Project title:	Pea Downy Mildew diversity in the UK
Project number:	FV 436
Project leader:	Dr Kerry Maguire, Processors and Growers Research Organisation (PGRO)
Report:	Annual report, March 2015
Previous report:	N/A
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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:	01 April 2014
Expected project completion date:	31 March 2018

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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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CONTENTS

GROWER SUMMARY

Headline

Selected pea (*Pisum)* lines showing strong or partial resistance to downy mildew, a major disease in peas, provide a resource for experimental field tests at different sites in the UK, alongside the collection and identification of current downy mildew isolates.

Background

Pea downy mildew 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 and picking 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 downy mildew race differentiation causes variable levels of tolerance.

The primary infection of the young seedling 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 downy mildew 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. A descriptive list is produced annually to indicate relative tolerance of current varieties (PGRO Vining Pea Growers 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 downy mildew.

Varieties may be more or less susceptible in different areas than expected. This is the result of the both the varied nature of the downy mildew population and the genetic interaction between the variety and the pathogen. The UK downy mildew population is made up of a number of genetically distinct races. A study carried out in 1989 identified 11 UK pathotypes

(Taylor et al., 1989). No studies have been undertaken since 1989 to establish dynamics and geographic spread of these populations. The project will investigate diversity and spread of the differential populations across the UK.

Summary

The project aims to provide growers with information about downy mildew race structure, geographic spread in the UK and varietal tolerance to races. Current conventional control options are limited to a seed treatment, rotational management and varietal tolerance. Varietal tolerance, however, may vary in different regions as race structure of downy mildew changes. Little is known about current race structure and the investigation will identify races in the UK and map their distribution to allow growers to utilise varietal information to greater benefit. Information from the project will feed into the breeding industry to develop improved resistance in pea varieties.

A significant body of historical literature has been collated and reviewed relating to downy mildew research and UK field trials and tests alongside the identification of early resistant germplasm. The work in the 1970-80's predates the advent of molecular markers, so there is considerable scope to re-visit and re-examine the various sources of earlier resistant germplasm and further characterise these.

Financial Benefits

Recommendations will be provided towards the end of the project and, as such, cost-benefit has not been calculated at this stage.

Action Points

Action points cannot be recommended at this stage.

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 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.

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 1 year in 5, 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. Vining and picking peas are harvested fresh and the disease causes pod and seed blemishing.

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.

Downy mildew is both soil and air borne and survives 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 results in yield reduction of up to 55% in the UK (Biddle et al., 1988) and poor produce quality.

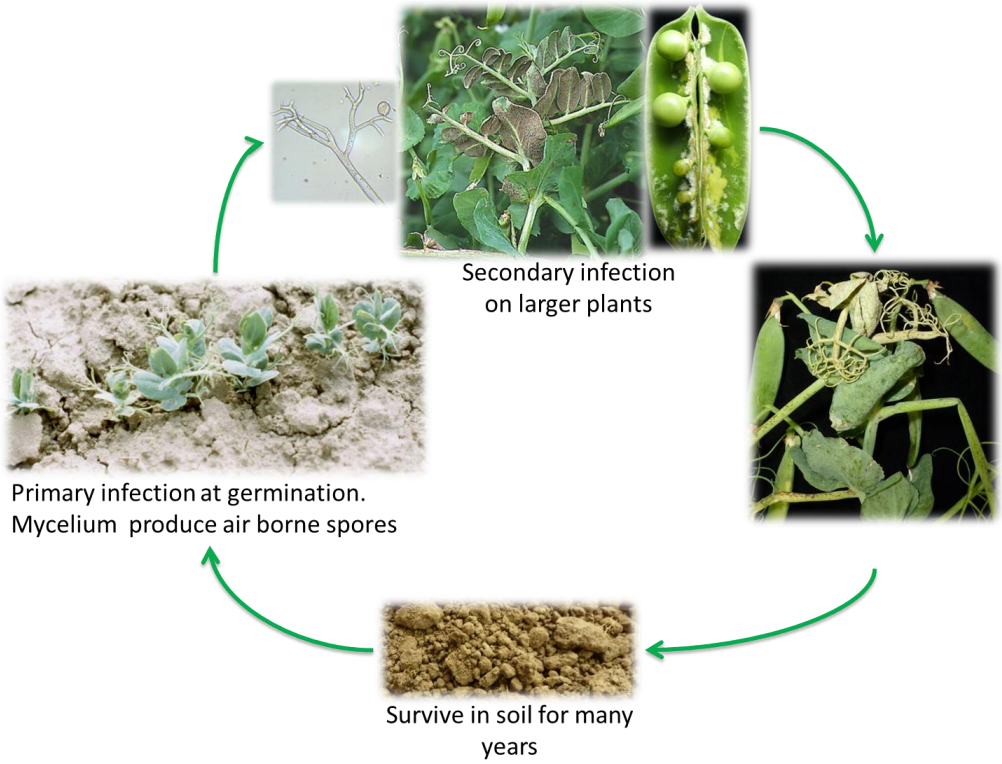


Figure 1. The life cycle of pea downy mildew.

Downy mildew produces large quantities of airborne spores and is able to evolve very quickly. 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. 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.

This project aims to study the genetic diversity of UK pea downy mildew populations by sampling across a range of different regions and to review the literature and past work on downy mildew in the UK to inform and help shape future actions undertaken within this project and beyond.

A significant body of historical literature has been collated and reviewed relating to downy mildew research and UK field trials and tests alongside the identification of early resistant germplasm. The work in the 1970-80's predates the advent of molecular markers, so there is considerable scope to re-visit and re-examine the various sources of earlier resistant germplasm and further characterise these.

1. A downy mildew host differential set described by Taylor *et al.* (1989) is available for *Pisum* through the Germplasm Resource Unit (GRU) at JIC. (See below: these lines are being multiplied up in 2014/15 to ensure their further availability for checking newly collected downy mildew race isolates).
2. Resistant germplasm from earlier studies has been identified.
3. JI 85 (a wild accession from Afghanistan) had not been pursued as a source of resistance in earlier work. Sufficient seed of JI 85 is available for further studies.

Materials and methods

Isolate collection

Requests for downy mildew infected plants were made to growers via PGRO road shows, open days, website, Pulse Magazine and requests made to specific growers.

Plant material was stored in the fridge. The material with active sporulation was placed in 20 ml SDW and 1 drop Tween 20 was added. This was agitated for 30 sec on a Whirl mixer. The resulting spore suspension was spray inoculated onto trays of pea seedlings cv. Avola at the 2-3 leaf stage. A propagator lid was placed over the seedlings to maintain humidity. These were placed at 5ºC for 24 h dark, and then moved to 20ºC 12h light with the lids removed. The lids were replaced after 5 d. Plants were inspected for signs of downy mildew sporulation between 7 and 14 d.

Literature review and seed multiplication

Literature and past work on Downy Mildew in the UK was reviewed to inform and help shape future actions undertaken within this project and beyond and is included in Appendix 1.

Two rounds of seed multiplication have been carried out at JIC in 2014/15 on germplasm lines identified in earlier studies as showing partial or strong resistance. The first of these was in the field in 2014 and the second under glasshouse conditions in 2014/15. For the latter, 12 lines were sown: JI 15 (13 plants), JI 85 (14 plants), JI 411 (15 plants), JI 441 (7 plants), JI 540 (12 plants), JI 560 (14 plants), JI 584 (15 plants), JI 758 (14 plants), JI 952 (15 plants), JI 1215 (12 plants), JI 1272 (7 plants), and JI 1273 (12 plants).

Results

Isolate Collection

Seventy plant samples were received. Twenty six of these came from variety trial sites. The material received had very low infection levels and most was not actively sporulating. Some of the samples were confused with leaf miner damage and did not have downy mildew symptoms. The prevalence of tissue rotting diseases resulted in the inability to successfully inoculate downy mildew onto new plant material in year 1.

Literature review and seed multiplication

Figure 2 shows lines mapped onto the structure analysis of the JI *Pisum* collection as published by Jing *et al.* 2010. Figure 2 indicates that accessions scored at the John Innes Institute in the early 1980's with high/moderate resistance are spread across group 1 (majority landrace group), group 2 (majority modern cultivar group) and their associated sub-groups (1.1-1.7 and 2.1 and 2.2). A significant number of lines also fall into the U and group 3 (wilder taxa including *P. fulvum*, *P. elatius* and *P. abyssinicum*).

Progress with the development of gene-specific markers will be available through the next project report.

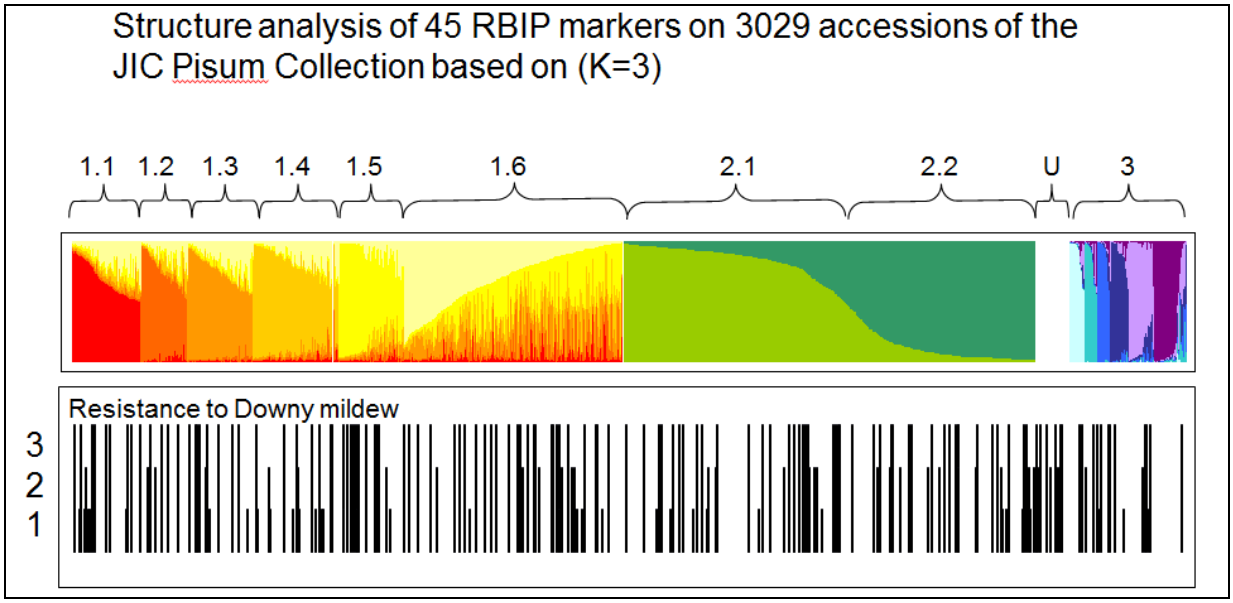


Figure 2. Downy mildew scores of 1-3 for JI *Pisum* germplasm (Table 4 Appendix 1) set against the molecular marker-based structure analysis of the JI *Pisum* collection published by Jing *et al.* 2010

A Downy Mildew host differential set described by Taylor *et al.* (1989) is available for *Pisum* through the Germplasm Resource Unit (GRU) at JIC. These lines are being multiplied up in 2014/15 to ensure their further availability for checking newly collected downy mildew race isolates.

JI 85 (a wild accession from Afghanistan) had not been pursued as a source of resistance in earlier work. Sufficient seed of JI 85 is available for further studies.

Discussion

Disease levels were low in 2014 and susceptible varieties developed little disease in field trials sown in downy mildew prone areas. It was therefore not surprising to receive few samples that were actively sporulating. In 2015, fields will be surveyed for downy mildew starting with areas with existing populations. Current knowledge of varietal tolerance will be used to target varieties where infection would be less expected. Information from the literature review carried out at JIC has identified lines showing partial or strong resistance to downy mildew that have been multiplied and will be available for checking on newly collected downy mildew race isolates.

Conclusions

The results obtained from the early *Pisum* germplasm screening described in Appendix 1 should inform further study within AHDB Horticulture and Pulse Crop Genetic Improvement Network (PCGIN – DEFRA) projects.

- Selected *Pisum* lines showing strong/partial resistance should be bulked to provide a resource for field tests at different sites in the UK, alongside the collection and identification of current downy mildew isolates.
- The older screening did not identify JI 15 as a source of major resistance. This line, along with JI 85 (Tables 1, 4 and 5), should be added to the resource above.
- The downy mildew resistance locus identified in the Holden (2009) and PCGIN (2009) studies are likely both derived from JI 15. Gene-specific markers should be developed to this region of Linkage Group I, exploiting the synteny with *Medicago truncatula* to facilitate gene identification.
- The early identified sources of resistance can now be compared in terms of common alleles at particular genetic loci (for example, the linkage group I locus above). This work has been initiated by looking at the distribution of lines within groupings of the JIC germplasm (Figure 2).

Downy mildew isolate collection in 2014 was constrained by poor conditions for disease development and sporulation, hence limited success in generating a downy mildew isolate collection. For 2015 collections will be systematically undertaken across the UK from plant emergence, and further investigation of alternative techniques for plant inoculation carried out to ensure success.

Knowledge and Technology Transfer

PGRO Open day 2014 (Oral and Poster presentation)

VAA Meeting November 2014 (Oral presentation)

Holbeach Marsh Pea Growers Technical Meeting 2014 (Oral presentation)

The Pulse Magazine Spring 2014 (Article)

PGRO Staff Away day 2014 (Oral presentation)

Bruce Farms Technical meeting 2014 (Oral presentation)

References

Anon[a] (2015). PGRO Pulse Agronomy Guide.

Anon[b] (2015) PGRO Vining Pea Growers Guide

Biddle et al., PGRO Pea Growing Handbook 1988

Taylor P.N., Lewis B.G. and Matthews P. (1989) Pathotypes of *Peronospora viciae* in Britain. J. Phytopathology 127: 100-106

Appendix 1: Literature review 2014-15

Report for Project: Pea Downy Mildew diversity in the UK (AHDB reference: FV 436/31304360; JIC reference: HDC_DM14)

Funder Details: Horticultural Development Company on behalf of AHDB

Supplier details: John Innes Centre, March 2015

In fulfilment of M1 (to review current literature on genetic stock-downy mildew race interactions) and M5 (to multiply genetic stocks of pea)

Objectives of this review

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 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.

This report aims to review the literature and past work on Downy Mildew in the UK to inform and help shape future actions undertaken within this project and beyond.

Summary

A significant body of historical literature has been collated and reviewed relating to downy mildew research and UK field trials and tests alongside the identification of early resistant germplasm. The work in the 1970-80's predates the advent of molecular markers, so there is considerable scope to re-visit and re-examine the various sources of earlier resistant germplasm and further characterise these.

1. A Downy Mildew host differential set described by Taylor *et al.* (1989) is available for *Pisum* through the Germplasm Resource Unit (GRU) at JIC. (See below: these lines are being multiplied up in 2014/15 to ensure their further availability for checking newly collected downy mildew race isolates).
2. Resistant germplasm from earlier studies has been identified.
3. JI 85 (a wild accession from Afghanistan) had not been pursued as a source of resistance in earlier work. Sufficient seed of JI 85 is available for further studies.

We report on these key areas below and provide recommendations for taking the project forward.

Early germplasm screening

Screening of the JI *Pisum* germplasm collection was initiated in 1982 by the then JI germplasm curator and pathologist, Peter Matthews. This work was in support of the pea breeding programme, based at the John Innes at that time, to identify useful genetic materials, to undertake crossing experiments to determine the genetic basis of the resistance, and to identify suitable resistant material as sources of resistance for the breeding programme.

Pathotype
The basis of the inoculum used for this screen has not been established, although reference to UK races 1-7 in the comments and notes indicates that use was being made of the extensive race collections that had been in use through the 1970's at NIAB.

A later compilation by Ambrose and Matthews (1991) provides details that might cross reference to this study (Table 1).

Table 1. Host differential set for *Peronospora pisi* and supporting references

Peronospora pisi

Hosts	Pathotypes							
	1	* 2	UK 3	* 4	5	* 6	* 7	* 8
JI 1272 cv. Katinka		4		1		1	4	4
JI 1273 cv. Heralda		1		1		4	1	4
JI 560 cv. Clause 50		1		2		4	3	4
JI 411 cv. Cobri	R	1	R	2	R	4	3	4
JI 540 cv. Dark Skinned Perfection	s	3	R	4	s	4	3	4
JI 584 cv. Recette	s	4	s	4	R	4	4	4
JI 758 cv. Starnairn	R	4	R	4	R	4	4	4
JI 441 cv. Puget		1		4		4	3	4
JI 952 cv. Koroza	R	1	R	1	R	4	4	4
JI 1215 cv. Cicero	R	4		1	R	4	3	4
JI 85 wild-type Afghanistan		1		1		1	4	1

* Hubelling, N. Med. Fac. Landbouww. Rijksuiv. Gent 40, 539-543, 1975.

* Ester & Ger1.agh, M. Zaadbelangen 33, 146-147, 1979

UK Taylor, P. PhD. Thesis University of East Anglia, 1984.

von Heyendorf, R. & Hoffman, G.M.Z. Pflkankh,Pflpath.PflSchwz.

Dow, P. Unpublished.

R: Resistant, s: Susceptible (Hubelling, 1975; Ester & Gerlagh, 1979)
1: resistant, 2,3,4: susceptible (Taylor, 1984)

Pea material

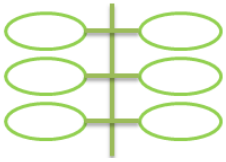
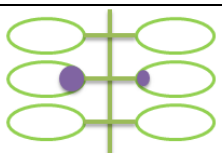
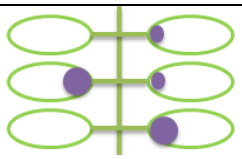
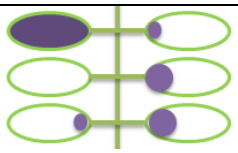
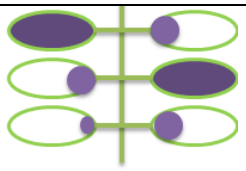
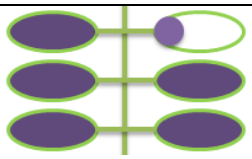
Eight seeds were sown in small Jiffy pots (GH 5s) into peat and sand. Jiffy pots were placed in gravel trays. Seedlings were inoculated at around 20 days old. Test plants were scored 10-20 days after inoculation.

Seedlings were scored using two scales. The first is a score of the observed phenotype (Table 2) and the second is the % of leaf area infected on the three inoculated nodes (Table 3).

Table 2. Scale for scoring Downy Mildew (Matthews 1984)

Score	Value judgement	Observed phenotype
0	Resistant	No infection
1	Hypersensitive	No sporulation, necrosis only
2	Slightly susceptible	Slight sporulation, some mycelial cover
3	Medium susceptibility	Medium sporulation on inoculated nodes
4	Very susceptible	Heavy sporulation (spread of infection from inoculated nodes)

Table 3. Percentage leaf area infection with illustration of three inoculated nodes

% infection	Scale	Phenotype at 3 inoculated nodes
0	0 (no infection)	
1	0-5%	
2	5-25%	
3	25-50%	
4	50-75%	
5	75-100%	

A total of 824 accessions were screened out of a total of 1400 accession which represented the collection at that time. Eighty-three accessions (10% of those screened) were found to be resistant to one or more races (races 3, 2, 5 or mixtures). It is clear from the documentation that genetic lines of interest were not exclusively those showing the highest possible resistance score (score=0 or 1). Results are therefore presented for accessions with scores of 1, 2 or 3 (Table 4) to provide a wider context for discussion with breeders. The breadth is noted to be important in the context of current discussions on the value of generating durable resistance, based on combining partial or moderate scores, as opposed to high resistance based on a major gene which might quickly break down.

Table 4. Resistant germplasm accessions for scores 1 to 3 from the JI *Pisum* collection identified in 1982

Score 1: Hypersensitive		Score 2: Slightly Susceptible		Score 3: Medium Susceptibility	
LINE	**NAME**	**LINE**	**NAME**	**LINE**	**NAME**
7	DE HAAN 201.1(DWARF)	2	P. ABYSSINICUM	15	WBH 1458
14	CAERULICANS-ar	17	LAMPRECHT 232	16	EXTRA RAPID
18	WBH 21	26	STIPULA-IMMINUATA-stim	33	CRYPTO-DWARF-le,la,cry/c
20	WBH 1089	73	WBH 1238	34	LAMM 30
21	BLEEKBLOIER	90	P.SATIVUM-AFGHANISTAN	44	WBH 974
22	FRUHE GELBE THYRINGER	99	P.SATIVUM-AFGHANISTAN	45	P.TRANSCAUCASICUM
23	PARVUS- ORANGE TESTA	118	WBH 22	46	WBH 1221
24	WBH 1510	125	GRISEOSTRIATA-gri	47	WBH 1276
25	WBH 1511	128	WBH 741	48	WBH 1303
74	ALTERNO-MARMORATA-mex	195	AROUS EL SHOAG	52	P. ASIATICUM
85	P.SATIVUM-AFGHANISTAN	250	P. JOMARDII	55	WBH 1288
86	P.SATIVUM-AFGHANISTAN	295	P.SATIVUM-GREECE	56	WBH 1080
87	P.SATIVUM-AFGHANISTAN	296	CHEMIN LONG	57	REDUCTUS-red
95	P.SATIVUM-AFGHANISTAN	310	ONWARD	58	WBH 1073
114	L 5618-af,tl	313	DUKE OF ALBANY	59	TENUIFOLIUS-ten
124	PINKISH WHITE-am1	316	LITTLE MARVEL	60	WBH 577
127	WBH 592	473	MARKET GEM	61	WBH 761
134	NAVICULA APERTUS-nap	540	PERFECTED FREEZER-70A	62	AUREA-au
138	CHLOROTICA-chi	607	EARLY PERFECTION-3040	65	WBH 1454
185	WIRAIG	751	P.SATIVUM-AFGHANISTAN	75	WBH 1470
194	AROUS EL SHOAG	793	WBH 578	83	P.SATIVUM-AFGHANISTAN
200	P.THEBAICUM	799	GOLDKONIG	84	P.SATIVUM-AFGHANISTAN
201	P.THEBAICUM	802	WINGES-37-red1-1	91	P.SATIVUM-AFGHANISTAN
228	P.SATIVUM-BOLIVIA	804	P.TIBETANICUM	93	P.SATIVUM-AFGHANISTAN
241	P. HUMILE	812	BLACK EYED SUSAN	102	P.SATIVUM-AFGHANISTAN
251	P.SATIVUM-ETHIOPIA	828	MAXIMO-REDUCTUS-mare	106	P.SATIVUM-AFGHANISTAN
301	TELEGRAPH	835	MAXIMO-REDUCTUS-mare	120	SABEL
322	CHENILLE	844	NILSSONS ANGUSTIFOLIA	123	P.STRIATA
366	MARVILLA DA AMERICA	846	HORIZONTALIS-ho	130	P. ABYSSINICUM
378	HERO	869	ONWARD ROGUE	131	PROCUMBENS-pro
411	COBRI	871	ACHIEVEMENT ROGUE	133	GRAY RADICULA
466	EARLY THIRTY DAYS	872	DUKE OF ALBANY ROGUE	137	CHLOROTICA-chi-3
467	DWARF SUGAR	879	PILOT ROGUE	156	P.SATIVUM-SUDAN
560	CLAUSE-50	887	BLACK EYED SUSAN ROGUE	157	P.SATIVUM-SUDAN
574	POLARETTE	926	MARKET GEM ROGUE	181	KEERAU PEA
657	MANSHOLTS IVORA	935	DELWICHE COMMANDO	184	KHADRAA
734	RUDUKAI	957	TRIUMPH	188	WIRAIG
758	STARNAIN	1206	MISOG-2:af,st	191	WIRAIG
795	WBH 1307	1228	WELLENSIEK'S DOMINANT	193	WIRAIG
800	P. ELATIUS	2370	LUD	202	THOMAS LAXTON ROGUE
807	P.SATIVUM-TURKEY	2371	LUD ROGUE	212	P.SATIVUM ARVENSE
833	MAXIMO-REDUCTUS-red1-4			214	P.SATIVUM ARVENSE
877	PIONEER ROGUE			218	FIELD PEA-INDIA
922	HUNDREDFOLD ROGUE			221	FIELD PEA-INDIA

1713	GOLD.STRAW x MUMMY BICOL.		227 P. ABYSSINICUM 271 P.SATIVUM-ETHIOPIA 297 THOMAS LAXTON 299 PEERLESS 303 GRADUS 305 FELTHAM FIRST 307 PILOT 309 IMPROVED HARBINGER 312 ACHIEVEMENT 330 PEERLESS ROGUE 333 KELVEDON TRIUMPH 335 SUTTONS EARLY GIANT ROGUE 342 CEFALONIA 377 MARMA 379 WEITOR 380 VALOR 390 CANNERS PERFECTION REG 423 DIK TROM 424 PAULI 445 NEW ERA 453 COMIRE 463 MAMMOTH MELTING SUGAR 486 DART 516 MARO 654 P. SATIVUM -UNKNOWN 691 SMALL BLACK PEA 794 WBH 774 796 CHLOROTICA-chi-3 803 WBH 680 805 WELLENSIEK'S WHITE INDENT, di 806 NAVICULA APERTUS-nap 813 YELLOW POLLEN-yp 815 UNDULATIFOLIUS-un 817 WISCONSIN-711 820 STIFF STRAW 829 MAXIMO-REDUCTUS-mare 832 MAXIMO-REDUCTUS-mare 842 LAMM 105 843 LAMM 105 845 DE HAANS SLENDER 868 IMPROVED HARBINGER ROGUE 870 GRADUS ROGUE 878 ONWARD ROGUE 882 HUNDREDFOLD ROGUE 884 FELTHAM FIRST ROGUE 885 CEFALONIA ROGUE 886 GREENGOLT ROGUE

		888	LAXTON SUPERB ROGUE
		891	GOLDKONIG ROGUE
		892	KINNAURI LOCAL ROGUE
		893	KINNAURI LOCAL ROGUE
		903	LIGHT GREEN CHLOROTICA-chi-5
		905	ALBINA-alb
		918	CHLOROTICA-chi
		920	AUREA-au
		927	PILOT ROGUE
		929	NUNHEMS 55/69
		931	TELEGRAPH ROGUE
		952	KOROZA
		956	TRAPPER
		958	IHAR-16
		960	P.SATIVUM-TURKEY
		966	PROCO
		1027	RAMTO
		1077	P.SATIVUM-TURKEY
		1194	MISOG-1:CONVENTIONAL
		1200	MISOG-1:st,tl
		1205	MISOG-2:tl
		1208	MISOG-2:tl,st
		1210	ERYGEL
		1219	HJA 51277-af
		1227	VILMORINS ACACIA
		1239	DSP/PMR
		1271	ORUS
		1314	PEE WEE
		1704	P. JOMARDII
		1705	P. JOMARDII
		1706	CLAMART x P.ELATIUS
		1707	P. ELATIUS x 1/3870
		1709	MUMMY BICOLOUR
		1710	MUMMY BICOLOUR
		1712	MUMMY x MUMMY BICOL. UMBELLATE
		1716	PURP.FLD.ACACIA

The range of diversity for each of the resistance scores (1, 2 and 3) in Table 4 covers a wide range of taxa within *Pisum* and does not appear to discriminate any particular subset of genetic material.

Based on the studies at JI in the 1980's, six accessions were selected for use in crossing experiments and as parents in the breeding programme (Table 5).

Table 5. Germplasm accessions used at JI for genetic studies and as parents in the breeding programme

JI Number	Name	Comments
85	*P. sativum* Afghanistan	Resistant to all pathotypes except 7
882	Hundredfold Rogue	
1181	Laga	Has shown high resistance in Russia
1182	Ukishyi	Has shown high resistance in Russia
1195	MISOG 1 (af)	
1273	Heralda	Resistant to pathotype 7

Published set of host differentials

Data of pathotype differentials of *P. viciae* published during the 1970-80's, and from a recent study in Canada (Liu *et al.* 2013), are summarised here.

Method

Leaves of pea cultivars, each bearing a single lesion of *Peronospora viciae* f. sp. *pisi*, were collected in widely distributed locations around the UK (Taylor 1984; Taylor *et al.* 1989), Germany (Gunther and Jaiser 1989), Netherlands (Ester and Gerlagh 1979), Sweden (Stegmark 1990) and Canada (Liu *et al.* 2013).

A solution of sporangia was used to inoculate plants of every host line. Inoculum was deposited on the plants with atomising spray. Inoculated plants were enclosed in plastic propagator boxes and covered with black plastic bags to maintain dark conditions. After 16-18 h the bags and propagator tops were removed and for the next 4-7 days the plants were exposed to ambient glasshouse humidity (60-70% relative humidity) with nature daylight.

Table 6. Lines/cultivars used as host differentials in the pathotype analysis of *P. viciae*

Line	Cultivar name
JI 85	*P. sativum* Afghanistan
JI 411	Cobri
JI 441	Puget
JI 540	Perfected Freezer/ Dark skin perfection
JI 560	Clause-50
JI 584	Recette
JI 758	Starnain
JI 952	Koroza
JI 1215	Cicero
JI 1272	Katinka
JI 1273	Heralda

Table 7. The disease severity score and origin of isolates used in the pathotype analysis

		Host differential line											
Isolate	Origin	UI 85	UI 411	UI 441	UI 540	UI 560	UI 584	UI 758	UI 952	UI 1215	UI 1272	UI 1273	Reference
2	see Hubbeling (1975)	1	1	1	3	1	4	2	1	4	4	1	Ester & Gerlagh 1979
4	see Hubbeling (1975)	1	2	4	4	2	4	1	1	1	1	1	
6	see Hubbeling (1975)	1	4	4	4	4	4	1	4	4	1	4	
7	Steenbergen, Netherlands	4	3	3	3	3	4	1	4	3	4	1	
8	Lelystad in Netherlands	1	4	4	4	4	4	3	4	4	4	4	
3	Taylor PhD thesis		R		R		S	R	R				Taylor 1984
1	North-West Germany		M	M		R	S	R	R	S	S	R	Gunther & Jaiser 1989
2	North-West Germany		M	S		M	S	M	R	S	S	M	
3	North-West Germany		M	R		R	S	M	R	S	S	R	
4	North-West Germany		S	S		S	S	R	M	M	R	S	
5	North-West Germany		R	R		R	R	R	R	R	R	R	
6	North-West Germany		S	S		M	S	M	S	S	S	S	
0S1	Norwich, Norfolk		0	2	2	0	3	1	0	3	4	0	Taylor *et al.* 1989
0S2	Norwich, Norfolk		1	3	3	1	4	1	0	4	4	1	
0S3	Norwich, Norfolk		0	1	2	0	2	1	0	3	3	0	
0S4	Norwich, Norfolk		0	2	0	0	3	1	0	3	3	1	
0S5	Norwich, Norfolk		0	3	3	0	4	1	0	4	4	1	
0S6	Norwich, Norfolk		1	2	1	0	2	1	0	2	4	1	
0S7	Norwich, Norfolk		0	0	2	0	2	0	0	2	2	0	
P1	Norwich, Norfolk		1	2	2	0	2	1	0	3	4	1	
P2	Norwich, Norfolk		3	2	0	3	2	2	0	2	2	0	
P3	Norwich, Norfolk		2	2	2	0	3	0	0	3	4	2	
P5	Norwich, Norfolk		0	2	3	2	2	0	0	3	3	2	
P6	Norwich, Norfolk		0	1	2	0	3	1	0	1	2	1	
P7	Norwich, Norfolk		1	2	3	2	3	2	0	4	3	3	
P8	Norwich, Norfolk		0	1	3	2	3	1	0	4	4	2	
P10	Norwich, Norfolk		1	2	4	0	4	1	1	4	4	1	
P11	Norwich, Norfolk		0	1	2	0	2	0	0	2	2	0	
P12	Norwich, Norfolk		0	2	2	0	2	1	0	3	4	1	
NAS3	Morley, Norfolk		1	4	2	1	2	0	0	3	3	3	
NAS4	Morley, Norfolk		0	0	2	0	2	0	0	2	2	0	
NAS5	Morley, Norfolk		1	4	3	1	4	0	0	3	2	4	
PM1	Morley, Norfolk		0	2	0	0	0	0	0	2	0	0	
PM2	Morley, Norfolk		0	2	3	0	0	0	0	1	0	0	
PM3	Morley, Norfolk		0	0	0	0	2	0	0	2	2	0	
PG5/7	Norfolk		0	0	2	0	2	0	0	4	2	0	
PG13	Norfolk		2	2	3	1	2	1	0	3	0	1	
PG14	Norfolk		0	2	3	0	2	0	0	3	2	2	

Table 7: continued

Isolate	Origin	Host differential line: JI 85	JI 411	JI 441	JI 540	JI 560	JI 584	JI 758	JI 952	JI 1215	JI 1272	JI 1273	Reference
BH1	Kent		2	4	3	2	2	1	1	3	4	3	Taylor *et al.* 1989
PD1	Kent		0	2	2	2	2	2	0	0	0	0	
PD2	Kent		2	4	3	2	2	2	2	3	3	4	
PD3	Kent		0	2	3	0	3	1	0	2	3	0	
PD4	Kent		1	4	2	3	4	2	1	2	4	3	
PG1	Essex		0	1	0	0	3	1	0	3	4	1	
PG2	Essex		2	2	2	1	1	1	0	2	0	1	
FH1	Suffolk		0	2	3	0	0	0	0	2	0	0	
MC5	Nottinghamshire		0	0	0	0	2	0	0	2	2	0	
AB2	Lincolnshire		0	0	0	0	2	0	0	3	4	0	
AB3	Lincolnshire		0	0	3	0	3	0	0	2	2	0	
AB4	Lincolnshire		0	0	2	0	2	0	0	3	2	0	
AB6	Lincolnshire		0	2	3	0	2	0	0	3	2	2	
BC1	Lincolnshire		0	0	3	0	2	0	0	3	3	0	
BC4	Lincolnshire		0	0	2	0	2	1	0	2	3	0	
BC5	Lincolnshire		0	2	3	0	2	0	0	3	2	2	
BC6	Lincolnshire		0	0	3	0	2	0	0	4	3	0	
CS2	Lincolnshire		0	0	3	0	3	0	0	3	3	0	
CS5	Lincolnshire		0	1	3	0	2	0	0	3	2	0	
JW1	Lincolnshire		2	4	2	3	2	2	1	3	3	3	
JW2	Lincolnshire		1	3	4	0	4	1	1	4	4	1	
JW3	Lincolnshire		1	3	3	0	3	1	1	3	4	1	
JW4	Lincolnshire		0	3	2	1	2	0	0	2	2	2	
JW5	Lincolnshire		1	3	2	2	3	0	0	3	4	2	
AB1	Bedfordshire		4	4	4	2	4	2	0	4	3	0	
URL1	Bedfordshire		0	0	0	0	2	0	0	2	4	0	
TB4/6	Angus		0	0	3	0	3	0	0	3	2	0	
PG3/4	Cambridgeshire		0	0	2	0	2	0	0	3	3	0	
PG9	Cambridgeshire		2	2	2	2	2	0	0	2	3	2	
PG10/11	Cambridgeshire		1	2	2	1	2	1	0	2	3	1	
N1	Vestfold, Norway			15	32								Stegmark 1990
N2	Vestfold, Norway			6	32								
N3	Vestfold, Norway			10	76								
N4	Vestfold, Norway			9	46								
S1	Skane, Sweden			61	52								
S2	Skane, Sweden			56	30								
S3	Skane, Sweden			28	73								
S4	Skane, Sweden			7	64								

Table 7: continued

		Host differential line										
Isolate	Origin	JI 85	JI 411	JI 441	JI 540	JI 560	JI 584	JI 758	JI 952	JI 1215	JI 1272	JI 1273	Reference
													Stegmark
S4	Skane, Sweden			11	70								1990
S5	Skane, Sweden			7	68								
S6	Skane, Sweden			4	59								
S7	Skane, Sweden			5	58								
S8	Skane, Sweden			8	63								
S9	Skane, Sweden			2	41								
S10	Skane, Sweden			57	60								
S11	Skane, Sweden			5	17								
S12	Skane, Sweden			21	52								
S13	Skane, Sweden			22	67								
S14	Skane, Sweden			8	62								
S15	Skane, Sweden			5	52								
81-1	Skane, Sweden		1	4	54			2					
83-1	Skane, Sweden		83	60	73			55					
Race 8	Wageningen, Netherlands		71	62	48			44					
													Liu *et al.*
M9ID24	Alberta, Canada		S			S		S			S		2013
M10ID25	Alberta, Canada		S			S		S			S		
M10ID26	Alberta, Canada		S			S		S			S		
M11ID27	Alberta, Canada		S			S		S			S		
Veg1ID28	Alberta, Canada		S			S		S			S		
Veg2ID29	Alberta, Canada		S			S		S			R		
Veg2ID30	Alberta, Canada		S			S		S			R		
Veg3ID31	Alberta, Canada		S			S		S			S		
Veg3ID32	Alberta, Canada		R			R		R			R		

Scoring:

Ester and Gerlagh 1979; Taylor *et al.* 1989: 0 = resistant, no visible symptoms, 1 = local necrosis of leaves, no sporulation, 2 = limited amount of sporangial production on some leaves, followed by local necrosis, 3 = abundant sporulation production but confined mainly to inoculated leaves, 4 = abundant sporangial production on leaves and stems;
Taylor 1984; Gunther and Jaiser 1989; Liu *et al.* 2013: R = Resistant, M = Moderately resistant, S = Susceptible;
Stegmark 1990: Values are the percentage area affected by sporulation on the most severely affected leaf and are the means of 10 plants.

Studies conducted by David Holden

In the PhD study conducted by David Holden, genetic maps were produced from crosses between a *P. abyssinicum* line (JI 2202) and a *Pisum sativum* line (JI 2822) (Holden 2009). JI 2822 is a recombinant inbred line derived from a mapping population derived from JI 15 × JI 399, where the JI 399 parent is a garden pea line. Markers (454) were mapped in the JI 2202 × JI 2822 F7 population, using predominantly SSR (simple sequence repeat) and SSAP (sequence-specific amplification polymorphism) markers.

Analysis of the JI 2202 × JI 2822 population revealed a strong partial resistance to downy mildew (*Peronospora viciae* f. sp. *pisi*), which was mapped using a quantitative trait locus (QTL) approach. Here approximately 52% of trait variance was explained by a single locus on linkage group I. The leaf infection assay was used to measure susceptibility to downy mildew infection in the JI 2202 × JI 2822 F5-7 bulk populations. Pathotypes A and B which differ in their ability to infect accessions and show race-specific resistance to downy mildew were used in a combined inoculum on the 101 F5-7 bulk lines. At the time of inoculation, all seedlings had leaves at node 4. Plants were inoculated by spraying both leaf surfaces; this was followed by a period of incubation and then scoring for disease severity. Each individual line was replicated in two separate trays (two replicates). Disease severity was scored for each individual seedling using a score of 0-9, based on the coverage of the worst-affected leaves (Figure 1).

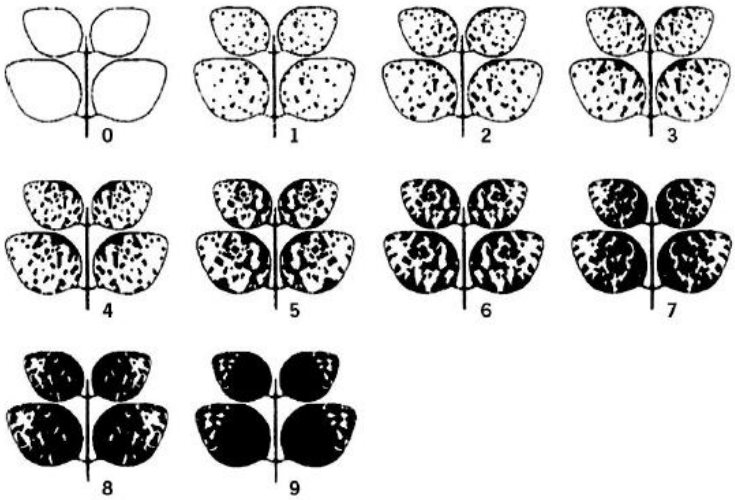


Figure 1. Cartoon showing disease severity key for downy mildew scores in the pea population JI 2202 x JI 2822. Severity scores are indicated: dark areas on the leaflets represent downy mildew lesions (Holden 2009, Figure 64)

Differences in susceptibility to downy mildew infection were detected between JI 2202 and JI 2822. Average disease severity scores were JI2202: 7.6, JI2822: 2.2 (Table 5; Figure 1). For some individual bulk lines, the severity score of two replicates was not consistent. It was or is suggested that, in these cases, there were a number of possibilities: a) some plants died before inoculation so that the score average was increased or decreased by having a low sample number compared with other replicates and lines, b) technically, the pathogen inoculation performed was not very uniform for the two replicates and this resulted in one replica showed apparent resistance whereas the other showed susceptibility, or c) the line could have been heterozygous for loci involved in resistance. As a result, only individual lines that were equally resistant or susceptible in both replications were selected for mapping quantitative trait loci to downy mildew resistance (Table 8).

Resistance to infection was found to be strongly associated with the aa98-560 locus on linkage group I, with a LOD score of 19.56, explaining 52% of trait score variance. Plants bearing the *P. sativum* marker genotype showed low susceptibility to infection compared with *P. abyssinicum* genotype lines: genotype means were 1.18 (standard deviation (s.d.) = 1.28) compared with 4.49 (s.d. = 1.75), respectively. This locus and linkage group I map are shown below, in Figures 2 and 3.

Table 8. Downy mildew resistance score on JI 2202 x JI 2822 F5 bulk lines

Plant	Ave REP1	Ave REP2	DM	Plant	Ave REP1	Ave REP2	DM
JI 2822	2.8	1.7	R	88	3.7	7.5	S
JI 2202	7.6	7.3	S	91	5.0	8.0	S
JI 281	8.1	7.5	S	94	7.0	8.3	S
1	1.5	0.0	R	95	4.4	7.3	S
2	2.4	0.7	R	96	0.3	3.2	R
6	0.5	1.8	R	97	0.0	0.0	R
11	3.7	4.4	S	100	4.0	7.3	S
14	5.1	7.0	S	101	0.4	2.3	R
15	0.7	0.3	R	103	0.3	0.5	R
16	2.7	1.4	R	105	3.4	5.8	S
17	0.7	2.8	R	106	1.1	1.6	R
18	3.8	6.5	S	108	0.0	0.3	R
19	3.1	4.9	S	111	0.7	2.1	R
22	0.5	1.6	R	113	0.2	1.2	R
23	5.7	6.9	S	117	0.3	0.3	R
25	0.6	2.2	R	120	1.0	2.3	R
26	0.8	1.0	R	121	0.5	0.4	R
27	7.8	7.2	S	122	3.4	5.6	S
29	5.7	7.2	S	123	0.3	1.0	R
31	6.8	5.6	S	130	0.9	1.3	R
36	3.2	3.3	S	132	2.8	2.6	R
39	3.2	3.7	S	133	0.7	0.8	R
44	3.9	2.8	R	134	0.0	0.0	R
45	5.8	4.3	S	135	0.3	0.3	R
46	3.7	2.7	R	136	0.8	0.0	R
47	1.8	2.5	R	137	1.9	1.6	R
49	0.3	0.0	R	140	1.2	2.1	R
50	3.8	2.5	R	142	3.1	5.5	S
52	3.8	5.5	S	143	1.1	1.3	R
54	0.1	0.1	R	145	1.6	0.3	R
58	4.5	4.5	S	147	0.0	0.9	R
59	3.3	6.1	S	149	5.5	5.8	S
60	0.2	0.0	R	151	7.3	5.0	S
62	4.0	6.4	S	155	0.8	0.7	R
63	0.4	0.3	R	160	0.5	0.2	R
66	0.1	0.3	R	162	8.0	4.3	S
67	0.3	0.5	R	166	6.8	5.0	S
73	1.0	2.5	R	170	0.8	1.6	R
74	0.0	1.4	R	173	1.0	0.3	R
76	0.2	0.9	R	176	3.6	6.0	S
80	0.4	1.8	R	180	0.7	0.4	R
81	0.2	1.0	R	181	7.3	5.2	S
82	0.2	0.2	R	183	6.4	6.0	S
84	0.1	1.8	R	185	8.4	8.2	S
87	1.8	1.8	R	191	1.8	1.7	R
				196	0.7	0.1	R

Ave = Average, DM = Downy mildew resistance (Holden 2009)

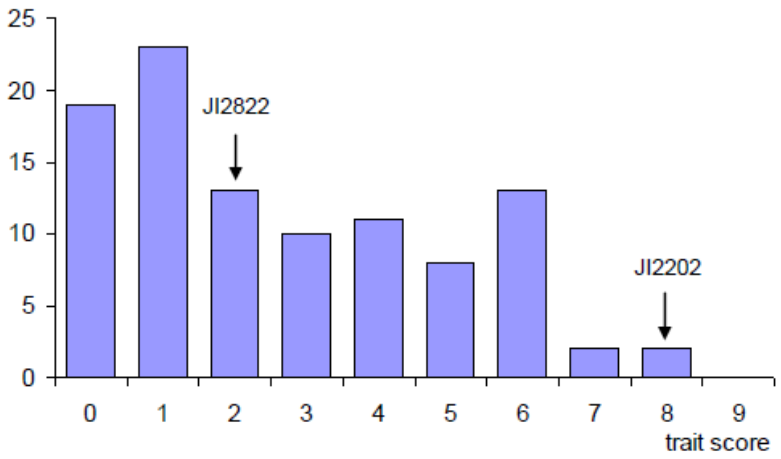


Figure 2. Susceptibility to downy mildew infection in the JI 2202 x JI 2822 population. Frequency distribution of trait scores on JI 2202 x JI 2822 F5-7 bulks, using pooled pathogen isolates; average trait scores per line are grouped as integer values. The distribution of susceptibility scores suggests an underlying bimodal distribution (Holden 2009, Figure 65).

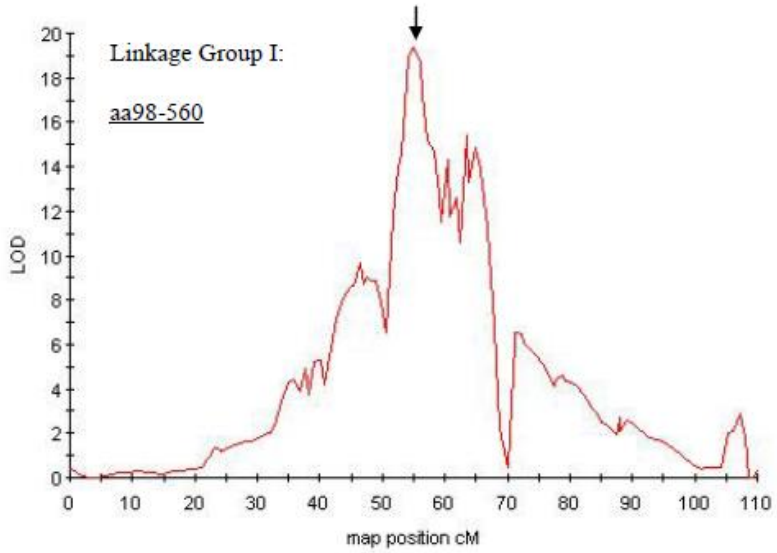


Figure 3. MapQTL output for resistance to downy mildew in the JI 2202 x JI 2822 mapping population, showing results for linkage group I. Arrow indicates the map position of marker locus aa98-560, where association with downy mildew resistance/susceptibility has a LOD score of 19.56 (Holden 2009, Figure 66).

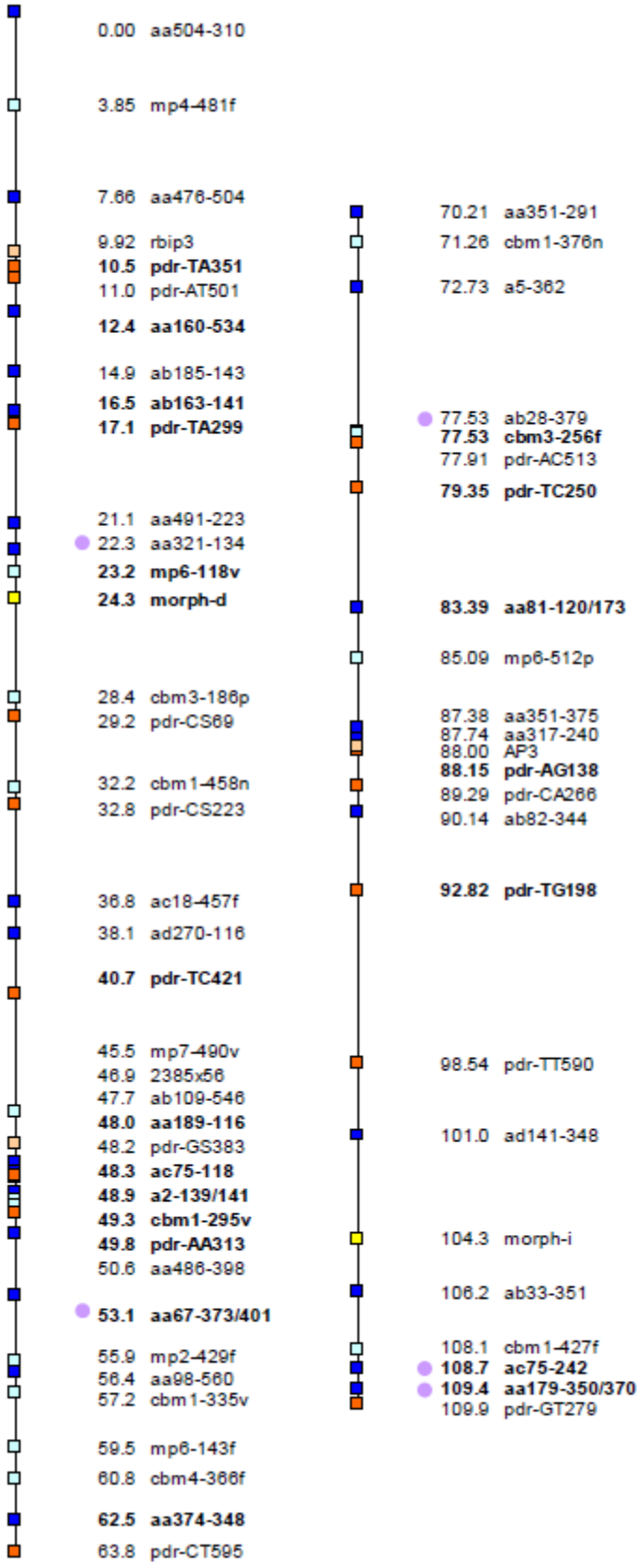


Figure 4. Genetic map of JI 2202 × JI 2282 F7 population: Linkage group I, part 1 (left) and continued as part 2 (right). SSR marker aa98-560 (close to end of left part of linkage group) was strongly associated with downy mildew resistance (Holden 2009, Figure 24).

Studies conducted within PCGIN

In parallel with the preparation for publication of the PhD thesis above (Holden 2009), analysis of a set of traits using a range of pea lines was carried out within PCGIN (Pulse Crop Genetic Improvement Network). Initial tests carried out at NIAB indicated that JI 15 showed high resistance to downy mildew. The resistance was also evident in JI 15 and in two of the three RILs derived from JI 15 x JI 1194 that were grown outside in plots at NIAB in the late 2000's. To further characterise the genetic basis for this downy mildew resistance trait, an extended set of pea RILs from the cross JI 15 x JI 1194 was tested. Since the cross JI 15 x JI 1194 already contained genetically marked lines, mapping disease resistance loci could proceed more rapidly than in the combining pea crosses claimed by breeders to be segregating for resistance. The JI 15 x JI 1194 population (60 RILs) was bulked to provide a minimum of 80 seeds per line, to enable a study of the population using two pathotypes of the fungus. Of 54 lines screened using the two pathotypes, 27 showed resistance to the fungus, whereas 27 showed susceptibility. These data showed perfect linkage to an SSAP marker, Tps1/211+ on the pea linkage group I (PCGIN 2009).

Recommendations and current progress

The results obtained from the early *Pisum* germplasm screening described above should inform further study within AHDB Horticulture and PCGIN projects.

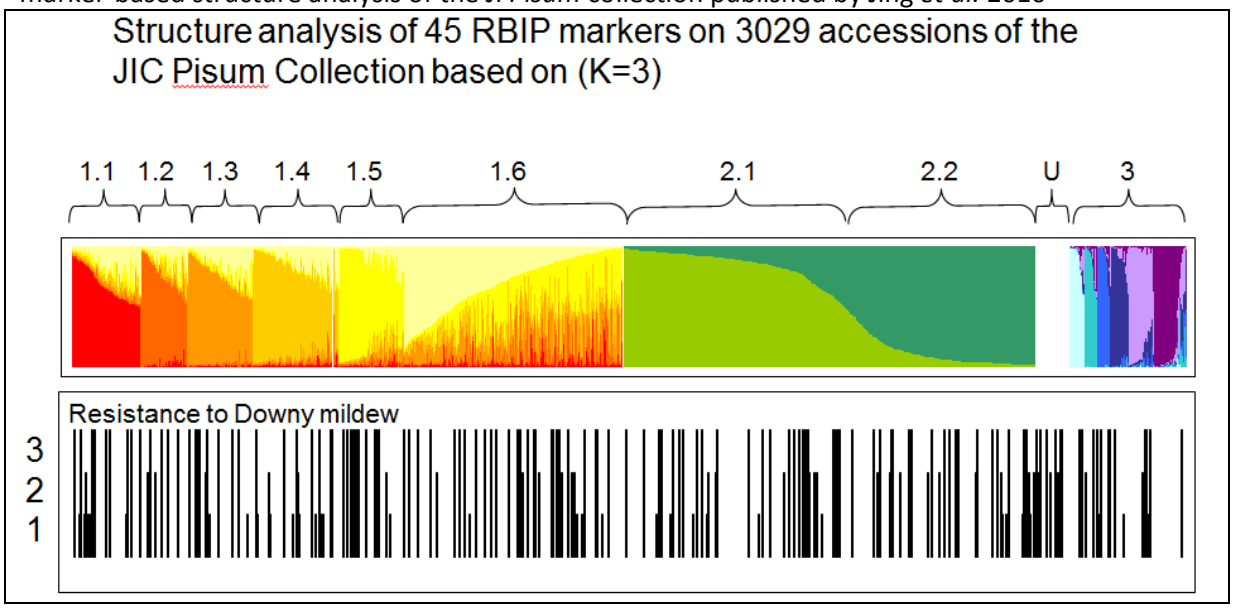
- Selected *Pisum* lines showing strong/partial resistance should be bulked to provide a resource for field tests at different sites in the UK, alongside the collection and identification of current downy mildew isolates.
- The older screening did not identify JI 15 as a source of major resistance. This line, along with JI 85 (Tables 1, 4 and 5), should be added to the resource above.
- The downy mildew resistance locus identified in the Holden (2009) and PCGIN (2009) studies are likely both derived from JI 15. Gene-specific markers should be developed to this region of Linkage Group I, exploiting the synteny with *Medicago truncatula* to facilitate gene identification.
- The early identified sources of resistance can now be compared in terms of common alleles at particular genetic loci (for example, the linkage group I locus above). This work has been initiated by looking at the distribution of lines within groupings of the JIC germplasm (Figure 5).

Two rounds of seed multiplication have been carried out in 2014/15 on germplasm lines identified in earlier studies as showing partial or strong resistance. The first of these was in the field in 2014 and the second under glasshouse conditions in 2014/15. For the latter, 12 lines were sown: JI 15 (13 plants), JI 85 (14 plants), JI 411 (15 plants), JI 441 (7 plants), JI 540 (12 plants), JI 560 (14 plants), JI 584 (15 plants), JI 758 (14 plants), JI 952 (15 plants), JI 1215 (12 plants), JI 1272 (7 plants), and JI 1273 (12 plants).

Figure 5 shows lines mapped onto the structure analysis of the JI *Pisum* collection as published by Jing *et al.* 2010. Figure 5 indicates that accessions with high/moderate resistance are spread across group 1 (majority landrace group), group 2 (majority modern cultivar group) and their associated sub-groups (1.1-1.7 and 2.1 and 2.2). A significant number of lines also fall into the U and group 3 (wilder taxa including *P. fulvum*, *P. elatius* and *P. abyssinicum*).

Progress with the development of gene-specific markers will be available through the next project report.

Figure 5. Downy mildew scores of 1-3 for JI *Pisum* germplasm (Table 4) set against the molecular marker-based structure analysis of the JI *Pisum* collection published by Jing *et al.* 2010



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