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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

At least 15 different pea downy mildew races exist in the UK currently. Five of these have been described for the first time in this project, whereas ten races had already been found during the 1980's (Taylor, 1986). Races 1, 3 and 10 seem to be slightly dominant across the UK over the last 30 years. The races collected in this project are stored in a culture collection at PGRO, currently holding 114 downy mildew isolates. Two pea germplasm accessions, JI 15 and JI 85, identified as carriers of resistance genes, have been used to generate crosses to dissect and understand the genetic basis of disease resistance and assist breeding. All downy mildew isolates were inoculated onto seedlings of JI 15 and JI 85 to test the durability of the resistance. The resistance of JI 15 was overcome both by individual races under growth room conditions and by natural populations in the field. Resistance of JI 85 was consistent in the majority of tests carried out.

A selection of vining and combining pea varieties were planted at several locations across the UK to identify regional differences of infection levels. Vining pea varieties show strong differences in infection levels depending on growing location whereas combining peas show more equal levels of infection.

Background

Pea downy mildew was first reported as a serious problem in pea crops in the UK in the 1960's, and yield losses between 45 and 80% are reported (Stegmark, 1994; Chang *et al.*, 2013). Downy mildew compromises the growth of the plants, and later spreads into the pods where it directly affects the quality of the developing seeds. Primary infection, which frequently results in plant death, is caused by soil borne oospores. Infected seedlings show grey mycelial growth on the underside of the leaves. Neighbouring and distant plants are infected by air borne spores. 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. 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. A new directive, affecting growers in 2018, has restricted the use of Wakil XL to pea seeds planted between

the 1st of April and the 29th of September. Peas sown early in February and March are therefore at greatest risk to the disease. There are currently no foliar-applied products to control downy mildew. Choice of variety can also reduce the risk of disease. Disease resistance exists in many combining pea varieties and ratings can be found in the PGRO Pulse Recommended List tables (<http://www.pgro.org/recommended-lists-2017/>). However, there is less varietal disease resistance available in vining peas and ratings can be found in the PGRO Vining Pea Descriptive List tables (<http://www.pgro.org/downloads/PGRO-GUIDE-2018-VINING-PEA.pdf>).

To understand downy mildew race diversity in the UK, downy mildew isolates were collected in 2016 and 2017 and their races determined. Field trials were carried out to investigate if pea varieties showed differences in severity of downy mildew infection at different locations and if these differences can be related to the occurrence of downy mildew races.

Summary

During the 2016 and 2017 growing season, downy mildew isolates were collected from across the UK. The race of these isolates was determined (Table A). Races 1, 3, 10 and 11 were the most prominent overall. Five so far unknown, potentially new races have been identified. PGRO holds 114 of these races in a culture collection. The geographical distribution of the races is shown in Figure A. Most isolates are distributed widely across the UK but dominance of races varies by location.

Table A: Number of isolates of each downy mildew race and isolate collection location in 2016 and 2017. Races 1 to 11 had already been described in work carried out in the 1980's. Races Unk 1 to Unk 5 are so far unknown and potentially new races identified in this project.

| Race | Number of isolates collected in 2016 | Location | UK County | Number of isolates collected in 2017 | Location | UK County | Total number of isolates |
|------|--------------------------------------|--|---|--------------------------------------|--|---|--------------------------|
| 1 | 2 | Stockbridge, Perth | Hampshire, Perth and Kinross | 13 | Alyth, Holbeach, Huggate, North Wootton(5), Sledmere, Stubton(3), Thorney | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 15 |
| 2 | 0 | | | 2 | Kilham, North Wootton | Yorkshire, Norfolk | 2 |
| 3 | 8 | Chatteris, Stockbridge, Howden (3), Perth, Sledmere, Kirton | Cambridgeshire, Hampshire, Yorkshire, Perth and Kinross, Lincolnshire | 19 | Alyth(3), Holbeach(2), Huggate, Kilham(2), North Wootton(2), Sledmere(2), Stubton(4), Thorney(2), Walcot | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 27 |
| 4 | 1 | Howden | Yorkshire | 4 | Chatteris, North Wootton, Stubton, Thorney | Cambridgeshire, Norfolk, Lincolnshire | 5 |
| 5 | 2 | Perth (2) | Perth and Kinross | 4 | Chatteris(2), Huggate, Thorney | Cambridgeshire, Yorkshire | 6 |
| 6 | 1 | Stockbridge | Hampshire | 1 | Huggate | Yorkshire | 2 |
| 7 | 0 | | | 0 | | | 0 |
| 8 | 5 | Howden (5) | Yorkshire | 4 | Chatteris, Sledmere, Stubton, Walcot | Cambridgeshire, Yorkshire, Lincolnshire | 9 |
| 9 | 1 | Stockbridge | Hampshire | 6 | Alyth(3), Ancaster, Kilham, Stubton | Perth and Kinross, Lincolnshire, Yorkshire | 7 |
| 10 | 9 | Chatteris (3), Stubton (2), Stockbridge, Sledmere, Kirton, Stratford upon Avon | Lincolnshire, Cambridgeshire, Hampshire, Yorkshire, Warwickshire | 7 | Alyth(3), Ancaster, Chatteris(2), Holbeach | Perth and Kinross, Lincolnshire, Cambridgeshire, Lincolnshire | 16 |
| 11 | 6 | Chatteris (2), Stubton (3), Sledmere | Cambridgeshire, Lincolnshire, Yorkshire | 8 | Alyth, Ancaster, Chatteris(2), Kilham, | Perth and Kinross, Lincolnshire, Cambridgeshire, Yorkshire | 14 |

| | | | | | | | |
|--------------|---|---------------|----------------|---|---|---|---|
| | | | | | Nocton, Thorney(2) | | |
| Unk 1 | 2 | Chatteris (2) | Cambridgeshire | 4 | Holbeach, Huggate, Kilham, Stubton | Lincolnshire, Yorkshire | 6 |
| Unk 2 | 1 | Stockbridge | Hampshire | 5 | Chatteris, Holbeach, Kilham, Stubton, Thorney | Cambridgeshire, Lincolnshire, Yorkshire | 6 |
| Unk 3 | 0 | | | 2 | Alyth, North Wootton | Perth and Kinross, Norfolk | 2 |
| Unk 4 | 0 | | | 3 | Kilham, Stubton, Thorney | Yorkshire, Lincolnshire, Cambridgeshire | 3 |
| Unk 5 | 0 | | | 1 | Huggate | Yorkshire | 1 |

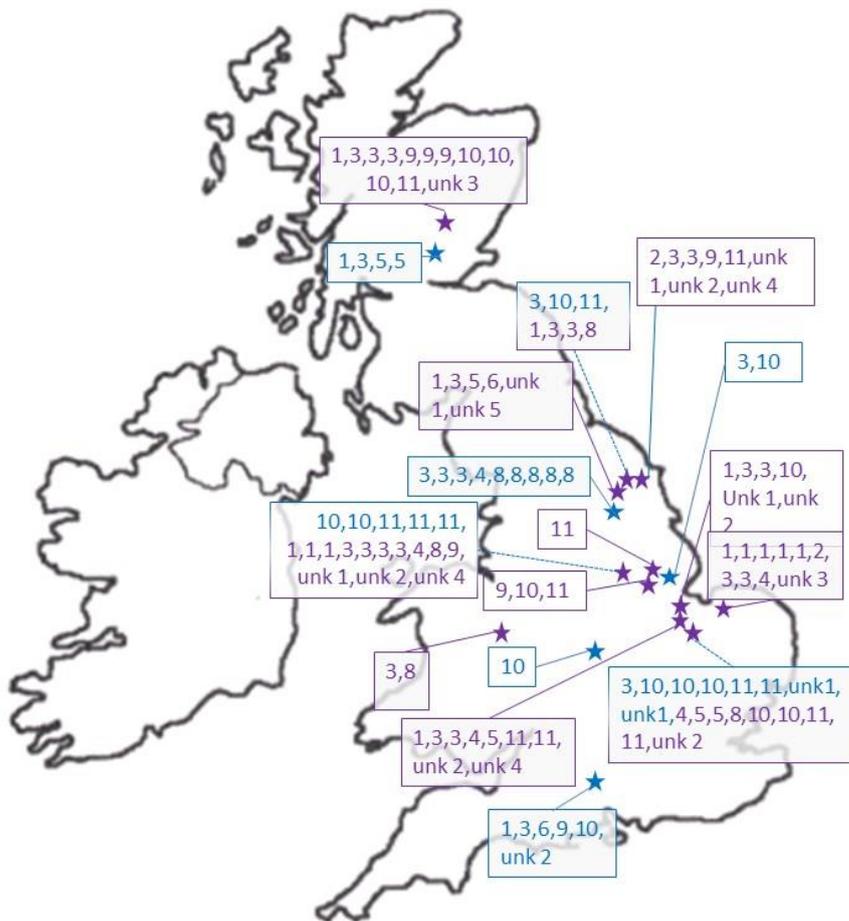


Figure A: Geographical distribution of the downy mildew races identified in 2016 and 2017. Boxes containing text in blue represent races collected in 2016 and boxes containing text in purple represent races collected in 2017. Races Unk 1 to Unk 5 were previously unknown and potentially new races identified in this project.

Two pea germplasm accessions, JI 15 and JI 85, identified as carriers of resistance genes to pea downy mildew, have been used to create new crosses to support genetic studies of disease resistance and support future breeding. In addition, the isolates collected have been inoculated onto JI 15 and JI 85 seedlings to monitor their performance (Table B). The resistance of JI 15 was overcome both by individual races under growth room conditions and by natural downy mildew populations in the field. Resistance of JI 85 was also overcome but only by a few races and it generally maintained good field resistance.

Table B: Susceptibility of germplasm accessions JI 15 and JI 85 to downy mildew isolates in 2016 and 2017, together with isolate number, collection location, collection date, pea host variety, and race. s = susceptible, r = resistant.

| <u>Isolate</u> | <u>UK County</u> | <u>Location</u> | <u>Date Collected</u> | <u>Pea variety source</u> | <u>Race</u> | <u>JI 15</u> | <u>JI 85</u> |
|----------------|-------------------|-------------------|-----------------------|---------------------------|-------------|--------------|--------------|
| I 130 | Hampshire | Stockbridge | 2016 | Kingfisher | 1 | s | s |
| I 226 | Lincolnshire | Holbeach, Koppert | 2017 | Wav 106 | 1 | s | r |
| I 264 | Norfolk | North Wootton | 2017 | ? | 1 | s | r |
| I 277 | Perth and Kinross | Alyth | 2017 | Maro | 1 | s | r |
| I 317 | Yorkshire | Sledmere | 2017 | Maro | 1 | s | r |
| I 246 | Cambridgeshire | Thorney | 2017 | ? | 3 | s | r |
| I 222 | Lincolnshire | Holbeach, Koppert | 2017 | Wav 106 | 3 | s | r |
| I 231 | Lincolnshire | Stubton | 2017 | ? | 3 | s | r |
| I 164 | Perth and Kinross | Perth | 2016 | JI 1272 | 3 | s | r |
| I 303 | Cambridgeshire | Chatteris | 2017 | Ida | 5 | s | r |
| I 328 | Yorkshire | Huggate | 2017 | 04555315N | 5 | s | r |
| I 202 | Lincolnshire | Stubton | 2017 | 06S55519A | 8 | s | r |
| I 127 | Hampshire | Stockbridge | 2016 | Greenwood | 9 | s | r |
| I 115 | Cambridgeshire | Chatteris | 2016 | JI 1272 | 10 | s | r |
| I 185 | Lincolnshire | Kirton | 2016 | Waverex | 10 | s | r |
| I 323 | Lincolnshire | Ancaster | 2017 | Kingfisher | 10 | s | r |
| I 209 | Lincolnshire | Nocton | 2017 | LG Element | 11 | s | r |
| I 223 | Lincolnshire | Holbeach, Koppert | 2017 | Wav 106 | 13 | s | r |
| I 241 | Lincolnshire | Stubton | 2017 | ? | 13 | s | r |
| I 337 | Yorkshire | Kilham | 2017 | Celebration | 13 | s | r |
| I 129 | Hampshire | Stockbridge | 2016 | Crackerjack | Unk 2 | s | r |
| I 162 | Perth and Kinross | Perth | 2016 | Avola | 1 | r | s |
| I 159 | Perth and Kinross | Perth | 2016 | JI 560 | 5 | r | s |
| I 174 | Cambridgeshire | Chatteris | 2016 | JI 85 | 10 | r | s |
| I 179 | Cambridgeshire | Chatteris | 2016 | JI 85 | 11 | r | s |
| I 176 | Cambridgeshire | Chatteris | 2016 | JI 85 | Unk 1 | r | s |

A selection of vining pea varieties was planted at six locations in 2016 and nine locations in 2017 and their infection levels monitored. Generally, Aloha, Anna, Ida and Maurice showed very low levels of infection regardless of location. In 2017, many vining pea varieties showed greatest relative infection levels at Chatteris or Stubton but Waverex and 06S54009A showed greatest levels of infection at Holbeach and Alyth, respectively (Figure B). In common with Ida, 08S01030, Anna and 04S51315N, Maurice had average levels of infection below 2% across all sites. Avola had relatively high levels of infection at Stubton, Chatteris and Thorney (each being over 6.5%) but fared better at Alyth (0.42%) and North Wootton (1.19%) with

hardly any infection at Holbeach (0.12%). Figure B provides an illustration of varietal performance at each site in 2016 and 2017, each pie chart representing a single variety and split to show % leaf area infection of that variety at each site. Figure B indicates that highest levels of infection were recorded at Chatteris, Thorney and Stubton.

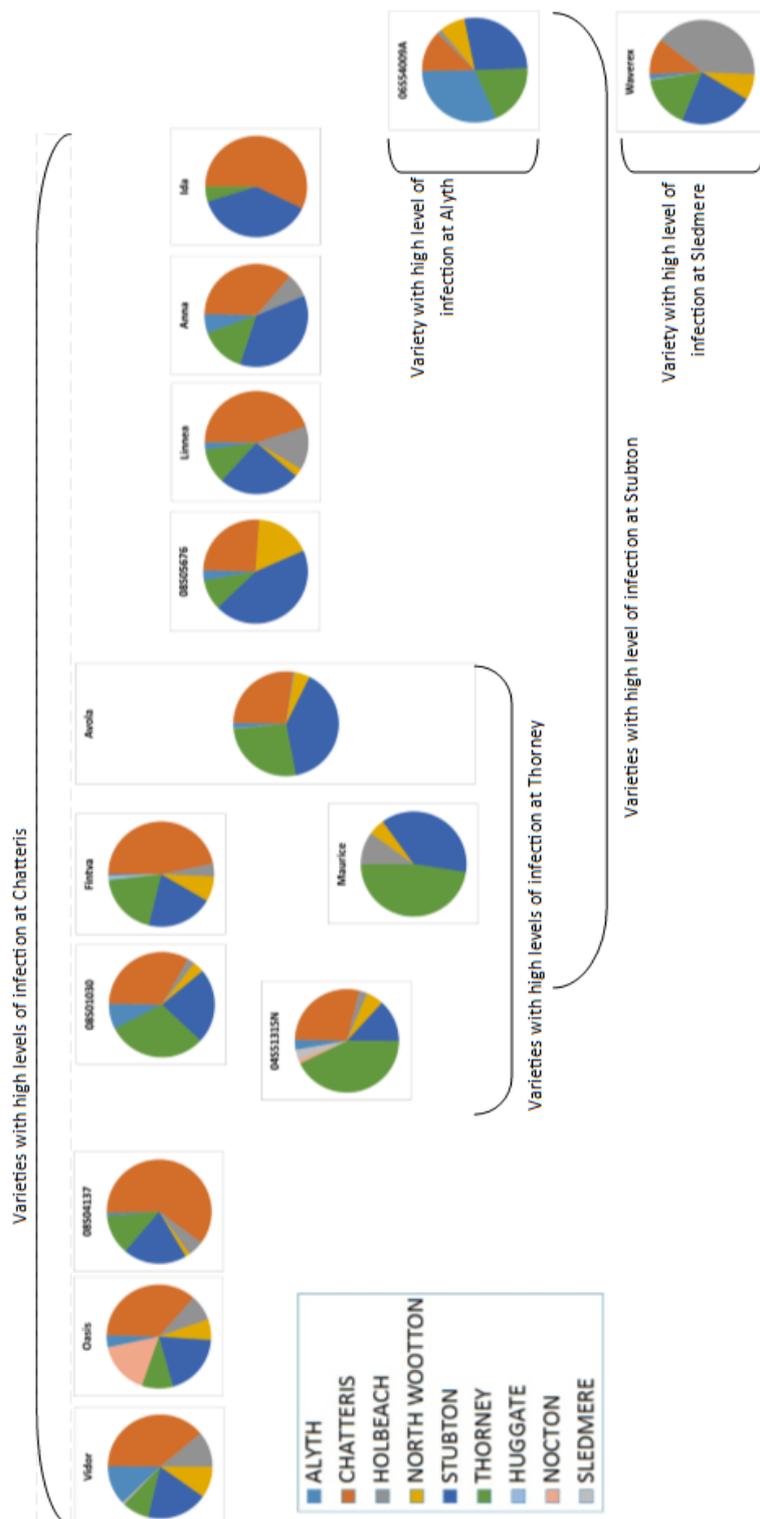


Figure B: Percentage of downy mildew for each variety at each site, illustrating varieties that performed better at each site in 2016 and 2017. Each pie chart represents a single variety and is split to show a comparison of % leaf area infection at each site as a proportion of the total infection at all sites.

Financial Benefits

Better management of downy mildew can reduce seedling losses caused by primary soil-borne infection and reduce secondary disease infection that is spread from primary inoculum sources. The use of varieties with tolerance to downy mildew will lower the risk of disease development and encourage improved yield and quality of produce. Increased yield and reduced factory losses due to improved quality will increase profits, and managing downy mildew using knowledge of varietal tolerance and field history does not increase cost. It is estimated that downy mildew causes yield loss of between 45 and 80% when high levels of infection occur between flowering and pod formation. The area of vining peas in 2017 was 33,500 hectares, producing 142,000 tons of frozen and canned peas (BGA, 2018). 44,000 hectares of combining peas were grown, producing 169,000 tons of harvested peas (EUROSTAT, 2018). The current farmgate value of vining peas is approximately £350 per ton and combining peas are currently around £180 per ton depending on end-use (Farmers Weekly, 2018). Total value of vining peas in 2017 was approximately £50M and the value of combining peas was approximately £30.5M. In years when infection by downy mildew is high, using the lower figure of 45% yield loss if disease occurs between flowering and pod formation, the estimated financial loss to growers could be £22.4M for vining peas and £13.7M for combining peas. The financial benefits to growers provided by the selection of the best performing varieties by region are therefore very high. The further financial benefit to processors by 1% improvement in factory throughput ensuing from the reduction of waste and quality loss, has been estimated to be 0.25 Euros per ton produced.

The JI germplasm lines that are carriers of resistance have been shared with breeding companies. The resistance of JI 15 has been overcome under field conditions and JI 15 will have to be combined with other lines that are carriers of resistance for durable resistance breeding. The downy mildew isolate collection at PGRO is used to screen new breeding lines to select promising candidates for resistance.

Action Points

- Select varieties with higher tolerance to downy mildew for your location to reduce disease impact (Tables 9 and 10).
- Contact PGRO to enquire about the performance of specific varieties in your area.
- Take field history into account and avoid planting peas early in fields with a known history of downy mildew.

- Maintain a rotation where peas are grown one year in five at most to avoid rapid build-up of soil-borne downy mildew.
- Treat seed that is to be planted after 1st April with Wakil XL seed treatment if varieties are susceptible to downy mildew.
- There are no effective foliar fungicides to control downy mildew in peas.

PGRO will repeat field trials across the UK and evaluate varietal performance, providing up-to-date information to growers and monitoring changes in race structure in the future.

SCIENCE SECTION

Introduction

Pea downy mildew (DM), caused by *Peronospora viciae* f. sp. *pisi*, is a 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; Chang *et al.*, 2013; Taylor, 1986). Despite the development of more resistant modern cultivars, DM remains a significant cause of losses to the profitability of the pea crop, firstly by compromising the growth of the plants through lesions on 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, primary infection which frequently results in plant death. Infected seedlings show grey mycelial growth on the underside of the leaves. Conidia released onto air currents infect neighbouring and distant plants. This secondary infection causes disease on flowering plants and pods. Infected plants have reduced photosynthetic area which results in substantial yield reduction and poor produce quality (Stegmark, 1994).

Some control of primary DM can be achieved through use of cultural practices and fungicidal seed treatments. Downy mildew is not transmitted via the seed but seed treatments protect the germinating seedling from primary infection by soil-borne oospores. 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 resistance exists in many combining pea varieties and ratings can be found in the PGRO Pulse Recommended List tables (<http://www.pgro.org/recommended-lists-2017/>). However, there is less varietal disease resistance available in vining peas and ratings can be found in the PGRO Vining Pea Descriptive List tables (<http://www.pgro.org/downloads/PGRO-GUIDE-2018-VINING-PEA.pdf>). 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. From 2018, the use of Wakil XL is restricted to peas planted between April 1st and September 29th. Peas sown earlier (in February and March) are therefore at greatest risk of downy mildew infection. There are currently no foliar-applied products to control DM.

Downy mildew produces large quantities of airborne spores and can evolve very quickly (Liu *et al.*, 2013). This results in the development of genetically diverse populations. 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 marrowfat variety Sakura scored a 7 for DM resistance 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). Trials are repeated annually within the same areas in Lincolnshire and the observed variation in scores may be due to changes in DM populations. Environmental conditions and field history also have an influence on disease development.

To understand downy mildew race diversity in the UK, DM isolates were collected in 2016 and 2017 and their races determined. Field trials were carried out to investigate if pea varieties show differences in severity of DM infection at different locations and if these differences can be related to the occurrence of DM races. Germplasm, identified as carrying genetic resistance to DM, was used in field trials.

Materials and methods

Literature review (JIC)

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

Seed multiplication (JIC)

Twelve pea germplasm accessions were sown in 2015 and 2016 for seed multiplication and seeds shared with PGRO: JI 15, JI 85, JI 411, JI 441, JI 540, JI 560, JI 584, JI 758, JI 952, JI 1215, JI 1272 and JI 1273. These accessions include those described as host differential variants (by which isolates may be identified) as well as germplasm having potential as sources of resistance genes for breeding programmes.

Isolate collection

During the 2016 and 2017 growing seasons, field samples of pea DM were collected by PGRO. The samples were obtained either by collection of infected pea plants during field visits by staff from PGRO or from pea growers across the UK who sent infected pea plants to PGRO. Most DM isolates were obtained from single lesions on individual leaves to increase the likelihood of obtaining pure rather than mixed races. Some samples were collected from

whole plants. Samples were collected from a wide area across the UK. To make sure that the isolates were of pure race, either three or five purification cycles were performed for each isolate under growth room conditions (three cycles for the isolates obtained from individual leaves, five cycles for whole plant collected samples). Great care was taken to only use individual pea stems as inoculum for the next cycle, at each stage.

Pisum sativum cv. Avola seeds were surface sterilised for 15 minutes using a 10% bleach solution and germinated 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. The seedlings were placed into compost in plastic propagator trays, covered with compost and the tray covered with a clear propagator lid. The trays were kept in a growth room at 15°C with alternating 16 hours light and 8 hours darkness. Infected plant samples were kept in trays covered with lids at all times 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 most DM samples was stored at -80°C for long-term storage of the culture collection.

Definitions of 'infected', 'susceptible' and 'resistant':

In the work conducted at PGRO any pea plant which showed any sporulation from DM was classified as infected, regardless of the presence or lack of necrosis. If any pea plant from a particular host line was classified as infected, then that host line was defined as susceptible to the particular isolate to which it had been exposed. If sporulation did not occur, then the plant was deemed to be non-infected. If all the plants in a host line which had been exposed to a particular isolate were deemed to be uninfected, then that host line was classified as being resistant to that particular isolate.

Pea differential host accessions

To determine the race of the DM isolates, four differential pea host accessions (JI 411, JI 560, JI 758 and JI 1272) with recorded resistances and susceptibilities to UK DM races were used (Taylor, 1986; Table 1). The pea differential accessions were inoculated with the DM isolates as described above. Eight seedlings per pea accession were inoculated with each DM isolate and presence or absence of DM infection recorded. Over the 2016 and 2017 seasons, five previously unknown races were identified. These races showed combinations of infection patterns on the four differential accessions, which were not recorded in the work of Taylor

(1986) (Table 1). In addition, two pea germplasm accessions with potentially strong resistance to DM (JI 15, identified by research within the Defra-funded Pulse Crop Genetic Improvement Network (PCGIN; www.pcg.in.org) and JI 85, identified within the literature review and desk study carried out within this project) were inoculated with each of the DM isolates to determine whether the isolates could overcome the resistance of these pea accessions. The purpose was to determine whether these two pea germplasm accessions maintained their DM resistance and showed potential to be used in DM resistance breeding.

Table 1: Susceptibility (S) or resistance (R) of four pea differential host accessions (JI 411, JI 560, JI 758, JI 1272) to 16 races of downy mildew. The differential hosts were used to determine race. Races 1 to 11 were identified by Taylor (1986). Races Unk 1 to Unk 5 are previously unknown races that were identified in this study.

| 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 |
| Unk 1 | R | S | R | R |
| Unk 2 | S | S | R | R |
| Unk 3 | R | R | S | R |
| Unk 4 | S | R | R | R |
| Unk 5 | S | R | S | R |

Field experiments

To investigate whether pea varieties show differences in infection levels in different areas of the UK, field trials were established in 2016 and 2017. Combining pea varieties, vining pea varieties, the four differential host accessions (JI 411, JI 560, JI 758, JI 1272) and both germplasm accessions (JI 15, JI 85) were planted at each location, replicated three times in each trial. All seed was untreated or, in case of combining peas, treated with thiram only. Thiram seed treatment controls damping off but does not influence downy mildew infection. Thiram treated seed was used because untreated seed of these varieties was not available. In 2016, 20 pea varieties were planted at six different locations across the UK. In 2017, 32

pea varieties were planted at nine different locations across the UK (Tables 2 and 3). Fifty seeds were planted per variety and replicate and netted to avoid bird damage. The leaves were assessed for downy mildew infection approximately six weeks after planting and at flowering, and pods assessed for infection levels just prior to maturity. Leaf infection was recorded as percentage of plants with percentage leaf infection; pod infection was recorded as percentage of pods infected.

Table 2: Location of field trials in 2016 and 2017, with grid reference and county.

| Field site | Grid ref | UK County | Year |
|---------------|----------|-------------------|------|
| Chatteris | TL422887 | Cambridgeshire | 2016 |
| Howden | SE737265 | Yorkshire | 2016 |
| Nocton | TF036638 | Lincolnshire | 2016 |
| Perth | NO061209 | Perth and Kinross | 2016 |
| St. Germans | TF611128 | Norfolk | 2016 |
| Stubton | SK884910 | Lincolnshire | 2016 |
| Alyth | NO282475 | Perth and Kinross | 2017 |
| Chatteris | TL416880 | Cambridgeshire | 2017 |
| Holbeach | TF397278 | Lincolnshire | 2017 |
| Huggate | SE888550 | Yorkshire | 2017 |
| Nocton | TF025633 | Lincolnshire | 2017 |
| North Wootton | TF610263 | Norfolk | 2017 |
| Sledmere | SE957633 | Yorkshire | 2017 |
| Stubton | SK893478 | Lincolnshire | 2017 |
| Thorney | TF283075 | Cambridgeshire | 2017 |

Table 3: Pea variety and type established in field trials in 2016 and 2017.

| Variety | Type | Year grown |
|-------------|-------------------------------------|------------|
| 06S54009A | Vining pea | 2017 |
| 06S55519A | Vining pea | 2017 |
| 04S51315N | Vining pea | 2017 |
| 08S01030 | Vining pea | 2017 |
| 08S05676 | Vining pea | 2017 |
| 08S04137 | Vining pea | 2017 |
| Aikido | Combining pea (marrowfat) | 2016, 2017 |
| Aloha | Vining pea | 2016 |
| Anna | Vining pea | 2017 |
| Avola | Vining pea | 2016, 2017 |
| Crackerjack | Combining pea (large blue) | 2016, 2017 |
| Fintva | Vining pea | 2017 |
| Genki | Combining pea (large blue) | 2017 |
| Greenwood | Combining pea (small blue) | 2017 |
| Gregor | Combining pea (white) | 2016, 2017 |
| Ida | Vining pea | 2017 |
| Jl 15 | Germplasm (resistance gene carrier) | 2016, 2017 |
| Jl 85 | Germplasm (resistance gene carrier) | 2016, 2017 |
| Jl 411 | DM race differential | 2016, 2017 |
| Jl 560 | DM race differential | 2016, 2017 |
| Jl 758 | DM race differential | 2016, 2017 |
| Jl 1272 | DM race differential | 2016, 2017 |
| Linnea | Vining pea | 2017 |
| Mantara | Combining pea (maple) | 2016, 2017 |
| Maro | Combining pea (marrowfat) | 2016, 2017 |
| Mascara | Combining pea (white) | 2016, 2017 |
| Maurice | Vining pea | 2016, 2017 |
| Oasis | Vining pea | 2016, 2017 |
| Prophet | Combining pea (large blue) | 2016, 2017 |
| Rose | Combining pea (maple) | 2017 |
| Sakura | Combining pea (marrowfat) | 2016, 2017 |
| Tomahawk | Vining pea | 2016 |
| Waverex | Petits pois vining pea | 2016, 2017 |
| Vidor | Vining pea | 2017 |

Results

New crosses (JIC)

Based on the desk study (Appendix 1), new crosses were established, to generate genetic material to study: a) combinations of resistance genes; and b) the genetic location of novel resistance genes. Several reciprocal crosses were carried out to generate multiple F1 seeds for the combinations listed below.

- JI 15 x JI 85-Cx-F1
- JI 85 x JI 15-Cx-F1
- JI 399 x JI 85-Cx-F1
- JI 85 x JI 399-Cx-F1
- JI 1194 x JI 15-Cx-F1
- JI 15 x JI 1194-Cx-F1
- JI 1194 x JI 85-Cx-F1
- JI 15 x JI 411-Cx-F1

All crosses were verified by phenotypic analysis of F1 plants, where possible. For example, the genetic status of F1 plants derived by 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 could 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 could be verified by genotyping, using amplification of one or more genes to identify sequence-based or amplicon size polymorphism between the parent lines or simply by phenotypic observations of the resultant F2 plants.

The source of resistance (JI 15) identified within Pulse Crop Genetic Improvement Network (PCGIN) is unlikely to offer durable resistance since one major genetic locus is implicated in the resistance derived from this accession. 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 accession, had been reported to be resistant to all downy mildew races, except for race 7. Neither JI 85, nor JI 15 (see above), had been included in further DM research. We proposed that the cross between JI 15 and JI 85 would be of particular interest with respect to developing durable resistance. This has been validated by the further study of this cross (F3:4) within PCGIN,

indicating that two independent resistance genetic loci are segregating (this was reported at the PCGIN stakeholder meeting, December 2017). Further crosses were established between JI 15 and JI 1194 to enlarge the small population (60 recombinant inbred lines) that was used in the exploitation of the legume genome sequences and in generating marker data for DM resistance breeding (see below). Higher numbers of recombinant inbred lines (RILs) will enable refinement of the marker data generated (ongoing within PCGIN). The cross established between JI 85 and JI 1194 was generated to enable the genetic mapping of any novel resistance locus/loci present in JI 85 (ongoing within PCGIN). The cross between JI 15 and JI 411 (the latter is one of the host differential resistant accessions) is available as F2 seeds (88) with further F1 seeds (6), should there be a later interest in studying this cross. Depending on the results of further work, the cross between JI 85 and JI 399 (together with the extant JI 15 x JI 399 RILs) are likely to be informative with regards to mapping. (JI 399 showed good resistance in PCGIN DM assays; it should be noted that the JI 1194 and the JI 399 are vining pea types).

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, using candidate genes based on synteny with the model genome sequence of *Medicago truncatula* and emerging transcriptome data for pea. Two gene-specific markers were shown to provide breeder relevant information linked to DM resistance: a serine/threonine kinase (STK) and a second gene (Function Unknown Protein, FUP) on pea LG I (see 2016 project report). The data support a position for the disease resistance locus between these two markers and suggest that using these two gene-specific markers will allow the LG 1 resistance gene to be followed in crosses. 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. The LG I map position for DM resistance (PCGIN) was originally determined by variation in repetitive DNA around the locus (a consequence of variation in retrotransposon insertion sites across the pea genome), where assays generated a complex array of fluorescently-labelled DNA fragments, which were analysed using specialist software (see 2016 project report). In contrast, the assays described in this project are based on simplex PCR (two primers),

enzyme digestion and gel electrophoresis, as may be conducted in a basic molecular biology laboratory, and hence are much more likely to be adopted by breeders. The protocols for the assays have been shared with breeders (two), along with relevant germplasm (vining type RILs) from the JI 15 x JI 1194 cross. Crosses established between vining pea lines and RILs have been tested within one breeding programme and the marker data shown to be validated in the selection of relevant resistant breeding lines.

Further refinement of the LG I genetic locus has identified a set of polymorphic genes within the genetic interval between the STK and FUP genes, research that will continue within PCGIN.

Race determination of the DM isolates using the pea differential host accessions

During 2015, as part of the method development, twelve DM field samples were successfully inoculated onto seedlings of JI 411, JI 560, JI 758 and JI 1272. Results were inconsistent, and the same JI accession varied in response to the same DM field sample. It was concluded that this observation was probably due to the fact that the DM field samples were not of a pure race. If several races coexisted within one DM field sample, it is possible that JI accessions were infected by different races, making race determination impossible. The hypothesis was tested using several inoculation steps and single lesions on single seedlings to purify isolates in 2016. Purified isolates were inoculated onto seedlings of JI 411, JI 560, JI 758 and JI 1272 and results were consistent. This illustrated the importance of purifying isolates to obtain pure races. The twelve DM field samples collected in 2015 did not undergo the purification stage, resulting in them being potentially mixtures of races, and were therefore excluded from this report.

During 2016, 84 DM samples were collected and inoculated onto pea seedlings for isolate purification. Furthermore, 17 samples that had been stored in 2015 were inoculated onto pea seedlings for isolate purification. Of these 101 samples, 42 isolates survived the purification process and their race was determined (3 from 2015 and 39 from 2016). These isolates were obtained from eight locations in the UK and from 21 different pea varieties (Table 4).

In 2017, 160 DM samples were collected across the UK and inoculated onto pea seedlings for isolate purification. Eighty-three isolates survived the purification process and their race was determined. These isolates were obtained from fourteen locations in the UK and from at least 25 different pea varieties (Table 5).

In both years, all isolates were inoculated onto the germplasm accessions, JI 15 and JI 85, as likely carriers of novel DM resistance genes (Table 6).

In total, six isolates from 2016 (15% of isolates collected in 2016) overcame the resistance of JI 15, and in 2017 fifteen of the isolates (18% of isolates collected in 2017) overcame the resistance of JI 15. Overall, the isolates that overcame the resistance of JI 15 came from several races (Races 1 [five], 3 [four], 5 [two], 8, 9, 10 [three], 11, and Unk 2 [four]). In 2016, the isolates which overcame the resistance of JI 15 were from races 1, 3, 9, 10 and Unk 2. In 2017, the isolates which overcame the resistance of JI 15 were from races 1, 3, 5, 8, 10, 11 and Unk 2. These isolates were collected from several locations within the UK (Table 6).

Although the resistance from JI 85 was overcome by six isolates in 2016, there was no infection of this accession by isolates collected in 2017. One isolate in 2016 infected both JI 15 and JI 85 (I 130 from Stockbridge, race 1). The other isolates which infected JI 85 were race 1 (I 162) and race 5 (I 159) from Perth, and one isolate each of races 10 (I 174), 11 (I 179) and unk 1 (I 176) from Chatteris.

Table 4: Characteristics of downy mildew isolates collected in 2016, with location, date of collection, variety, DM race, tolerance of the germplasm accessions JI 15 and JI 85, and storage. r = resistant, s = susceptible.

| Isolate | UK County | Location | Date Collected | Pea variety | Race | JI 15 | JI 85 | Storage |
|---------|-------------------|---------------------|-------------------|-----------------|-------|-------|-------|---------|
| I 085 | Lincolnshire | Donington | collected in 2015 | ? | 10 | r | r | no |
| I 094 | Kent | Romney Marsh | collected in 2015 | Kelvedon Wonder | 10 | r | r | yes |
| I 100 | Lincolnshire | West Ashby | collected in 2015 | ? | 5 | r | r | yes |
| I 107 | Cambridgeshire | Chatteris | 20/05/2016 | ? | 3 | r | r | yes |
| I 112 | Cambridgeshire | Chatteris | 31/05/2016 | JI 758 | 11 | r | r | no |
| I 113 | Cambridgeshire | Chatteris | 31/05/2016 | Prophet | Unk 1 | r | r | no |
| I 115 | Cambridgeshire | Chatteris | 31/05/2016 | JI 1272 | 10 | s | r | no |
| I 117 | Lincolnshire | Stubton | 31/05/2016 | Sakura | 11 | r | r | no |
| I 118 | Lincolnshire | Stubton | 31/05/2016 | Tomahawk | 10 | r | r | no |
| I 119 | Lincolnshire | Stubton | 31/05/2016 | Mascara | 11 | r | r | no |
| I 120 | Lincolnshire | Stubton | 31/05/2016 | Crackerjack | 10 | r | r | yes |
| I 123 | Lincolnshire | Stubton | 31/05/2016 | Maro | 11 | r | r | no |
| I 127 | Hampshire | Stockbridge | 03/06/2016 | Greenwood | 9 | s | r | yes |
| I 128 | Hampshire | Stockbridge | 03/06/2016 | Sakura | 6 | r | r | yes |
| I 129 | Hampshire | Stockbridge | 03/06/2016 | Crackerjack | Unk 2 | s | r | yes |
| I 130 | Hampshire | Stockbridge | 03/06/2016 | Kingfisher | 1 | s | s | yes |
| I 139 | Hampshire | Stockbridge | 03/06/2016 | Prophet | 10 | r | r | no |
| I 140 | Hampshire | Stockbridge | 03/06/2016 | Mascara | 3 | r | r | yes |
| I 146 | Yorkshire | Howden | 30/06/2016 | Tomahawk | 8 | r | r | yes |
| I 147 | Yorkshire | Howden | 30/06/2016 | JI 1272 | 8 | r | r | yes |
| I 148 | Yorkshire | Howden | 30/06/2016 | Maro | 3 | r | r | yes |
| I 149 | Yorkshire | Howden | 30/06/2016 | Gregor | 8 | r | r | yes |
| I 150 | Yorkshire | Howden | 30/06/2016 | Sakura | 3 | r | r | yes |
| I 151 | Yorkshire | Howden | 30/06/2016 | Oasis | 8 | r | r | yes |
| I 152 | Yorkshire | Howden | 30/06/2016 | Avola | 4 | r | r | yes |
| I 153 | Yorkshire | Howden | 30/06/2016 | JI 560 | 8 | r | r | yes |
| I 156 | Yorkshire | Howden | 30/06/2016 | JI 411 | 3 | r | r | yes |
| I 159 | Perth and Kinross | Perth | 30/06/2016 | JI 560 | 5 | r | s | yes |
| I 162 | Perth and Kinross | Perth | 30/06/2016 | Avola | 1 | r | s | no |
| I 163 | Perth and Kinross | Perth | 30/06/2016 | Waverex | 5 | r | r | yes |
| I 164 | Perth and Kinross | Perth | 30/06/2016 | JI 1272 | 3 | s | r | no |
| I 170 | Yorkshire | Sledmere | 07/07/2016 | Amalfi | 10 | r | r | yes |
| I 171 | Yorkshire | Sledmere | 07/07/2016 | Amalfi | 3 | r | r | yes |
| I 172 | Yorkshire | Sledmere | 07/07/2016 | Amalfi | 11 | r | r | yes |
| I 174 | Cambridgeshire | Chatteris | 08/07/2016 | JI 85 | 10 | r | s | yes |
| I 176 | Cambridgeshire | Chatteris | 08/07/2016 | JI 85 | Unk 1 | r | s | yes |
| I 178 | Cambridgeshire | Chatteris | 08/07/2016 | JI 85 | 10 | r | r | yes |
| I 179 | Cambridgeshire | Chatteris | 08/07/2016 | JI 85 | 11 | r | s | yes |
| I 184 | Lincolnshire | Kirton | 12/07/2016 | Waverex | 3 | r | r | yes |
| I 185 | Lincolnshire | Kirton | 12/07/2016 | Waverex | 10 | s | r | yes |
| I 188 | Warwickshire | Stratford upon Avon | 20/07/2016 | Legacy | 10 | r | r | yes |

Table 5: Characteristics of downy mildew isolates collected in 2017, with location, date of collection, variety, DM race, tolerance of the germplasm accessions JI 15 and JI 85, and storage. r = resistant, s = susceptible.

| Isolate | UK County | Location | Date Collected | Pea variety | Race | JI 15 | JI 85 | Storage |
|---------|----------------|---------------|----------------|-------------|-------|-------|-------|---------|
| I 201 | Cambridgeshire | Chatteris | 28/04/2017 | Prophet | 10 | r | r | yes |
| I 202 | Lincolnshire | Stubton | 02/05/2017 | 06S55519A | 8 | s | r | yes |
| I 203 | Lincolnshire | Stubton | 02/05/2017 | Oasis | 1 | r | r | yes |
| I 204 | Lincolnshire | Stubton | 02/05/2017 | Maro | 3 | r | r | yes |
| I 205 | Lincolnshire | Stubton | 02/05/2017 | JI 1272 | 1 | r | r | yes |
| I 206 | Lincolnshire | Stubton | 02/05/2017 | 06S54009A | 3 | r | r | yes |
| I 207 | Lincolnshire | Stubton | 02/05/2017 | Avola | 1 | r | r | yes |
| I 209 | Lincolnshire | Nocton | 09/05/2017 | LG Element | 11 | s | r | yes |
| I 222 | Lincolnshire | Holbeach | 16/05/2017 | Wav 106 | 3 | s | r | yes |
| I 223 | Lincolnshire | Holbeach | 16/05/2017 | Wav 106 | Unk 2 | s | r | yes |
| I 225 | Lincolnshire | Holbeach | 16/05/2017 | Wav 106 | 10 | r | r | yes |
| I 226 | Lincolnshire | Holbeach | 16/05/2017 | Wav 106 | 1 | s | r | yes |
| I 227 | Cambridgeshire | Chatteris | 18/05/2017 | JI 1272 | 4 | r | r | yes |
| I 229 | Lincolnshire | Stubton | 23/05/2017 | Linnea | Unk 4 | r | r | yes |
| I 231 | Lincolnshire | Stubton | 23/05/2017 | ? | 3 | s | r | yes |
| I 232 | Lincolnshire | Stubton | 23/05/2017 | ? | 9 | r | r | yes |
| I 234 | Lincolnshire | Stubton | 23/05/2017 | ? | 3 | r | r | yes |
| I 238 | Lincolnshire | Stubton | 23/05/2017 | ? | 4 | r | r | yes |
| I 240 | Lincolnshire | Stubton | 23/05/2017 | ? | Unk 1 | r | r | yes |
| I 241 | Lincolnshire | Stubton | 23/05/2017 | ? | Unk 2 | s | r | yes |
| I 243 | Cambridgeshire | Thorney | 24/05/2017 | ? | Unk 4 | r | r | yes |
| I 244 | Cambridgeshire | Thorney | 24/05/2017 | ? | 5 | r | r | yes |
| I 246 | Cambridgeshire | Thorney | 24/05/2017 | ? | 3 | s | r | yes |
| I 247 | Cambridgeshire | Thorney | 24/05/2017 | ? | Unk 2 | r | r | yes |
| I 248 | Cambridgeshire | Thorney | 24/05/2017 | ? | 1 | r | r | yes |
| I 249 | Cambridgeshire | Thorney | 24/05/2017 | ? | 11 | r | r | yes |
| I 251 | Cambridgeshire | Thorney | 24/05/2017 | ? | 4 | r | r | yes |
| I 252 | Cambridgeshire | Thorney | 24/05/2017 | ? | 3 | r | r | yes |
| I 253 | Cambridgeshire | Thorney | 24/05/2017 | ? | 11 | r | r | yes |
| I 254 | Norfolk | North Wootton | 24/05/2017 | ? | 3 | r | r | yes |
| I 255 | Norfolk | North Wootton | 24/05/2017 | ? | 1 | r | r | yes |
| I 256 | Norfolk | North Wootton | 24/05/2017 | ? | 1 | r | r | yes |
| I 258 | Norfolk | North Wootton | 24/05/2017 | ? | 1 | r | r | yes |
| I 259 | Norfolk | North Wootton | 24/05/2017 | ? | 3 | r | r | yes |
| I 260 | Norfolk | North Wootton | 24/05/2017 | ? | 4 | r | r | yes |
| I 261 | Norfolk | North Wootton | 24/05/2017 | ? | Unk 3 | r | r | yes |
| I 262 | Norfolk | North Wootton | 24/05/2017 | ? | 1 | r | r | yes |
| I 263 | Norfolk | North Wootton | 24/05/2017 | ? | 2 | r | r | yes |
| I 264 | Norfolk | North Wootton | 24/05/2017 | ? | 1 | s | r | yes |

Table 5: continued.

| Isolate | UK County | Location | Date Collected | Pea variety | Race | Jl 15 | Jl 85 | Storage |
|---------|-------------------|-----------|----------------|-------------|-------|-------|-------|---------|
| I 268 | Perth and Kinross | Alyth | 01/06/2017 | Linnea | 10 | r | r | yes |
| I 269 | Perth and Kinross | Alyth | 01/06/2017 | 08501030 | 9 | r | r | yes |
| I 270 | Perth and Kinross | Alyth | 01/06/2017 | Gregor | 3 | r | r | yes |
| I 271 | Perth and Kinross | Alyth | 01/06/2017 | Avola | 3 | r | r | yes |
| I 272 | Perth and Kinross | Alyth | 01/06/2017 | Mascara | 11 | r | r | yes |
| I 274 | Perth and Kinross | Alyth | 01/06/2017 | Crackerjack | 3 | r | r | yes |
| I 275 | Perth and Kinross | Alyth | 01/06/2017 | 06554009A | Unk 3 | r | r | yes |
| I 277 | Perth and Kinross | Alyth | 31/05/2017 | Maro | 1 | s | r | yes |
| I 278 | Perth and Kinross | Alyth | 01/06/2017 | Vidor | 10 | r | r | yes |
| I 279 | Perth and Kinross | Alyth | 01/06/2017 | 06554009A | 10 | r | r | yes |
| I 280 | Perth and Kinross | Alyth | 01/06/2017 | 06555519A | 9 | r | r | yes |
| I 281 | Perth and Kinross | Alyth | 01/06/2017 | Oasis | 9 | r | r | yes |
| I 282 | Yorkshire | Huggate | 01/06/2017 | Oasis | 3 | r | r | yes |
| I 283 | Yorkshire | Huggate | 01/06/2017 | Oasis | 1 | r | r | yes |
| I 284 | Yorkshire | Kilham | 01/06/2017 | Celebration | Unk 4 | r | r | yes |
| I 285 | Yorkshire | Kilham | 01/06/2017 | Celebration | 11 | r | r | yes |
| I 287 | Yorkshire | Kilham | 01/06/2017 | Celebration | 3 | r | r | yes |
| I 288 | Yorkshire | Kilham | 01/06/2017 | Celebration | 3 | r | r | yes |
| I 289 | Yorkshire | Kilham | 01/06/2017 | Celebration | 9 | r | r | yes |
| I 291 | Lincolnshire | Holbeach | 02/06/2017 | Fintra | Unk 1 | r | r | yes |
| I 300 | Lincolnshire | Holbeach | 02/06/2017 | 08504137 | 3 | r | r | yes |
| I 302 | Cambridgeshire | Chatteris | 05/06/2017 | Oasis | 10 | r | r | yes |
| I 303 | Cambridgeshire | Chatteris | 05/06/2017 | Ida | 5 | s | r | yes |
| I 304 | Cambridgeshire | Chatteris | 05/06/2017 | Jl 15 | 5 | r | r | yes |
| I 305 | Cambridgeshire | Chatteris | 05/06/2017 | Prophet | 11 | r | r | yes |
| I 310 | Cambridgeshire | Chatteris | 05/06/2017 | 0804137 | 8 | r | r | yes |
| I 311 | Cambridgeshire | Chatteris | 05/06/2017 | Maro | Unk 2 | r | r | yes |
| I 313 | Cambridgeshire | Chatteris | 05/06/2017 | Mantara | 11 | r | r | yes |
| I 316 | Yorkshire | Sledmere | 09/06/2017 | 04551315N | 8 | r | r | yes |
| I 317 | Yorkshire | Sledmere | 09/06/2017 | Maro | 1 | s | r | yes |
| I 320 | Yorkshire | Sledmere | 09/06/2017 | Oasis | 3 | r | r | yes |
| I 321 | Yorkshire | Sledmere | 09/06/2017 | Jl 1272 | 3 | r | r | yes |
| I 323 | Lincolnshire | Ancaster | 09/06/2017 | Kingfisher | 10 | s | r | yes |
| I 324 | Lincolnshire | Ancaster | 09/06/2017 | Kingfisher | 11 | r | r | yes |
| I 326 | Lincolnshire | Ancaster | 09/06/2017 | Kingfisher | 9 | r | r | yes |
| I 327 | Yorkshire | Huggate | 09/06/2017 | Oasis | Unk 5 | r | r | yes |
| I 328 | Yorkshire | Huggate | 09/06/2017 | 04555315N | 5 | s | r | yes |
| I 329 | Yorkshire | Huggate | 09/06/2017 | Genki | Unk1 | r | r | yes |
| I 330 | Yorkshire | Huggate | 09/06/2017 | Maro | 6 | r | r | yes |
| I 331 | Yorkshire | Kilham | 09/06/2017 | Celebration | Unk 1 | r | r | yes |
| I 337 | Yorkshire | Kilham | 09/06/2017 | Celebration | Unk 2 | s | r | yes |
| I 338 | Yorkshire | Kilham | 09/06/2017 | Celebration | 2 | r | r | yes |
| I 341 | Lincolnshire | Walcot | 26/06/2017 | Knight 1 | 8 | r | r | yes |
| I 356 | Lincolnshire | Walcot | 27/06/2017 | Knight 1 | 3 | r | r | yes |

Table 6: Characteristics of downy mildew isolates collected in 2016 and 2017 to which JI 15 or JI 85 germplasm showed susceptibility, with location, date of collection, variety and DM race. r = resistant, s = susceptible.

| Isolate | UK County | Location | Date Collected | Pea variety | Race | JI 15 | JI 85 |
|---------|-------------------|---------------|----------------|-------------|-------|-------|-------|
| I 130 | Hampshire | Stockbridge | 2016 | Kingfisher | 1 | s | s |
| I 226 | Lincolnshire | Holbeach | 2017 | Wav 106 | 1 | s | r |
| I 264 | Norfolk | North Wootton | 2017 | ? | 1 | s | r |
| I 277 | Perth and Kinross | Alyth | 2017 | Maro | 1 | s | r |
| I 317 | Yorkshire | Sledmere | 2017 | Maro | 1 | s | r |
| I 246 | Cambridgeshire | Thorney | 2017 | ? | 3 | s | r |
| I 222 | Lincolnshire | Holbeach | 2017 | Wav 106 | 3 | s | r |
| I 231 | Lincolnshire | Stubton | 2017 | ? | 3 | s | r |
| I 164 | Perth and Kinross | Perth | 2016 | JI 1272 | 3 | s | r |
| I 303 | Cambridgeshire | Chatteris | 2017 | Ida | 5 | s | r |
| I 328 | Yorkshire | Huggate | 2017 | 04555315N | 5 | s | r |
| I 202 | Lincolnshire | Stubton | 2017 | 06S55519A | 8 | s | r |
| I 127 | Hampshire | Stockbridge | 2016 | Greenwood | 9 | s | r |
| I 115 | Cambridgeshire | Chatteris | 2016 | JI 1272 | 10 | s | r |
| I 185 | Lincolnshire | Kirton | 2016 | Waverex | 10 | s | r |
| I 323 | Lincolnshire | Ancaster | 2017 | Kingfisher | 10 | s | r |
| I 209 | Lincolnshire | Nocton | 2017 | LG Element | 11 | s | r |
| I 223 | Lincolnshire | Holbeach | 2017 | Wav 106 | Unk 2 | s | r |
| I 241 | Lincolnshire | Stubton | 2017 | ? | Unk 2 | s | r |
| I 337 | Yorkshire | Kilham | 2017 | Celebration | Unk 2 | s | r |
| I 129 | Hampshire | Stockbridge | 2016 | Crackerjack | Unk 2 | s | r |
| I 162 | Perth and Kinross | Perth | 2016 | Avola | 1 | r | s |
| I 159 | Perth and Kinross | Perth | 2016 | JI 560 | 5 | r | s |
| I 174 | Cambridgeshire | Chatteris | 2016 | JI 85 | 10 | r | s |
| I 179 | Cambridgeshire | Chatteris | 2016 | JI 85 | 11 | r | s |
| I 176 | Cambridgeshire | Chatteris | 2016 | JI 85 | Unk 1 | r | s |

Geographical distribution and frequency of occurrence of UK pea DM isolates

Table 7: Geographical location of origin of downy mildew races collected during 2016 and 2017.

| Race | Number of isolates collected in 2016 | Location | UK County | Number of isolates collected in 2017 | Location | UK County | Total number of isolates |
|------|--------------------------------------|--|---|--------------------------------------|--|---|--------------------------|
| 1 | 2 | Stockbridge, Perth | Hampshire, Perth and Kinross | 13 | Alyth, Holbeach, Huggate, North Wootton(5), Sledmere, Stubton(3), Thorney | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 15 |
| 2 | 0 | | | 2 | Kilham, North Wootton | Yorkshire, Norfolk | 2 |
| 3 | 8 | Chatteris, Stockbridge, Howden (3), Perth, Sledmere, Kirton | Cambridgeshire, Hampshire, Yorkshire, Perth and Kinross, Lincolnshire | 19 | Alyth(3), Holbeach(2), Huggate, Kilham(2), North Wootton(2), Sledmere(2), Stubton(4), Thorney(2), Walcot | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 27 |
| 4 | 1 | Howden | Yorkshire | 4 | Chatteris, North Wootton, Stubton, Thorney | Cambridgeshire, Norfolk, Lincolnshire | 5 |
| 5 | 2 | Perth (2) | Perth and Kinross | 4 | Chatteris(2), Huggate, Thorney | Cambridgeshire, Yorkshire | 6 |
| 6 | 1 | Stockbridge | Hampshire | 1 | Huggate | Yorkshire | 2 |
| 7 | 0 | | | 0 | | | 0 |
| 8 | 5 | Howden (5) | Yorkshire | 4 | Chatteris, Sledmere, Stubton, Walcot | Cambridgeshire, Yorkshire, Lincolnshire | 9 |
| 9 | 1 | Stockbridge | Hampshire | 6 | Alyth(3), Ancaster, Kilham, Stubton | Perth and Kinross, Lincolnshire, Yorkshire | 7 |
| 10 | 9 | Chatteris (3), Stubton (2), Stockbridge, Sledmere, Kirton, Stratford upon Avon | Lincolnshire, Cambridgeshire, Hampshire, Yorkshire, Warwickshire | 7 | Alyth(3), Ancaster, Chatteris(2), Holbeach | Perth and Kinross, Lincolnshire, Cambridgeshire, Lincolnshire | 16 |

| | | | | | | | |
|-------------|---|--|---|---|--|---|----|
| 11 | 6 | Chatteris (2), Stubton (3), Sledmere | Cambridgeshire, Lincolnshire, Yorkshire | 8 | Alyth, Ancaster, Chatteris(2), Kilham, Nocton, Thorney(2) | Perth and Kinross, Lincolnshire, Cambridgeshire, Yorkshire | 14 |
| Unk1 | 2 | Chatteris (2) | Cambridgeshire | 4 | Holbeach, Huggate, Kilham, Stubton | Lincolnshire, Yorkshire | 6 |
| Unk2 | 1 | Stockbridge | Hampshire | 5 | Chatteris, Holbeach, Kilham, Stubton, Thorney | Cambridgeshire, Lincolnshire, Yorkshire | 6 |
| Unk3 | 0 | | | 2 | Alyth, North Wootton | Perth and Kinross, Norfolk | 2 |
| Unk4 | 0 | | | 3 | Kilham, Stubton, Thorney | Yorkshire, Lincolnshire, Cambridgeshire | 3 |
| Unk5 | 0 | | | 1 | Huggate | Yorkshire | 1 |

The most frequently recorded races in 2016 were races 3 and 10, comprising 46% of the isolates collected during the year (Figure 1). Races 8 and 11 comprised 28% of the isolates collected during the year. Several races occurred in more than one location in the UK but some races were collected from one just location: race 4 (Howden, Yorkshire), race 6 (Stockbridge, Hampshire), race 9 (Stockbridge, Hampshire), Unk 1 (Chatteris, Cambridgeshire) and Unk 2 (Stockbridge, Hampshire). Race 7 was not recorded in 2016.

The most frequently recorded races in 2017 were races 1 and 3, comprising 39% of the isolates collected during the year (Figure 2). Races 10 and 11 comprised 18% of the isolates collected during the year. Several races occurred in more than one location in the UK but race 6 and Unk 5 were each only found at Huggate (Yorkshire). Race 7 was not recorded in 2017.

Nearly a quarter (23%) of all isolates from 2016 and 2017 were race 3 (Figure 3); races 10 and 1 comprised 25% of the isolates collected. There was only 1 isolate obtained of Unk 5, and race 7 was not recorded in either 2016 or 2017.

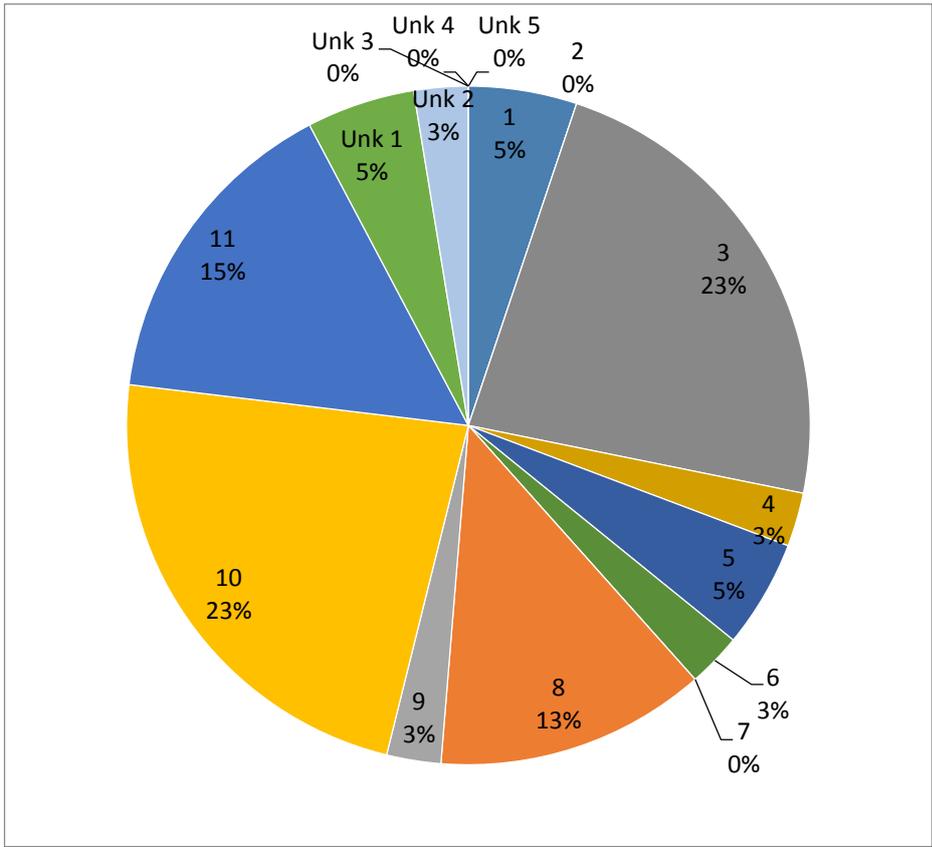


Figure 1: Isolates collected, as a percentage of total collected in 2016, categorised by race.

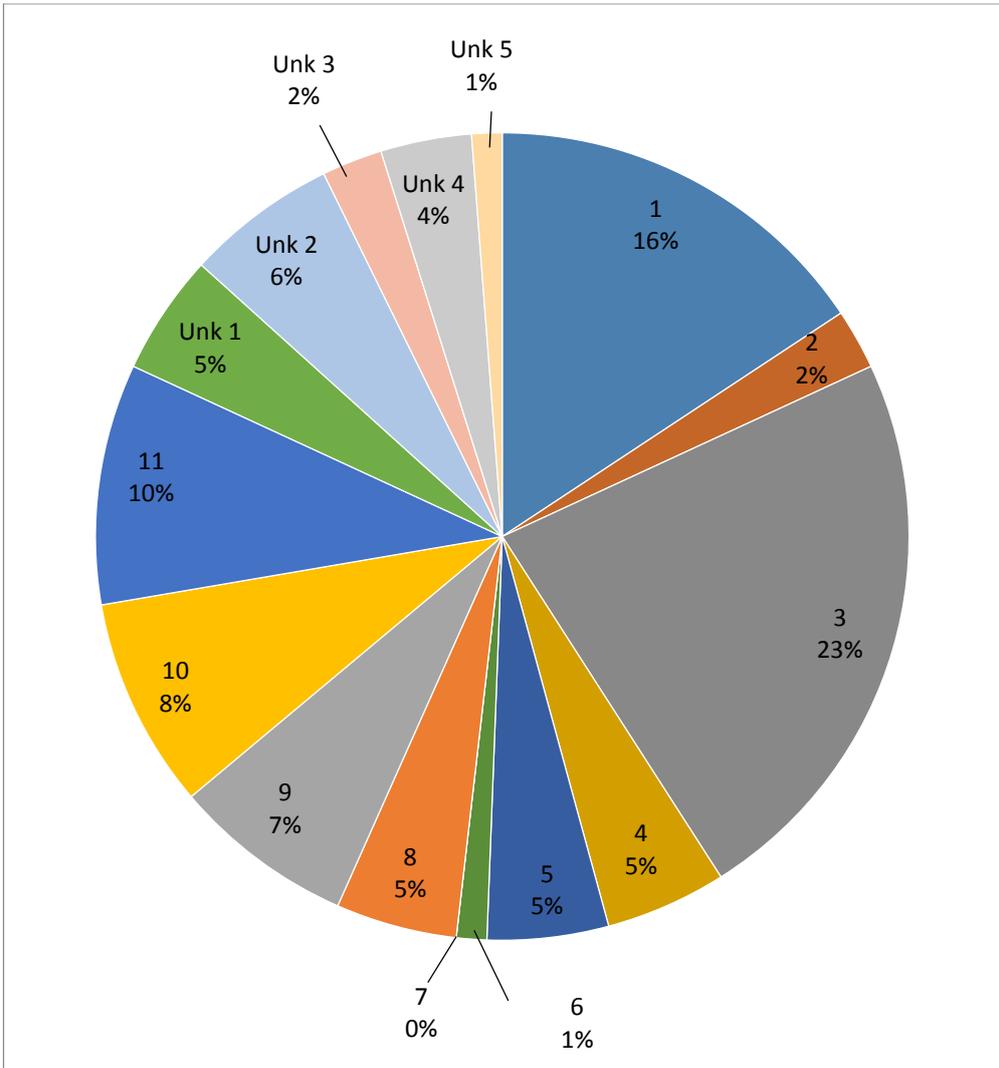


Figure 2: Isolates collected, as a percentage of total collected in 2017, categorised by race.

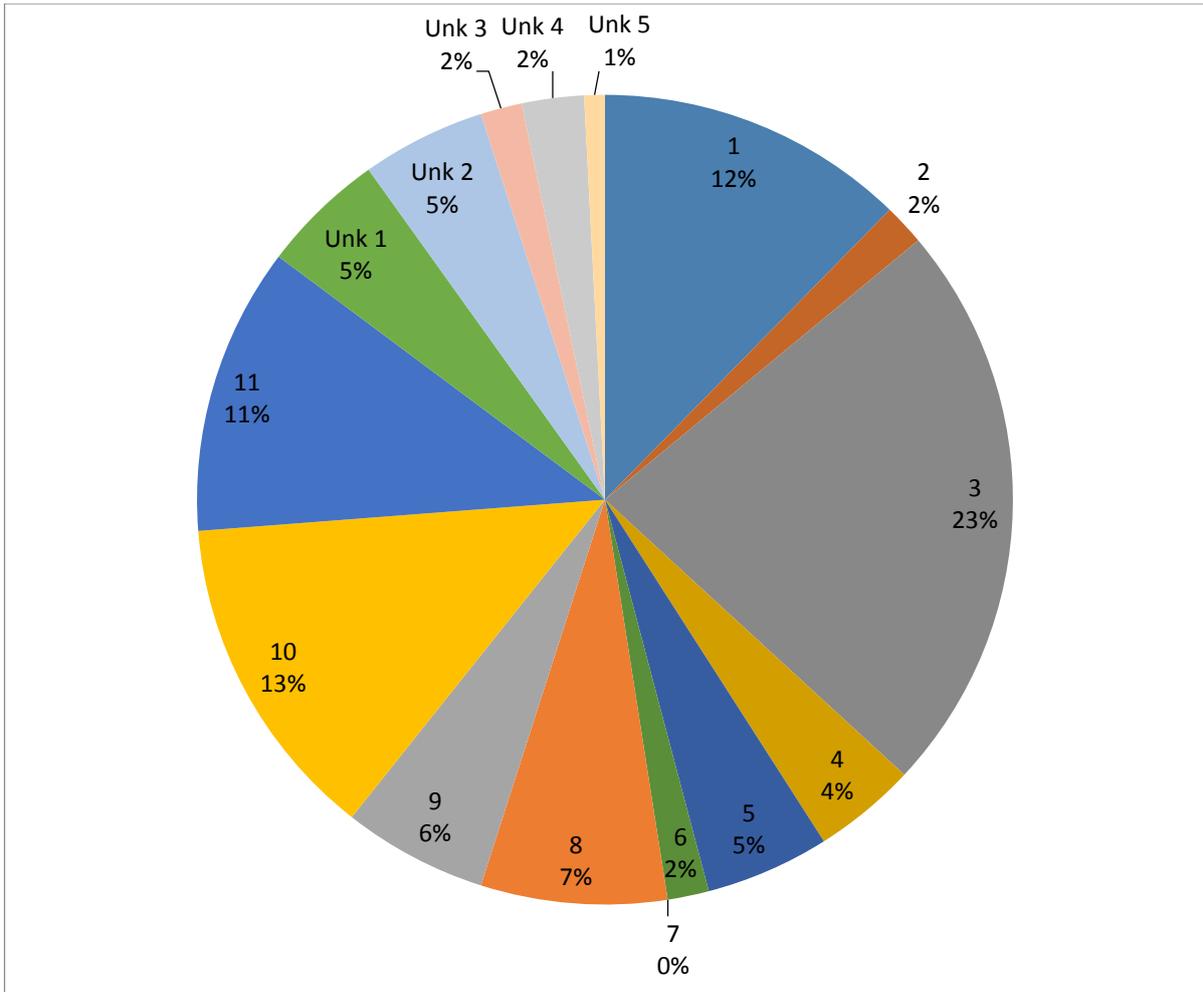


Figure 3: Isolates collected, as a percentage of total collected in 2016 and 2017, categorised by race.

Distribution of downy mildew races across the UK

The locations from which downy mildew isolates were collected, including field trial locations in 2016 and 2017, are shown in Figure 4.

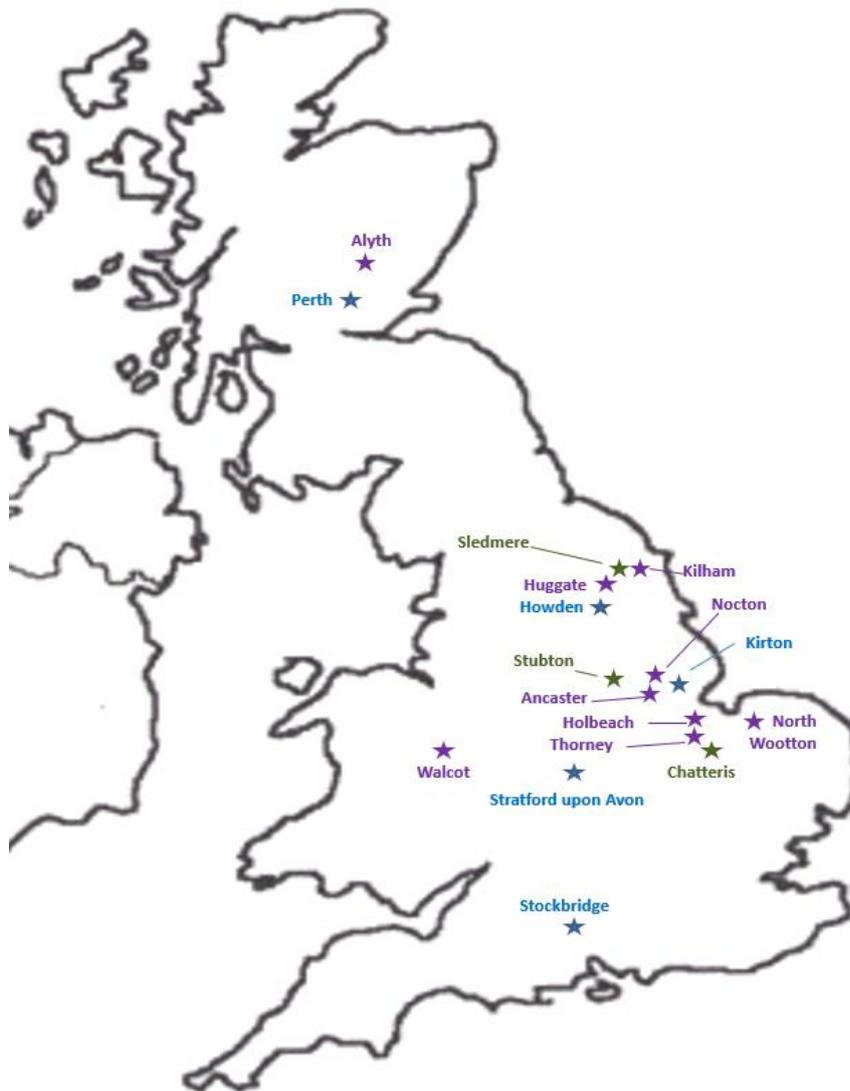


Figure 4: Locations of downy mildew isolates collected in 2016 and 2017. Blue text represents samples collected in 2016, purple text represents samples collected in 2017, green text represents samples collected in both 2016 and 2017.

Figure 5 shows the geographical distribution of the DM races found in this study.

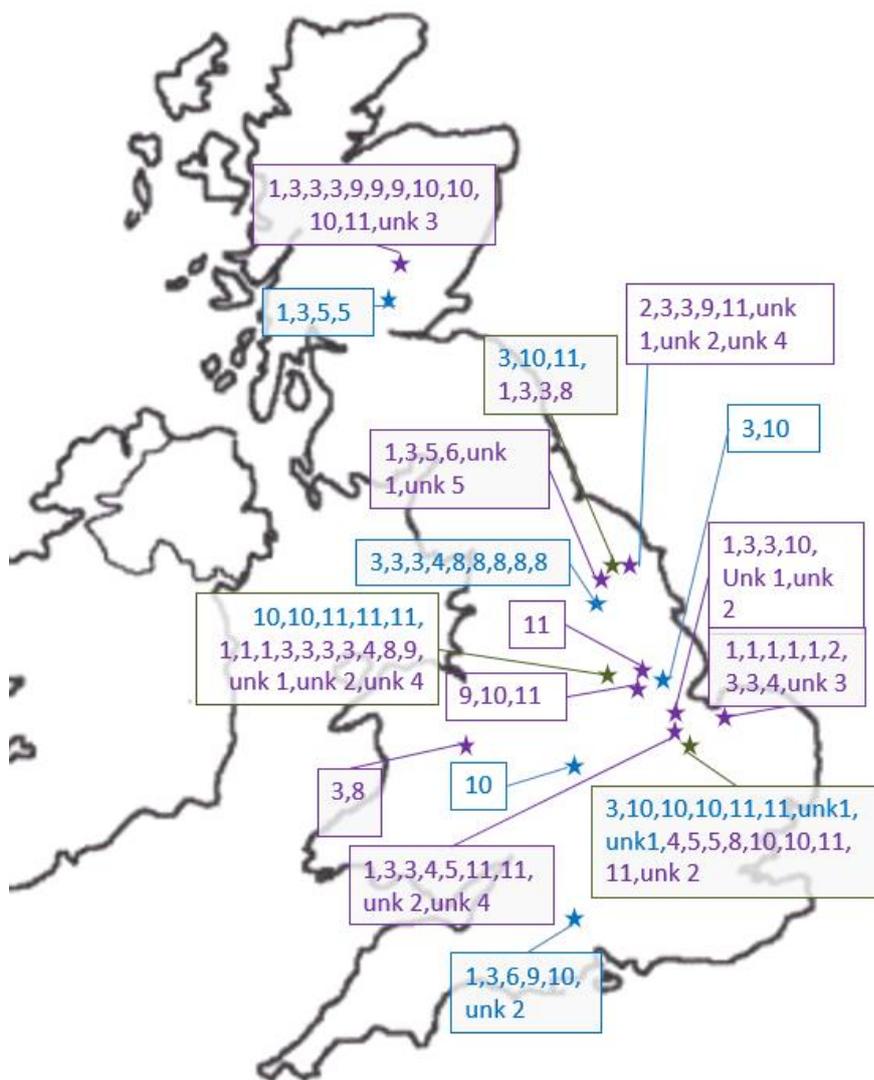


Figure 5: Geographical distribution of DM races collected in 2016 and 2017. Blue text within boxes represents isolates collected in 2016, purple text within boxes represents isolates collected in 2017. Green boxes represent sites where isolates were collected in both 2016 and 2017.

A direct comparison of DM race frequency in 2016 and 2017 can be made at three locations in England, Chatteris (Cambridgeshire), Sledmere (Yorkshire) and Stubton (Lincolnshire)

(Table 8). At Chatteris, similar numbers of isolates from race 10 and 11 were found in both years. At Sledmere, race 3 was present in both years. At Stubton, the isolates were different in both years. Isolates of race 10 were found at Sledmere and Stubton in 2016 but not in 2017. There was a much greater diversity of races found at Stubton in 2017 than in 2016; the greatest number of isolates came from race 3 (no isolates of race 3 were found in 2016). This difference might partly be explained by the fact that more samples were collected in 2017 than in 2016. Overall there are marked differences in the races of DM found at each location between the two years.

Table 8: Comparison of DM race frequency in 2016 and 2017 at Chatteris, Sledmere and Stubton.

| Year | Location | Race |
|------|-----------|--|
| 2016 | Chatteris | 3,10,10,10,11,11, Unk 1, Unk 1 |
| 2017 | Chatteris | 4,5,5,8,10,10,11,11, Unk 2 |
| 2016 | Sledmere | 3,10,11 |
| 2017 | Sledmere | 1,3,3,8 |
| 2016 | Stubton | 10,10,11,11,11 |
| 2017 | Stubton | 1,1,1,3,3,3,3,4,8,9, Unk 1, Unk 2, Unk 4 |

Differences in varietal susceptibility to downy mildew populations at different geographical locations

Downy mildew disease occurrence in field trials in 2016:

Twenty pea varieties were planted at six locations across the UK in 2016 (Tables 2 and 3). At three of the field trial locations, St. Germans, Perth and Nocton, only low levels of DM infection occurred (% leaf area infection is an average for all plants of each variety evaluated at each location) (Tables 9, 10).

St. Germans field trial site:

- Only the variety Oasis showed any DM infection (0.33% leaf area infection).
- The race composition of the DM population remains unknown because the DM samples taken from the field did not survive the purification process under growth room conditions.

Perth field trial site:

- Only Avola showed infection (0.33% leaf area infection).
- The DM population comprised of at least three different races, race 1, 3 and 5.

Nocton field trial site:

- The highest infection levels of just under 2% were seen in Avola and JI 560.
- Low levels of infection appeared in most of the other lines, except for JI 15, JI 85, Mantara, Maurice, Oasis, Prophet, Sakura and Waverex, which were completely uninfected.
- There is no race data for Nocton, because DM samples taken from the field did not survive the purification process under growth room conditions.

The other three field trial locations, Chatteris, Howden and Stubton, showed higher levels of DM infection.

Chatteris field trial site:

- Only Aloha and Maurice were free of leaf infection.
- Avola, Gregor, Oasis, Prophet, Sakura, Tomahawk and Waverex had medium levels of approximately 4% infection, and infection levels of greater than 5% were recorded for JI 1272, Maro and Mascara.
- The DM population at Chatteris comprised of at least four different races, 3, 10, 11 and Unk 1.

Howden field trial site:

- Overall infection levels at Howden were greatest compared to all other sites in 2016.
- Avola had the highest level of infection at 15% leaf area, followed by Sakura, Maro, Tomahawk and JI 560 with 10%, 7%, 6% and 6%, respectively.
- No variety was completely free of leaf infection.
- Races 3, 4 and 8 were present.

Stubton field trial site:

- Highest infection levels of greater than 3% were recorded for Avola, JI 560, JI 1272 and Maro.
- Six varieties were free of infection and the remainder had infection levels of 0.1 to 2%.
- The DM population of Stubton comprised two different races, races 10 and 11.

Downy mildew disease occurrence in field trials in 2017:

In 2017, the field trials were expanded to use a total of 32 combining pea varieties, vining pea varieties and pea research lines (the same research germplasm lines used in 2016), planted at nine different locations across the UK (including some locations which were used in 2016; Tables 11 and 12).

Nocton field trial site:

- Race 11 was present.
- Only very low levels of DM infection were present with the only infection recorded for JI 411 (0.03%) and Fintva (0.1%) lines.
- There was no infection in variety Avola.

Huggate field trial site:

- Races 1, 3, 5, 6, Unk 1 and Unk 5 were present.
- Only a few varieties of pea were affected by DM but one of these varieties had greater levels of infection relative to the others at this site (Oasis 3%).
- There was no infection in variety Avola.

Sledmere field trial site:

- Races 1, 3 and 8 were present.
- There were generally low levels of infection at this site.

Alyth field trial site:

- Races 1, 3, 9, 10, 11 and Unk 3 were present.
- There were generally low levels of infection at this site.

North Wootton field trial site:

- Races 1, 2, 3, 4 and Unk 3 were present.
- There were generally low levels of infection at this site.

Holbeach field trial site:

- Races 1, 3, 10, Unk 1 and Unk 2 were present.
- There were generally low levels of infection at this site.

Stubton field trial site:

- Races 1, 3, 4, 8, 9, Unk 1, Unk 2 and Unk 4 were present.
- Avola had the highest rate of infection (10%).
- All varieties had some infection.
- There were generally high levels of infection at this site.

Thorney field trial site:

- Races 1, 3, 4, 5, 11, Unk 2 and Unk 4 were present.
- All varieties had some infection.

Chatteris field trial site:

- Races 1, 3, 4, 5, 11, Unk 2 and Unk 4 were present.
- All varieties had some infection.
- Overall, the highest total average infection was recorded at this site.

The variety Maurice performed well at all sites, having 0% infection at five of the sites and the highest rate of infection for Maurice was 0.16% (Thorney).

Table 9 shows the difference in infection levels of each variety by location.

Table 9: Mean percentage leaf infection of 20 pea varieties grown at six different locations in 2016. Red background represents varieties with the highest infection levels at each trial site; Orange background represents varieties with medium infection levels at each trial site; Blue background represents varieties with the lowest infection levels at each trial site; No colour represents varieties with no infection. VP = Vining pea, CP = combining pea, JI = John Innes race differential or germplasm.

| | | Chatteris | Howden | Nocton | Perth | St. Germans | Stubton |
|-------------|-----------|-----------|--------|--------|-------|-------------|---------|
| Variety | Type | %DM | | | | | |
| Aikido | CP | 1.17 | 2.22 | 0.13 | 0.00 | 0.00 | 0.00 |
| Aloha | VP | 0.00 | 0.03 | 0.04 | 0.00 | 0.00 | 0.00 |
| Avola | VP | 4.17 | 15.33 | 1.75 | 0.33 | 0.02 | 3.50 |
| Crackerjack | CP | 1.58 | 1.57 | 0.08 | 0.00 | 0.00 | 2.18 |
| Gregor | CP | 4.89 | 1.59 | 0.38 | 0.00 | 0.00 | 1.25 |
| JI 1272 | JI | 8.03 | 4.38 | 0.14 | 0.01 | 0.00 | 3.62 |
| JI 15 | JI | 0.96 | 0.37 | 0.00 | 0.00 | 0.00 | 0.23 |
| JI 411 | JI | 1.03 | 0.71 | 0.21 | 0.00 | 0.00 | 0.00 |
| JI 560 | JI | 1.88 | 5.75 | 1.68 | 0.00 | 0.00 | 5.00 |
| JI 758 | JI | 1.31 | 3.72 | 0.25 | 0.00 | 0.00 | 1.85 |
| JI 85 | JI | 0.01 | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mantara | CP | 1.75 | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 |
| Maro | CP | 5.83 | 9.58 | 0.17 | 0.01 | 0.00 | 3.52 |
| Mascara | CP | 6.25 | 0.20 | 0.08 | 0.00 | 0.00 | 0.02 |
| Maurice | VP | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 |
| Oasis | VP | 4.25 | 4.10 | 0.00 | 0.00 | 0.33 | 1.65 |
| Prophet | CP | 4.60 | 0.35 | 0.00 | 0.00 | 0.00 | 0.04 |
| Sakura | CP | 3.62 | 6.90 | 0.00 | 0.00 | 0.00 | 0.43 |
| Tomahawk | VP | 4.10 | 5.73 | 0.31 | 0.00 | 0.00 | 0.96 |
| Waverex | VP | 4.12 | 1.75 | 0.00 | 0.00 | 0.00 | 0.17 |
| | Site mean | 2.98 | 3.25 | 0.26 | 0.02 | 0.02 | 1.22 |

The greatest levels of infection at the PGRO trial sites in 2017 occurred at Chatteris, Stubton and Thorney (Table 10).

Table 10: Mean percentage leaf infection of 32 pea varieties grown at nine different locations in 2017. Red background represents varieties with the infection levels at each trial site; Orange background represents varieties with medium infection levels at each trial site; Blue represents varieties with the lowest infection levels at each trial site. No colour represents varieties with no infection. VP = Vining pea, CP = combining pea, JI = John Innes race differential or germplasm.

| | | ALYTH | CHATTERIS | HOLBEACH | HUGGATE | NOCTON | NORTH WOOTTON | SLEDMERE | STUBTON | THORNEY |
|-------------|-----------|-------|-----------|----------|---------|--------|---------------|----------|---------|---------|
| Variety | Type | %DM | | | | | | | | |
| Aikido | CP | 0.67 | 1.56 | 0.08 | 0.00 | 0.00 | 0.49 | 0.70 | 1.62 | 0.37 |
| Crackerjack | CP | 0.67 | 3.37 | 0.03 | 0.00 | 0.00 | 0.81 | 0.00 | 2.03 | 0.85 |
| Genki | CP | 0.03 | 1.74 | 0.12 | 0.03 | 0.00 | 0.99 | 0.03 | 0.80 | 1.33 |
| Greenwood | CP | 0.08 | 0.93 | 0.00 | 0.00 | 0.00 | 0.39 | 0.03 | 2.17 | 0.79 |
| Gregor | CP | 0.17 | 4.73 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 1.90 | 1.07 |
| Mantara | CP | 0.25 | 1.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.25 | 0.13 |
| Maro | CP | 1.37 | 6.93 | 4.70 | 0.03 | 0.00 | 1.92 | 2.00 | 4.45 | 4.38 |
| Mascara | CP | 0.08 | 1.50 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 1.45 | 1.01 |
| Prophet | CP | 0.25 | 2.31 | 0.17 | 0.00 | 0.00 | 0.00 | 0.07 | 1.18 | 0.24 |
| Rose | CP | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.42 | 0.21 |
| Sakura | CP | 0.33 | 2.58 | 0.08 | 0.00 | 0.00 | 0.29 | 0.00 | 1.64 | 0.86 |
| JI 1272 | JI | 0.12 | 4.00 | 0.20 | 0.00 | 0.00 | 2.47 | 0.17 | 9.13 | 1.95 |
| JI 15 | JI | 0.00 | 0.40 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.50 | 0.46 |
| JI 411 | JI | 0.03 | 1.96 | 0.03 | 0.00 | 0.03 | 0.04 | 0.08 | 3.28 | 2.16 |
| JI 560 | JI | 0.15 | 3.29 | 0.00 | 0.00 | 0.00 | 1.68 | 0.08 | 7.95 | 4.36 |
| JI 758 | JI | 0.00 | 1.17 | 0.00 | 0.00 | 0.00 | 0.04 | 0.07 | 1.15 | 1.53 |
| JI 85 | JI | 0.00 | 0.04 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 | 0.18 |
| 04S51315N | VP | 0.08 | 0.75 | 0.07 | 0.03 | 0.00 | 0.14 | 0.08 | 0.35 | 1.13 |
| 06S54009A | VP | 3.50 | 1.42 | 0.17 | 0.00 | 0.00 | 0.88 | 0.00 | 3.08 | 2.11 |
| 06S55519A | VP | 0.70 | 9.18 | 0.17 | 0.00 | 0.00 | 0.03 | 0.00 | 2.87 | 3.27 |
| 08S01030 | VP | 0.28 | 1.21 | 0.08 | 0.00 | 0.00 | 0.13 | 0.00 | 0.84 | 1.12 |
| 08S04137 | VP | 0.07 | 4.46 | 0.37 | 0.00 | 0.00 | 0.10 | 0.00 | 1.45 | 0.91 |
| 08S05676 | VP | 0.20 | 1.60 | 0.00 | 0.00 | 0.00 | 1.08 | 0.00 | 2.77 | 0.58 |
| Anna | VP | 0.20 | 1.18 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 1.20 | 0.48 |
| Avola | VP | 0.42 | 6.79 | 0.12 | 0.00 | 0.00 | 1.19 | 0.03 | 10.00 | 6.68 |
| Fintva | VP | 0.07 | 5.04 | 0.43 | 0.00 | 0.10 | 0.83 | 0.03 | 2.20 | 2.09 |
| Ida | VP | 0.00 | 1.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.83 | 0.11 |
| Linnea | VP | 0.17 | 3.27 | 1.00 | 0.00 | 0.00 | 0.18 | 0.00 | 1.86 | 0.80 |
| Maurice | VP | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.02 | 0.00 | 0.13 | 0.16 |
| Oasis | VP | 0.70 | 6.68 | 1.50 | 3.00 | 0.00 | 1.19 | 0.03 | 3.63 | 1.79 |
| Vidor | VP | 1.67 | 5.23 | 1.50 | 0.00 | 0.00 | 1.30 | 0.08 | 2.55 | 1.13 |
| Waverex | VP | 0.17 | 1.17 | 4.17 | 0.00 | 0.00 | 0.83 | 0.03 | 2.33 | 1.69 |
| | Site Mean | 0.39 | 2.72 | 0.49 | 0.10 | 0.00 | 0.53 | 0.11 | 2.41 | 1.43 |

Varietal susceptibility to DM infection varied by location (Figures 6 (vining peas) and 7 (combining peas)).

In 2017, many vining peas varieties showed greatest relative infection levels at Chatteris and Stubton although Waverex and 06S54009A showed greatest levels of infection at Holbeach and Alyth, respectively. Maurice had lower levels of infection at Chatteris than most varieties and generally low infection across all sites. Performance of Maurice appeared to be good regardless of location in 2017, which concurred with trials conducted in 2016. In common with Ida, 08S01030, Anna and 04S51315N, mean percentage infection for Maurice was below 2% across all sites (for Maurice the highest average level of infection was only 0.16%). Avola had relatively high levels of infection at Stubton, Chatteris and Thorney (each being over 6.5%) but fared better at Alyth (0.42%) and North Wootton (1.19%) with hardly any infection at Holbeach (0.12%).

In 2017, there was a higher rate of infection of combining peas (CP) at Chatteris than at other sites. Exceptions to this were the varieties Greenwood and Rose which had the highest rate of infection at Stubton. In general, combining peas grown in Holbeach, Nocton and Alyth showed very low infection levels. Only 2 varieties showed any significant infection at Sledmere: Maro (2%) and Aikido (0.7%). Rose had generally low levels of infection (0.41% maximum). Maro had relatively high levels of infection across all sites with the greatest infection rate for combining peas at each site. The infection at Holbeach was over 21 times greater than for any other combining pea variety (infection was less than 0.23% for all other varieties at Holbeach). Overall, there was less influence of location on infection occurring in combining peas than in vining peas.

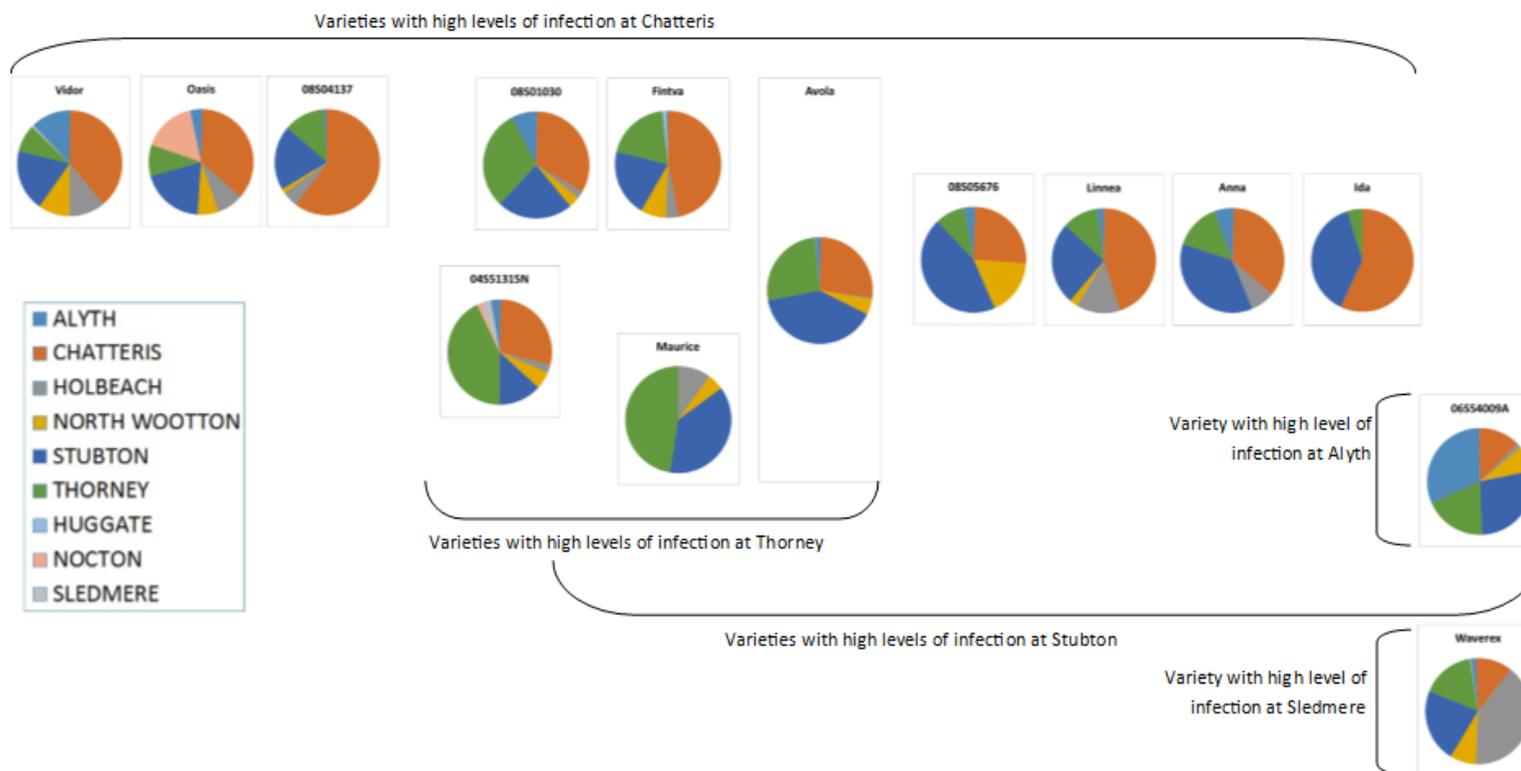


Figure 6: Percentage of downy mildew for each vining pea variety at each site, illustrating those that performed better at each site in 2016 and 2017. Each pie chart represents a single variety and is split to show a comparison of % leaf area infection at each site as a proportion of the total infection at all sites.

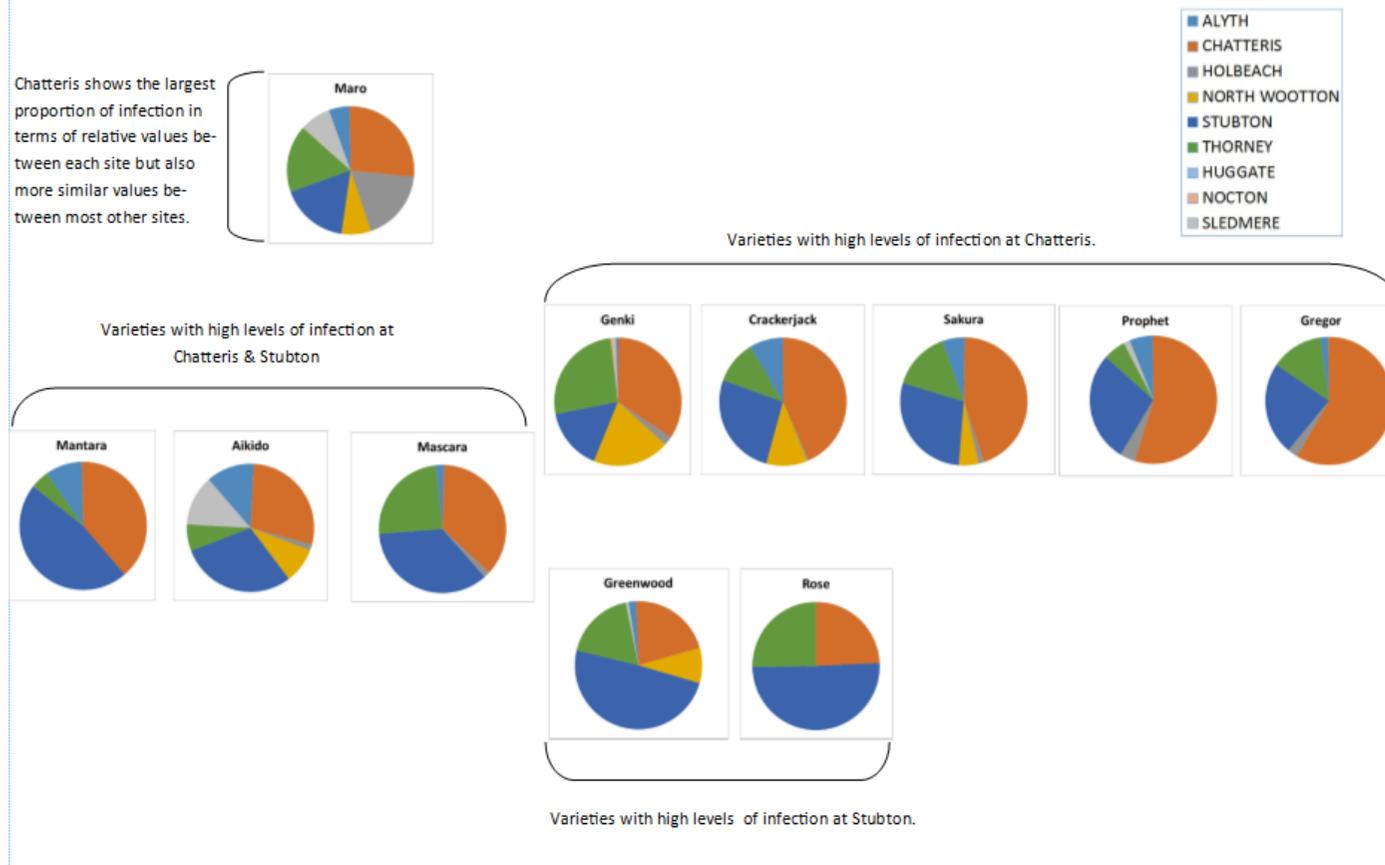


Figure 7: Percentage of downy mildew for each combining pea variety at each site, illustrating those that performed better at each site in 2016 and 2017. Each pie chart represents a single variety and is split to show a comparison of % leaf area infection at each site as a proportion of the total infection at all sites.

Discussion

Long term storage

Overall, 114 downy mildew isolates are in long term storage at PGRO which holds, to the best of our knowledge, the major *Peronospora viciae* f. sp. *pisi*, (downy mildew) culture collection in the UK. It was shown that DM isolates can be infectious after storage at -80°C for one year, allowing the use of specific isolates of DM races for further studies.

Resistance screening of germplasm accessions JI 15 and JI 85

Several isolates overcame the resistance of JI 15 both in the field and in the inoculation experiment under growth room conditions in both 2016 and 2017. Since the resistance identified in JI 15 is based on one major genetic locus, it is unlikely to be durable. The results indicated that the JI 15 resistance locus should be combined with other resistance genes to provide long-term value in breeding for pea downy mildew resistance.

The desk study identified JI 85 as a germplasm accession with resistance to all downy mildew races except race 7. Clarification of the genetic loci implicated in the resistance of JI 85 is the subject of ongoing research within PCGIN. Only six isolates overcame resistance of JI 85 in the growth room and little infection at field sites occurred in 2016 (maximum mean percentage infection 0.38%; Table 9) and 2017 (maximum mean percentage infection 0.18%; Table 10), indicating that JI 85 holds great potential for breeding resistance to DM in both vining and combining peas.

Race distribution in 2016 and 2017

In 2017, races 5, 10 and 11 were found at Chatteris but not Stubton whilst races 1, 3, 9, Unk 1 and Unk 4 were found at Stubton but not Chatteris. Races 4, 8 and Unk 2 were found at both sites. It is possible that a large difference in infection rate between Stubton and Chatteris indicated a differing level of susceptibility to one or more of these races. There were several vining peas that had a much greater level of infection at Chatteris than Stubton: 06S55519A, 08S04137, Fintva, 04S51315N, and Vidor (relatively more than 50% greater levels of infection) and Oasis, Linnea, Ida and 08S01030 (relatively 30% to 45% greater levels of infection). These may be more susceptible to races 5, 10 and/or 11 than to races 1, 3, 9, Unk 1, Unk 4, 4, 8 or Unk 2. Anna had very similar levels of infection at Chatteris and Stubton

which may indicate that it is most susceptible to one or more of those races appearing at both sites (4, 8 and Unk 2). Avola, 08S05676, Waverex and 06S54009A had much greater levels of infection at Stubton than Chatteris which could suggest a greater susceptibility to races 1, 3, 9, Unk 1 and/or Unk 4 than to races 4, 8, Unk 2, 5, 10 and 11.

The high rates of infection at Chatteris compared to other locations, mean infection including all varieties and lines being 2.72%, may indicate that races 5, 10 and 11 were more aggressively infectious compared to races 4, 8 and Unk 2, which were also found at the Stubton and/or Sledmere sites. Observations in 2016 appear to support this, with races 10 and 11 being the most frequently recorded at Stubton which had a high level of infection, with mean infection including all varieties and lines of 2.41% (Table 10).

Overall, races found at Chatteris were similar in 2016 and 2017 but differed greatly between years at Stubton (Table 11). Differences in one location could be due to pea variety, weather conditions, differences in population size or dynamic, or random sampling effects. More data would be needed to identify whether the Stubton population is generally more dynamic than the Chatteris population.

Table 11: Isolates that were common to each site, Chatteris, Sledmere and Stubton in 2016 and 2017. The numbers in the blue boxes indicate the quantity of each isolate recorded at each site.

| Site | Chatteris | | Sledmere | | Stubton | |
|-------|-----------|------|----------|------|---------|------|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| Race | | | | | | |
| 1 | | | | 1 | | 3 |
| 2 | | | | | | |
| 3 | 1 | | 1 | 2 | | 5 |
| 4 | | 1 | | | | 1 |
| 5 | | 2 | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | 1 | | 1 | | 1 |
| 9 | | | | | | 1 |
| 10 | 3 | 2 | 1 | | 2 | |
| 11 | 2 | 2 | 1 | | 3 | |
| Unk 1 | 2 | | | | | 1 |
| Unk 2 | | 1 | | | | 1 |
| Unk 3 | | | | | | |
| Unk 4 | | | | | | 1 |
| Unk 5 | | | | | | |

Varietal performance in 2016 and 2017

Eighteen varieties were planted at both Chatteris and Stubton in 2016 and 2017, allowing direct comparisons to be made between years and varieties (Figures 8 and 9).

Downy mildew incidence at the Chatteris trial site:

There is no clear trend for 2016 and 2017 in infection levels of combining pea varieties. Figure 8 shows that some varieties have substantially more infection (e.g. Crackerjack and Maro), others have substantially less infection (e.g. Mantara, Mascara, Prophet) and some have similar levels of infection (e.g. Gregor). JI 411 and JI 560 had increases in infection, JI 15 had a reduction in infection and JI 758 had little difference in infection in 2017, compared with 2016. No infection was recorded on JI 85 in either year.

Avola and Oasis vining pea varieties had an increase in infection in 2017, Avola increasing from 4.17% in 2016 to 6.79% in 2017, and Oasis increasing from 3.95% in 2016 to 6.68% in 2017. Waverex had a reduction in infection in 2017 (1.17%) compared to 2016 (4.12%) and Maurice had no infection in either year.

Lines with less than 1% infection in both years included John Innes differential accessions JI 15 and JI 85 and the vining pea Maurice. There were no combining peas with infection less than 1%. Lines with infection of less than 2% across both years included the combining peas Aikido and Mantara and the John Innes differential accession JI 411.

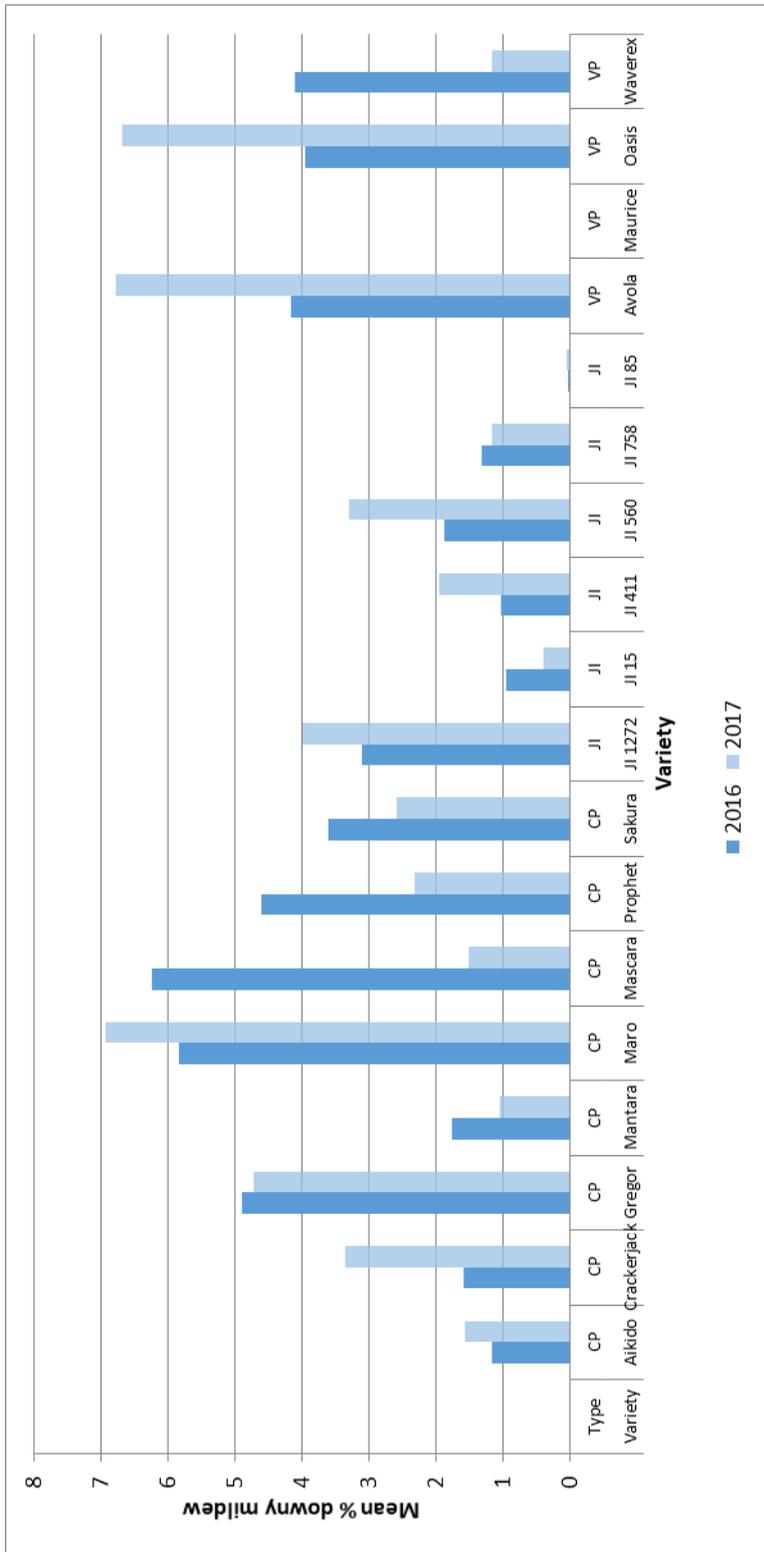


Figure 8: Mean percentage downy mildew infection of varieties at Chatteris in 2016 and 2017. CP = combining peas, JI = John Innes accession, VP = vining peas.

Downy mildew incidence at the Stubton trial site:

At Stubton, there was an increase in infection in 2017 compared to 2016 (Figure 9), and many varieties had double the percentage of leaf area infection with downy mildew in 2017. The only exception was Crackerjack, a combining pea that had a small decrease in the percentage of infection in 2017 (0.15%). JI 560, JI 1272 and Avola had large increases in infection, all three varieties having greater than 7.5% mean leaf area infection in 2017.

Several varieties of combining pea had DM infection in 2017 even though infection was at or close to zero in 2016, including Aikido, Mantara, Mascara and Prophet (Figure 9). Sakura had a large increase in 2017 compared to 2016, whilst Gregor and Maro had smaller relative increases.

There was an increase in infection levels of the John Innes differential accessions in 2017. JI 411 had an infection rate greater than 3% in 2017 compared to 0% in 2016.

The vining pea Maurice had 0.13% infection in 2017 compared to 0% in 2016. Infection of Oasis increased from 1.65% in 2016 to 3.63% in 2017. Infection of Avola increased from 3.5% in 2016 to 10% in 2017, and infection of Waverex increased from 0.17% in 2016 to 2.33% in 2017.

JI 15 and JI 85 had small increases in percentage leaf area infection in 2017 compared to 2016, incidence increasing from 0.23% to 0.5% for JI 15 and 0.02% to 0.17% for JI 85.

JI 15, JI 85 and Maurice all performed exceptionally well in both years with less than 1% infection. Varieties with less than 2% infection in both years included the combining peas Aikido, Gregor, Mantara, Mascara, Prophet and Sakura and the John Innes differential accession JI 758.

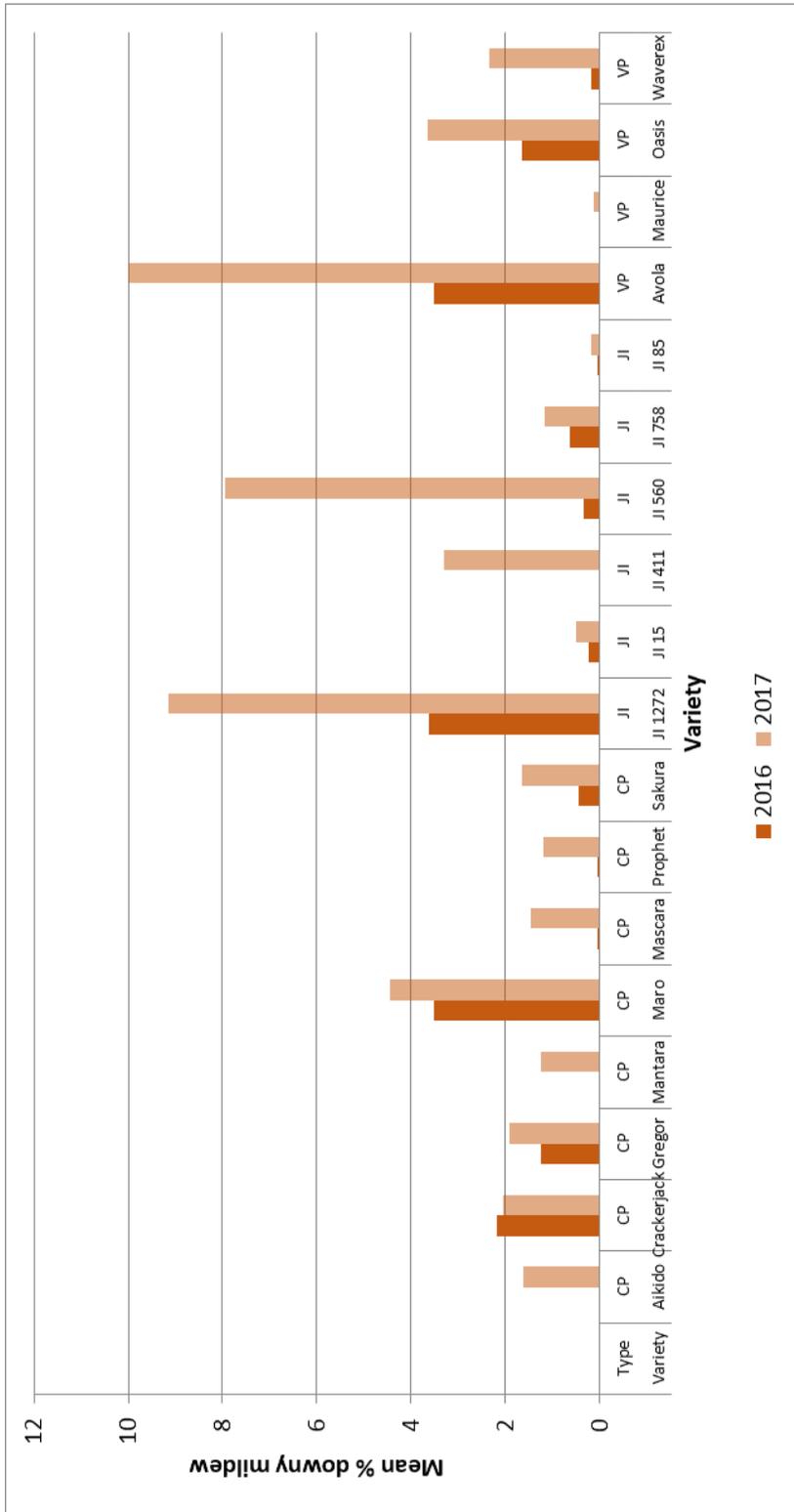


Figure 9: Mean percentage downy mildew infection of varieties at Stubton in 2016 and 2017. CP = combining peas, JI = John Innes accession, VP = vining peas.

Comparisons of downy mildew infection of pea varieties between the sites Chatteris and Stubton in 2016 and 2017

At both sites, less than 1% infection was recorded in both years for the John Innes germplasm accessions JI 15 and JI 85 and the vining pea variety Maurice. Varieties with less than 2% infection in both years included combining peas Aikido, Mantara and the John Innes differential accession JI 758.

Gregor, Maro, Mascara, Prophet, Sakura and Oasis had higher levels of infection at Chatteris than Stubton in both years. Only JI 1272 had higher rates of infection at Stubton compared to Chatteris in each year.

The combining pea Aikido had higher levels of infection at both sites in 2017, as did Maro, John Innes accessions JI 1272, JI 411, JI560, JI 85, and the vining peas Avola and Oasis. No variety had reduced infection levels at both sites in 2017. The only variety which had an increase in infection at Chatteris but a decrease at Stubton in 2017 was the combining pea Crackerjack. Varieties that showed a decrease in infection at Chatteris and an increase at Stubton were the combining peas Gregor, Mantara, Mascara, Prophet, Sakura, John Innes accessions JI 15, JI 758 and the vining pea Waverex.

There may be a link between the presence of specific races that appear at one location in one year and not the other, and those varieties showing greatest levels of infection at the time and place where the specific races were found. For example in 2017, races 5, 10 and 11 were found at Chatteris but not Stubton (Table 12) whilst races 1, 3, 9, Unk 1 and Unk 4 were found at Stubton and not Chatteris. It is possible that the varieties Crackerjack, Gregor, Maro, Prophet, Sakura and Oasis, which had greater levels of infection at Chatteris than Stubton in 2017 (Table 13), were more susceptible to one of the races 5, 10 and 11. Crackerjack had a slightly higher level of infection at Stubton in 2016 than in 2017 (Table 14) and races 10 and 11 were recorded in 2016 but not 2017 (Table 15).

Table 12: Races that were recorded at single sites only, in either 2016 or 2017

| Site | Races found in 2016 | Races found in 2017 |
|-----------|---------------------|-------------------------|
| Chatteris | 3, Unk 1(2) | 5(2),10,11 |
| Stubton | None | 1(3),3(5),9,Unk 1,Unk 4 |

Table 13: Varieties with the highest levels of infection by location, in each year. Example: Crackerjack had higher infection in 2016 at Stubton than Chatteris.

| Year | 2016 | 2017 |
|------------------|--|---|
| Site | | |
| Chatteris | Aikido, Gregor, Maro, Mascara, Prophet, Sakura, JI 15, JI 411, JI 560, JI 758, Avola, Oasis, Waverex | Crackerjack, Gregor, Maro, Prophet, Sakura, Oasis |
| Stubton | Crackerjack, JI 1272 | JI 1272, JI 411, JI 560, JI 85, Avola |

Table 14: Varieties with the highest levels of infection by year, at the same location. Example: Crackerjack had higher infection at Stubton in 2016 than 2017.

| Site | Chatteris | Stubton |
|-------------|---|--|
| Year | | |
| 2016 | Gregor, Mantara, Mascara, Prophet, Sakura, JI 15, JI 758, Waverex | Crackerjack |
| 2017 | Aikido, Crackerjack, Maro, JI 1272, JI 411, JI 560, Avola, Oasis | Aikido, Gregor, Mantara, Maro, Mascara, Prophet, Sakura, JI 1272, JI 15, JI 411, JI 560, JI 758, JI 85, Avola, Maurice, Oasis, Waverex |

Table 15: Races that only appeared in an individual location in one of the two years. Brackets indicate the quantity of isolates recorded per race.

| Site | Races found in 2016 | Races found in 2017 |
|------------------|---------------------|-----------------------------------|
| Chatteris | 3, Unk 1(2) | 4, 5(2), 8, Unk 2 |
| Stubton | 10(2), 11(3) | 1(3),3(5),4,8,9,Unk 1,Unk 2,Unk 4 |

Comparison of downy mildew races collected by Taylor (1986) with those collected in 2016 and 2017

56 isolates were categorised into races by Taylor in 1986 (Table 16). Most of the isolates collected by Taylor (1986) were collected in Norfolk, and some in the south-east of England. In 2016 and 2017 the collection area was larger, and isolates were collected from the main UK pea growing area, including the east of England and Scotland. In 2016, 42 isolates were characterised by race, and in 2017 83 isolates were characterised (Table 16).

Table 16: Number of DM isolates of each race determined in 1986 by Taylor, and in 2016 and 2017 within this project, with percentage of total number of isolates characterised in each year.

| Race identification number | Number of isolates | Percentage of isolate total in 1986 | Number of isolates | Percentage of isolate total in 2016 | Number of isolates | Percentage of isolate total in 2017 | Total number of isolates |
|----------------------------|--------------------|-------------------------------------|--------------------|-------------------------------------|--------------------|-------------------------------------|--------------------------|
| | Taylor 1986 | Taylor 1986 | Herold 2016 | Herold 2016 | Herold 2017 | Herold 2017 | Herold 2016 and 2017 |
| 1 | 9 | 16 | 2 | 5.1 | 13 | 15.7 | 15 |
| 2 | 2 | 4 | 0 | 0.0 | 2 | 2.4 | 2 |
| 3 | 4 | 7 | 9 | 23.1 | 19 | 22.9 | 28 |
| 4 | 5 | 9 | 1 | 2.6 | 4 | 4.8 | 5 |
| 5 | 1 | 2 | 2 | 5.1 | 4 | 4.8 | 6 |
| 6 | 1 | 2 | 1 | 2.6 | 1 | 1.2 | 2 |
| 7 | 1 | 2 | 0 | 0.0 | 0 | 0.0 | 0 |
| 8 | 2 | 4 | 5 | 12.8 | 4 | 4.8 | 9 |
| 9 | 9 | 16 | 1 | 2.6 | 6 | 7.2 | 7 |
| 10 | 19 | 34 | 9 | 23.1 | 7 | 8.4 | 16 |
| 11 | 3 | 5 | 6 | 15.4 | 8 | 9.6 | 14 |
| Unk 1 | 0 | 0 | 2 | 5.1 | 4 | 4.8 | 6 |
| Unk 2 | 0 | 0 | 1 | 2.6 | 5 | 6.0 | 6 |
| Unk 3 | 0 | 0 | 0 | 0.0 | 2 | 2.4 | 2 |
| Unk 4 | 0 | 0 | 0 | 0.0 | 3 | 3.6 | 3 |
| Unk 5 | 0 | 0 | 0 | 0.0 | 1 | 1.2 | 1 |
| Total number of isolates | 56 | | 39 | | 83 | | 122 |

Within this project, ten of the eleven races identified by Taylor (1986) were identified, but not race 7. In 2016, two previously unknown races (Unk 1 and Unk 2) were found. Both these races were also found in 2017 and three additional races (Unk 3, Unk 4 and Unk 5) were found in 2017.

The frequency of DM races in the samples collected in this project was quite different in 2016 and 2017 (Figures 10, 11, 12). Race 10 occurred most frequently in the samples collected in 1986 and in 2016, although a lower proportion of the total samples collected in 2016 were of race 10. In 2017 the frequency of isolates in race 10 in the samples collected was much lower. There was a very similar frequency of isolates collected from race 1 in 1986 and 2017 (16%) but in 2016 frequency was lower (5%). The frequency of race 3 isolates found increased from 7% in 1986 to 23% in both 2016 and 2017.

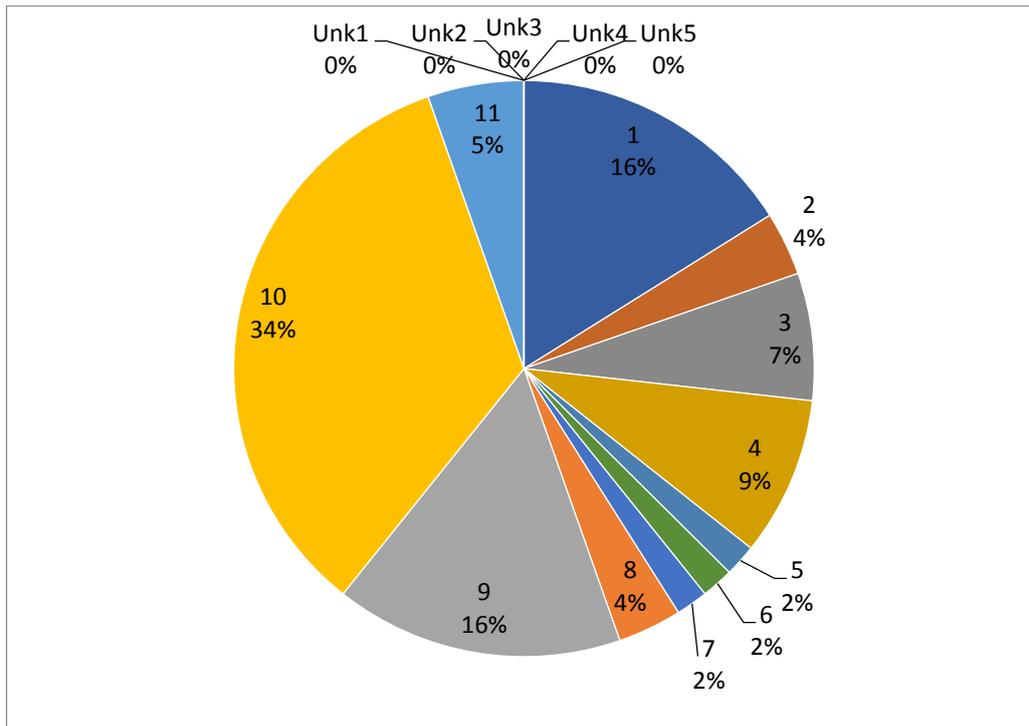


Figure 10: Frequency of downy mildew races, with identification number of isolate and percentage of total number of isolates (Taylor, 1986).

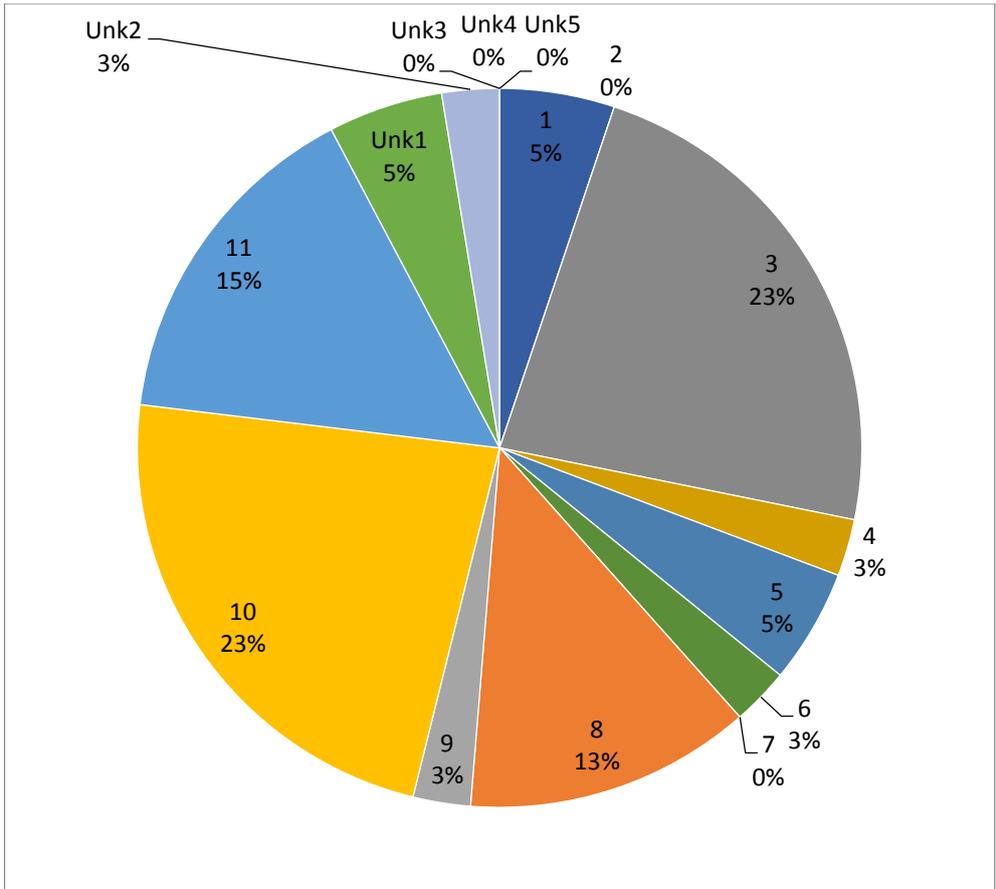


Figure 11: Frequency of downy mildew races, with identification number of isolate and percentage of total number of isolates, found in this project in 2016 (Herold, 2016).

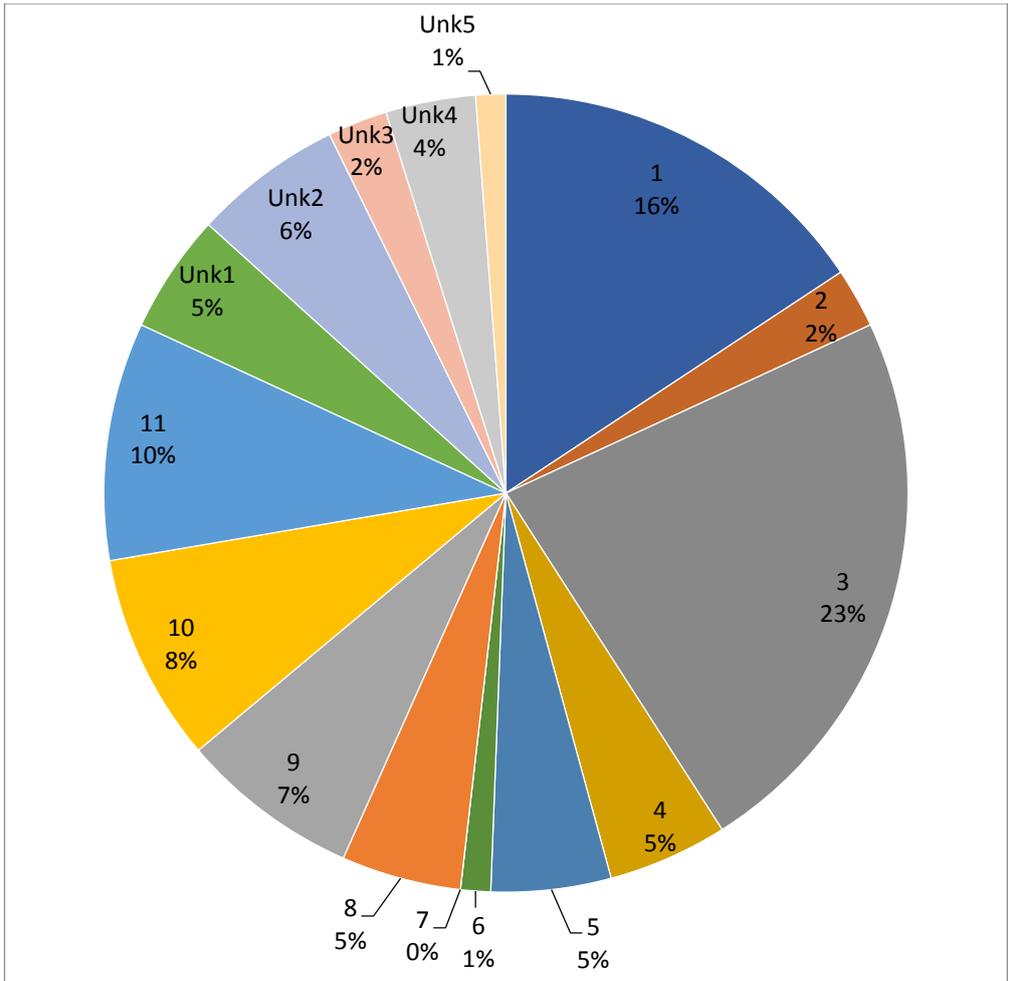


Figure 12: Frequency of downy mildew races, with identification number of isolate and percentage of total number of isolates, found in this project in 2017 (Herold, 2017).

Some races have remained stable since the 1980's, demonstrated by the existence of those that were identified in the 1980's in the present study. Race 10 has been consistently of importance, but other races have increased or decreased in presence over the last 30 years. It is possible that changes in race structure over the years may be explained by the difference in varieties grown now compared to those in the 1980's. This may have contributed to the selection of certain races over the years. Older pea varieties that are most affected by DM are no longer grown on a large scale, which may be reflected in the genetic background and relative susceptibility of potential host crops. For example, Avola, a vining pea variety that was in commercial use by 1986 (S. Belcher, Personal Communication, 2018), was infected with races 1 and 4 in 2016 and races 1 and 3 in 2017. Another factor could be that evolution has naturally selected for the success of those races which are more 'fit', i.e. more competitive

and hence prevalent. The pattern of races could also reflect external stimuli such as environmental factors (e.g. warming climate, local weather patterns, availability of water, degradation of soils) and interactions with changing microflora and fauna, in turn influenced by differing farming techniques and availability of soil treatments and pesticides. Some of these variables may have an effect over several years whilst others may have a much quicker effect (e.g. some races may be able to multiply under drought conditions better than others). Due to the method of spread by spores, prevailing wind may play a part in the movement of different races in conjunction with the use of farm machinery and the movement of animals and birds.

World-wide distribution of DM races

A few publications have looked at DM races across the world (Ester and Gerlagh, 1979; Taylor 1986; Gunther and Jaiser, 1989; Liu et al., 2013). These have been compared to races identified in this project between 2015 and 2017 (Table 19 and Figure 13).

Table 17: Combined current and historic information, showing number of isolates of each DM race and location of isolate collection point (international data). Where a figure in a column is followed by brackets, this indicates the number of isolates greater collected, if greater than 1.

| Race | Number of isolates (Ester and Gerlagh, 1979) | Location | Number of isolates (Taylor, 1986) | UK County | Number of isolates (Gunther & Jaiser, 1989) | Location | Number of isolates (Liu et al., 2013) | Location | Number of isolates (Herold 2015) | UK County | No. of isolates (Herold 2016) | Location (UK) | UK County | No. of isolates (Herold 2017 data) | Location (UK) | UK County | Total number of isolates |
|------|--|-----------------------------|-----------------------------------|--|---|------------|---------------------------------------|-----------------|----------------------------------|--------------------|-------------------------------|--|---|------------------------------------|--|---|--------------------------|
| 1 | 1 | Lelystad The Netherlands | 9 | Norfolk (3), Kent(3), Lincolnshire, Bedfordshire, Cambridgeshire | 2 | NW Germany | 6 | Alberta, Canada | | | 2 | Stockbridge, Perth | Hampshire, Perth and Kinross | 13 | Alyth, Holbeach, Huggate, North Wootton(5), Sledmere, Stubton(3), Thorney | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 15 |
| 2 | | | 2 | Norfolk, Essex | | | 2 | Alberta, Canada | | | 0 | | | 2 | Kilham, North Wootton | Yorkshire, Norfolk | 2 |
| 3 | 1 | Steenbergen The Netherlands | 4 | Norfolk (2), Lincolnshire, Cambridgeshire | | | | | | | 8 | Chatteris, Stockbridge, Howden (3), Perth, Sledmere, Kirton | Cambridgeshire, Hampshire, Yorkshire, Perth and Kinross, Lincolnshire | 19 | Alyth(3), Holbeach(2), Huggate, Kilham(2), North Wootton(2), Sledmere(2), Stubton(4), Thorney(2), Walcot | Perth and Kinross, Lincolnshire, Yorkshire, Norfolk, Cambridgeshire | 27 |
| 4 | | | 5 | Norfolk (3), Lincolnshire (2) | 1 | NW Germany | | | | | 1 | Howden | Yorkshire | 4 | Chatteris, North Wootton, Stubton, Thorney | Cambridgeshire, Norfolk, Lincolnshire | 5 |
| 5 | | | 1 | Norfolk | 1 | NW Germany | | | 1 | Lincolnshire | 2 | Perth (2) | Perth and Kinross | 4 | Chatteris(2), Huggate, Thorney | Cambridgeshire, Yorkshire | 6 |
| 6 | | | 1 | Norfolk | | | | | | | 1 | Stockbridge | Hampshire | 1 | Huggate | Yorkshire | 2 |
| 7 | | | 1 | Kent | | | | | | | 0 | | | 0 | | | 0 |
| 8 | | | 2 | Norfolk, Lincolnshire | | | | | | | 5 | Howden (5) | Yorkshire | 4 | Chatteris, Sledmere, Stubton, Walcot | Cambridgeshire, Yorkshire, Lincolnshire | 9 |
| 9 | | | 9 | Norwich(6), Kent, Essex, Lincolnshire | | | | | | | 1 | Stockbridge | Hampshire | 6 | Alyth(3), Ancaster, Kilham, Stubton | Perth and Kinross, Lincolnshire, Yorkshire | 7 |
| 10 | | | 19 | Norfolk (2), Norfolk(2), Nottinghamshire, Lincolnshire(9), Bedfordshire, Angus, Cambridgeshire | | | | | 2 | Lincolnshire, Kent | 9 | Chatteris (3), Stubton (2), Stockbridge, Sledmere, Kirton, Stratford upon Avon | Lincolnshire, Cambridgeshire, Hampshire, Yorkshire, Warwickshire | 7 | Alyth(3), Ancaster, Chatteris(2), Holbeach | Perth and Kinross, Lincolnshire, Cambridgeshire, Lincolnshire | 16 |
| 11 | | | 3 | Norfolk (2), Suffolk | 1 | NW Germany | 1 | Alberta, Canada | 1 | Yorkshire | 6 | Chatteris (2), Stubton (3), Sledmere | Cambridgeshire, Lincolnshire, Yorkshire | 8 | Alyth, Ancaster, Chatteris(2), Kilham, Necton, Thorney(2) | Perth and Kinross, Lincolnshire, Cambridgeshire, Yorkshire | 14 |
| Unk1 | | | | | | | | | | | 2 | Chatteris (2) | Cambridgeshire | 4 | Holbeach, Huggate, Kilham, Stubton | Lincolnshire, Yorkshire | 6 |
| Unk2 | | | | | 1 | NW Germany | | | | | 1 | Stockbridge | Hampshire | 5 | Chatteris, Holbeach, Kilham, Stubton, Thorney | Cambridgeshire, Lincolnshire, Yorkshire | 6 |
| Unk3 | | | | | | | | | | | 0 | | | 2 | Alyth, North Wootton | Perth and Kinross, Norfolk | 2 |
| Unk4 | | | | | | | | | | | 0 | | | 3 | Kilham, Stubton, Thorney | Yorkshire, Lincolnshire, Cambridgeshire | 3 |
| Unk5 | | | | | | | | | | | 0 | | | 1 | Huggate | Yorkshire | 1 |

Comparison of race diversity between Taylor (1986) and Herold (2016 and 2017)

Taylor started his work using ten differential pea lines for the characterisation of downy mildew races and identified 22 different races (Table 18).

Table 18: Tolerance of ten pea differential host lines to 22 races of downy mildew (Taylor, 1986). S = susceptible, R = resistant. Orange boxes represent differential lines used in the 2016 and 2017 study.

| Taylor race number | Number of Isolates | Host differential line (JI line) | | | | | | | | | |
|--------------------|--------------------|----------------------------------|-----|-----|-----|-----|-----|-----|------|------|------|
| | | 411 | 441 | 540 | 560 | 584 | 758 | 952 | 1215 | 1272 | 1273 |
| Taylor 1 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Taylor 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| Taylor 3 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| Taylor 4 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Taylor 5 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |
| Taylor 6 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| Taylor 7 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| Taylor 8 | 4 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| Taylor 9 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| Taylor 10 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| Taylor 11 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Taylor 12 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| Taylor 13 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |
| Taylor 14 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| Taylor 15 | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| Taylor 16 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| Taylor 17 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Taylor 18 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Taylor 19 | 2 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |
| Taylor 20 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| Taylor 21 | 10 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| Taylor 22 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |

Taylor (1986) discovered that the isolates he had tested could be grouped into eleven different races using just four differential pea accessions – JI 411, JI 560 JI 758 and JI 1272 (Tables 18 and 19). These four pea accessions defined by Taylor were used in this project. Table 19 shows the susceptibility or resistance of the four differential pea accessions to the 22 races identified by Taylor (1986).

Table 19: Susceptibility of the four differential host accessions to races identified by Taylor (1986) and Herold (2016 and 2017).

| Taylor race number | Host differential line (JI line) | | | | Equivalent Herold race |
|--------------------|----------------------------------|-----|-----|------|------------------------|
| | 411 | 560 | 758 | 1272 | |
| Taylor 1 | S | S | S | S | 1 |
| Taylor 2 | S | S | S | S | 1 |
| Taylor 3 | S | S | S | R | 2 |
| Taylor 4 | S | R | S | S | 4 |
| Taylor 5 | S | R | S | S | 4 |
| Taylor 6 | S | R | R | S | 5 |
| Taylor 7 | S | S | S | S | 1 |
| Taylor 8 | S | S | R | S | 3 |
| Taylor 9 | S | S | S | S | 1 |
| Taylor 10 | R | S | S | S | 6 |
| Taylor 11 | R | S | S | R | 7 |
| Taylor 12 | R | S | R | S | 8 |
| Taylor 13 | R | R | S | S | 9 |
| Taylor 14 | R | R | S | S | 9 |
| Taylor 15 | R | R | R | S | 10 |
| Taylor 16 | R | R | R | S | 10 |
| Taylor 17 | R | R | R | R | 11 |
| Taylor 18 | R | R | R | R | 11 |
| Taylor 19 | R | R | S | S | 9 |
| Taylor 20 | R | R | S | S | 9 |
| Taylor 21 | R | R | R | S | 10 |
| Taylor 22 | R | R | R | S | 10 |

The information in Table 19 was used to produce a summary of equivalent races (Table 20). This analysis shows that races 1, 9 and 10 identified in this study could potentially be several different races if 10 differential accessions had been used for identification. This might partly explain why races 1 and 10 are quite frequent in some years. The analysis also shows that the unknown races identified here do not correspond to any of the 22 races characterised by Taylor (1986). This might mean that these races are newly identified in the UK.

Table 20: Possible Taylor races representing races identified by Herold (2016 and 2017).

| Herold pathotype | Equivalent Taylor Pathotype number |
|------------------|---|
| | 1 Taylor 1, Taylor 2, Taylor 7, Taylor 9 |
| | 2 Taylor 3 |
| | 3 Taylor 8 |
| | 4 Taylor 4, Taylor 5 |
| | 5 Taylor 6 |
| | 6 Taylor10 |
| | 7 Taylor 11 |
| | 8 Taylor 12 |
| | 9 Taylor 13, Taylor 14, Taylor 19, Taylor 20 |
| | 10 Taylor 15, Taylor 16, Taylor 21, Taylor 22 |
| | 11 Taylor 17, Taylor 18 |
| Unk 1 | n/a |
| Unk 2 | n/a |
| Unk 3 | n/a |
| Unk 4 | n/a |
| Unk 5 | n/a |

Conclusions

- PGRO has a pea downy mildew culture collection of 114 isolates belonging to fifteen different races
- The resistance of JI 15 has been overcome both by six individual races under growth room conditions and by natural populations in the field, which may suggest that this line is not suitable as an only source of resistance to downy mildew in breeding material
- Resistance of JI 85 to downy mildew was observed in most tests
- Combining pea varieties show less variation in infection levels at different locations in the UK
- Vining pea varieties show greater variation in infection levels depending on growing location
- Ten DM races which were identified in the 1980's are still present in the UK today
- Five DM races were identified for the first time in the UK in this project

- Races 1, 3 and 10 occurred more frequently across the UK during the studies described here, including Taylor (1986) (Figures 3 and 10), compared to all other races

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: AHDB-Horticulture

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. *pisii*) 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

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

* Hubelling, N. Med. Fac. Landbouww. Rijksuiv. Gent 40, 539-543, 1975.
 * Ester & Gerl. 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 & Gerl. agh, 1979)
 1: resistant, 2,3,4: susceptible (Taylor, 1984)

Pea material

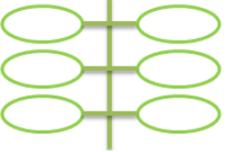
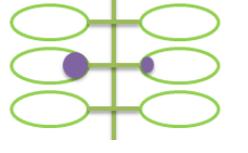
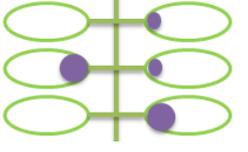
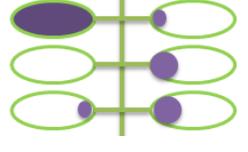
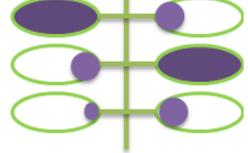
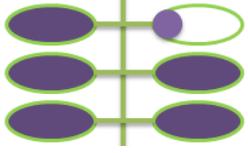
Eight seeds were sown in small Jiffy pots (GH 5s) into peat and sand. Jiffy pots were placed in gravel trays. Seedlings were inoculated at around 20 days old. Test plants were scored 10-20 days after inoculation.

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 accessions 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
922 HUNDREDFOLD ROGUE
1713 GOLD.STRAW x MUMMY BICOL.

218 FIELD PEA-INDIA
221 FIELD PEA-INDIA
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
SUTTONS EARLY GIANT
335 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
WELLENSIEK'S WHITE INDENT,
805 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
IMPROVED HARBINGER
868 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 |
| | | 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 | <i>P. sativum</i> 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 1986; 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 | <i>P. sativum</i> 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

| Isolate | Origin | Host differential line | | | | | | | | | | | Reference |
|---------|-----------------------------|------------------------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|------------------------------|
| | | JI 85 | JI 411 | JI 441 | JI 540 | JI 560 | JI 584 | JI 758 | JI 952 | JI 1215 | JI 1272 | JI 1273 | |
| 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 1986 |
| 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 | |
| OS1 | Norwich, Norfolk | | 0 | 2 | 2 | 0 | 3 | 1 | 0 | 3 | 4 | 0 | Taylor <i>et al.</i> 1989 |
| OS2 | Norwich, Norfolk | | 1 | 3 | 3 | 1 | 4 | 1 | 0 | 4 | 4 | 1 | |
| OS3 | Norwich, Norfolk | | 0 | 1 | 2 | 0 | 2 | 1 | 0 | 3 | 3 | 0 | |
| OS4 | Norwich, Norfolk | | 0 | 2 | 0 | 0 | 3 | 1 | 0 | 3 | 3 | 1 | |
| OS5 | Norwich, Norfolk | | 0 | 3 | 3 | 0 | 4 | 1 | 0 | 4 | 4 | 1 | |
| OS6 | Norwich, Norfolk | | 1 | 2 | 1 | 0 | 2 | 1 | 0 | 2 | 4 | 1 | |
| OS7 | 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 | | | | | | | | | | | Reference |
|---------|-----------------|------------------------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|------------------------------|
| | | J1 85 | J1 411 | J1 441 | J1 540 | J1 560 | J1 584 | J1 758 | J1 952 | J1 1215 | J1 1272 | J1 1273 | |
| BH1 | Kent | | 2 | 4 | 3 | 2 | 2 | 1 | 1 | 3 | 4 | 3 | Taylor <i>et al.</i> 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 |
| | | | | | | | | | | | Stegmark 1990 |
| N1 | Vestfold, Norway | 15 | 32 | | | | | | | | |
| 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

| Isolate | Origin | Host differential line | | | | | | | | | | | Reference | | |
|----------|----------------------------|------------------------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|-----------|--|---------------------------|
| | | J185 | J1411 | J1441 | J1540 | J1560 | J1584 | J1758 | J1952 | J11215 | J11272 | J11273 | | | |
| S4 | Skane, Sweden | | | 11 | 70 | | | | | | | | | | Stegmark 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 | | | | | | |
| M9ID24 | Alberta, Canada | | S | | | S | | S | | | S | | | | Liu <i>et al.</i> 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 1986; 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 2009

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 x JI 399, where the JI 399 parent is a garden pea line. Markers (454) were mapped in the JI 2202 x JI 2822 F7 population, using predominantly SSR (simple sequence repeat) and SSAP (sequence-specific amplification polymorphism) markers. Analysis of the JI 2202 x JI 2822 population revealed a strong partial resistance to downy mildew (*Peronospora viciae* f. sp. *psii*), 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 x 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).

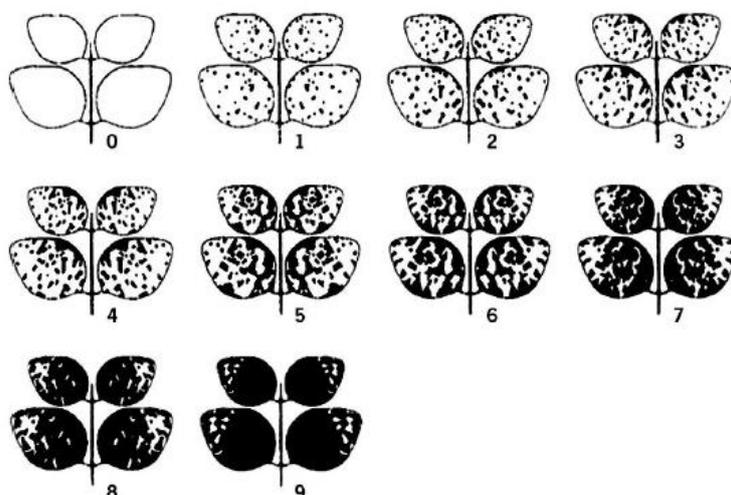


Figure 1. Cartoon showing disease severity key for downy mildew scores in the pea population JI 2202 x JI 2822. Severity scores are indicated: dark areas on the leaflets represent downy mildew lesions (Holden 2009, Figure 64)

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 |
|---------|-------------|-------------|----|-------|-------------|-------------|----|
| Jl 2822 | 2.8 | 1.7 | R | 88 | 3.7 | 7.5 | S |
| Jl 2202 | 7.6 | 7.3 | S | 91 | 5.0 | 8.0 | S |
| Jl 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)

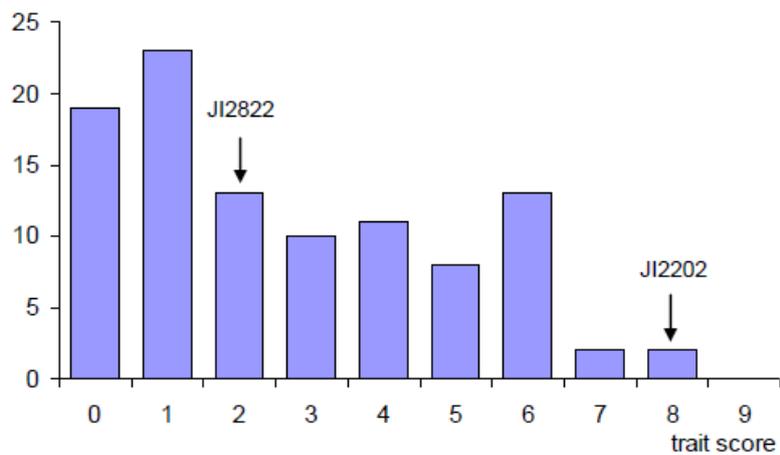


Figure 2. Susceptibility to downy mildew infection in the JI 2202 x JI 2822 population. Frequency distribution of trait scores on JI 2202 x JI 2822 F5-7 bulks, using pooled pathogen isolates; average trait scores per line are grouped as integer values. The distribution of susceptibility scores suggests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

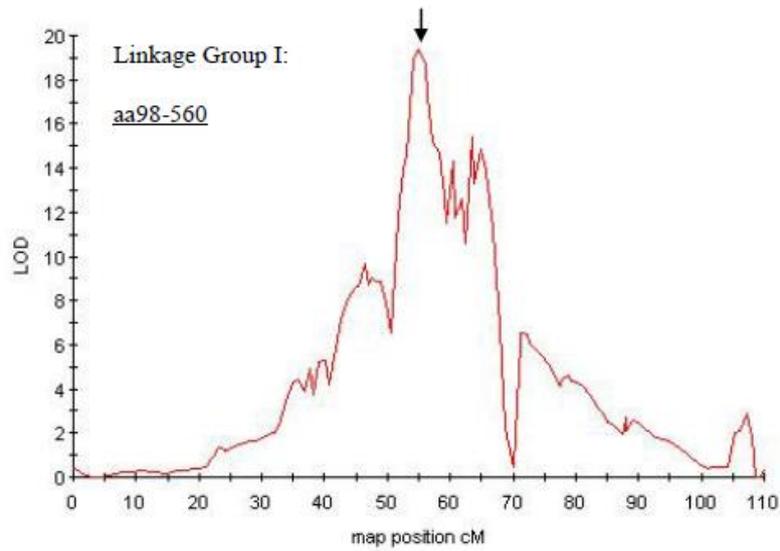


Figure 3. MapQTL output for resistance to downy mildew in the JI 2202 x JI 2822 mapping population, showing results for linkage group I. Arrow indicates the map position of marker locus aa98-560, where association with downy mildew resistance/susceptibility has a LOD score of 19.56 (Holden 2009, Figure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

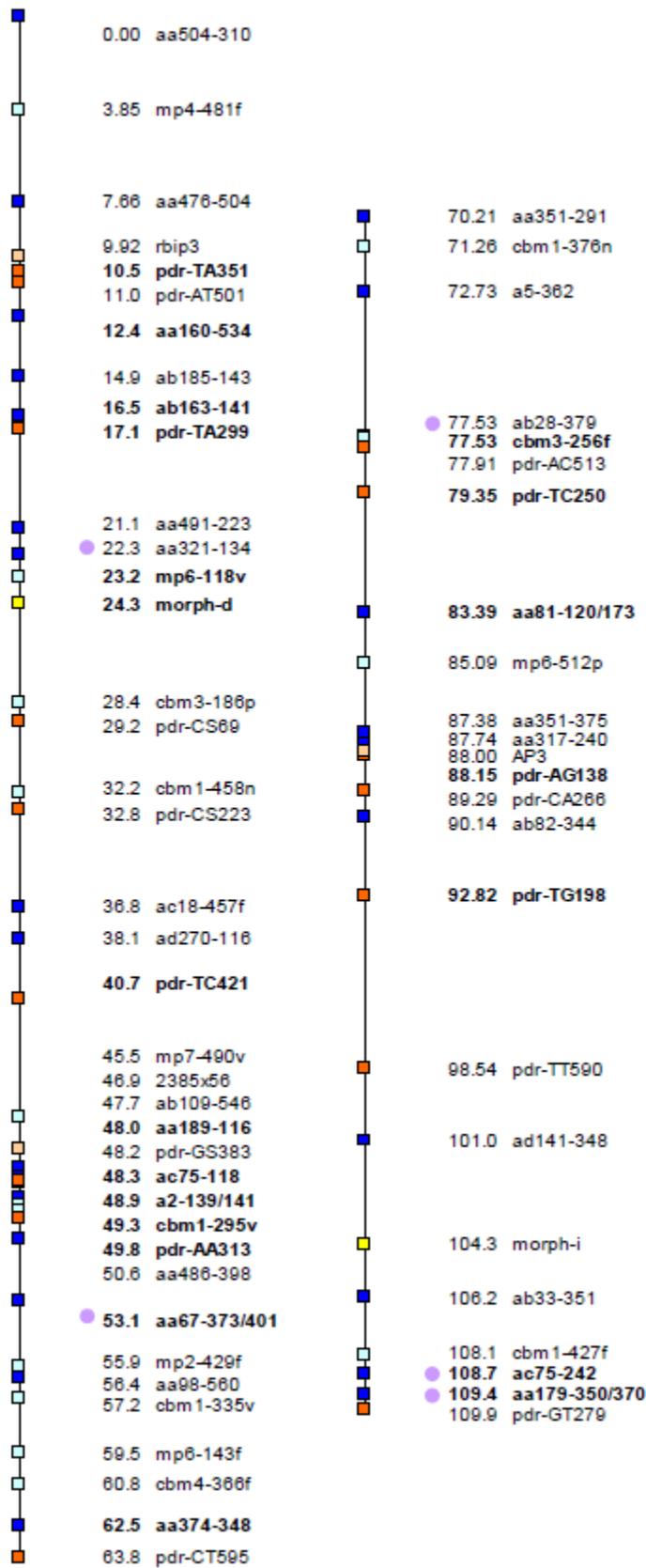


Figure 4. Genetic map of JI 2202 × JI 2282 F7 population: Linkage group I, part 1 (left) and continued as part 2 (right). SSR marker aa98-560 (close to end of left part of linkage group) was strongly associated with downy mildew resistance (Holden 2009, Figure 24).

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 × 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 × JI 1194 was tested. Since the cross JI 15 × 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 × 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 and PCGIN projects.

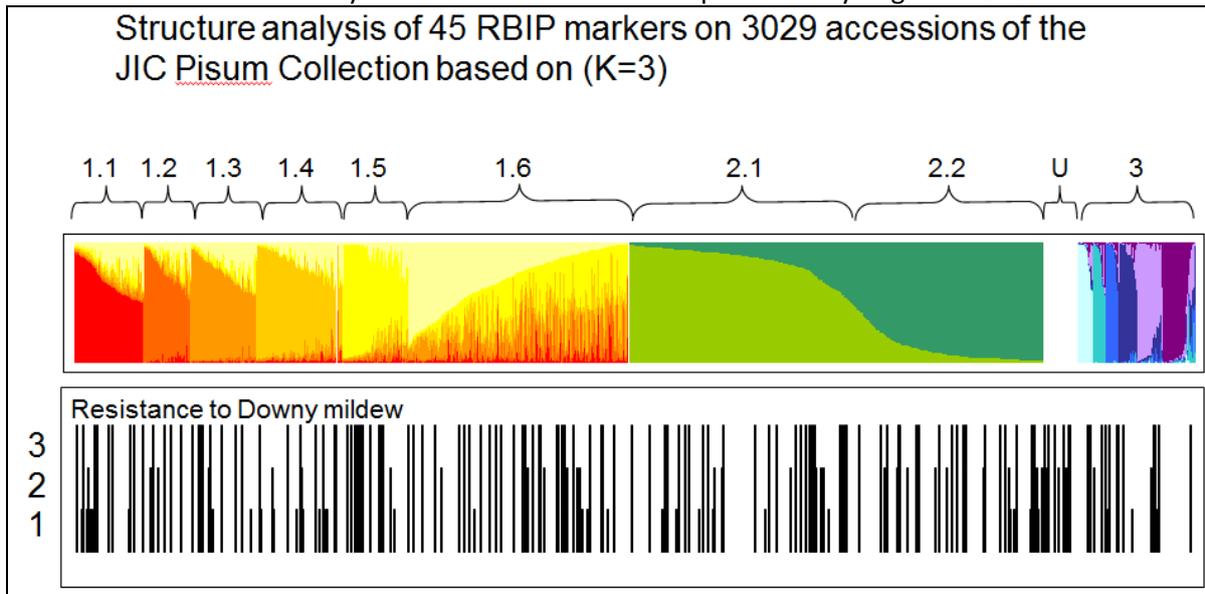
- Selected *Pisum* lines showing strong/partial resistance should be bulked to provide a resource for field tests at different sites in the UK, alongside the collection and identification of current downy mildew isolates.
- The older screening did not identify JI 15 as a source of major resistance. This 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 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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Personal Communications:

S. Belcher (2018). Vining Pea Variety Historic Data. PGRO.

Knowledge and Technology Transfer

- Article in AHDB Grower, The technical guide for horticulture, April 2017; Herold, L., Mildew resistance in its place.
- Agrovista Crop Protection Course, presentation, PGRO, December 2017
- Cereals 2017, discussions with stakeholders, June 2017
- CropTech 2017, discussions with stakeholders, November 2017
- Grower meeting Bruce Farms, discussion with grower manager, Perth and Kinross, June 2017
- Grower meeting Green Pea Group, discussion with grower manager and growers, November 2017
- Grower meeting Holbeach Marsh Pea Growers, discussion with grower manager and growers, February 2018
- Legume panel meetings, presentation to panel members, PGRO, October 2017 and January 2018
- PGRO Crop Protection course, presentation, PGRO, February 2018
- PGRO Vining Pea Open Day, Nocton, discussions with stakeholders, June 2017
- Pulse panel meetings, presentation to panel members, PGRO, November 2017 and February 2018