host lines to the different field samples were observed (see sample 101 for example, Table 7) which indicates that the DM populations differ in different UK regions. This has implications for tolerances of pea varieties to DM because tolerance levels will depend on the composition of each DM population. A pea variety might be more tolerant to a DM population in Yorkshire than to a DM population in Kent. To further investigate this point and in order to inform growers which pea varieties perform best in their individual growing regions small scale field experiments will be performed in 2016. The field trials will replace the proposed pot tests (M13, M16) because growing peas in pots for field inoculation carries the great risk of plant death due to dried out

pots. A selection of combining and vining pea lines plus the four pea host lines and the two germplasm lines (Table 7) will be grown in fields at six different locations along the East Coast of the UK and DM infection will be assessed. The four differential host lines will be included to see whether DM populations differ in the different locations and the two germplasm lines will be included to further test the strength of their resistance.

To test the broad utility of the molecular assays developed for the JI 15 x JI 1194 population which segregates for DM resistance, the two genetic markers shown in Figures 5 and 6 have been deployed in a second mapping population generated at JIC (JI 15 x JI 399). Progeny lines carrying the JI 15 LG I resistance locus have been identified, thus generating two groups which may be tested for their resistance. In this cross, however, unlike the situation with the JI 15 x JI 1194 population, there may be additional resistance arising from JI 399 (Ref PCGIN annual report 2006/7, which showed that, of the JI lines tested, JI 1194 was the most susceptible to the NIAB DM pathogen samples tested whereas, in contrast, JI 399 showed low infection at < 10%). Crosses between JI 15 and elite lines have been established by industry. The two closely linked markers shown may now be deployed in screening progeny lines, in conjunction with disease assays based on newly acquired pathogen samples. This activity will be coordinated with JIC and PGRO.

Conclusions

- A method to cultivate pea DM was optimised by PGRO
- PGRO has a DM culture collection of 16 different field samples (M6)
- Downy mildew field samples seem to be a mixture of several DM races (M10)
- Pea differential host lines have been multiplied by the JIC in sufficient numbers for glasshouse and field trials to be carried out (M5)
- Two gene-specific markers will allow the LG 1 resistance gene to be followed in crosses, based on JI 15 x JI 1194 (M8)
- Gene markers for a second mapping population (JI 15 x JI 399) have been developed by JIC (M7, M8)
- New crosses have been established at JIC (M9)

Knowledge and Technology Transfer

1) Cereals 2015 (June 2015). Discussion with growers, breeders and other stakeholder that visited PGRO's stand at Cereals 2015.
2) DEFRA GINs Conference: Uncorking the genetic 'GINie' for British crops (February 2016). Presentation by Dr Claire Domoney, JIC: Unlocking nature's diversity for UK pulse crops. Audience: Pulse Crop Genetic Improvement Network (PCGIN) and other GIN stakeholders, policy-makers, scientists and radio and news press.
3) Legume panel meetings (November 2015 and February 2016). Update on project given by Becky Ward.
4) PGRO and Syngenta Roadshows (4 meetings in January and February 2016). Presentations by Dr Lea Wiesel, PGRO: Pea downy mildew and beneficial microbes. Audience: Pea growers, pea breeders, scientists and other stakeholders.
5) PGRO Crop Protection course (February 2016). Presentation by Becky Ward, PGRO: Diseases and fungicides 2016. Audience: Pea growers and agronomists.
6) PGRO Pulse Open Day Stubton (July 2015). Discussion with growers, breeders and other stakeholder that visited PGRO's open day.
7) PGRO Vining pea Open Day Nocton (June 2015). Discussion with growers, breeders and other stakeholder that visited PGRO's open day.
8) The Pulse Magazine (December 2015). Article by Dr Lea Wiesel, Pea downy mildew diversity. Processors and Growers Research Organisation, Peterborough, UK.
9) The Vegetable Magazine (December 2015) Article by Dr Lea Wiesel, Pea downy mildew diversity. Processors and Growers Research Organisation, Peterborough, UK.

References

Biddle, A.J., Knott, C.M., Gent, G.P. (1988) Pea Growing Handbook. Processors and Growers Research Organisation, Peterborough, UK.

Maguire, K. (2015). Pea Downy Mildew diversity in the UK. AHDB Horticulture Annual Report 2015 for project FV 436.

Liu, J., Cao, T., Chang, K.-F., Hwang, S.-F., Strelkov, S.E. (2013) Virulence and diversity of *Peronospora viciae* f. sp. *pisi* in Alberta, Canada. Crop Protection 43, 18-26.

PGRO Pulse Agronomy Guide (2010) Advice on agronomy and varieties of combining peas, field beans, and lupins. Processors and Growers Research Organisation, Peterborough, UK.

PGRO Pulse Agronomy Guide (2013) Advice on agronomy and varieties of combining peas, field beans, and lupins. Processors and Growers Research Organisation, Peterborough, UK.

PGRO Pulse Agronomy Guide (2016) Advice on agronomy and varieties of combining peas, winter and spring field beans, and other pulse crops including latest PGRO Recommended Lists. Processors and Growers Research Organisation, Peterborough, UK.

Taylor, P.N. (1986). Resistance of *Pisum sativum* to *Peronospora pisi*. PhD thesis, University of East Anglia

