Grower Summary

HNS 196a

Identification of factors which influence infection and control of the newly emerged Peronospora causing downy mildew on aquilegia

Final 2016

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AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

AHDB Horticulture is a Division of the Agriculture and Horticulture Development Board.

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Project leader:	Dr Philip Jennings, Fera
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Key staff:	Ms Gilli Thorp
Location of project:	Fera
Industry Representative:	Mr Toby Marchant, Orchard Dene Nurseries, Lower Assendon, Henley-on-Thames, Oxfordshire, RG9 6AL
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SCIENCE SECTION

Introduction

Aquilegia downy mildew (ADM) is caused by a species of *Peronospora* which is currently unnamed and has not been reported outside of the UK. The first reported occurrence of the downy mildew appears to have been on a nursery in 2011. Since this report the disease has become more widespread causing plant loss both on nursery and in 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 whitish/purple fungal growth can often be seen. The disease can spread quickly and lead to rapid plant death, which generally occurs when the disease moves from the leaves into the crown, leading to crown and then plant death

Little or no work has been published on the disease, and as a result it is difficult to establish from the literature why the disease has become more prevalent. This study aimed to establish the conditions required for infection and sporulation, the type of spores involved and the conditions required for their survival. Strategies for disease control (cultural and chemical) will be developed.

Materials and methods

Unless otherwise stated *Aquilegia* Mckana Giant Hybrids were used in all studies within this project.

1) Conditions required for infection of aquilegia by downy mildew

a) Leaf wetness

Five replicate aquilegia leaves were placed in humidity chambers maintained at 19, 49, 81, 97 and 100% using concentrated salt solutions (**Table 1**). Leaves were misted with water to run-off and leaf wetness recorded every half hour until leaves were dry or 48 hrs which ever was the longest.

Table 1. Solutions used to achieve different moisture chamber relative humidity.

Solution	Recorded humidity (%)
Lithium chloride	19.9
Potassium carbonate	49.0
Sodium chloride	81.4
Potassium chloride	97.5
Water	100

b) Temperature

Conidial germination

Conidial germination of the peronospora responsible for ADM was examined at five temperatures (5, 10, 15, 20, and 25°C) and four incubation times (2, 4, 6, and 24 hours). ADM conidia were washed from previously infected leaves and adjusted to a concentration of 10^4 conidia/ml^{-1}. Conidial suspensions were placed at the different temperatures, with 20 µl removed on completion of each incubation period and germination counts carried out on 100 spores. Conidia were classed as germinated when the length of the germ tube was greater than the diameter of the conidia. For each time and temperature three replicate counts were made.

Plant infection

Infection of aquilegia plants by downy mildew was examined at five temperatures (5, 10, 15, 20, and 25°C) and six leaf wetness periods (1, 2, 4, 6, 10 and 18 hours). At each temperature and timing three replicate pots containing 20 aquilegia plants at the first true leaf stage were inoculated to run off with a spore suspension containing 10^4 ADM conidia/ml^{-1} water and incubated at 100% humidity. At the end of the incubation period plants were removed from the incubator and leaves air dried to prevent further infection (penetration of the leaf epidermis by an infection peg produced by a germinating spore on the leaf surface). Plants were transferred to the glasshouse and grown at 18°C for 10 days and plant infection assessed based on leaf yellowing and sporulation.

Results from the leaf wetness and temperature experiments were combined to establish a risk grid for aerial infection of aquilegia by downy mildew.

2) Survival of conidia

a) Temperature

For each test, aquilegia plants inoculated 10 to 14 days earlier were wetted overnight to stimulate conidia production. Sporulating leaves were removed from the plant and three replicate leaves exposed to temperatures of 5, 10, 15, 20 and 25°C. Conidia were exposed to the different temperatures for periods of 24 and 48 hours. Following completion of each exposure conidia were washed from the three replicate leaves and spore germination assessed following incubation at 20°C for 24 hours. Germination was assessed by counting 100 conidia per replicate. Conidia were classed as germinated when the length of the germ tube was greater than the diameter of the conidia.

b) Humidity

The experiment described in 2a was repeated under four humidity regimes (19, 49, 81 and 100%) and three exposure times (6, 24 and 48 hours).

3) Control

A small scale trial was carried out on the five fungicide products known to have activity against oomycetes (**Table 2**). Eight week old aquilegia plants were treated with fungicide either 2 or 10 days prior to inoculation with the ADM pathogen. All products were applied according to the manufacturer's recommended rate. Control plants were sprayed with an equivalent volume of sterile distilled water. Plants were inoculated with a conidial suspension at a concentration of 10^4 conidia/ml to run off and incubated at 100% humidity overnight to ensure infection. Plants were maintained at 18ºC for 15 days and then the number of leaves per plant showing downy mildew symptoms assessed. Each treatment was replicated five times. Data were analysed using a two way analysis of variance.

Table 2. Fungicides used in efficacy testing against the *Peronospora* species responsible for aquilegia downy mildew.

Product	Active ingredient	Application	Application rate (per L)
Percos	Ametoctradin (300 g/kg) + dimethomorph (225 g/L)	Foliar spray	0.4 ml
Fenomenal	Fenamidone (60 g/kg) + fosetyl-aluminium (600 g/kg)	Foliar spray/	2.25 g
Fubol Gold	Mancozeb (640 g/kg) + metalaxyl-M (40 g/kg)	Foliar spray	0.95 g
Revus	Mandipropamid (250 g/L)	Foliar spray	0.3 ml
Signum	Boscalid (267 g/kg) + pyraclostrobin (67 g/kg)	Foliar spray	0.75 g

Insufficient plants were obtained with a crown infection so trials to control this type of infection were not carried out.

Results

1) Conditions required for infection of aquilegia by downy mildew

a) Leaf wetness

Increasing humidity increased the length of time it took for water to dry from the surface of aquilegia leaves (**Figure 1**). Leaves dried in 1.5 hours at a humidity of 20%, 3 hours at 50% humidity and 8 hours at 80% humidity. Leaves subjected to 100% humidity remained wet for the duration of the test.

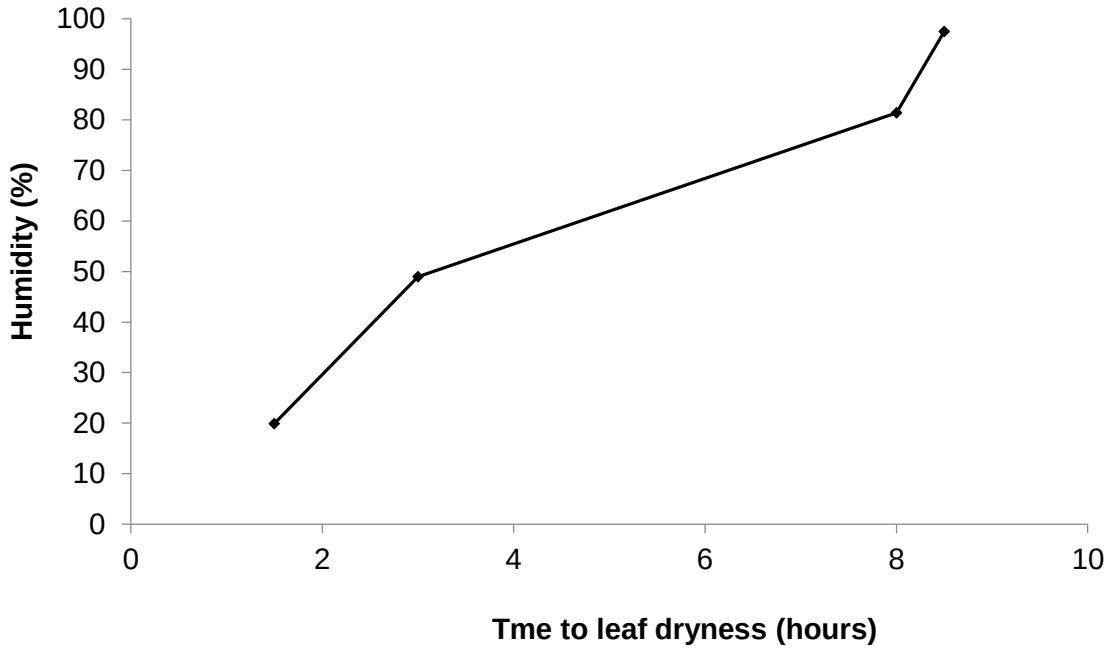

Figure 1. The effect of humidity on the drying time of aquilegia leaves.

b) Temperature

Conidial germination

Conidial germination occurred at all the test temperatures, however the level of germination at 25ºC was very low (2%) even after 24 hours (**Figure 2**). Germination first occurred after 2 hours at 10 and 15ºC with 2% and 19% germination respectively. After 4 hours incubation conidia had also germinated at 5 and 20ºC, with a significantly higher germination rate at 5ºC.

A high level of conidial germination (90%) was recorded for temperatures between 5 and 20ºC after 24 hours incubation.

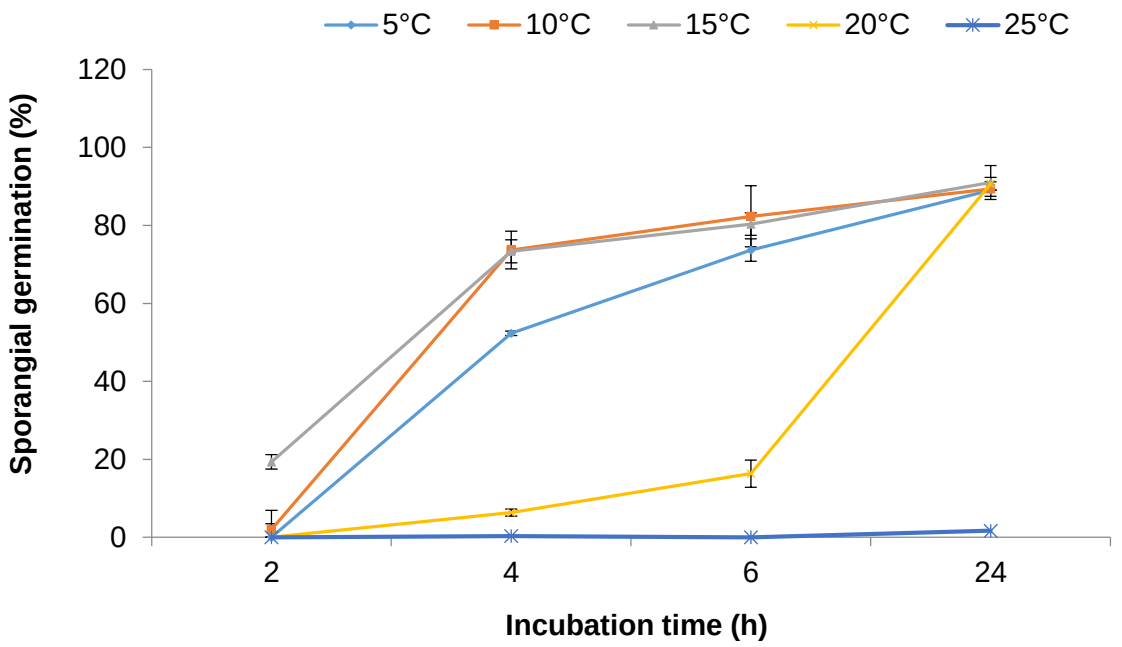

Figure 2. Effect of temperature on germination of conidia produced by the *Peronospora* species responsible for aquilegia downy mildew. Error bars represent standard error of the mean.

Plant infection

Infection of aquilegia by downy mildew occurred at temperatures between 5 and 20ºC dependant on the period of leaf wetness, no infection of aquilegia plants occurred at 25ºC (**Figure 3**). No infection occurred at any temperature where leaf wetness was less than 6 hours. An a leaf wetness period of 6 hours resulted in infection of aquilegia plants at 15 and 20ºC; however the level of infection was low with only 2% of plants infected. After 10 hours of leaf wetness the level of infection at 15 and 20ºC increased to 26%, infection also occurred after 10 hours of leaf wetness at 10ºC with 23% of plants infected. For infection to occur at 5ºC at least 18 hours of leaf wetness was required.

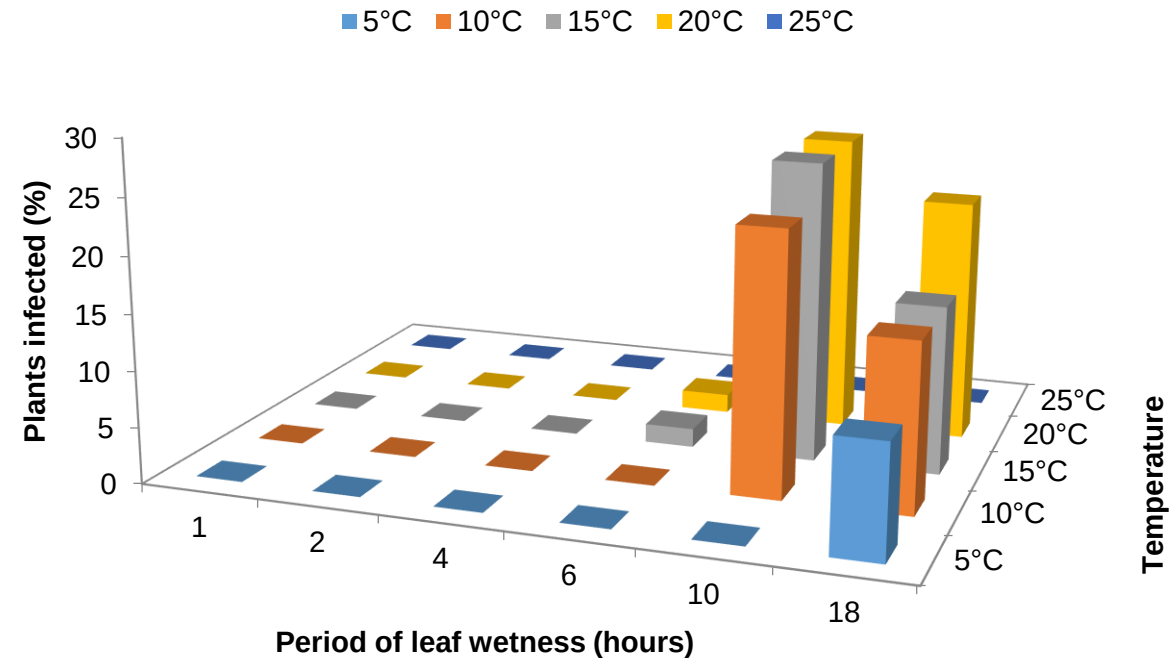

Figure 3. Effect of temperature and length of leaf wetness on infection of aquilegia by downy mildew.

Based on the leaf wetness and infection data a risk grid was created (**Figure 4**) to provide an easy guide to the risk of aquilegia infection by downy mildew. These data indicated that the risk of infection is highest when humidity was greater than 90%. Infections could occur down to 70% humidity where temperatures were between 15 and 20ºC. When humidity was lower than 70% the risk of downy mildew infection of aquilegia was negligible.

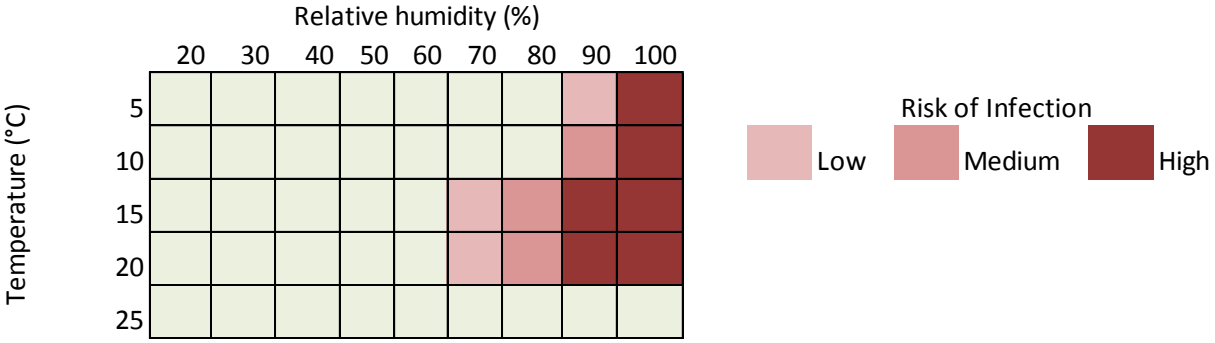

Figure 4. Temperature and humidity risk grid for the infection of aquilegia plants by the *Peronospora* species responsible for downy mildew.

2) Survival of conidia

a) Temperature

Conidia survived at temperatures between 5 and 25ºC when exposed for 48 hours. There was no significant (p = 0.05) difference in germination (and hence survival) of conidia following exposure at 5, 10 or 15ºC for 24 and 48 hours, however the longer exposure time at 20 and 25ºC resulted in a decrease in conidial germination.

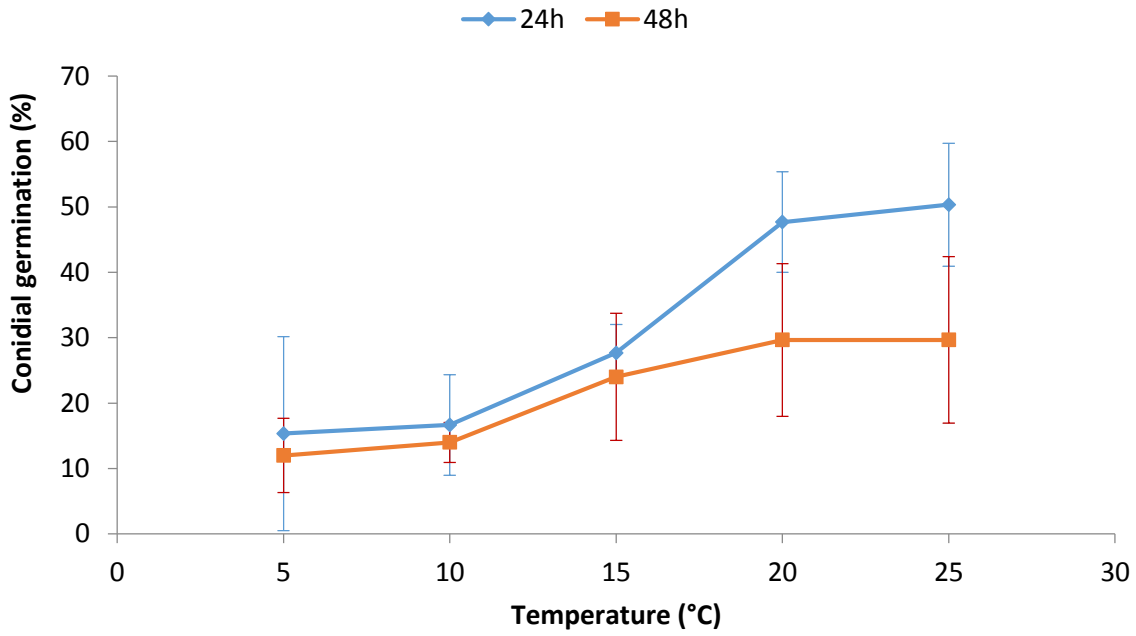

Figure 5. The effect of temperature on survival of conidia produced by the *Peronospora* species responsible for aquilegia downy mildew. Error bars represent standard error of the mean.

b) Humidity

Exposure of conidia to 100% humidity did not have an adverse effect on conidial germination over the three exposure periods examined (Figure 6). Reducing the humidity to 50% or less reduced conidial germination to less than 1% for exposure times of 24 and 48 hours. Exposure of conidia to 20% humidity for 6 hours reduced germination compared to exposure at 50, 80 or 100% humidity. Although differences in mean germination were seen at the different humidity levels and exposure times the wide variability in germination across the replicates meant the differences were not significant (p = 0.05).

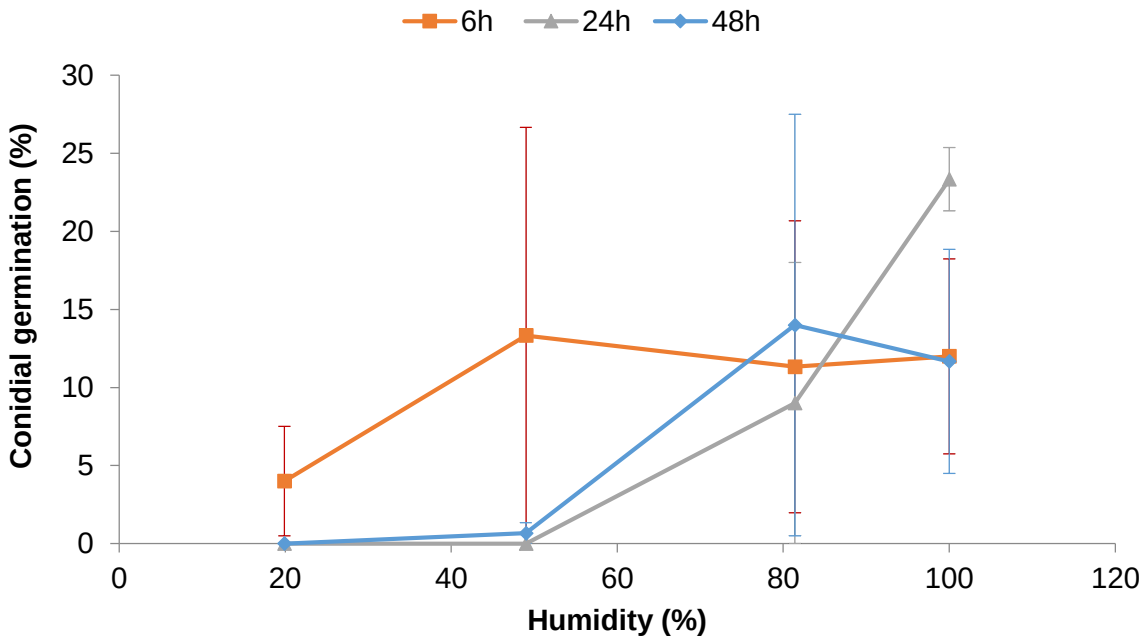

Figure 6. The effect of humidity regime and exposure time on the survival of conidia produced by the *Peronospora* species responsible for aquilegia downy mildew. Error bars represent standard error of the mean.

3) Control

Five products were tested as protectant foliar sprays for efficacy against aquilegia downy mildew. The sprays were applied as a protectant treatment either 2 or 10 days before plant inoculation. When applied as a protectant 2 days prior to inoculation, Fubol Gold (metalaxyl-M), Fenomenal (fenamidone and fosetyl-aluminium), Signum (boscalid and pyraclostrobin) and Revus (mandipropamid) all gave 100% control of downy mildew infections (**Figure 7**). The application of Percos (ametoctradin and dimethomorph) was less effective than the other products; however the control achieved was still over 90%. The application of Fubol Gold 10 days prior to inoculation with downy mildew still gave 100% protection of aquilegia. The level of control achieved by Fenomenal, Signum, Percos and Revus were all lower than when the same products were applied 2 days prior to inoculation and ranged from 73% for Signum to 21% for Percos.

No phytotoxicity effects were observed following any of the treatments applied.

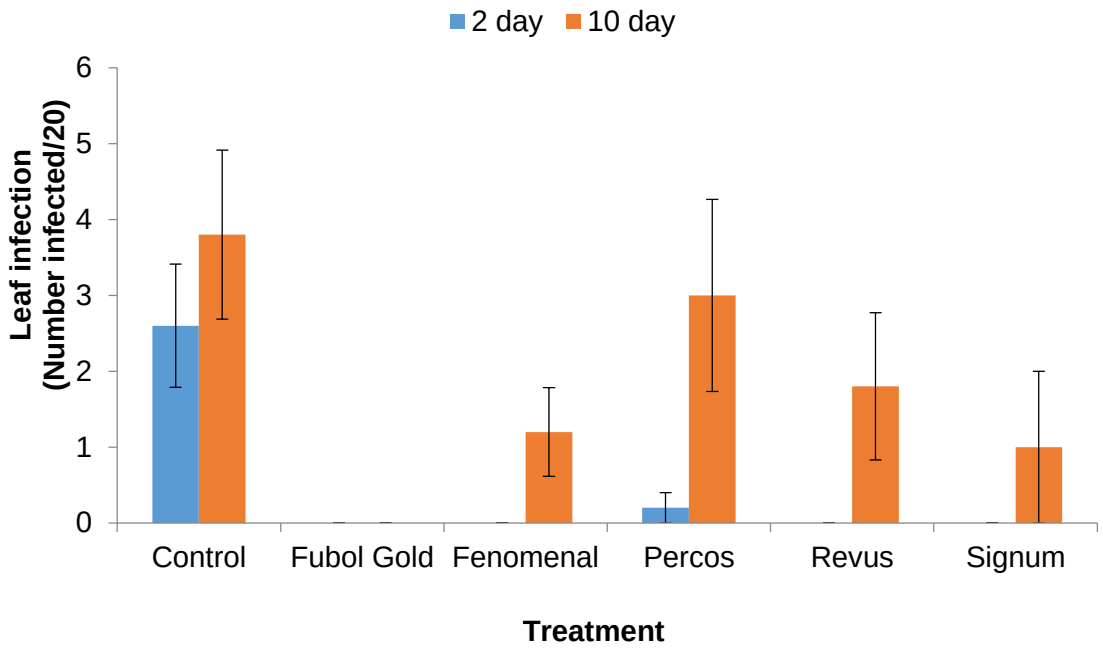

Figure 7. The effect of preventative fungicide application timings on aquilegia downy mildew symptoms. Error bars represent standard error of the mean. The least significant difference (5%) = 1.99

Discussion

In this project studies have focused on the effect of temperature and period of leaf wetness on infection of aquilegia by downy mildew, the effect of temperature and humidity on survival of conidia and methods of control.

Experiments to determine the effect of temperature on infection of aquilegia by downy mildew showed that symptoms were seen for temperatures between 5 and 20ºC, with no infection of aquilegia at 25ºC. This is a narrower infection window than reported for downy mildew pathogens such as *Peronospora belbahrii* (basil downy mildew) where infection occurred between 5 and 25ºC (Jennings *et al*., 2016). Within this project the optimum temperature for infection of aquilegia was around 20ºC, which is consistent with the results for *P. belbahrii* (Jennings *et al*., 2016; Garibaldi *et al*., 2007). A minimum period of 6 hours of leaf wetness was required for aquilegia to become infected. This was similar to the work carried out by Garibaldi *et al*. (2007) on Basil downy mildew, but longer than reported by Jennings (2016) where infection occurred after 4 hours leaf wetness under optimum temperature conditions. Combining humidity leaf wetness data with the infection data indicated that the risk of aquilegia infection was highest where the humidity was greater than 90%, as infection could occur at temperatures between 5 and 20ºC. Infections could also occur at a humidity of 70% at temperatures between 15 and 20ºC. Where the humidity was lower than 70% the risk of downy

mildew infection of aquilegia was negligible. These data may help explain why ADM is more prevalent in gardens during the autumn than the summer when plant leaves tend to be wetter as a result of heavy dews etc.

Within this project the effect of light on infection was not investigated however this has been examined for *Peronospora* species such as *P. belbahrii* and *P. violae* (Pansy downy mildew). For these pathogens it was shown that infection only occurred in the dark, any incubation in the light totally inhibited infection (Jennings *et al*., 2016; Jennings *et al*., 2009). This is also consistent with work undertaken by Cohen *et al*. (2013a) who demonstrated that exposure of *P. belbahrii* infections to light supressed the formation of conidia. As infection of aquilegia is also caused by a *Peronospora* species, it is likely that light will have a similar negative effect on infection and sporulation. Both these findings suggest that the manipulation of the light wavelength that crops are grown under could result in reduced infection levels.

The foliar application of Fubol Gold, Fenomenal, Signum and Revus 2 days prior to inoculation gave 100% control of Aquilegia downy mildew. Increasing the gap between fungicide application and inoculation to 10 days reduced the activity of all but the Fubol Gold treatment. The level of control achieved by Fenomenal, Signum, Percos and Revus ranged from ranged from 73% for Signum to 21% for Percos. These levels of control were similar to results seen for Impatiens downy mildew caused by *Plasmopara obducens* (Jennings *et al.*, 2011) and Pansy downy mildew (Jennings *et al*., 2009). In these studies application timings were 3, 7 and 14 days prior to inoculation with 100% control seen for both downy mildew pathogens when applied 3 and 7 days pre-inoculation. In this project, the downy mildew control achieved with a 10 day interval between fungicide treatment and inoculation was midway between the control achieved for the 7 and 14 day intervals seen for *P. obducens* and *P. violae*. This suggests that, as with other downy mildew pathogens, a 7 day interval between fungicide treatments would be optimal to ensure aquilegia plants stay clear of downy mildew.

For other downy mildew pathogens curative fungicide treatments have not been particularly effective at controlling disease. Based on this it is unlikely that treatment of plants with a crown infection will results in a control of ADM.

The range of active ingredients showing protectant activity against aquilegia downy mildew suggest that effective spray programmes could be developed which do not rely on a single mode of action or active ingredient thus reducing the risk of fungicide resistance developing.

Conclusions

- Conidia germinated over a wide range of temperatures, with the optimum germination temperature between 10 and 15ºC. Germination of conidia occurred after 2 hours.
- Plant infection occurred at temperatures between 5 and 20ºC and required at least six hours of leaf wetness. No infection occurred at 25ºC.
- Infection could occur at temperatures between 5 and 10ºC when the humidity was greater than 90%. At temperatures between 15 and 20ºC infection could occur at humidity levels greater than 70%.
- A range of fungicides gave effective control when applied as a preventative treatment 2 days prior to inoculation. Only Fubol Gold gave 100% control where the gap between spray and inoculation was increased to 10 days.
- The range of active ingredients showing effective protectant control of ADM suggests that effective spray programmes could be identified which do not rely on a single mode of action or active ingredient. This should reduce the risk of resistant populations developing.

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.

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