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Management of Botrytis neck rot in onions – second year results

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Contents

1 Executive summary

This report outlines work carried out in the 2005-06 growing season on onion Botrytis neck rot, caused by *Botrytis allii*, for the MAF Sustainable Farming Fund project on Allium pests and diseases. The present onion seed testing methodology to detect *Botrytis* spp was reviewed during 2004. A method from the Department of Primary Industries, Plant Disease Diagnostic Laboratory, Australia, was selected to conduct seed tests. Nine seed lines were received from three different seed companies and tested for level of *B. allii* infection. Levels of *B. allii* infection varied from 0 to 1.8%.

Interviews were conducted with growers about their onion management practices and the impacts these had on the final levels of onion neck rot in their crops. A small sample of growers and crops showed that lack of crop rotation may have a severe impact on the final levels of neck rot, especially if neck rot had been present in previous years. In general, a healthy onion with a well-cured neck is seldom affected by neck rot after storage. In order to avoid Botrytis neck rot, it is important to follow these management guidelines:

- Use certified, disease-free, treated seed;
- Choose a quick-maturing cultivar so neck tissues dry before storage;
- Select light, well-drained, well-prepared fertile seedbed;
- Use a minimum of a 2-year rotation, and avoid using paddocks that have had neck rot;
- Use fertilisers sparingly and on basis of soil tests;
- Do not irrigate excessively especially when tops are drying;
- Ensure neck tissues are dry before topping;
- Avoid injury to bulbs during harvest and storage;
- Eradicate weeds, remove plant debris after harvest.

Isolates of *B. allii* were obtained from seed lines tested and from onion bulbs sent to the plant pathology laboratory during 2005 and 2006. A total number of 62 isolates were obtained from these two sources. Isolates were transferred to potato-dextrose agar (PDA) slants and kept at 5°C in the plant pathology reference collection. In an additional study (funded and reported separately), 50 isolates from the culture collection were tested for sensitivity to carbendazim (chemical group: benzimidazole).

We recommend that the culture collections will be maintained and more *B. allii* isolates collected, more seed lines will be tested and more growers will be interviewed regarding their management practices to control neck rot.

2 Objectives

The objectives for the Onion Botrytis neck rot project for the 2005–06 season were:

- Continue work on establishing a baseline of Botrytis infection in New Zealand onion seed lines by surveying approximately 25 onion seed lines from different seed companies and relating this to the subsequent levels of bulb infection.
- Continue grower surveys on current protocols and management practices for onion neck rot control, especially in Canterbury. Collect weather data from nearby weather stations so that comparisons between weather conditions and subsequent disease levels can be made.
- Establish a reference collection of *Botrytis allii* isolates from a range of New Zealand onion-growing areas and from different cultivars of onion and maintain this collection in the Crop & Food Research Plant Pathology Laboratory for further studies.

3 Materials and methods

3.1 Seed line tests

A total of nine seed line samples were received from three seed companies in 2005.

Currently, ISTA (International Seed Testing Association) is developing a method for onion seed testing. However this method is not available yet. Contacts were established with seed testing laboratories at Massey (Seed Tech Services and National Seed Laboratory) and Seed Technology at Lincoln University during 2004 to review the current international recommendations for *B. allii* testing in seed. We identified a standardised procedure being used by the Department of Primary Industries, Plant Disease Diagnostic Laboratory, Australia, which was used for all seed lines tested during this study.

3.1.1 *Procedure for testing onion seed for* Botrytis allii

1. Weigh 3.0 g of onion seed and place in a tea strainer or similar wire mesh device.
2. Measure temperature of sodium hypochlorite and sterile distilled water: 22°C.
3. Immerse seed in strainer in 80 ml of 3% sodium hypochlorite for 1 minute. Gently shake the strainer.

4. Immerse seed in strainer in two changes of 100 ml sterile distilled water for 1 minute each (the vessel for this should be sterile prior to adding water).

5. Empty seed from strainer into a sterile petri plate.

6. Using tweezers, place 25 seeds per plate individually on petri plates containing half-strength lactic acid potato-dextrose agar (LPDA). Tweezers should be dipped in alcohol and flame sterilised every five seeds before dipping in sterile distilled water. Tweezer tips should not be allowed to become heated, as this could eradicate *B. allii* from seed.

7. Incubate seed at 25°C for 7 days (it may be necessary to mark colony origin at 5 days) before identification of *Botrytis* colonies.

Notes

- A minimum of 550 seeds should be tested. If no *B. allii* colonies are detected in 550 seeds there is a 99% probability that the seed line is free of *B. allii.*
- All sterilisation and plating procedures are to be performed within a laminar flow cabinet.
- Sodium hypochlorite should be stored under refrigeration to avoid degradation and not used more than 6 months beyond the date of purchase.
- LPDA is composed of 19.5 g Sigma Potato Dextrose Agar (PDA), 2.5 g Sigma Agar, made up to a volume of 1 litre, autoclaved and cooled to 50°C before adding 1.4 ml lactic acid stock solution (AnalaR) per litre, resulting in a pH of 4.0. Thickness of agar is not critical, but thinner agar is easier for inspection (100 plates/l).

3.2 Establishment of a reference culture collection at the plant pathology laboratory

Isolates of *B. allii* were obtained from seed lines tested and from onion bulbs sent by industry contacts to the plant pathology laboratory during 2005 and 2006. Isolates obtained from seed lines were transferred to PDA slants and kept at 5°C. Isolates were obtained from onion bulbs received from growers using the following methods:

1. Bulbs were incubated in a moist container for 7 days to promote conidiophore production by *Botrytis* spp. Conidia were removed from conidiophores with a sterile needle and placed onto prune agar in a petri plate. Plates were incubated in the dark at 20°C. Pure isolate colonies were obtained and transferred to PDA slants for long-term storage at 5°C.

2. Tissue was removed from the neck area of onion bulbs, surface sterilised using a 0.5% solution of sodium hypochlorite for 1 minute and rinsed twice in sterile water. Segments were placed on petri plates containing prune extract agar and incubated in the dark for 7 days.

Pure isolate colonies were obtained and transferred to PDA slants for long-term storage at 5°C.

3.3 Grower interviews

Two growers with several onion crops each were interviewed at the end of the 2005–06 growing season. Detailed questions were asked about a crop or crops with high levels of Botrytis neck rot at harvest and about a crop or crops with low levels of neck rot. Information was asked about paddock history, previous crops, sowing date, sowing density, seed line used, whether seed line was tested for *B. allii* infection prior to sowing, fungicide applications during the season (date, product, rate), fertiliser inputs, irrigation, harvest details (method, date, storage), final Botrytis neck rot levels in the crop and final destination of the bulbs. Weather data (hourly temperature, rainfall, relative humidity and leaf wetness) were collected from a nearby Crop & Food Research weather station. The Kerrytown weather station was the closest station to grower 1 and the Pendarves weather station was closest to grower 2.

4 Results

4.1 Seed lines

Nine seed lines were received from three different seed companies and tested for level of *B. allii* infection following the methodology in the section 3.1.1. Levels of infection ranged from 0 to 1.8% (Table 1).

Table 1: Percentage of onion seed infected with B. allii *in tests conducted during 2005.*

Origin	Seed line	Received	Test set up	Assessment	% infection
A	1	Aug 2005	17/08/05	24/08/05	0.5
A	2	Aug 2005	17/08/05	24/08/05	0.2
A	3	Aug 2005	17/08/05	24/08/05	0
B	1	Nov 2005	24/11/05	1/12/05	0
B	2	Nov 2005	24/11/05	1/12/05	1.8
C	1	Dec 2005	1/12/05	8/12/05	0
C	2	Dec 2005	1/12/05	8/12/05	0.5
C	3	Dec 2005	1/12/05	8/12/05	1.6
C	4	Dec 2005	1/12/05	8/12/05	0

4.2 *Establishment of a reference culture collection*

A total of 62 isolates were obtained from onion seed lines tested and onion bulbs received from growers during 2005 and 2006 (Table 2).

Table 2: List of isolates obtained from onion seed or bulbs during 2005–06 and kept in the Crop & Food plant pathology culture collection.

Isolate	Sender	Source	Isolation date	To collection
1	1	bulb	25/11/2005	12/12/2005
2	2	seed	1/12/2005	12/12/2005
3	2	seed	1/12/2005	12/12/2005
4	2	seed	1/12/2005	12/12/2005
5	2	seed	1/12/2005	12/12/2005
6	2	seed	1/12/2005	12/12/2005
7	2	seed	1/12/2005	12/12/2005
8	2	seed	1/12/2005	12/12/2005
9	2	seed	1/12/2005	12/12/2005
10	2	seed	1/12/2005	12/12/2005
11	1	bulb	22/11/2005	12/12/2005
12	1	bulb	22/11/2005	12/12/2005
13	1	bulb	22/11/2005	12/12/2005
14	1	bulb	22/11/2005	12/12/2005
15	1	bulb	22/11/2005	12/12/2005
16	1	bulb	22/11/2005	12/12/2005
17	1	bulb	22/11/2005	12/12/2005
18	1	bulb	22/11/2005	12/12/2005
19	1	bulb	25/11/2005	12/12/2005
20	1	bulb	25/11/2005	12/12/2005
21	1	bulb	25/11/2005	12/12/2005
22	1	bulb	25/11/2005	12/12/2005
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25	1	bulb	25/11/2005	12/12/2005
26	1	bulb	25/11/2005	12/12/2005
27	1	bulb	25/11/2005	12/12/2005
28	1	bulb	25/11/2005	12/12/2005
29	1	bulb	25/11/2005	12/12/2005
30	2	seed	25/11/2005	12/12/2005
31	1	bulb	25/11/2005	12/12/2005
32	1	bulb	25/11/2005	12/12/2005

Isolate	Sender	Source	Isolation date	To collection
33	1	bulb	25/11/2005	12/12/2005
34	3	seed	8/12/2005	19/12/2005
35	3	seed	8/12/2005	19/12/2005
36	3	seed	8/12/2005	19/12/2005
37	3	seed	8/12/2005	19/12/2005
38	3	seed	8/12/2005	19/12/2005
39	3	seed	8/12/2005	19/12/2005
40	3	seed	8/12/2005	19/12/2005
41	3	seed	8/12/2005	19/12/2005
42	3	seed	8/12/2005	19/12/2005
43	3	seed	8/12/2005	19/12/2005
44	3	seed	8/12/2005	19/12/2005
45	3	seed	8/12/2005	19/12/2005
46	2	seed	1/12/2005	19/12/2005
47	4	bulb	1/12/2005	4/11/2005
48	4	bulb	1/10/2005	4/11/2005
49	4	bulb	1/10/2015	4/11/2005
50	5	seed	1/10/2005	4/11/2005
51	5	seed	1/10/2005	4/11/2005
52	6	bulb	30/01/2006	27/02/2006
53	6	bulb	30/01/2006	27/02/2006
54	6	bulb	30/01/2006	27/02/2006
55	6	bulb	7/02/2006	27/02/2006
56	6	bulb	7/02/2006	27/02/2006
57	6	bulb	7/02/2006	27/02/2006
58	6	bulb	7/02/2006	27/02/2006
59	6	bulb	7/02/2006	27/02/2006
60	6	bulb	7/02/2006	27/02/2006
61	6	bulb	7/02/2006	27/02/2006
62	6	bulb	7/02/2006	27/02/2006

4.3 Grower interviews

4.3.1 Grower 1

Three paddocks were included from grower 1 in this survey. In all crops, sowing density was 65 seeds/m^2 and a base dressing of 50 kg N was used, with a top dressing of 400 kg of 12-10-10 during the growing season. Irrigation at 20 mm each time was used every 6-7 days when neutron probe assessment showed water deficit. Seed is generally tested for *B. allii* levels before sowing and sometimes rejected due to high levels of *B. allii* detected in the tests.

Paddock 1 was on its fourth year of onions. Previously the cultivar Red Beauty had been planted on this paddock and that cultivar had high levels of Botrytis neck rot. The cultivar in the 2005–06 season was ELK. This crop received the following fungicides (number of applications in parenthesis): carbendazim (4), tebuconazole (2), mancozeb (5), and iprovaliacarp & propineb (1). The crop had about 12% infection level of neck rot in the paddock. It was harvested in early February and had high neck rot levels of between 20-30%.

Paddock 2 was also on its fourth year of onions. The cultivar in the 2005–06 season was PLK. This crop received the following fungicides (number of applications): carbendazim (4), tebuconazole (3), mancozeb (7), iprovaliacarp & propineb (1), copper (1), procymidone (1) and didecyldimethyl-ammonium chlorine (1). Part of the crop had about 12% infection level of neck rot in the paddock. It was topped and lifted on 10 February, taken to the shed about 2 weeks later, and it had final neck rot levels of 20-30%. The remainder of the crop was barred on 10 February and left to dry for approximately 6 weeks before being taken to the shed. The final levels of Botrytis neck rot were low in this crop. Weather conditions taken from the nearby Kerrytown weather station during the growing season are outlined in Figures 1 and 2. From February 10 to 24 there was no rain, the mean temperature varied between 13 and 19.6°C and mean relative humidity was between 61 and 86%. From February 10 to March 27 there were 7 rainy days with a total rainfall of 20 mm, the mean temperature varied between 9.1 and 19.6°C and the mean relative humidity was between 53 and 90%.

Paddock 3 was on its second year of onions, including cultivars Red Beauty, Patagonia and CLK. This crop received the following fungicides (number of applications): carbendazim (6), tebuconazole (4), mancozeb (9), iprovaliacarp & propineb (1), copper (2), procymidone (1) and didecyldimethyl-ammonium chlorine (1). Red Beauty was affected by neck rot and received a further application of carbendazim, mancozeb, copper and prosymidone. This crop was harvested and had no botrytis apart from low levels in Red Beauty.

4.3.2 Grower 2

Three paddocks were included from grower 2 in this survey. Sowing density was 76 seeds/m^2. Irrigation at 25 mm each time was used five or six times during the growing season unless otherwise stated. Seed is generally not tested for *B. allii* levels before sowing.

Paddock 1 was in its second year of onions, with cultivar Franklin sown on 30 September 2005. This crop received the following fungicides (number of applications in parenthesis): mancozeb (7), copper hydroxide (1) and mefenoxam + mancozeb (1). It was irrigated five times, receiving 30 mm each time. The crop was lifted in early March 2006 (no topping) and harvested in early April. It was completely free of Botrytis neck rot and it was exported. Weather conditions at a nearby weather station are outlined in Figures 3 and 4. During March when the crop was drying, the mean daily temperature ranged from 9.9 to 18.7°C, relative humidity ranged from 51 to

91% and there were 7 rainy days with a total of 17 mm rain (no weather data were obtained beyond 31 March 2006).

Paddock 2 was on its second year of onions, with cultivar Red Keep sown on 25 August 2005. This crop received four applications of thiophanate-methyl during the growing season. The crop was lifted in mid-February 2006 (no topping) and harvested a month later. It was completely free of Botrytis neck rot and it was exported. The mean daily temperature during drying ranged from 9.9 to 2.05°C, relative humidity ranged from 48 to 75.9% and there were 7 rainy days with a total of 23 mm rain.

Paddock 3 was also on its second year of onions, with cultivar Red Beauty sown early September 2005. This crop was damaged on 5 February by a freak hail storm which stripped all leaves and damaged bulb shoulders. It was lifted 10 days later and harvested in mid-March 2006. The final level of Botrytis neck rot in this crop was 5% and the crop was exported to Australia.

In general, no mechanical clipping is carried out, but some paddocks are hand-clipped. The crops that are clipped green then lifted are then dried in a dryer heated to 25–30°C for 3 days. Usual rotation is 2 years on onions, and 3 years if no major onion disease issues have been present, but if possible, only paddocks with no known history of onion cropping are used. If Botrytis neck rot has been present, then onions will not be grown in this paddock again. Carbendazim is used only as a seed treatment, not during crop growth. The main aim of the grower is to have undamaged plants (no hail, thrips, chemical damage, etc), otherwise neck rot may be a problem, especially during growing seasons with high rainfall and humidity. High levels of nitrogen fertiliser are avoided as this makes the crop more prone to neck rot. Weed control is an essential part of neck rot management so that air flow through the crop is improved and high moisture levels conducive to the development of neck rot are avoided.

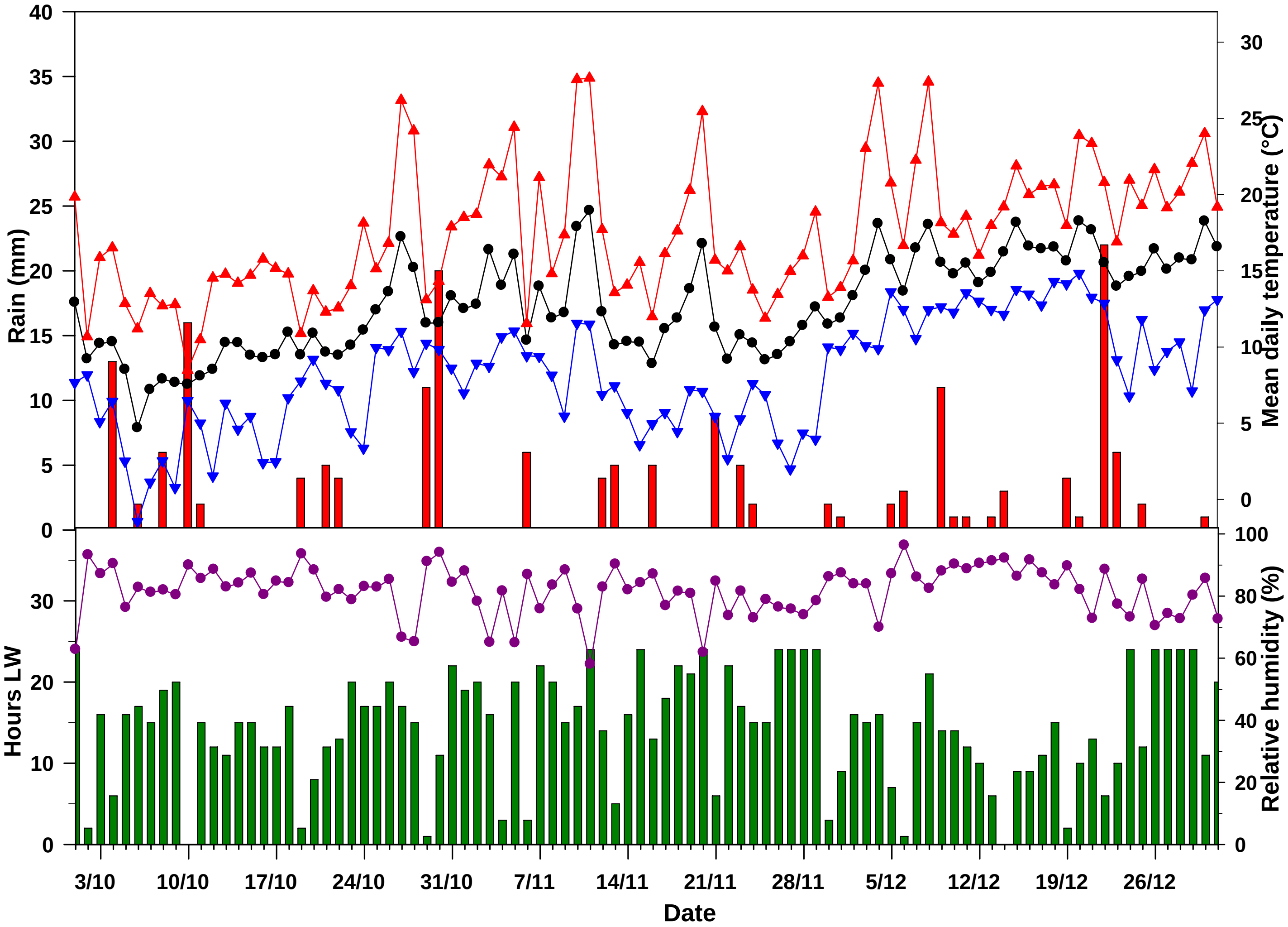

Figure 1: Weather data from the Kerrytown weather station from 1 October to 31 December 2005. The top graph shows daily total rainfall (red bars) on left axis and minimum (blue), maximum (red) and mean temperatures on the right axis. The bottom graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

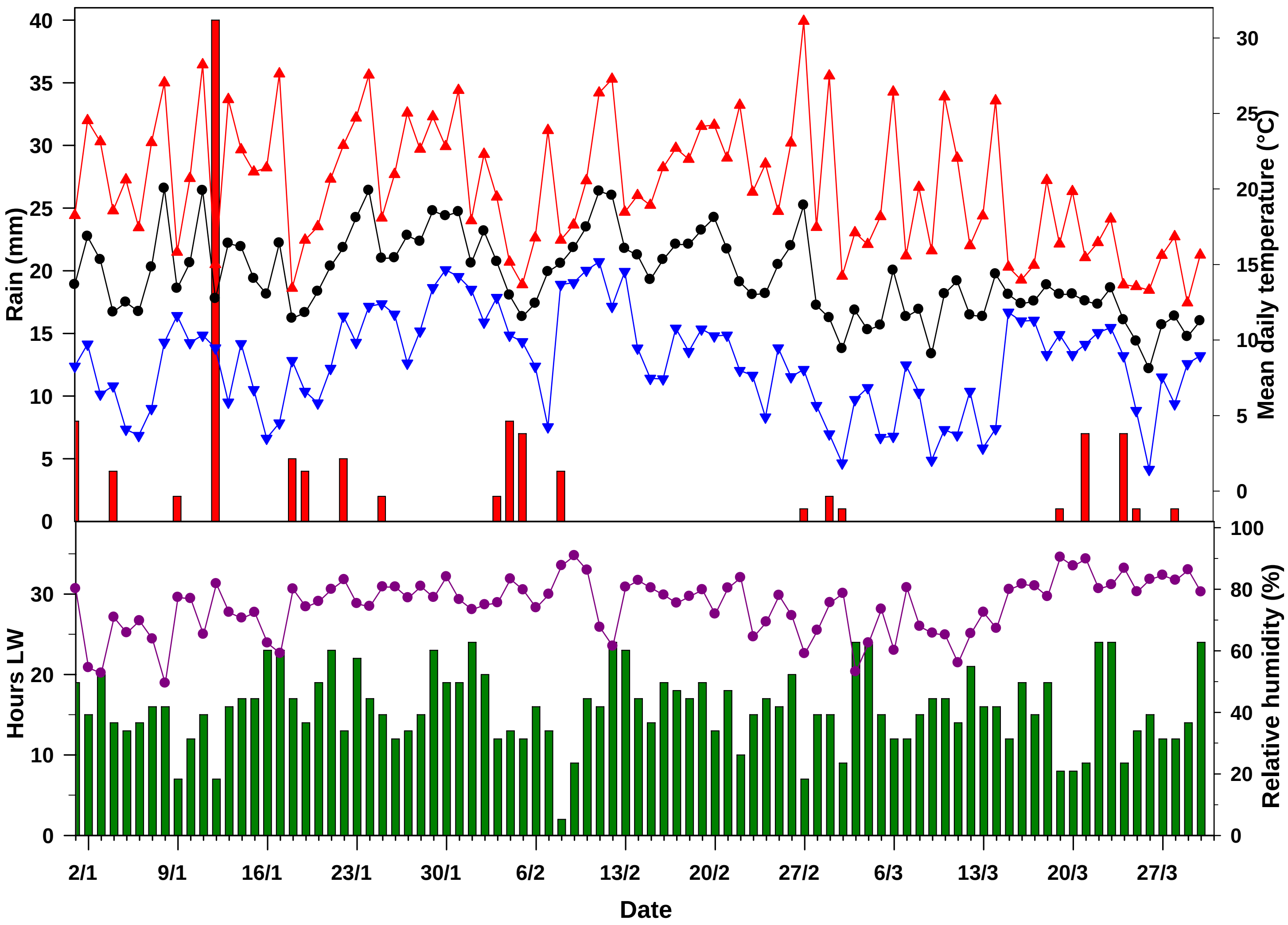

Figure 2: Weather data from the Kerrytown weather station from 1 January to 30 March 2006. The top graph shows daily total rainfall (red bars) on left axis and minimum (blue), maximum (red) and mean temperatures on the right axis. The bottom graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

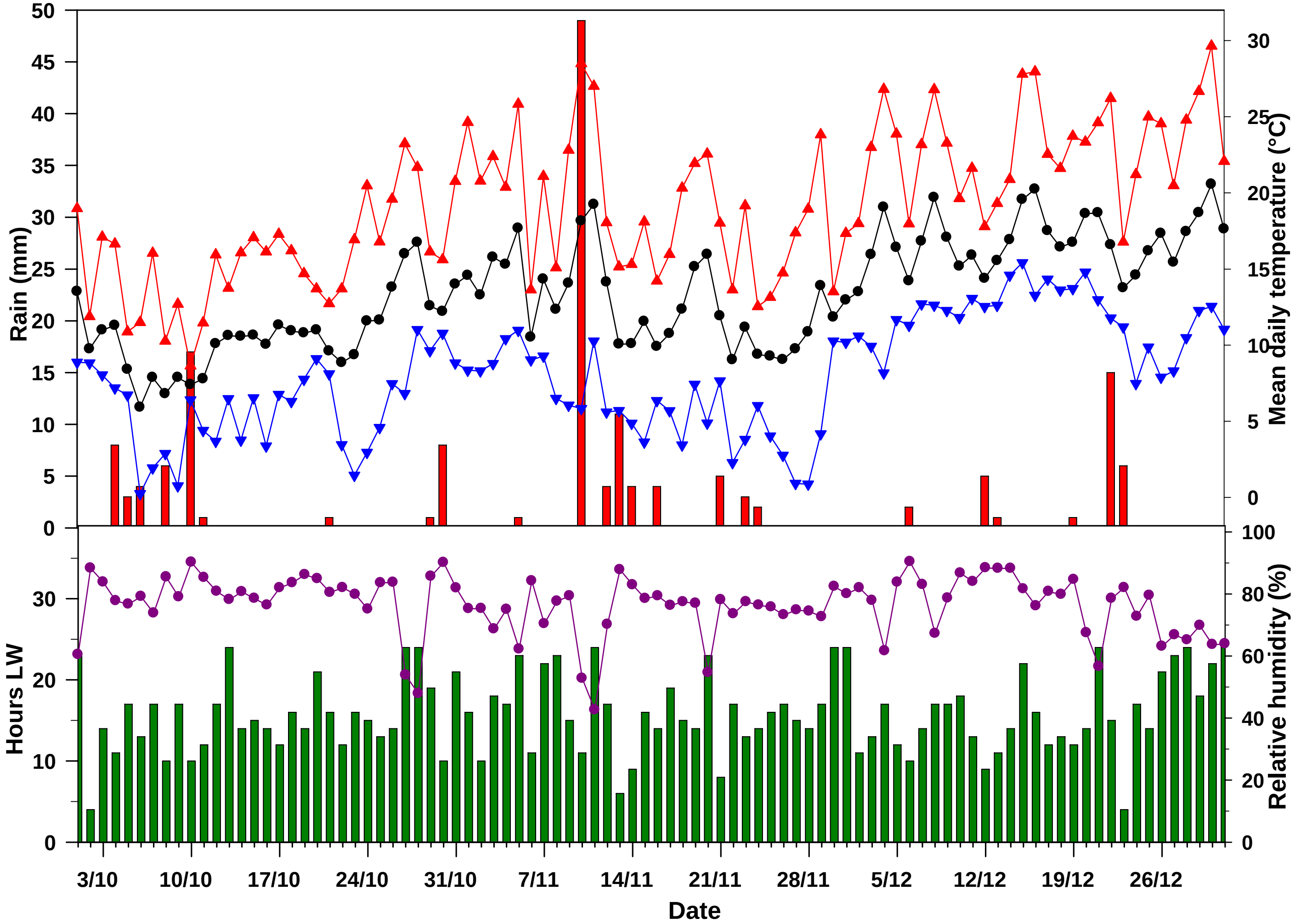

Figure 3: Weather data from the Pendarves weather station from 1 October to 31 December 2005. The top graph shows daily total rainfall (red bars) on left axis and minimum (blue), maximum (red) and mean temperatures on the right axis. The bottom graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

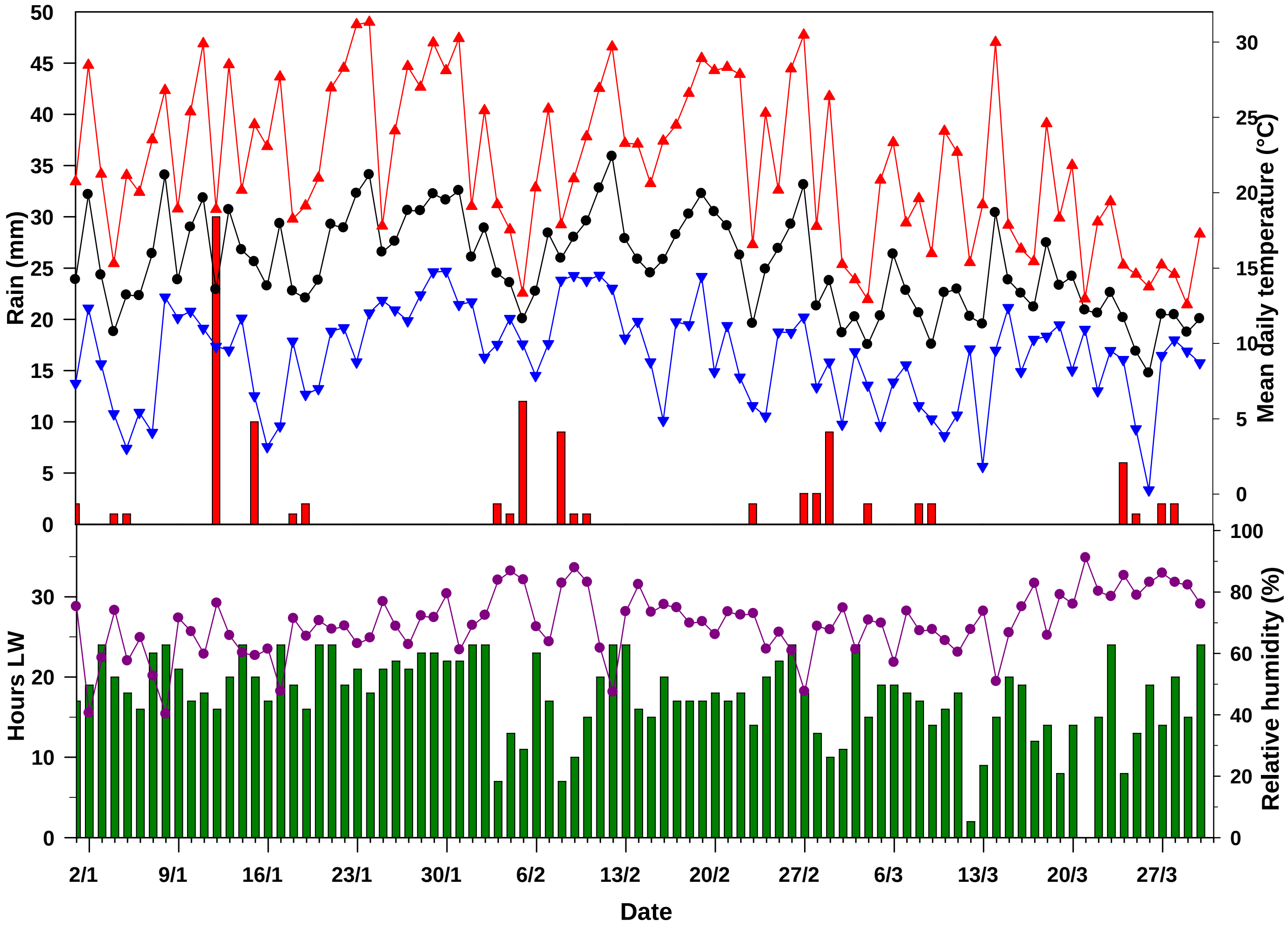

Figure 4: Weather data from the Pendarves weather station from 1 January to 30 March 2006. The top graph shows daily total rainfall (red bars) on left axis and minimum (blue), maximum (red) and mean temperatures on the right axis. The bottom graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

5 Discussion

The levels of *B. allii* on tested seed lines on this study were low and varied from 0 to 1.8%. However, even low levels of seed infection can provide initial inoculum to the paddock and spread the disease to other surrounding plants although weather conditions during the season are also important in disease development. Infected seed has been indicated as the main source of infection of onion bulbs in storage as levels of seed infection can correlate with subsequent levels of bulb disease. Stewart & Franicevic (1994) found a relationship between levels of seed infection by *B. allii* and bulb rot in store, with higher seed infections resulting in greater levels of bulb rot. Sowing of uninfected seed did not result in bulb rot attributable to *B. allii*. However, two seed lines known to be infected with *B. allii* at levels of 10.3% and 30.3%, produced 3.2 and 10.2% Botrytis bulb rot in store, respectively. The fungus invades seeding cotyledons and remains symptomless in the leaf tissue until it colonises the tissue at the necks of bulbs, causing neck rot.

At the property of grower 1, Botrytis neck rot levels ranged from nil to approximately 30%. The paddock (cultivar ELK) with the most severe neck rot was in its fourth year of onions and the previous season's onions in this paddock had high levels of neck rot. In the same paddock, the cultivar PLK had less neck rot and more fungicide applications. In paddock 3, the red cultivar had some neck rot and the other cultivars had none. Fungicides were used frequently. At the property of grower 2, a red cultivar that suffered a severe hailstorm close to harvest was most seriously affected by neck rot at a final level of 5%. The other paddocks were free of neck rot. Fungicides were used sparingly and carbendazim was used only as a seed treatment. The grower aimed to use paddocks with no known history of onions, but usual rotation was 2 years or 3 years if no major onion disease issues were present. The main aim of the grower was to have undamaged plants (no hail, thrips, chemical damage, etc), use nitrogen fertiliser sparingly and follow rigorous weed control programme.

It is difficult to make firm conclusions from the limited amount of grower interviews, but it appeared that crop rotation is very important in avoiding disease development in the crop, especially if neck rot has been present previously. Plant debris from previous crops harbours sclerotia of *B. allii* and can act as initial inoculum even if the seed is clean (Lorbeer et al. 2004). There are also cultivar differences, with some cultivars more susceptible to neck rot than others. The general health of the crop is important aspect of avoiding neck rot: if a crop is healthy and there is no damage (e.g. hail), it will not be affected by neck rot. Weather conditions during the growing season and during harvest can have an effect on final neck rot levels. Infection by *B. allii* can occur at any stage of the onion life cycle. Other potential sources of infection in onion crops are airborne conidia originating from volunteer onions, infected debris and cull-piles. Primary infection can also originate from sclerotia (resting bodies) surviving in plant debris and soil. Where infected bulbs are used for seed production, the disease can infect flowers, which leads to *B. allii* infected seed.

Disease progress is affected by the weather conditions during the season. Conidia disperse when rain showers are frequent. Free water is required for conidium germination, so most infection events will occur during moderate temperatures with high humidity (80% RH or over) after prolonged wet conditions caused by rainfall or overhead irrigation. The fungus grows optimally at 21ºC and invades the leaves in which it develops until they senesce and become necrotic. The conidiophores are then produced which continue to infect onion crops. Neck rot can be particularly severe if prolonged wet periods occur during curing when onion necks are still succulent. The presence of wounds also provides entry points for the pathogen. However, it is very difficult to pinpoint the effect of individual weather conditions, such as rain showers, to the final levels of onion neck rot because disease expression occurs such a long time after harvest.

A healthy onion with a well-cured neck is seldom affected by neck rot after storage. The following management issues are important in preventing neck rot:

- Seed quality: Use certified disease-free treated seed or treat seed before planting. When onion bulbs are used to establish seedlings they should be heat-treated to kill infection by exposing them to the sun for 12 days during which the temperature must exceed 40°C for about 4 hours. Infected onions should not be used to establish seedlings.
- Cultivar: quick-maturing cultivars are less prone to neck rot as neck tissues dry before storage.
- Soil quality: Always plant seeds or set in light, well drained, well-prepared fertile seedbed. If feasible, sterilise seedbed before planting. Avoid heavy soils, heavy seeding rate, overcrowding, poor air circulation, planting too deep.
- Rotation: practice a minimum of 2-year crop rotation.
- Fertilization: strive for steady vigorous plant growth, not soft luxuriant growth. Use fertiliser sparingly on the basis of a soil test but not at the end of the season.
- Irrigation: do not irrigate excessively and especially not when tops are drying.
- Harvest: follow practices that help plants fully dry down at the end of the season, allow tops to mature well before harvest, undercut and windrow onions until inside neck tissues are dry before topping close to the neck, dry before storing or during the first few days of storage. Avoid injury during harvest and storing.
- Sanitation: eradicate weeds, especially perennial and wild onions in and near paddocks. Remove unharvested plant parts and destroy infected plant debris after harvest.
- Storage: Cure onions with forced heated air at 27-35°C for a few days at the beginning of the storage period. Ideal storage conditions are 0-1°C at 65-75% humidity. Do not circulate warm air over cold onions as this will cause sweating with resultant mould problems. Open the storage doors when the air outside is cool and dry to exhaust warm moist air.

6 Recommendations for future work

- Maintenance of culture collection and collection of more isolates for the isolate collection.
- Further seed line testing.
- Further interviews with growers.

7 Acknowledgements

We thank the seed companies, onion industry personnel and growers who contributed samples and information for this study. MAF Sustainable Farming Fund funded this project.

8 References

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