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Management of Botrytis neck rot in onions – results from 2006-07 season

SLH Viljanen-Rollinson, EL Parr, VM Marroni, Butler RC

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Contact: Paul Munro, Alpha Research Ltd

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New Zealand Institute for Crop and Food Research Limited
Private Bag 4704, Christchurch, 8140, New Zealand.

Contents

1 Executive summary

This report outlines work carried out in the 2006-07 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 (DPI), Plant Disease Diagnostic Laboratory, Australia, was selected to conduct seed tests. A total of 39 seed lines from four different companies were tested in 2006-07 for level of *B. allii* infection following the DPI methodology. Infected seed was detected in 35 seed lines (73%). Levels of infection ranged from 0.2 to 24.5%. The infection levels were considerably higher than those tested in 2005, which varied from 0 to 1.8%. The high levels of seed infection detected in these tests should be of serious concern to the onion industry.

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. 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 a 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 the 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, and remove plant debris after harvest.

1.1 Future directions

This project was found to be beneficial to securing funding in the 2007 FRST funding round for the programme Future Vegetables. *Botrytis allii* is included in two parts of the programme, with aims to detect and measure levels of *B. allii* DNA in onions, to determine the viability of the pathogen and to develop molecular markers for determining fungicide resistance of *B. allii.*

2 Objectives

The objectives for the Onion Botrytis neck rot project for the 2006-07 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 (completion of this milestone requires sourcing information from growers about the onion neck rot levels in their crops).
- Continue grower interviews in Canterbury to obtain more reliable information on management practices that are likely to be effective in controlling onion neck rot. Include this season's data from the growers interviewed last season. Collect weather data from nearby weather stations so that comparisons between weather conditions and subsequent disease levels can be made.
- Maintain existing culture collection of *Botrytis allii* in the Crop & Food Research plant pathology collection and add to the collection from seed and infected bulbs collected in the field.

3 Materials and methods

3.1 Seed line tests

A total of 39 seed lines from four different companies were tested in 2006-07 (results also included for earlier seed tests). Currently, ISTA (the 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.

3.2 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

8. 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.*
9. All sterilisation and plating procedures are to be performed within a laminar flow cabinet.
10. Sodium hypochlorite should be stored under refrigeration to avoid degradation and not used more than 6 months beyond the date of purchase.
11. LPDA is composed of 19.5 g Sigma Potato Dextrose Agar (PDA), 2.5 g Sigma Agar, made up to a volume of 1 L, 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).

3.3 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.

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.

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.

Currently there are over 80 isolates of *B. allii* in the culture collection.

3.4 Grower interviews

Four growers were interviewed after the 2006-07 growing season. Detailed questions were asked about the management of Botrytis neck rot in their crops: 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, fertiliser inputs, irrigation, harvest details (method, date, storage), final Botrytis neck rot levels and destination of the bulbs. Weather data (hourly temperature, rainfall, relative humidity and leaf wetness) were collected from two Crop & Food Research weather stations. The Kerrytown weather station was the closest station to grower 1 and the Pendarves weather station was closest to grower 2. No weather data were available for Growers 3 or 4.

4 Results

4.1 Seed lines

A total of 39 seed lines from 4 different companies were tested in 2006-2007 (results also included for earlier seed tests) for level of *B. allii* infection following the methodology in the section 3.1.1. Infected seed was detected in 35 seed lines. Levels of infection ranged from 0.2 to 24.5% (Table 1).

Table 1: Percentage of onion seed infected with *B. allii* in tests conducted from 2005 to 2007.

Date	Test	Sender	Seeds/550	% infection
17/08/2005	1	1	3	0.5
17/08/2005	2	1	1	0.2
17/08/2005	3	1	0	0
24/11/2005	4	2	0	0
24/11/2005	5	2	10	1.8
1/12/2005	6	3	0	0
1/12/2005	7	3	3	0.5
1/12/2005	8	3	9	1.6
1/12/2005	9	3	0	0
18/10/2006	10	2	135	24.5
18/10/2006	11	2	23	4.2
18/10/2006	12	2	6	1.1
19/10/2006	13	2	83	15.1
8/11/2006	14	2	1	0.2
9/11/2006	15	2	9	1.6
9/11/2006	16	2	3	0.5
21/11/2006	17	2	3	0.5
11/12/2006	18	2	0	0.0
11/12/2006	19	2	74	13.5
11/12/2006	20	2	44	8.0
20/02/2007	21	2	2	0.4
8/03/2007	22	2	29	5.3
8/03/2007	23	2	11	2.0
16/03/2007	24	2	12	2.2
16/03/2007	25	2	0	0.0
22/03/2007	26	2	4	0.7
22/03/2007	27	2	0	0.0
26/03/2007	28	2	0	0.0
26/03/2007	29	2	70	12.7
12/04/2007	30	2	0	0.0
12/04/2007	31	4	77	14.0
13/04/2007	32	4	22	4.0
13/04/2007	33	4	36	6.5

Date	Test	Sender	Seeds/550	% infection
3/05/2007	34	4	16	2.9
3/05/2007	35	4	18	3.3
8/05/2007	36	4	2	0.4
23/05/2007	37	4	4	0.7
20/06/2007	38	4	17	3.1
20/06/2007	39	4	9	1.6
22/06/2007	40	4	3	0.5
16/07/2007	41	4	10	1.8
18/07/2007	42	4	7	1.3
20/07/2007	43	4	11	2.0
27/07/2007	44	4	0	0.0
27/07/2007	45	4	1	0.2
8/08/2007	46	4	0	0.0
9/08/2007	47	4	0	0.0
9/08/2007	48	4	0	0.0

4.2 Grower interviews

Four growers were interviewed about the practices they have used to manage Botrytis onion neck rot in their crops during the 2006-07 growing season.

4.2.1 Grower 1

Grower 1 was also interviewed in the previous year (2005-06). Four paddocks from Grower 1 were included in this year's survey. Sowing density was again 65 seeds/m^2. Irrigation records were not available but a neutron probe is used on the property to indicate water deficit and irrigation is applied as required. All seed is treated with a mixture of procymidone (1%) and carbendazim (12.5%). Seed is also tested prior to sowing for *B. allii* levels by an independent seed-testing station and seed is rejected if high levels are present. The final neck rot levels in the crops varied from 0 to 10%.

Paddock 1 was in the first year of onions (barley and peas prior to onions). The cultivar was ELK, sown on 20 August 2006. This crop received the following fungicides (number of applications in parentheses): carbendazim (4), mancozeb (8), tebuconazole (3), metalaxyl-M (2) and copper hydroxide (4). The crop was lifted in early March 2007 and harvested on 17 March.

Paddock 2. The cultivar was Canterbury/Patagonia, sown on 5 September 2006. This crop received the following fungicides (number of applications in parentheses): carbendazim (3), mancozeb (7), tebuconazole (6), metalaxyl-M (1), copper hydroxide (4), procymidone (1) and didecyldimethyl-ammonium chlorine (2). The crop was lifted in early March 2007 and harvested on 17 March.

Paddock 3. The cultivar was Red Beauty/Red Emperor, sown on 7 September 2006. This crop received the following fungicides (number of applications in parentheses): carbendazim (3), mancozeb (8), tebuconazole (7), metalaxyl-M (2), copper hydroxide (5), procymidone (1) and didecyldimethyl-ammonium chlorine (2). The crop was lifted in mid March 2007, and harvested on 30 March.

Paddock 4. The cultivar was Tilbury, sown on 9 September 2006. This crop received the following fungicides (number of applications in parentheses): carbendazim (4), mancozeb (7), tebuconazole (6), metalaxyl-M (1), copper hydroxide (6) and didecyldimethyl-ammonium chlorine (3). The crop was lifted in mid April 2007, and harvested on 5 May.

4.2.2 Grower 2

Grower 2 was also interviewed in the previous year (2005-06). Sowing density was 76 seeds/m^2. Irrigation was used as required. No particular paddocks were identified for this survey. The management of onion neck rot on this property followed the same principles as the previous year. In general, the fungicide programme consisted of mancozeb, copper hydroxide and occasionally mefenoxam and mancozeb or thiophanate-methyl if neck rot was present while the crop was still growing. Carbendazim was used as a seed treatment but not usually during crop growth. Usual rotation was 2 years on onions if there had been no major onion disease issues in the past. The aim of the grower was to have undamaged plants (no hail, thrips, chemical or wind damage), otherwise neck rot may be a problem, especially during growing

seasons with high rainfall and humidity at the time of lifting and harvest. Last season one crop received wind damage at the second and third true leaf stage, and fungicides (carbendazim, thiphanate-methyl and mancozeb) were applied within 24 h of the damage occurring. High levels of nitrogen fertiliser were avoided as this makes the crop more prone to neck rot. Weeds were carefully controlled as good air flow through the crop is improved and high moisture levels conducive to the development of neck rot were then avoided. Generally no mechanical clipping was carried out, but some paddocks (especially the reds) are hand-clipped. Most onions were taken to the dryer heated to 25-30°C for 2 days. The grower felt that heating was especially important for hand-clipped onions and for crops that had been exposed to wet and moist weather conditions between lifting and harvest. The final neck rot levels in the 2006-07 season varied from 0 to 10%.

4.2.3 Grower 3

This grower was interviewed for the first time for the management of onion neck rot during the 2006-07 season. Untreated seed of hybrid onions was used at the sowing density of 60 seeds/m^2. In the future, the grower hopes to obtain untreated organically grown seed. Onions were sown to paddocks with no previous history of onions. No fungicides were used except for biological control products. Weeds were generally a major problem, and flame weeding was used at the first true leaf stage. Although this damaged the leaves slightly, generally the plants recovered well. In addition, inter-row cultivation and hand-weeding were used to control weeds. Some trial work was carried out in the past when tops were mulched and a flame weeder passed across the onions which “seared” the crop. This killed the weeds and cured the neck area, and the results were promising in reducing the final level of neck rot. Irrigation was carried out 5 or 6 times, usually after Christmas, applying 20 mm at a time. Onions were lifted and left to dry on the paddock until dry, were not clipped and were not dried in the drier. All onions were sold at the local market, and none were exported. During the 2006-07 growing season, the final neck rot levels were about 20%. The grower felt that the final onion neck rot levels were highly dependent on the weather conditions during harvest. Some seasons ago, when moist, cloudy weather conditions were prevailing, the grower lost the entire onion crop to neck rot, but in most years, the final neck rot levels are about 20%.

4.2.4 Grower 4

Grower 4 grows mainly ELK, PLK, Franklin and Tilbury onions sown at 2.5 kg per ha. Untreated seed was first given hot water treatment, and then treated with a mixture of carbendazim and procymidone. The grower followed a new harvesting regime from the 2007 season onwards, and this has changed some of the management issues. The grower used to have onions in the same paddock for up to 3 years, but now onions are grown in the same paddocks for longer. Crops were irrigated with a centre pivot system delivering 20 mm each time. The aim was to give the crops a total of 600-700 mm irrigation (including rainfall). Mancozeb was applied every 7-10 days from the third true leaf growth stage onwards (a total of approximately 10 applications per season). Carbendazim was applied mainly at flag leaf dieback stage, midway through dieback, with a third application 10-14 days later and a final application approximately 5 days prior to topfall. Amistar was used occasionally for downy mildew control. Weed control consisted of ioxynil, fluroxypul and methabenzthiazuron depending on the specific weed problem.

In previous years, harvesting was done by topping the crop green and leaving it to dry on the ground. Since the 2007 growing season, the harvesting technique was changed to first barring the crop out without topping, leaving them to dry on the ground for about 14 days, then mechanically topping and within 4 hours taking them to the drying shed at 28°C for 48 hours. Prior to the new harvest technique, final onion neck rot levels typically reached 10-20%, and in some years, the majority of the crop was lost due to neck rot damage, especially if the weather conditions were moist during harvest. Since the adoption of the new harvest technique, the levels of onion neck rot have been from 0 to negligible. Adoption of the new technique has meant that area of onions grown by this grower has decreased, because he felt that it was more important to have harvest capacity than quantity, to allow harvesting as quickly as possible. One area of a paddock was left to dry on the ground for nearly 1 month, and the final neck rot levels in this crop reached 5%. The majority of the crop was exported, but occasionally it was sent to local or Asian markets. Sheep were grazed on paddocks after harvest to try to reduce the level of onion debris carrying botrytis to the next crop.

4.3 Weather data from Kerrytown and Pendarves weather stations

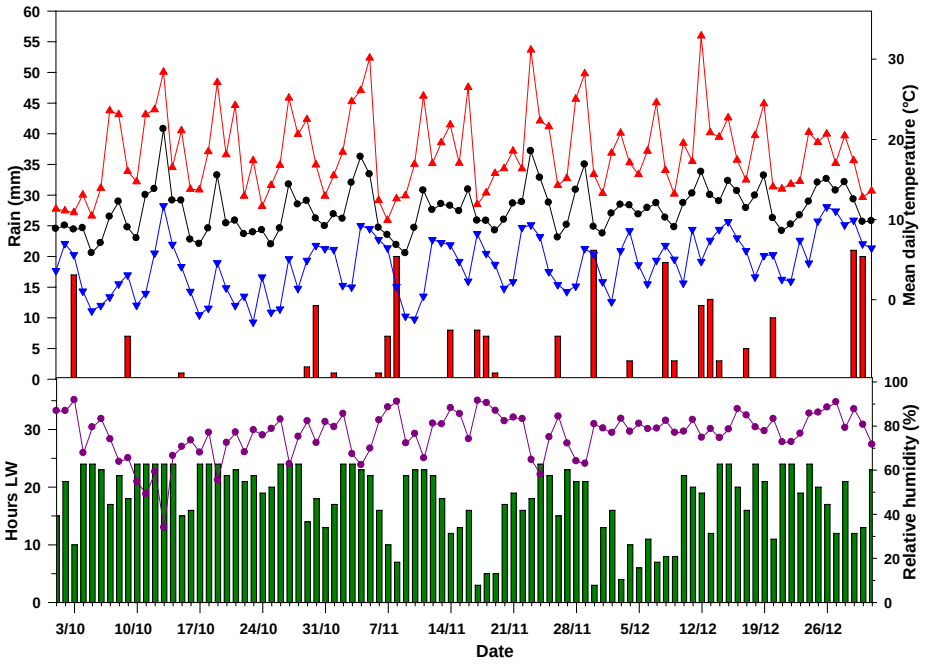

Figure 1: Weather data from the Kerrytown weather station from 1 October to 31 December 2006. The top graph shows daily total rainfall (red bars) on the left axis and minimum (blue), maximum (red), and mean temperatures on the right axis. The lower graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

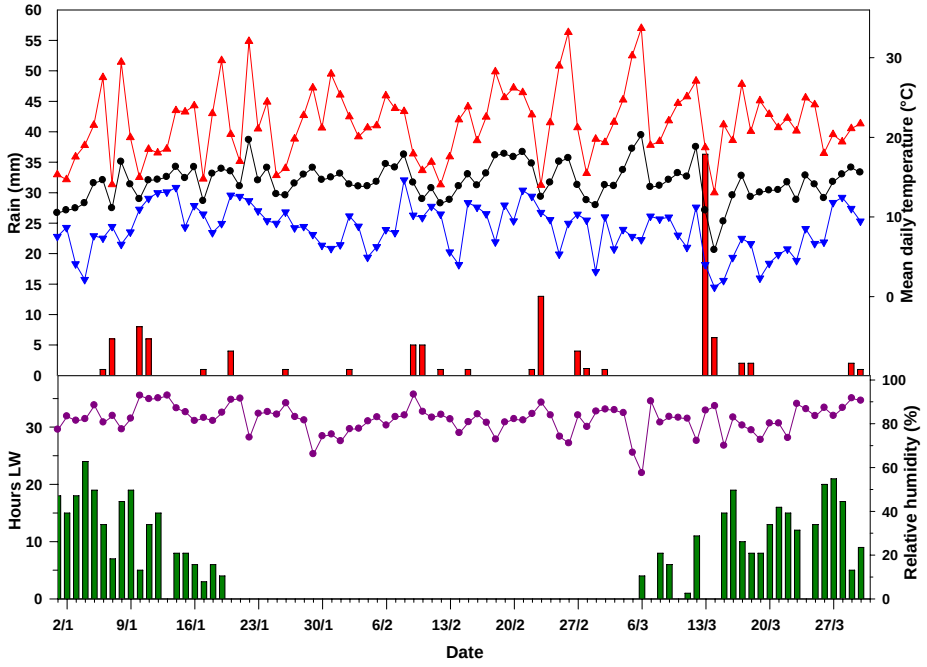

Figure 2: Weather data from the Kerrytown weather station from 1 January to 30 March 2007. The top graph shows daily total rainfall (red bars) on the left axis and minimum (blue), maximum (red), and mean temperatures on the right axis. The lower graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis; note that leaf wetness sensor was malfunctioning some of the time) and the mean relative humidity (%) for each day (purple line, right axis).

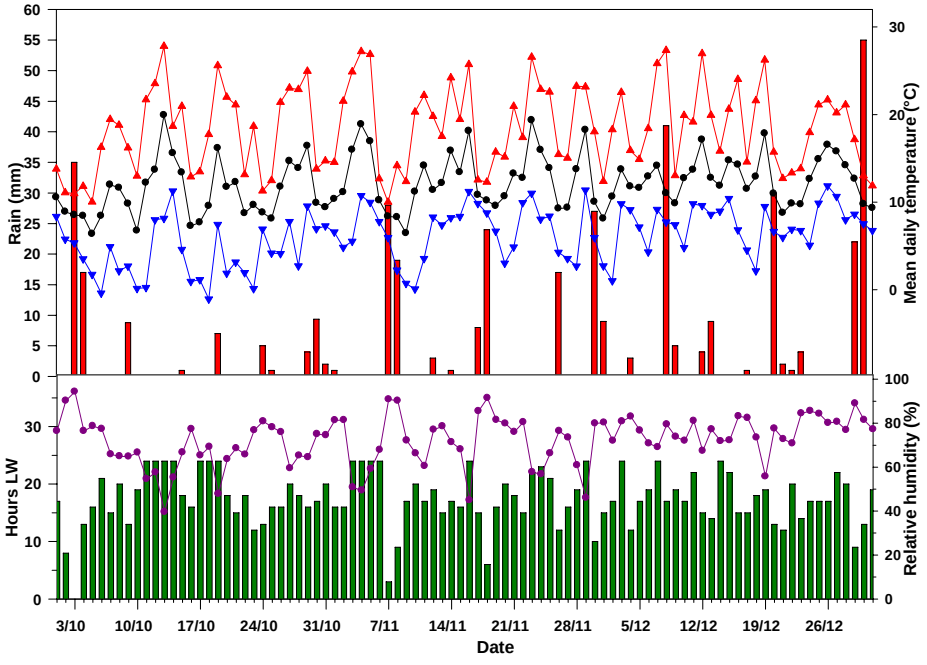

Figure 3: Weather data from the Pendarves weather station from 1 October to 31 December 2006. The top graph shows daily total rainfall (red bars) on the left axis and minimum (blue), maximum (red), and mean temperatures on the right axis. The lower graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

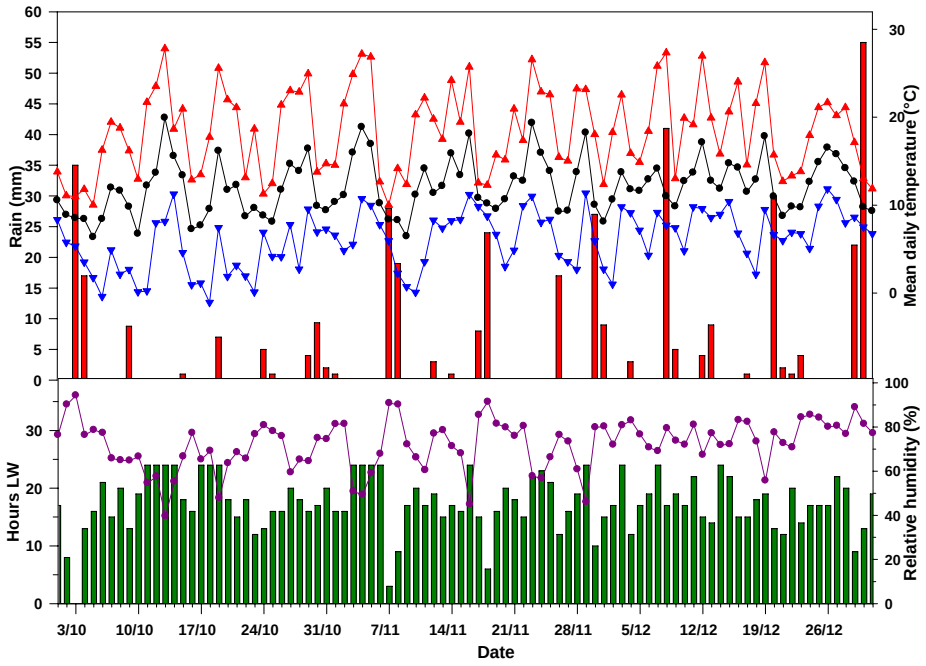

Figure 4: Weather data from the Pendarves weather station from 1 January to 30 March 2007. The top graph shows daily total rainfall (red bars) on the left axis and minimum (blue), maximum (red), and mean temperatures on the right axis. The lower graph shows the number of hours each day when a leaf wetness sensor recorded that leaves were wet (green bars, left axis) and the mean relative humidity (%) for each day (purple line, right axis).

5 Discussion

From a total of 39 seed lines, infected seed was detected in 35 seed lines (73%). Levels of infection ranged from 0.2 to 24.5%. From the infected seed lines, 34% had infection levels of less than 1%, 43% had infection levels of between 1 and 5%, 9% had infection levels of between 5 and 10% and 14% had infection levels of more than 10%. The infection levels were considerably higher than those tested in 2005, which varied from 0 to 1.8% (Marroni et al. 2006). The high levels of seed infection are a major concern to the industry, because even low levels of seed infection can provide initial inoculum to the paddock and spread the disease to the surrounding plants. 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.

From the grower interviews, a common theme for onion neck rot control was to avoid build-up of inoculum and to follow correct harvest procedures so that the necks of onions are cured, not allowing entry of the pathogen into the neck area while drying. Some growers had changed the rotation and did not plant onions in the same paddock for more than 2 consecutive years. 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). Some growers were concentrating on improving harvest procedures, with good results. The general health of the crop is an 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. 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. 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.

Overall, neck rot management in onions is based on the principle of a healthy onion with a well-cured neck is seldom affected by neck rot after storage. The following management issues may help in preventing neck rot:

- Seed quality: Use certified disease-free treated seed or treat seed before planting.
- Cultivar: select quick-maturing cultivars so neck tissues dry before storage.
- Soil quality: Always plant seeds or set in light, well drained, well-prepared fertile seedbed. Avoid heavy soils, heavy seeding rate, overcrowding, poor air circulation, and planting too deep.
- Rotation: practice a minimum of 2-year crop rotation.
- Fertilisation: 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 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.

This project was found to be beneficial to securing funding in the 2007 FRST funding round for the programme Future Vegetables. *Botrytis allii* is included in two parts of the programme, in “Molecular tools for detecting intractable pests and pathogens of export onions” and in “Best management practices, tools for pesticide resistance”.

In the project “Molecular tools for detecting intractable pests and pathogens of export onions”, we aim to detect and measure levels of *B. allii* DNA in onions and to determine the viability of the pathogen. Viability information would provide the basis for a decision support system to minimise rejection of export consignments.

In the project “Tools for pesticide resistance” we aim to develop molecular markers for determining fungicide resistance of *B. allii.* This will provide the underlying technology for developing resistance management tools for onion crops, which will enable the vegetable industry to improve their stewardship of agrichemical technologies.

6 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.

7 References

Lorbeer JW, Seyb AM, Boer Md, Ende JEvd 2004. Botrytis species on bulb crops. In: Elad Y, WIlliamson B, Tudzynski P, Delen N ed. Botrytis: biology, pathology and control. Dordrecht, The Netherlands, Kluwer Academic. Pp. 273• 294.

Marroni MV, Viljanen-Rollinson SLH, Butler RC. 2006. Management of Botrytis neck rot in onions – second year results. Crop & Food Research Confidential Report No. 1710.

Stewart A, Franicevic SC 1994. Infected seed as a source of inoculum for Botrytis infection of onion bulbs in store. Australasian Plant Pathology 23: 36–40.