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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

In 2016, 42 downy mildew (DM) isolates were collected across the UK and their races determined. These 42 isolates belonged to thirteen different races, nine of which had been defined by work performed in the 1980's (Taylor, 1986). Races 10 and 11 were most frequent in 2016 and races varied at different locations. Twenty pea varieties were planted at six locations across the UK to investigate whether severity of disease symptoms varied with geographical location. Some pea varieties showed differences in DM infection in different locations. However, one year's data is not enough to define any relationship between race occurrence and disease severity.

Background

Pea DM, caused by *Peronospora viciae* f. sp. *pisi*, is a major disease of both vining and combining peas in the UK. Primary infection caused by soil borne oospores can kill plants, while secondary infections caused by airborne spores can reduce yield by up to 55% in the UK. Quality standards for vining peas are high and blemish due to disease infection is not accepted by processors. Downy mildew invades pods, reducing the quality and visual appearance of the produce. Primary infection, caused by soil-borne oospores, can be supressed by the use of the seed treatment Wakil XL (metalaxyl-M, fludioxonil and cymoxanil) but this has been restricted to peas drilled from the 1st of April and will not be available for pea drilled in February or March. Disease tolerance is present in some varieties, and DM race differentiation leads to variable levels of disease occurrence at different growing locations.

Primary infection of young seedlings can be reduced by growing peas in a rotation of one year in five. Vining peas are intensively grown in the east of the UK where processing factories are located. In order to maintain 150 minutes from field to frozen for high quality peas the pea growing area is restricted. This intensity of pea cropping has led to the build-up of soil-borne inoculum. Wakil XL is used when there is a high risk of DM, either from early sowing into poor soil conditions when weather is suitable for disease development, or where disease pressure is high. Crop rotation and seed treatment reduce the incidence of primary infection by soil-borne oospores but secondary infection from airborne spores cannot be controlled in this way. Descriptive and recommended lists are produced annually to indicate relative tolerance of current pea varieties to DM (PGRO Vining Pea Growers Guide and PGRO Pulse Agronomy Guide) and growers use the lists to influence their choice of variety and seed treatment. No single option to reduce the risk of the disease gives complete control of DM.

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The UK DM population is made up of a number of genetically distinct races, shown by a study carried out in the 1980s, in which 11 UK races were identified (Taylor, 1986). No studies have been undertaken since then to establish dynamics and geographic spread of these races. In this project (FV 436), the current distribution of DM populations across the UK is being investigated. Differences in DM populations affect the susceptibility of individual pea varieties to the disease. This is due to differing interactions between the host variety and the pathogen race. In order to be able to recommend pea varieties with lower susceptibility to DM for specific regions of the UK, differences in pea varietal resistance to the different DM populations is also being studied.

Summary

In 2016, 42 DM isolates were obtained and their race determined (Table 1). These isolates were collected from eight different locations across the UK and from 21 different pea varieties. The 42 isolates comprised of thirteen different races, nine of which had been characterised by Taylor's study (for details refer to Table 4 the Science Section). Overall, comparison of project results and those obtained by Taylor appears to indicate that the occurrence of DM races has remained relatively similar over the last 30 years. For 2017 the aim is to collect isolates from the same locations as 2016 to determine whether the indication of race stability holds up.

All 42 isolates were also inoculated onto potential breeding lines JI 15 and JI 85 which are carriers of resistance genes as identified during the Pulse Crop Genetic Improvement Network (PCGIN) study. In total, six of the 42 isolates overcame the resistance of JI 15; one isolate each of race 1, 9 and an unknown race (unk 2) from Stockbridge, one isolate of race 3 from Perth, and two isolates of race 10 from Chatteris and Kirton. The resistance from JI 85 was also overcome by six isolates, all different except the race 1 isolate from Stockbridge (I 130), which also infected JI 15. The other five isolates were one race 1 and one race 5 from Perth, one isolate each of races 10 and 11 from Chatteris and one isolate of an unknown race (unk 1) from Chatteris.

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Table 1: Forty two 2016 Downy Mildew isolates. Isolate number, collection location, collection date, pea host variety, race, performance on germplasm lines JI 15 and JI 85 and availability in PGRO's long term storage.

Isolate	Location	Grid reference	Collected	Pea variety	Race	JI 15	JI 85	Storage
I 85	Donington	PE11 4TR (PC)	collected in 2015	?	10	r	r	no
I 94	Romney Marsh	TN28 8TS (PC)	collected in 2015	Kelvedon Wonder	10	r	r	yes
I 100	West Ashby	LN9 5PT (PC)	collected in 2015	Span	5	r	r	yes
I 107	Chatteris	TL422887	20/05/2016	?	3	r	r	yes
I 112	Chatteris	TL422887	31/05/2016	JI 758	11	r	r	no
I 113	Chatteris	TL422887	31/05/2016	Prophet	Unk 1	r	r	no
I 115	Chatteris	TL422887	31/05/2016	JI 1272	10	s	r	no
I 117	Stubton	SK884910	31/05/2016	Sakura	11	r	r	no
I 118	Stubton	SK884910	31/05/2016	Tomahawk	10	r	r	no
I 119	Stubton	SK884910	31/05/2016	Mascara	11	r	r	no
I 120	Stubton	SK884910	31/05/2016	Crackerjack	10	r	r	yes
I 123	Stubton	SK884910	31/05/2016	Maro	11	r	r	no
I 127	Stockbridge	SU335358	03/06/2016	Greenwood	9	s	r	yes
I 128	Stockbridge	SU335358	03/06/2016	Sakura	6	r	r	yes
I 129	Stockbridge	SU335358	03/06/2016	Crackerjack	Unk 2	s	r	yes
I 130	Stockbridge	SU335358	03/06/2016	Kingfisher	1	s	s	yes
I 139	Stockbridge	SU335358	03/06/2016	Prophet	10	r	r	no
I 140	Stockbridge	SU335358	03/06/2016	Mascara	3	r	r	yes
I 146	Howden	SE737265	30/06/2016	Tomahawk	8	r	r	yes
I 147	Howden	SE737265	30/06/2016	JI 1272	8	r	r	yes
I 148	Howden	SE737265	30/06/2016	Maro	3	r	r	yes
I 149	Howden	SE737265	30/06/2016	Gregor	8	r	r	yes
I 150	Howden	SE737265	30/06/2016	Sakura	3	r	r	yes
I 151	Howden	SE737265	30/06/2016	Oasis	8	r	r	yes
I 152	Howden	SE737265	30/06/2016	Avola	4	r	r	yes
I 153	Howden	SE737265	30/06/2016	JI 560	8	r	r	yes
I 156	Howden	SE737265	30/06/2016	JI 411	3	r	r	yes
I 159	Perth	NO061209	30/06/2016	JI 560	5	r	s	yes
I 162	Perth	NO061209	30/06/2016	Avola	1	r	s	no
I 163	Perth	NO061209	30/06/2016	Waverex	5	r	r	yes
I 164	Perth	NO061209	30/06/2016	JI 1272	3	s	r	no
I 170	Sledmere	SE929680	07/07/2016	Amalfi	10	r	r	yes
I 171	Sledmere	SE929680	07/07/2016	Amalfi	3	r	r	yes
I 172	Sledmere	SE929680	07/07/2016	Amalfi	11	r	r	yes
I 174	Chatteris	TL422887	08/07/2016	JI 85	10	r	s	yes
I 176	Chatteris	TL422887	08/07/2016	JI 85	Unk 1	r	s	yes
I 178	Chatteris	TL422887	08/07/2016	JI 85	10	r	r	yes
I 179	Chatteris	TL422887	08/07/2016	JI 85	11	r	s	yes
I 184	Kirton	TF316371	12/07/2016	Waverex	3	r	r	yes
I 185	Kirton	TF316371	12/07/2016	Waverex	10	s	r	yes
I 188	Stratford upon Avon	SP167522	20/07/2016	Legacy	10	r	r	yes
I 189	Mixture				3	r	r	yes

PC = post code; r = resistant; s = susceptible

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The geographical distribution of the different races is shown in Figure 1. DM isolates were collected from a total of eleven sites, ten in England and one in Scotland. Races 3, 10 and 11 occurred in several locations in the UK whereas races 6 and 9 were only found in Stockbridge, Hampshire and race 8 was only found in Howden, Yorkshire. Results of geographical distribution are based on one year of data only and more data are needed to draw conclusions distribution patterns. In 2017, more isolates will be collected from both new locations and locations already studied in 2016.

Figure 1. Distribution and race of 42 DM isolates in 2016.

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Field trials to evaluate infection levels of different pea varieties in six different locations across the UK were established in 2016 (Table 2). A total of 20 combining pea varieties, vining pea varieties and research pea lines were planted at each location, with three replicates (Table 3). The research lines were four differential pea lines (JI 411, JI 560, JI 758, JI 1272) and two germplasm lines (JI 15, JI 85) which are carriers of resistance genes. Fifty seeds were sown, per variety and replicate. Leaf infections were scored as percentage of plants with percentage leaf infection; pod infection was measured as percentage of pods infected over all 50 plants per variety.

Table 2. Locations of the six DM field trials in 2016

Field site	Grid ref	County
Chatteris	TL422887	Cambridgeshire
Howden	SE737265	Yorkshire
Nocton	TF036638	Lincolnshire
Perth	NO061209	Perthshire
St Germans	TF611128	Norfolk
Stubton	SK884910	Lincolnshire

Table 3. Pea varieties grown at the six field sites in 2016

Variety	Type
Aikido	Marrowfat (combining)
Aloha	Vining pea
Avola	Vining pea
Crackerjack	Large Blue (combining)
Gregor	White Pea (combining)
JI 1272	DM race differential
JI 15	Germplasm (resistance gene carrier)
JI 411	DM race differential
JI 560	DM race differential
JI 758	DM race differential
JI 85	Germplasm (resistance gene carrier)
Mantara	Maple Pea (combining)
Maro	Combining Pea
Mascara	White Pea (combining)
Maurice	Vining pea
Oasis	Vining pea
Prophet	Large Blue (combining)
Sakura	Marrowfat (combining)
Tomahawk	Vining pea
Waverex	Petis pois

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Table 4 shows an overview of results obtained in 2016. In three of the locations, St Germans, Perth and Nocton, only low levels of DM infection occurred. The other three locations, Chatteris, Howden and Stubton, showed higher levels of DM infection.

Table 4. Mean percentage leaf infection and pod infection of 20 pea varieties grown at six different locations.

	Chatteris	Howden	Nocton	Perth	St Germans	Stubton
Variety	**% leaf infection**					
Aikido	1.17	2.22	0.13	0.00	0.00	0.00
Aloha	0.00	0.03	0.04	0.00	0.00	0.00
Avola	4.17	15.33	1.75	0.33	0.02	3.50
Crackerjack	1.58	1.57	0.08	0.00	0.00	2.18
Gregor	4.89	1.59	0.38	0.00	0.00	1.25
JI 1272	8.03	4.38	0.14	0.01	0.00	3.62
JI 15	0.96	0.37	0.00	0.00	0.00	0.23
JI 411	1.03	0.71	0.21	0.00	0.00	0.00
JI 560	1.88	5.75	1.68	0.00	0.00	5.00
JI 758	1.31	3.72	0.25	0.00	0.00	1.85
JI 85	0.01	0.38	0.00	0.00	0.00	0.00
Mantara	1.75	0.18	0.00	0.00	0.00	0.00
Maro	5.83	9.58	0.17	0.01	0.00	3.52
Mascara	6.25	0.20	0.08	0.00	0.00	0.02
Maurice	0.00	0.17	0.00	0.00	0.00	0.00
Oasis	4.25	4.10	0.00	0.00	0.33	1.65
Prophet	4.60	0.35	0.00	0.00	0.00	0.04
Sakura	3.62	6.90	0.00	0.00	0.00	0.43
Tomahawk	4.10	5.73	0.31	0.00	0.00	0.96
Waverex	4.12	1.75	0.00	0.00	0.00	0.17
Variety	**% pod infection**					
Aikido	1.00	0.00	NA	NA	7.33	0.00
Aloha	1.00	1.67	NA	NA	7.33	0.00
Avola	71.67	15.00	NA	NA	91.67	1.67
Crackerjack	6.00	0.00	NA	NA	2.33	1.33
Gregor	13.00	0.00	NA	NA	3.00	0.00
JI 1272	5.33	0.00	NA	NA	8.00	4.00
JI 15	8.33	0.67	NA	NA	0.00	0.00
JI 411	11.33	0.33	NA	NA	11.67	5.00
JI 560	4.33	0.00	NA	NA	2.33	3.33
JI 758	4.33	0.00	NA	NA	0.00	1.67
JI 85	4.00	1.67	NA	NA	0.00	1.33
Mantara	2.67	0.00	NA	NA	0.33	0.00
Maro	7.33	0.00	NA	NA	4.00	2.33
Mascara	0.33	0.00	NA	NA	0.00	0.00
Maurice	1.33	0.00	NA	NA	1.67	0.00
Oasis	45.00	1.67	NA	NA	22.33	15.00
Prophet	8.67	0.00	NA	NA	1.33	1.67
Sakura	5.33	0.00	NA	NA	7.33	3.33
Tomahawk	46.00	10.00	NA	NA	31.67	0.67
Waverex	53.33	0.00	NA	NA	8.33	6.67

Infection severity was assessed for each individual location and split into three categories - bottom, middle and upper third. Range of percentage infection for each category (both leaves and pod) depends on the location. Green = bottom third; Yellow = middle third; red = upper third. No colour = free from infection. NA = not assessed

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During 2016, variety performance, location and DM population showed some potential relationships but more data will be needed to verify any trends. For example, varieties Maro and JI 1272 showed high infection levels at Chatteris and Stubton sites, where races 10 and 11 were dominant. Mascara, on the other hand, was severely infected in Chatteris but not in Stubton, and JI 560 in Stubton but not in Chatteris. This might indicate that other races than race 10 and 11 played a role in overall infection at these field sites. Four varieties, Aikido, Aloha, Mantara and Maurice only had very low levels of infection in all locations and might prove to be varieties of choice in fields with a history of DM. The overall goal would be to identify pea varieties that can show resistance to the majority of DM races making them less susceptible to changes in DM populations. Noticeable were the levels of pod infection in some varieties, which were relatively high even though leaf infection had been low. This is of importance, in particular, for the two potential breeding lines JI 15 and JI 85 both of which had relatively high pod infection levels in Chatteris and Howden although leaf infection had been low. Pod quality is of greatest importance in the vining pea industry because only unblemished pods contain high quality peas. If JI 15 and JI 85 are generally prone to pod infection under field conditions, their usefulness in resistance breeding might be reduced.

Financial Benefits

None at the moment. One year of data is insufficient for advice on which pea varieties would perform best in specific locations in the UK. More data are needed about downy mildew populations across the UK.

Action Points

Until more results from this project become available, please refer to the PGRO Vining Pea Grower Guide and the PGRO Pulse Agronomy Guide for information about resistance of different pea varieties to downy mildew.

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SCIENCE SECTION

Introduction

Downy mildew, caused by *Peronospora viciae* f. sp. *pisi*, (DM) 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; Taylor, 1986). Despite the development of more resistant modern cultivars, downy mildew remains a significant cause of losses to the profitability of the pea crop, firstly by compromising the growth of the plants through lesions of the stem leaves and stipules, and later by spreading into the pods where it directly affects the quality of the developing seeds. Downy mildew is both soil and air-borne, surviving in the soil as oospores. When peas are drilled, root leachates stimulate the germination of the oospores. These move to the seedlings and cause systemic, 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 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. Downy mildew is not transmitted via the seed but seed treatments protect the germinating seedling from 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 Agronomy Guide Recommended List tables. However, there is less varietal disease resistance available in vining peas and ratings can be found in the PGRO Vining Pea Descriptive List tables (https://horticulture.ahdb.org.uk/publication/vining-pea-growers-guide-%E2%80%93-2017)
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 April 2017 onwards, the new MAPP number of Wakil XL can only be used on peas planted from the 1st of April onwards. Early sown peas in the next growing season will potentially be at even greater risk of downy mildew infection. There are currently no foliar-applied products to control downy mildew.

Downy mildew produces large quantities of airborne spores and is able to evolve very quickly (Liu *et al*., 2013). This results in the development of genetically diverse populations. The

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constantly changing population can result in the development of new virulent races that are able to cause severe infections in varieties that were previously only mildly susceptible or moderately tolerant. For example, the marrow fat variety Sakura 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 yearly within the same areas in Lincolnshire and although changes in environmental conditions have an influence on disease development it is unlikely that environmental conditions alone have resulted in these differences in resistance.

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

Materials and methods

Isolate collection

During the 2016 growing season, field samples of pea DM were collected by PGRO. The samples were either obtained by collection of infected pea plants during field visits by staff from PGRO or were received from pea growers across the UK who sent infected pea plants to PGRO. Most DM isolates were isolated from single lesions on individual leaves to increase likelihood of obtaining isolates of pure rather than mixed races. Some samples were collected from whole plants. The samples covered a wide area across the UK. In order to make sure that the isolates are 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 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 in a tray filled with moist compost, covered with compost and the tray covered with a lid. The trays were kept in a growth room at 15°C with 16h light and 8h darkness each 24 hours. Infected plant samples were always kept in trays covered with lids to avoid cross contamination. Sub-culturing was performed in a cabinet enclosed on three sides with as little air movement as

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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.

Pea differential host lines

In order to determine the race of the DM isolates, four differential pea host lines with recorded resistances and susceptibilities to UK DM races were used (Taylor, 1986; Table 5). The pea differential lines were inoculated with the DM isolates as described above. Eight seedlings per pea line were inoculated with each DM isolate and presence or absence of DM infection recorded. In addition, two pea germplasm lines with potentially strong resistance to DM (JI 15 and JI 85) were also inoculated with each of the DM isolates to determine whether the isolates could overcome the resistance of these pea lines. If these two pea germplasm lines, which had been identified as carriers of resistance genes in the Pulse Crop Genetic Improvement Network (PCGIN) study, show resistance to a wide range of UK DM populations they have the potential to be used in DM resistance breeding.

Table 5. Susceptibility (S) or resistance (R) of four pea differential host lines (JI 411, JI 560, JI 758, JI 1272) to eleven races of downy mildew (UK pathotypes). The differential host lines can be used to determine the race of a DM isolate (Taylor, 1986).

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

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Field experiments

Field trials to evaluate infection levels of different pea varieties, in six different locations across the UK, were established in 2016 (Table 6). Combining pea varieties, vining pea varieties, the four differential host lines and the two germplasm lines (carriers of resistance genes) were planted at each location, with three replicates (Table 7). Fifty seeds were planted per variety and replicate, and netted to avoid bird damage. Figure 2 shows the layout of the trial at Nocton. The leaves were assessed for downy mildew infection around six weeks after planting and at flowering, and pods were assessed for infection levels just before maturity. Leaf infections were scored as percentage of plants with percentage leaf infection; pod infection was measured as percentage of pods infected.

Table 6. Locations of the six DM field trials in 2016.

Field site	Grid ref	County
Chatteris	TL422887	Cambridgeshire
Howden	SE737265	Yorkshire
Nocton	TF036638	Lincolnshire
Perth	NO061209	Perthshire
St Germans	TF611128	Norfolk
Stubton	SK884910	Lincolnshire

Table 7. Twenty pea varieties grown at the six field sites in 2016.

Variety	Type
Aikido	Marrowfat (combining)
Aloha	Vining pea
Avola	Vining pea
Crackerjack	Large Blue (combining)
Gregor	White Pea (combining)
JI 1272	DM race differential
JI 15	Germplasm (resistance gene carrier)
JI 411	DM race differential
JI 560	DM race differential
JI 758	DM race differential
JI 85	Germplasm (resistance gene carrier)
Mantara	Maple Pea (combining)
Maro	Combining Pea
Mascara	White Pea (combining)
Maurice	Vining pea
Oasis	Vining pea
Prophet	Large Blue (combining)
Sakura	Marrowfat (combining)
Tomahawk	Vining pea
Waverex	Petis pois

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Figure 2: Field trial at Nocton after pea emergence.

Results

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

During the 2016 season, 84 DM samples were collected and inoculated onto pea seedlings for purification. Furthermore, 17 samples that had been stored in 2015 have been inoculated onto pea seedlings for purification. Out of these 101 samples, 42 isolates survived the purification process and their race was determined. These isolates had been obtained from eight different locations across the UK and from 21 different pea varieties (Table 8).

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Table 8. Forty two 2016 Downy Mildew isolates. Isolate number, collection location, collection date, pea host variety, race, performance on germplasm lines JI 15 and JI 85 and availability in PGRO's long term storage.

Isolate	Location	Grid reference	Collected	Pea variety	Race	JI 15	JI 85	Storage
I 85	Donington	PE11 4TR (PC)	collected in 2015	?	10	r	r	no
I 94	Romney Marsh	TN28 8TS (PC)	collected in 2015	Kelvedon Wonder	10	r	r	yes
I 100	West Ashby	LN9 5PT (PC)	collected in 2015	Span	5	r	r	yes
I 107	Chatteris	TL422887	20/05/2016	?	3	r	r	yes
I 112	Chatteris	TL422887	31/05/2016	JI 758	11	r	r	no
I 113	Chatteris	TL422887	31/05/2016	Prophet	?	r	r	no
I 115	Chatteris	TL422887	31/05/2016	JI 1272	10	s	r	no
I 117	Stubton	SK884910	31/05/2016	Sakura	11	r	r	no
I 118	Stubton	SK884910	31/05/2016	Tomahawk	10	r	r	no
I 119	Stubton	SK884910	31/05/2016	Mascara	11	r	r	no
I 120	Stubton	SK884910	31/05/2016	Crackerjack	10	r	r	yes
I 123	Stubton	SK884910	31/05/2016	Maro	11	r	r	no
I 127	Stockbridge	SU335358	03/06/2016	Greenwood	9	s	r	yes
I 128	Stockbridge	SU335358	03/06/2016	Sakura	6	r	r	yes
I 129	Stockbridge	SU335358	03/06/2016	Crackerjack	?	s	r	yes
I 130	Stockbridge	SU335358	03/06/2016	Kingfisher	1	s	s	yes
I 139	Stockbridge	SU335358	03/06/2016	Prophet	10	r	r	no
I 140	Stockbridge	SU335358	03/06/2016	Mascara	3	r	r	yes
I 146	Howden	SE737265	30/06/2016	Tomahawk	8	r	r	yes
I 147	Howden	SE737265	30/06/2016	JI 1272	8	r	r	yes
I 148	Howden	SE737265	30/06/2016	Maro	3	r	r	yes
I 149	Howden	SE737265	30/06/2016	Gregor	8	r	r	yes
I 150	Howden	SE737265	30/06/2016	Sakura	3	r	r	yes
I 151	Howden	SE737265	30/06/2016	Oasis	8	r	r	yes
I 152	Howden	SE737265	30/06/2016	Avola	4	r	r	yes
I 153	Howden	SE737265	30/06/2016	JI 560	8	r	r	yes
I 156	Howden	SE737265	30/06/2016	JI 411	3	r	r	yes
I 159	Perth	NO061209	30/06/2016	JI 560	5	r	s	yes
I 162	Perth	NO061209	30/06/2016	Avola	1	r	s	no
I 163	Perth	NO061209	30/06/2016	Waverex	5	r	r	yes
I 164	Perth	NO061209	30/06/2016	JI 1272	3	s	r	no
I 170	Sledmere	SE929680	07/07/2016	Amalfi	10	r	r	yes
I 171	Sledmere	SE929680	07/07/2016	Amalfi	3	r	r	yes
I 172	Sledmere	SE929680	07/07/2016	Amalfi	11	r	r	yes
I 174	Chatteris	TL422887	08/07/2016	JI 85	10	r	s	yes
I 176	Chatteris	TL422887	08/07/2016	JI 85	?	r	s	yes
I 178	Chatteris	TL422887	08/07/2016	JI 85	10	r	r	yes
I 179	Chatteris	TL422887	08/07/2016	JI 85	11	r	s	yes
I 184	Kirton	TF316371	12/07/2016	Waverex	3	r	r	yes
I 185	Kirton	TF316371	12/07/2016	Waverex	10	s	r	yes
I 188	Stratford upon Avon	SP167522	20/07/2016	Legacy	10	r	r	yes
I 189	Mixture				3	r	r	yes

PC = post code; r = resistant; s = susceptible

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All 42 isolates were also inoculated onto the germplasm lines JI 15 and JI 85 which are carriers of DM resistance genes (Table 8). In total, six of the 42 isolates overcame the resistance of JI 15; race 1 (I 130) and 9 (I 127) and unk 2 (I 129) from Stockbridge, one race 3 (I 164) from Perth, and two race 10s from Chatteris (I 155) and Kirton (I 185). The resistance from JI 85 was overcome by six isolates, one of which, race 1 from Stockbridge (I 130), also infected JI 15. The other isolates to overcome the resistance of JI 85 were race 1 (I 162) and race 5 (I159) from Perth, one isolate each of races 10 (I 174), 11 (I 179) and unk 1 (I 176) from Chatteris.

The 42 isolates comprised of 13 different races (Table 9). Eleven races which were characterised by Taylor (1986) and two unknown races (unk 1 and unk 2) that have infection pattern on the four pea differential lines that are not defined by the work performed at the John Innes Centre in the 1980s.

Table 9. Number of isolates of each DM race and location of isolate collection.

Race	JI 411	JI 560	JI 758	JI 1272	No isolates	Location
1	S	S	S	S	2	Stockbridge, Perth
2	S	S	S	R	0	
3	S	S	R	S	9	Chatteris, Stockbridge, Howden (3), Perth, Sledmere, Kirton, Mixture
4	S	R	S	S	1	Howden
5	S	R	R	S	3	West Ashby, Perth (2)
6	R	S	S	S	1	Stockbridge
7	R	S	S	R	0	
8	R	S	R	S	5	Howden (5)
9	R	R	S	S	1	Stockbridge
10	R	R	R	S	11	Chatteris (3), Stubton (2), Stockbridge, Sledmere, Kirton, Stratford apon Avon
11	R	R	R	R	6	Chatteris (2), Stubton (3), Sledmere
Unk 1	R	S	R	R	2	Chatteris (2)
Unk 2	S	S	R	R	1	Stockbridge

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Distribution of downy mildew races across the UK

The geographical distribution of the DM races found in this study is shown in Figure 3. Races 3, 10 and 11 occurred in several locations in the UK. Races 6, 9 and unk 2 were only found in Stockbridge, Hampshire, race unk 1 was only found in Chatterris and race 8 was only found in Howden, Yorkshire.

Figure 3. Distribution and race of 42 Downy Mildew isolates in 2016.

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Differences in varietal susceptibility to downy mildew populations at different locations

Twenty pea varieties were planted at six locations across the UK (Table 10). At three of the locations, St Germans, Perth and Nocton, only low levels of DM infection occurred. At St Germans, only variety Oasis showed any DM infection and this at only 0.33% leaf area. This low leaf infection, however, did not translate to low pod infection. In contrast, pod infection was high. At St Germans, only four of the varieties were free from pod infection (JI 15, JI 85, JI 758, Mascara), the other 16 varieties showed 1 to 92% of pod infection. The highest infection of 92% was seen in Avola, and Tomahawk showed 32% infection. Low pod infections of less than 3% was seen in Crackerjack, JI 560, Mantara, Maurice and Prophet. At St Germans, 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. At the Perth site, only Avola showed infection, at 0.33% leaf area. Pod infection was not assessed in Perth because of time restrictions. In Perth, the DM population comprised of at least three different races, race 1, race 3 and race 5. At the Nocton 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 varieties, with the exception of JI 15, JI 85, Mantara, Maurice, Oasis, Prophet, Sakuar and Waverex which were completely clean. Pod infection was not assessed in Nocton because the field site was harvested before all varieties had finished pod set. Race occurrence in Nocton also remains unknown, because DM samples taken from the field did not survive the purification process under growth room conditions.

The other three locations, Chatteris, Howden and Stubton, showed higher levels of DM infection. In Chatteris, only Aloha and Maurice were free of leaf infection. Avola, Gregor, Oasis, Prophet, Sakura, Tomahawk and Waverex show medium levels of around 4% infection and high infection of greater than 5% were observed in JI 1272, Maro and Mascara. Pod infection in Chatteris were very high overall. Not a single variety was free of pod infection and most varieties showed infection levels of up to 10%. Four varieties had even greater infection levels (Oasis = 45%, Tomahawk = 46%, Waverex = 43%, Avola = 72%). The DM population of Chatteris comprised of at least four different races, 3, 10, 11 and unk 1. In Howden, overall infection levels were greatest. Avola showed 15% of leaf area infected followed by Sakura, Maro, Tomahawk and JI 560 with 10%, 7%, 6% and 6%, respectively. Not a single variety was completely free of leaf infection. Several varieties did not show any pod infection and most of the other varieties had less than 2%. Only Tomahawk at 10% and Avola at 15% were higher infected. In Howden, races 3, 4 and 8 were present. In Stubton, highest infection levels of greater than 3% were seen in Avola, JI 560, JI 1272 and Maro. Six varieties were free of infection and the remainder had infection levels of 0.1 to 2%. Pod infection in Stubton was

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less than 4% with the exception of Oasis and Waverex that showed 15 and 7% infection, respectively. The DM population of Stubton comprised of two different races, races 10 and 11.

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Table 10. Mean percentage leaf infection and mean percentage pod infection of 20 pea varieties grown at six different locations

	Chatteris	Howden	Nocton	Perth	St Germans	Stubton
Variety	**% leaf infection**					
Aikido	1.17	2.22	0.13	0.00	0.00	0.00
Aloha	0.00	0.03	0.04	0.00	0.00	0.00
Avola	4.17	15.33	1.75	0.33	0.02	3.50
Crackerjack	1.58	1.57	0.08	0.00	0.00	2.18
Gregor	4.89	1.59	0.38	0.00	0.00	1.25
JI 1272	8.03	4.38	0.14	0.01	0.00	3.62
JI 15	0.96	0.37	0.00	0.00	0.00	0.23
JI 411	1.03	0.71	0.21	0.00	0.00	0.00
JI 560	1.88	5.75	1.68	0.00	0.00	5.00
JI 758	1.31	3.72	0.25	0.00	0.00	1.85
JI 85	0.01	0.38	0.00	0.00	0.00	0.00
Mantara	1.75	0.18	0.00	0.00	0.00	0.00
Maro	5.83	9.58	0.17	0.01	0.00	3.52
Mascara	6.25	0.20	0.08	0.00	0.00	0.02
Maurice	0.00	0.17	0.00	0.00	0.00	0.00
Oasis	4.25	4.10	0.00	0.00	0.33	1.65
Prophet	4.60	0.35	0.00	0.00	0.00	0.04
Sakura	3.62	6.90	0.00	0.00	0.00	0.43
Tomahawk	4.10	5.73	0.31	0.00	0.00	0.96
Waverex	4.12	1.75	0.00	0.00	0.00	0.17
Variety	**% pod infection**					
Aikido	1.00	0.00	NA	NA	7.33	0.00
Aloha	1.00	1.67	NA	NA	7.33	0.00
Avola	71.67	15.00	NA	NA	91.67	1.67
Crackerjack	6.00	0.00	NA	NA	2.33	1.33
Gregor	13.00	0.00	NA	NA	3.00	0.00
JI 1272	5.33	0.00	NA	NA	8.00	4.00
JI 15	8.33	0.67	NA	NA	0.00	0.00
JI 411	11.33	0.33	NA	NA	11.67	5.00
JI 560	4.33	0.00	NA	NA	2.33	3.33
JI 758	4.33	0.00	NA	NA	0.00	1.67
JI 85	4.00	1.67	NA	NA	0.00	1.33
Mantara	2.67	0.00	NA	NA	0.33	0.00
Maro	7.33	0.00	NA	NA	4.00	2.33
Mascara	0.33	0.00	NA	NA	0.00	0.00
Maurice	1.33	0.00	NA	NA	1.67	0.00
Oasis	45.00	1.67	NA	NA	22.33	15.00
Prophet	8.67	0.00	NA	NA	1.33	1.67
Sakura	5.33	0.00	NA	NA	7.33	3.33
Tomahawk	46.00	10.00	NA	NA	31.67	0.67
Waverex	53.33	0.00	NA	NA	8.33	6.67

Infection severity was assessed for each individual location and split into three categories - bottom, middle and upper third. Range of percentage infection for each category (both leaves and pod) depends on the location. Green = bottom third; Yellow = middle third; red = upper third. No colour = free from infection. NA = not assessed

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Discussion

In 2016, the race of 42 DM isolates was determined. Thirty one of these isolates are in long term storage at PGRO which holds, to the best of our knowledge, the only DM culture collection in the UK. It was successfully shown that DM isolates can be revived from long term storage after being stored at -80°C for three months, giving the potential to use specific isolates of DM races for further studies.

During 2016, a total of 39 isolates could be compared with the 56 isolates which were categorised into races by Taylor (1986) (Table 11). Most of the isolates that were collected by Taylor were collected in the Norfolk area, with the majority of the remaining isolates collected from the south-east of England. In 2016, the collection area was larger. The majority of isolates collected from the east and south-east of England, in-part reflecting the UK pea growing area.

Within this project, nine of the eleven races identified by Taylor (1986) were identified, but not races 2 and 7. Figures 4 and 5 show the race frequency of the study by Taylor (1986) and this study, respectively. Race 10 was the most frequent race found in both studies with 34% and 28% of all isolates from Taylor (1986) and Herold (2017), respectively. This might indicate that there is some degree of race stability over the 30 year period. However, one year of data in both decades is not enough to come to a definite conclusion. Races 3, 8 and 11 showed higher frequency in 2017 in comparison to 1986, whereas races 1, 4 and 9 showed lower frequency in 2017 in comparison to 1986. In both years, races 2, 5, 6 and 7 only made up very low percentages of downy mildew isolates, if any. For the coming season of 2017 the aim is to collect isolates from the same locations as in 2016. In addition, isolates will be collected from additional areas of the UK to increase our knowledge of race distribution.

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Table 11. Number of isolates and percentage of each race found by Taylor (1986) and in this project (Herold, 2017).

	Number of isolates		Percentage	
Race	Taylor	Herold	Taylor	Herold
1	9	2	16	5
2	2	0	4	0
3	4	9	7	23
4	5	1	9	3
5	1	3	2	8
6	1	1	2	3
7	1	0	2	0
8	2	5	4	13
9	9	1	16	3
10	19	11	34	28
11	3	6	5	15
Total	56	39		

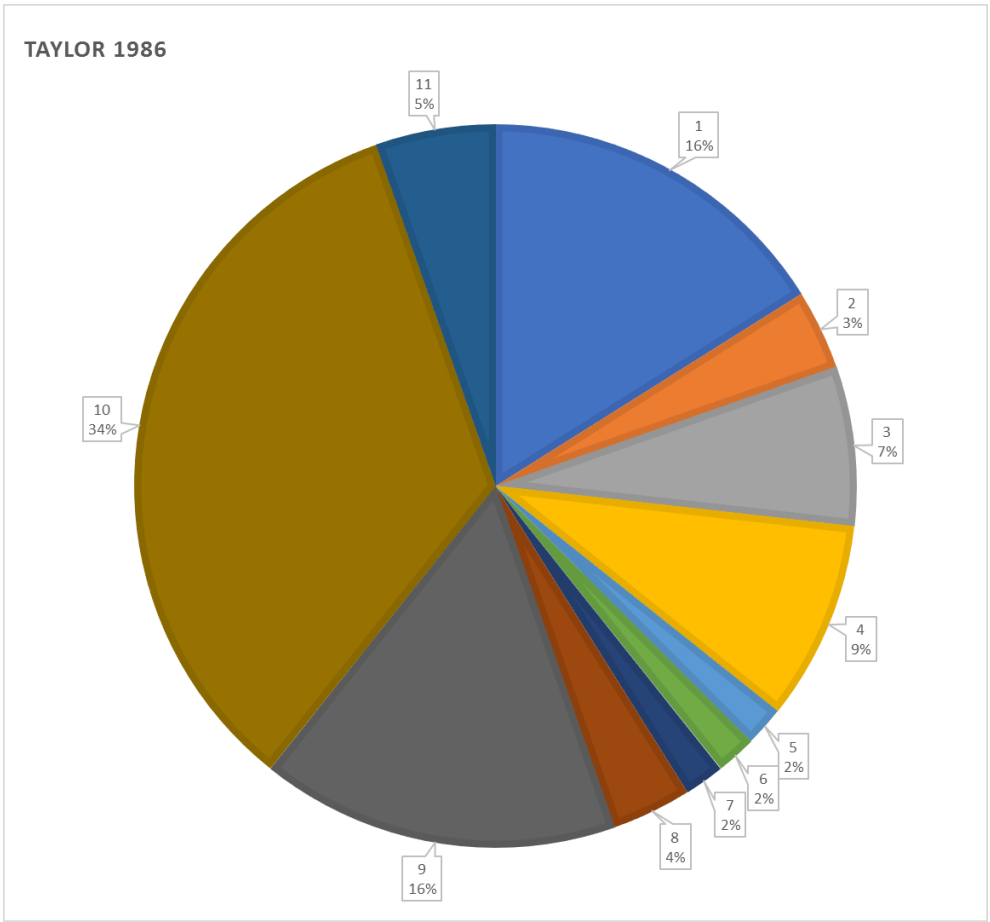

Figure 4. Frequency of downy mildew races (number of isolates and percentage) as reported by Taylor (1986).

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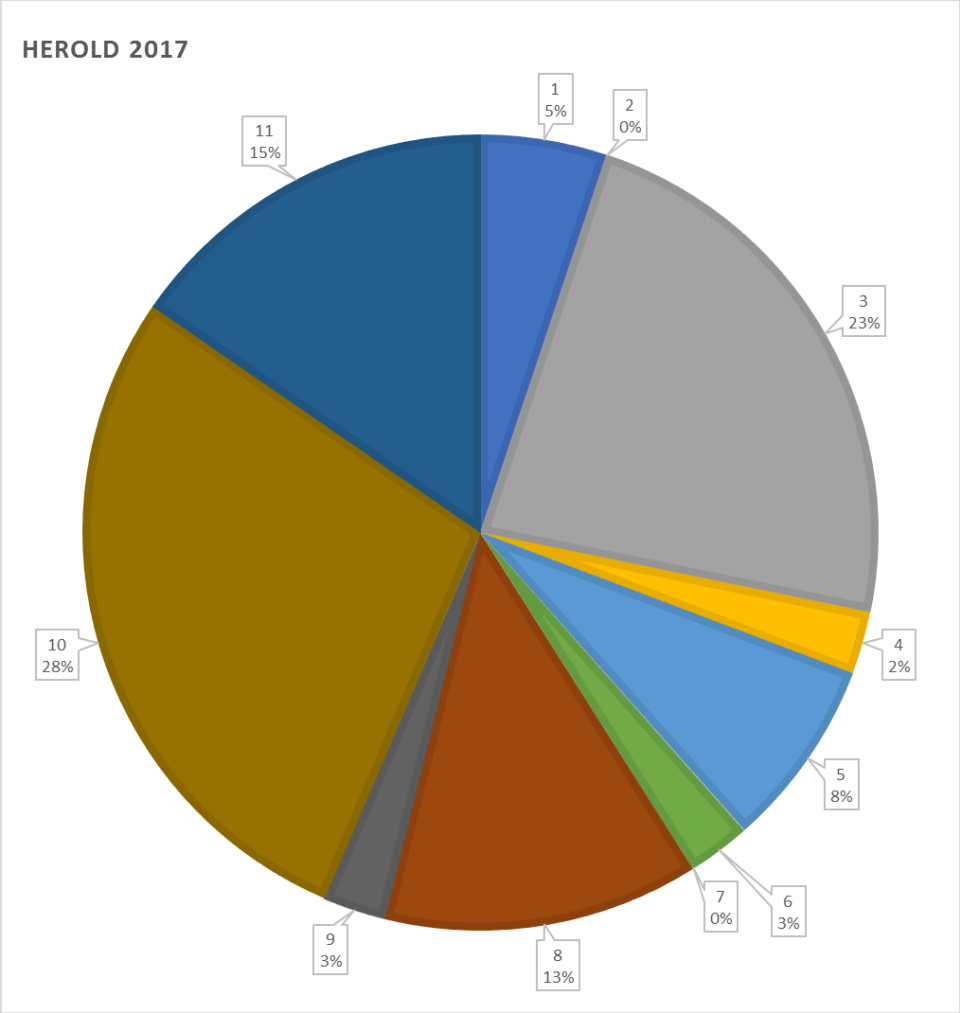

Figure 5. Frequency of downy mildew races (number of isolates and percentage) found in 2016 (Herold, 2017).

In addition to the races already known from the work performed by Taylor, two unknown races were found that show different infection patterns when inoculated onto the four differential pea lines. One unknown race (unk 1) infected JI 560 only but not the other three JI lines (Table 9). Two of the isolates, I113 and I176 from Chatteris showed this pattern. Isolate I129 from Stockbridge infected JI 411 and JI 560 but not JI 758 or JI 1272 and is therefore also of unknown race, but a different unknown race (unk 2).

Taylor started his work using ten differential pea lines for the characterisation of downy mildew races and identified 22 different races. He discovered that the isolates he had tested could be grouped into eleven different races using just four differential pea lines – JI 411, JI 560 JI 758 and JI 1272. These four pea lines were used by Taylor and in this project to study the geographical distribution of downy mildew races in the UK. It is therefore not known whether the two unknown races identified here are new races or had just not been detected by Taylor.

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Since total race number is dependent on the differential pea lines used – and greater diversity of races can be detected when using ten differential pea lines – it is possible these two unknown races detected in 2016 had not been detected by Taylor.

Eleven isolates overcame the resistance of either JI 15 or JI 85 (Table 8), one isolate was able to infect both. There was no clear pattern to the isolates that overcame the resistance. They were collected from different places and belong to different races (Table 12). With regards to JI 15, the six isolates were from four different locations, five different races, isolated from five different pea varieties. With regards to JI 85, the six isolates were from three different locations, five different races, isolated from four different pea varieties. Noticeable was that three isolates that overcame the resistance of JI 85 in the growth room had been collected from infected JI 85 plants in the field in Chatteris.

Table 12. Isolate number, location and pea variety of the isolates that overcame resistance of either JI 15 or JI 85.

Isolate	Location	Pea variety	Race	JI 15	JI 85
I 115	Chatteris	JI 1272	10	s	r
I 127	Stockbridge	Greenwood	9	s	r
I 129	Stockbridge	Crackerjack	Unk 2	s	r
I 130	Stockbridge	Kingfisher	1	s	s
I 159	Perth	JI 560	5	r	s
I 162	Perth	Avola	1	r	s
I 164	Perth	JI 1272	3	s	r
I 174	Chatteris	JI 85	10	r	s
I 176	Chatteris	JI 85	Unk 1	r	s
I 179	Chatteris	JI 85	11	r	s
I 185	Kirton	Waverex	10	s	r

The data presented here on the geographical distribution of different races is based on only one year of data and more data is needed to add to the robustness of these results. The same is true for differences of varietal susceptibilities in different locations. During 2016, variety performance, location and DM population showed some indication of a pattern but more data will be needed to justify any conclusions. For example, varieties Maro and JI 1272 showed high infection levels at Chatteris and Stubton where races 10 and 11 were dominant. In contrast, Mascara was severely infected in Chatteris, but not in Stubton and JI 560 in Stubton but not in Chatteris.

Four varieties, Aikido, Aloha, Mantara and Maurice only had very low levels of infection in all locations and may prove to be varieties of choice in fields with a history of DM. Of particular importance were the varieties which showed low levels of leaf infection, yet relatively high

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levels of pod infection. This is of particular importance for the two germplasm lines JI 15 and JI 85, both of which had relatively high pod infection levels in Chatteris (JI 15 and JI 85) with lower but still noticeable pod infection in Howden and Stubton (JI 85). Downy mildew not only impacts on overall yield but has major implications on product quality in vining peas. Pod infection leads to brown discolouration on pea seeds making them unsuitable for high quality produce. The fact that JI 15 and JI 85 have shown high levels of pod infection may have implication for their usefulness in resistance breeding where seed quality is of greatest importance.

Conclusions

- PGRO has a pea downy mildew culture collection of 31 isolates belonging to eleven different races
- Race 10 was most frequently occurring race in 2016, occurring in eight of twelve sites sampled
- Downy mildew populations (combinations of all races from each location) differed across the UK, eg. six isolates were collected from six different varieties at the Stockbridge site and all were different races. At no site where multiple samples were collected, were they all of the same race
- Resistance of both germplasm lines, JI 15 and JI 85, was overcome by six different isolates collected from four and three different sites respectively
- Several pea varieties differed in their downy mildew infection levels showing greater susceptibility to the disease in some geographical locations than in others eg. the variety Maurice was consistently resistant to DM across the UK whereas variety Mascara showed high DM infection at only one of the six locations.

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Knowledge and Technology Transfer

- Agrovista Crop Protection Course, presentation, PGRO, November 2016
- Cereals 2016, discussions with stakeholders, June 2016
- CropTech 2016, discussions with stakeholders, November 2016
- Grower meeting Bruce Farms (two meetings), discussion with grower manager, Perthshire, June 2016 and February 2017
- Grower meeting Dengie Crops, discussion with grower manager, January 2017
- Grower meeting Green Pea Group, discussion with grower manager and growers, February 2017
- Grower meeting Holbeach Marsh Pea Growers, discussion with grower manager and growers, February 2017
- Grower meeting Stemgold Peas, discussion with grower manager and growers, March 2017
- Legume panel meeting, presentation to panel members, PGRO, January 2017
- PGRO and Syngenta Roadshows (four meetings), presentation, January and February 2017
- PGRO Crop Protection courses, presentation, PGRO, February 2017
- PGRO Vining pea Open Day, Nocton, discussions with stake holders, June 2016
- Pulse panel meeting, presentation to panel members, PGRO, February 2017
- The Pulse Magazine, Spring 2017, article by Dr Lea Herold, 'Distribution of pea downy mildew races across the UK', Processors and Growers Research Organisation, Peterborough, UK

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