Project title:	Identification of inoculum sources and potential control strategies for the newly emerged Peronospora causing downy mildew on aquilegia
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Project leader:	Dr Philip Jennings, Fera
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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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CONTENTS

GROWER SUMMARY

Headline

Oospores were detected at low levels in aquilegia seed, suggesting that aquilegia downy mildew can be seed borne and that infected seed could be a potential source of the pathogen.

Background and expected deliverables

Aquilegia downy mildew (ADM) is caused by a currently unnamed *Peronospora* species which has not been reported outside of the UK. The first reported occurrence of ADM appears to be on a nursery in 2011. Since the first report, the disease has become widespread causing loss of plants both on nurseries and in public/private gardens. Some of these losses have led to coverage in the national gardening press.

Symptoms are typical of those produced by other downy mildews, with affected leaves developing angular, yellow patches which eventually turn brown. On the underside of the infected leaves a fine spore bearing fungal growth can often be seen. The disease spreads quickly and has led to rapid plant death.

A literature review revealed there was no information on the incidence, distribution and control of the disease either from the UK or overseas. However, there is an abundance of knowledge available for downy mildew diseases caused by other *Peronospora* species, which can be used to help determine potential sources of infection, the conditions required for both infection and sporulation, and control strategies.

Summary of the work and main conclusions

Grower survey

28 questionnaires were distributed with seven completed and returned. Of these, five respondents reported having had an outbreak of ADM on their nursery. Four of the seven respondents bought-in their plants as plug plants or liners, one bought-in both seed and young plants and two bought-in seed to raise their own plants. Of the three growers who raised plants from seed, all had purchased them from the same company and two reported an outbreak of ADM. There did not appear to be any link between the plant varieties grown and the prevalence of ADM on nurseries.

Seed contamination by the Peronospora responsible for aquilegia downy mildew

Seed samples from 14 aquilegia varieties were obtained to determine whether the pathogen responsible for ADM could be detected within or on the seed. The seed supplier and variety

of the seed were selected based on the results of the grower survey and in consultation with the grower co-ordinator.

Seed washing showed that oospores (resting spores) were present in one seed sample (*Aquilegia vulgaris* var. *plena* 'Christa Barlow') at a rate of 1 oospore per 100 seeds.

A primer set was produced which detected the DNA of the pathogen responsible for ADM. The primer set did not detect ADM DNA in any of the seed samples; however funding was not available to fully optimise the test, so low levels of ADM DNA in the sample may have been undetected.

A trial using the oospore-contaminated seed sample was established to determine whether infected seed would lead to infected plants, and if so, determine the conditions required for infection. By the end of the project no ADM symptoms were found on any plants in the trial. The trial is being continued and results reported for the follow-on project (HNS 196a).

Financial benefits

The average value of Aquilegia sales among the growers surveyed was £6,785.00, with high and low sales figures of £16,000 and £2,000 respectively. The number of commercial growers who have been adversely affected by ADM is unclear. Of those who responded to the survey, plant losses experienced due to the disease could be as high as 20%, with the aquilegia crop accounting for anything up to 10% of sales of herbaceous plants generally. Another issue was the level of press coverage warning of the dangers from the disease which led to concern among growers that a loss of retail sales may result. This would also be compounded by the inability of amateur growers to control the disease once established in gardens.

Understanding the pathogen responsible for aquilegia downy mildew, in particular through identifying pathways of introduction, will ensure effective management strategies can be developed to minimise future losses from the disease. Establishing an effective and economic strategy for the control of the downy mildew pathogen early in the supply chain will reduce the risk of financial loss through plant wastage and consumer dissatisfaction with the product. This is particularly important while the pathogen is at an early stage of establishment in the UK.

Action points for growers

- Even though there is no definitive data to suggest that seed is the main source of contamination, it is recommended that as the disease is in circulation, suppliers should check stock plants for symptoms and seed batches for contamination.
- As far as possible ensure any starting plant material is disease-free.
- Isolate and clearly label bought-in seed, young plants and liners to permit traceability should problems arise.
- Ensure adequate air circulation around plants to minimise prolonged periods of leaf wetness. If possible, avoid overhead irrigation as this is likely to exacerbate the disease. If it is necessary to irrigate using overhead systems then do this early on in the day to allow foliage to dry out quickly.
- Practice good nursery hygiene, clean up crop debris between crops and at the end of the season to minimise the risk of carry-over of the disease. Place any infected plants into covered bins prior to disposal.
- Maintain an effective preventative fungicide programme, ensuring a range of products with different modes of action are included to minimise the risk of resistance development.

SCIENCE SECTION

Introduction

Aquilegia downy mildew (ADM) is caused by a currently unnamed *Peronospora* species which has not been reported outside of the UK. The first reported occurrence of ADM appears to be on a nursery in 2011. Since its first report the disease has become more widespread causing loss of plants both on nursery and in public/private gardens. Some of these losses have led to extensive coverage in the national press.

Symptoms are typical of those produced by other downy mildews with affected leaves developing angular, yellow patches which eventually turn brown. On the underside of these leaves a fine spore bearing fungal growth (white/purple in colour) can often be seen. The disease spreads quickly and has led to rapid plant death.

A literature review revealed there was no epidemiological or control data available either from the UK or overseas on ADM. However, there is an abundance of knowledge available for downy mildews caused by other *Peronospora* species which can be used to help determine potential sources of infection, the conditions required for infection and sporulation and control strategies.

This project aimed to determine whether the *Peronospora* species responsible for ADM is present in seed batches and whether infected seed can act as a primary source of infection. It is hoped that the data produced will help growers to combat the threat from this newly emerging downy mildew.

Materials and methods

1. Grower survey

To explore the impact of ADM on the industry and the retail market, a grower questionnaire (see appendices) was produced by the project grower co-ordinator and distributed to the industry on request. The aim of the survey was to provide details on how widespread ADM was across the industry, the potential value of lost sales and whether the problem was focused around a particular plant or seed source.

2. Seed contamination by the Peronospora responsible for aquilegia downy mildew

    2.1. Seed washing

For each sample, 100 seed were counted into a 1.5 ml micro-centrifuge tube and 200 µl of sterile distilled water (SDW) added. To dislodge any oospores from the seed the tubes were shaken using a vortex mixer, set at maximum speed, for 5 minutes. At the end of shaking the

wash water was removed, placed into a fresh 1.5 ml micro-centrifuge tube and centrifuged at 16,000g for 5 minutes. Following removal of the supernatant the pellet was re-suspended in 40 µl of SDW and the number of spores present counted. Counts were made on five batches of 100 seed for each aquilegia variety. Where there were multiple packets of seed purchased, all packets were bulked together and thoroughly mixed before the start of testing.

2.2. Presence of aquilegia downy mildew DNA in seed

DNA extraction from seed

DNA was extracted from seed using a CTAB extraction method and Kingfisher™ mL magnetic particle processor. For each aquilegia variety, 5 replicates of 100 seed were counted into a labelled 5 ml capped transport tube containing a single 7/16 inch ball bearing and 2 ml of CTAB soil extraction lysis buffer with 2% antifoam B added. Two additional tubes were prepared, 1) minus seed and 2) with seed and ADM sporangia, these tubes acted as negative and positive controls respectively. All seed samples and controls were ground for 2 minutes using a Kleco 96 grinding machine; if required a further 2 ml of lysis buffer (with antifoam) was added and the sample ground for an extra 30 seconds. The ground sample and negative control were transferred from the 5 ml tubes to 2 ml centrifuge tubes containing 250 µl Buffer B (Wizard Magnetic DNA purification system for food) and 750 µl of precipitation solution (Wizard kit). The tubes were briefly vortex mixed, centrifuged at 13,000 rpm for 10 minutes and 750 µl of the supernatant transferred to a clean 2 ml centrifuge tube. To this, 50 µl of Magensil beads (Wizard kit) and 600 µl of isopropanol were added, the samples vortex mixed and incubated for 5 minutes at room temperature with occasional mixing by tube inversion. DNA was retrieved via the 'gDNA' program on the Kingfisher using an incubation period of 5 minutes at 65ºC. The extracted DNA was stored at -25ºC until required.

DNA extraction from leaf material

DNA from leaf material was extracted using a CTAB extraction method and Kingfisher™ mL magnetic particle processor. Two to three aquilegia leaves showing typical symptoms of a downy mildew infection were removed from a plant and placed in a labelled double mesh grinding bag containing 4 ml of CTAB soil extraction lysis buffer. An additional grinding bag was prepared, using uninfected leaves, as an extraction negative control. The sample, and negative control, was ground for approximately 15 seconds using a Homex flat bed grinder and the resulting liquid transferred into a 2 ml centrifuge tube. The lysate was separated from the debris by centrifugation at 10,000 rpm for 5 minutes and 1ml of the lysate transferred to

a fresh 2 ml centrifuge tube containing 250 µl Buffer B (Wizard Magnetic DNA purification system for food) and 750 µl of precipitation solution (Wizard kit). From this point the procedure used was the same as for the extraction from seed.

PCR primer set testing

The real time PCR primer set (Pfar), developed in the AHDB Horticulture funded project FV 228, to detect *Peronospora farinosa* f. sp. *betae* in red beet was known to detect a number of *Peronospora* species and so was identified as having potential for detecting the peronospora responsible for causing aquilegia downy mildew.

The existence of a DNA sequence for the peronospora responsible for ADM (GenBank® reference 242036_7C3leaf2013) meant that, in addition to testing the Pfar primer set, it was possible to design primer sets (Table 1) with the potential to specifically detect ADM.

Table 1. Description of primer sequence for the three primer sets identified as having potential for detecting the *Peronospora* species responsible for causing aquilegia downy mildew.

Primer set		Sequence (5' – 3')
Paq1	Forward	ATG TCT AGG CTC GCA CAT CG
	Reverse	CGC ACA GCA CAA TTT CCC AA
Paq2	Forward	TGA ACA TAC TGT GGG GAC GA
	Reverse	CTC GAC AGC CAA AAC TGT CA
Paq3	Forward	TTC CAC GTG AAC CGT ATC AA
	Reverse	ATA CCG CGA ATC GAA CAC TC

All primers were partially validated using DNA extracted from aquilegia plant material known to be infected by downy mildew and other oomycete pathogens including *Peronospora violae* (pansy downy mildew), *Peronospora belbahrii* (basil downy mildew), *Plasmopara obducens* (impatiens downy mildew), *Peronospora farinosa* (red beet downy mildew), *Phytophthora ramorum*, *Phytophthora kernoviae*, *Phytophthora cinnamomi*, *Phytophthora cactorum* and *Pythium ultimum*. Amplification reactions were carried out using GoTaq® G2 Flexi DNA Polymerase using the manufacturers recommended concentrations. The PCR reaction consisted of denaturation (94°C) for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 55°C for 60 seconds and 72°C for 90 seconds, with a final extension step of 72°C for 10 minutes. PCR products were separated by electrophoresis in a 2% agarose gel amended with ethidium bromide for DNA visualisation.

Quantitative DNA analysis

To obtain quantitative DNA data, the primer set showing the greatest potential in the traditional PCR was selected for further testing using a TaqMan® real time PCR assay. Amplification reactions were carried out using TaqMan environmental mastermix (Applied Biosystems) and Evagreen® fluorescent dye; this negated the need for a probe to be developed.

DNA was analysed using an Applied Biosystems 7900HT Fast Real-Time PCR machine with temperature cycles consisting of denaturation 50°C for 2 minutes, annealing or thickening at 95°C for 10 minutes and 40 amplification cycles alternating between 95°C for 15 seconds and 60°C for 1 minute.

2.3. Transmission of ADM from contaminated seed to infected plants.

A trial was established during November 2015 to determine 1) whether seed contaminated with ADM would lead to symptomatic plants and 2) the environmental conditions required for crop infection to occur.

The trial was established with the aquilegia variety *Aquilegia vulgaris* var. *plena* 'Christa Barlow' and employed three watering regimes 1) watering from below, 2) watering from above in the morning and 3) watering from above in the evening. For each watering regime 10 replicate seed trays were sown with each tray containing 50 seeds. Trays were transferred to the glasshouse and kept at a constant temperature of 18°C with a 16 hour day/8 hour night lighting set up. Half the replicates for each watering regime were covered each evening in order to increase the humidity with the remaining 5 trays left uncovered. Plants were checked daily for any symptoms of disease. Tinytag data loggers were placed within the trial to record temperature and humidity conditions. Any disease development was analysed in relation to watering regime, temperature and humidity.

Results

1. Grower survey

A total of 28 survey questionnaires were distributed with seven completed and returned. Of the returned questionnaires, 5 growers reported having had an outbreak of ADM. Five out of the seven also reported that a widespread outbreak of ADM would have a significant impact on their overall sales.

The average value of Aquilegia sales among these growers was £6,785.00, with high and low sales figures of £16,000 and £2,000 respectively.

Of the seven respondents, 1) 4 bought their plants as young plants or liners; 2) 1 bought both seed and young plants; and 3) 2 bought seed to raise their own plants.

Two of the growers who raised plants from seed reported an outbreak of ADM; both growers bought seed from Jelitto. Growers who bought young plants and liners did not know the origin of the seed used.

There did not seem to be any link between varieties grown and the prevalence of ADM on nurseries.

2. Seed contamination by the Peronospora responsible for aquilegia downy mildew

Seed samples from 14 aquilegia varieties (Table 2) were obtained to determine whether the pathogen responsible for ADM could be detected in seed. The seed producer and variety for twelve of the samples were selected based on results of the grower survey and in consultation with the grower co-ordinator. The remaining two samples were obtained from a site known to have high levels of ADM.

Table 2. Aquilegia varieties used in testing to determine whether aquilegia seed could be a source of aquilegia downy mildew infection.

Sample	Variety	Supplier	Quantity
1	*Aquilegia caerulea* hybrid 'Blue Star'	Jelitto	4g
2	*Aquilegia chrysantha* 'Yellow Queen'	Jelitto	4g
3	*Aquilegia vulgaris* 'Alba' [Munstead White]	Jelitto	4g
4	*Aquilegia vulgaris* var. *plena* 'Blue Barlow'	Jelitto	4g
5	*Aquilegia vulgaris* var. *plena* 'Ruby Port'	Jelitto	4g
6	*Aquilegia vulgaris* var. *plena* 'Christa Barlow'	Jelitto	4g
7	*Aquilegia flabellata* 'Mini-star'	Moles	2,500 seed
8	Aquilegia Mckana Giant Hybrids	Moles	4,000 seed
9	*Aquilegia vulgaris* 'William Guinness'	Moles	2,500 seed
10	*Aquilegia vulgaris* 'Winky Red & White'	Moles	1,000 seed
11	*Aquilegia vulgaris* 'Nora Barlow Mixed'	Johnsons	
12	*Aquilegia vulgaris* 'Biedermeier Mixed'	Johnsons	
13	*Aquilegia vulgaris* var. *stellata* – White	Touchwood Plants	100 seed
14	AAC Large Maroon & Yellow	Touchwood Plants	200 seed

2.1. Seed washing

Of the fourteen varieties tested, oospores were only found in the washings from the *A. vulgaris* var. *plena* 'Christa Barlow' sample bought from Jelitto (Figure 1). The overall level of oospore contamination in the Christa Barlow sample was low with an average of 1 oospore per 100 seed (Table 3). Oospores were found in two of the five replicate washings with two and three oospores present in the two replicates.

To check whether the oospores could be from non-obligate oomycete pathogens e.g. *Pythium* or *Phytophthora* species, 500 seed form the Christa Barlow seed sample were plated onto potato dextrose agar. No colonies resembling *Pythium* or *Phytophthora* species were observed emerging from any of the plated seed suggesting these species were not responsible for production of the oospores.

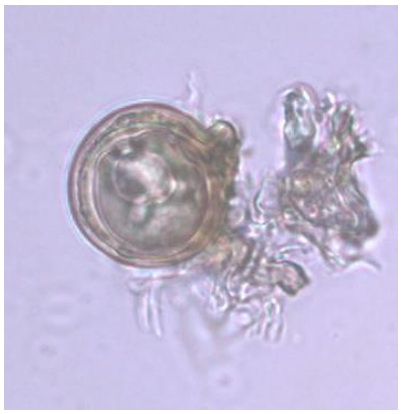

Figure 1. Image of oospore found in washings from seed of *Aquilegia vulgaris* var. *plena* 'Christa Barlow' bought from Jelitto seed company.

Table 3. Average oospore count from aquilegia seed washings of seed samples from 14 varieties.

Aquilegia variety	Average number of oospores/100 seed
A. caerulea hybrid 'Blue Star'	0
A. chrysantha 'Yellow Queen'	0
A. vulgaris 'Alba' [Munstead White]	0
A. vulgaris var. *plena* 'Blue Barlow'	0
A. vulgaris var. *plena* 'Ruby Port'	0
A. vulgaris var. *plena* 'Christa Barlow'	**1**
A. flabellata 'Mini-star'	0
A. Mckana Giant Hybrids	0
A. vulgaris 'William Guinness'	0
A. vulgaris 'Winky Red & White'	0
A. vulgaris 'Nora Barlow Mixed'	0
A. vulgaris 'Biedermeier Mixed'	0
A. vulgaris var. *stellata* – White	0
AAC Large Maroon & Yellow	0

2.2. Presence of aquilegia downy mildew DNA in seed

The primer set produced for the detection of *P. farinosa* (Paqfar) did not detect DNA of ADM extracted from infected aquilegia leaf material (Table 4); as a result this primer set was not taken any further in this project. The primer sets Paq1, Paq2 and Paq3 all produced PCR products of the correct length (572 base pairs (bp)) in the presence of ADM DNA indicating that they could be suitable for its detection. The specificity of these primers was determined by challenging them with DNA from other oomycete pathogens including the downy mildew pathogens *P. belbahrii*, *P. violae*, *P. obducens* and *P. farinosa* and a number of *Pythium* and *Phytophthora* species. Both Paq1 and Paq2 produced a PCR product 572 bp long in the presence of DNA from all the downy mildew pathogens tested. Paq3 produced a product for *P. violae*, *P. obducens* and *P. farinosa*, but not *P. belbahrii*. The challenge to phytophthora and pythium DNA showed that Paq2 did not detect DNA of any of the species tested whereas Paq1 and Paq3 both detected *P. ultimum*. As *P. ultimum* is a ubiquitous pathogen which could be found in seed samples both Paq1 and Paq3 were excluded from any further analysis.

Table 4. Detection of DNA of a panel of oomycete pathogens using primer sets Paq1, Paq2, paq3 and Pfar using a PCR agarose gel test.

Oomycete pathogen	Detection of pathogen DNA*			
	Paq1	Paq2	Paq3	Pfar
Peronospora (Aquilegia)	✓	✓	✓	x
Peronospora belbahrii	✓	✓	x	x
Peronospora violae	✓	✓	✓	✓
Plasmopara obducens	✓	✓	✓	✓
Peronospora farinosa	✓	✓	✓	✓
Phytophthora ramorum	x	x	x	x
Phytophthora kernoviae	x	x	x	x
Phytophthora cinnamomi	x	x	x	x
Phytophthora cactorum	x	x	x	x
Pythium ultimum	✓	x	✓	x

* ✓ indicates PCR product produced 572 bp long for P.aq1, P.aq2 and P.aq3, and 370 bp for Pfar; x indicates no PCR product produced.

The results from the PCR testing of Paq2 indicated it was the most suitable primer set for further testing using real time PCR and Evagreen® fluorescent dye. The results presented in Table 5 represent the mean cycle threshold (Ct) value for the testing of Paq2 against the oomycete pathogen panel previously described. The Ct value represents the number of amplification cycles after which fluorescence, and therefore DNA, can be detected above a background level. The Ct value is inversely proportional to the amount of target DNA, so the lower the Ct value the more target DNA is present in the sample. A value of 40 (the maximum number of amplification cycles) indicates a negative result (either no DNA present in the sample or the DNA present was not detected by the primer set). The Ct values presented in Table 5 indicated that Paq2 detected DNA of ADM and *P. belbahrii*, but did not detect DNA from any of the other oomycete pathogens tested. This result indicated that the specificity of Paq2 increased in the real time PCR test compared to the traditional PCR test. However, the high Ct value obtained (33), when Paq2 was tested against ADM DNA extracted from a heavily infected and sporulating leaf, suggested that the test needed further optimisation; a Ct value of 25 or less would be expected from this type of positive control using a fully optimised test.

When DNA, extracted from the aquilegia seed samples used in this project, was tested using Paq2 in the real time PCR test, no ADM DNA was detected in any of the samples i.e. all samples returned Ct values of 40. This either indicated that ADM DNA was not present in any of the seed samples tested or that the DNA present was at a level too low to be detected in the non-optimised test.

Table 5. Mean cycle threshold (Ct) value results for primer set Paq2 when used against DNA extracted from the Peronospora species responsible for aquilegia downy mildew and a panel of oomycete pathogens.

Oomycete Pathogen DNA	Ct value
Peronospora (Aquilegia)	33
Peronospora belbahrii	37
Peronospora violae	40
Plasmopara obducens	40
Peronospora farinosa	40
Phytophthora ramorum	40
Phytophthora kernoviae	40
Phytophthora cinnamomi	40
Phytophthora cactorum	40
Pythium ultimum	40

2.3. Transmission of ADM from contaminated seed to infected plants.

The trial established during November 2015 using the aquilegia variety *A. vulgaris* var. *plena* 'Christa Barlow' (potentially contaminated with oospores of the pathogen responsible for ADM) did not result in any plants showing symptoms of ADM by the end of this project. The trial will be continued as part of project HNS 196a with final results for the trial reported in the report for that project.

Discussion

The grower questionnaire provided information which suggested that ADM could be seed borne. Seed were tested for contamination by the ADM pathogen using two methods, 1) seed washing to determine the presence of surface contamination by oospores and 2) through analysis for peronospora DNA. The presence of oospores, and a good correlation between oospore number and level of peronospora DNA would provide an indication that the seed contamination was on the surface, however the absence of oospores and presence of peronospora DNA would suggest the seed contamination was more likely to be internal. The presence of oospores in one of the aquilegia seed samples tested suggested that a surface contamination was present and that seed could be a source of inoculum for the ADM pathogen. Research on basil downy mildew (Garibaldi *et al*., 2004) showed that *Peronospora belbahrii* was seed-borne, with seed contamination as low as 0.02% leading to visible infection of crops. If we assume that the oospores found in each replicate of the *A. vulgaris* var. *plena* 'Christa Barlow' seed sample came from one seed in that replicate then the overall level of contamination for the sample can be estimated at 0.2% (2 contaminated seed in 500 seed tested); a level of infection higher than that found to lead to crop infection for basil. Even though the contamination of aquilegia seed was at a low level, any unnoticed infections resulting from these seed could spread quickly leading to widespread infection of a nursery. To date, the trial to establish whether contaminated aquilegia seed leads to crop infection has not resulted in any diseased plants. The timing of the project meant that the trial was carried out in a glasshouse maintained at 18ºC, as little is known about germination of oospores of this particular *Peronospora* species it is possible that optimal germination of oospores occurs at temperatures lower than those used in the trial.

A primer set (Paq2) was produced which detected DNA of the *Peronospora* species responsible for ADM. A limited validation of this primer set in a PCR test indicated that it did not detect the non-obligate *Pythium* or *Phytophthora* species screened but did detect pathogens responsible for downy mildew infections on other crops. The use of Paq2 in a real time PCR assay improved the specificity of the primer set (only cross reacting with *P.*

belbahrii). The funding available within the project did not allow for optimisation of real time PCR test conditions which meant that high DNA levels were required before detection occurred. As a result it was not possible to determine whether the lack of a positive result from testing the aquilegia seed samples was because ADM DNA was not present in the sample or it was present but below the level of detection of the test.

Even though there is not definitive data to suggest that seed is the main source of contamination it is recommend that as the disease is in circulation suppliers should check stock plants for symptoms and seed batches for contamination.

Conclusions

- ADM was commonly reported by the respondents to the grower questionnaire.
- Reporting of disease was most frequent from nurseries growing plants from seed.
- There did not seem to be a link between varieties grown and the prevalence of ADM on nurseries.
- Oospores were found in seed washings suggesting there was surface contamination of seed with the pathogen responsible for ADM.
- Seed contamination rates were low but could still lead to infection on nurseries.
- Undetected low levels of infection can quickly spread across a nursery leading to widespread infection.

Knowledge and Technology Transfer

Herbaceous Perennials Technical Discussion Group, 'New pests and diseases of herbaceous plants', Winter meeting – RHS Wisley, Woking, Surrey, Tuesday 16 February 2016.

References

Garibaldi A, Minuto A, Minutto G, Gullino ML (2004). Seed transmission of *Peronospora* sp. of basil. Zeitschift fur pflanzenkrankheiten und pflanzenschutz, 111, 465-469.

Appendices

Aquilegia Downy Mildew
Grower Survey June/July 2015
Following the recent decision by the AHBD Hardy Nursery Stock Panel to fund research into this relatively new disease we are asking growers to contribute any experiences they may have had with the problem. Dr Phillip Jennings of FERA has been commissioned to begin his research by looking at the source of outbreaks and in particular at the likelihood of seed contamination being a contributory cause.
If you grow Aquilegias for any market sector we would be grateful if you could help the industry by answering the following questions even if you have not experienced any problems.
All answers will be held in confidence but if you would prefer not to answer any question please put N/A in the relevant box.

Have you experienced an outbreak of Aquilegia Downy Mildew (ADM) on your nursery?	Yes / No (Please circle)
What is the approximate wholesale value of your annual crop(s) of Aquilegia?	£_____
Would a wide spread outbreak of ADM have a significant financial impact on your overall sales?	Yes / No (Please circle)
Which of the following production methods do you use to raise Aquilegias? A Seed bought in and sown at the nursery. B Young plants (seedlings) bought in for potting. C Liners bought in for potting.	Please tick box (s) ☐ ☐ ☐
If you have had ADM on the nursery was it first seen on: A. Seed raised plants B. Seedlings bought in C. Liners bought in	 ☐ ☐ ☐
Please select from the following Aquilegia types that you grow on your nursery. A caerulae Origami Series B caerulea Musik series C caerulea Songbird or State series D flabellata Spring Magic or Cameo series E vulagaris Winky or Clementine series F Other vulgaris or caerulea species or hybrids Please indicate	Please tick ☐ ☐ ☐ ☐ ☐ ☐
If you grow plants from seed sown at your nursery please indicate the companies from whom you have purchased the seed?	
If you have observed any links between your supplies of either seed or production material and an outbreak of ADM please give details of this in the box e.g. all affected plants came from the same seed source.	

