Canadian Journal of Plant Science

Early detection of onion neck rot disease in Manitoba

Journal:	<i>Canadian Journal of Plant Science</i>
Manuscript ID	CJPS-2021-0064.R3
Manuscript Type:	Article
Date Submitted by the Author:	29-Aug-2021
Complete List of Authors:	Singh, Poonam; Assiniboine Community College, Agriculture and Environment Ahmad, Faiz; Brandon University, Biology; Brandon University, Biology Bisht, Vikram; Manitoba Agriculture Food and Rural Development Thakkar, Nevy; Assiniboine Community College, Agriculture and Environment Sajjad, Sumreen; Brandon University, Biology
Is the invited manuscript for consideration in a Special Issue?_:	Canadian Horticulture Special Issue
Keywords:	Allium cepa, Botrytis neck rot, Early detection, PCR

SCHOLARONE™
Manuscripts

Early detection of onion neck rot disease in Manitoba

Poonam Singh, Faiz Ahmad, Vikram Bisht, Nevy Thakkar, Sumreen Sajjad

Poonam Singh. School of Agriculture and Environment, Assiniboine Community College,
Brandon, Manitoba, R7A2A9, Canada.

Faiz Ahmad. Biology Department, 270-18th Street, Brandon, Manitoba, R7A 6A9, Canada

Vikram Bisht. Manitoba Agriculture and Resource Development, PO Box 1149, 65 - 3rd Avenue
NE, Carman, Manitoba, R0G 0J0, Canada.

Nevya Thakkar. School of Agriculture and Environment, Assiniboine Community College,
Brandon, Manitoba, R7A2A9, Canada.

Sumreen Sajjad. Biology Department, 270-18th Street, Brandon, Manitoba, R7A 6A9, Canada.

Corresponding author: Poonam Singh ([Email: singhp@assiniboine.net](mailto:singhp@assiniboine.net))

Competing interests: The authors declare there are no competing interests.

Abstract: Neck rot caused by the fungus *Botrytis allii* Munn. is one of the most devastating diseases of onions (*Allium cepa* L.) resulting in significant yield losses. This disease is latent in nature developing symptomless onion plants in the field with bulbs typically showing symptoms 1-2 months after harvest in the storage. Molecular studies were conducted to detect latent infections of *Botrytis* neck rot in the onion fields of Manitoba, Canada. Plant samples of onion cultivars Redwing and Pocono were collected every 10 days throughout the growing season starting from planting until bulb harvesting during 2018, 2019, and 2020 from a research farm in Brandon, Manitoba, and plant samples of cultivar Redwing were collected during 2019 and 2020 from a commercial vegetable farm in Portage La Prairie, Manitoba. The amplified DNA fragment of onion leaves and neck region of collected samples were subjected to polymerase chain reaction (PCR) using *Botrytis* specific primer pair BA2f/BA1r. *Botrytis allii* was detected on onion samples collected from the commercial farm as early as the end of June 2019 and 2020 when plants were at the 5-7 leaf stage. The majority of onion samples collected from the research farm also started testing positive for the pathogen from June (2019, 2020) and July (2018) onwards. This knowledge about the timing of infection in the field will be useful in helping farmers to develop and evaluate management strategies in the field, and also predict the storability and availability of quality bulbs for sale.

Keywords: *Allium cepa*, *Botrytis* neck rot, Early detection, PCR

Introduction

Onion (*Allium cepa* L.) is an economically important vegetable crop of Canada with a cultivated area of 5776 hectares and a farm gate value of Canadian \$ 91,36,30,00 (Government of Canada, 2018). Dry onions (yellow, white, red) are the most common type of onion produced on the prairies (Kaulbars et al. 2014) with an estimated consumption of 7 kilograms per person a year (Statistics Canada, 2019).

In the Canadian prairies, including Manitoba, onions are direct-seeded when the frozen ground is thawed (typically during the end of April in Manitoba), or the seeds are planted in the greenhouse and later transplanted into the field. The crop requires a growing period of at least 120 days from planting to harvest (Kaulbars et al. 2014). Onions are stored under a controlled environment after harvest until the bulbs are shipped for marketing, which starts after harvest and continues throughout winter until the beginning of spring. Many abiotic factors like temperature, relative humidity, irrigation, soil fertility, and biotic factors like insect pests, diseases, and physiological disorders affect the yield and quality of onions. Among these, neck rot caused by the fungus *Botrytis allii* Munn. (syn. *B. aclada*) (Maude 1990; Yohalem et al. 2003), is one of the most damaging diseases of onions; losses due to neck rot during storage can be significant ranging from 35% (Tietjen and Ceponis 1981; Woodward 2001) up to 70% (Bansal et al. 2015). Most storage losses of onions in Manitoba, Saskatchewan and Alberta are attributed to botrytis neck rot (Kaulbars et al. 2014). The inability to detect infected bulbs at the grading stage is also of concern to growers who intend to export onions, as crops that appear perfect when packed, may rot in transit and storage.

The initial symptoms of neck rot disease can typically be noticed after 1-2 months of storage (Maude and Presly 1977). The symptoms start at the neck of the onion and move

downwards into the scales, but infection may occur at any part of the bulb. The neck area of the infected onion becomes watery; the scale tissues become soft, grey, wet, and translucent with the fungal sclerotia developing between scales (Petropoulos et al. 2017). The wounds caused by the fungus also open the tissue for several other infections (Hafez et al. 2013; Maude and Presly 1977).

B. allii can infect onions at any stage during the growing season. Potential sources of inoculum are infected seeds, onion debris from previous crops, and alternate crops (Maude, 1990; Walcott et al. 2004). Contaminated seeds can pass infections to growing leaves which act as a site for developing fungal colonies. The weather conditions during the growing and curing period play an important role in disease occurrence and prevalence. Infection from spores produced on dead onion tissue (cull onions) occurs during moist cool weather when temperatures are between 10-24°C. Plants can be infected by wind-blown *B. allii* spores through leaves, but the infection often enters the onion bulb through the succulent neck at the time of harvest (Delahaut and Stevenson 2004). Sclerotia of *B. allii* can also be contained in contaminated soils (Alberta Agriculture and Rural Development 2014), where they are viable for more than two years even without the presence of a host. When onions are planted, the sclerotia germinate and grow hyphae that infect onion tissue through wounds (Alderman and Lacy 1984).

Several reports from North America have indicated that *B. allii* infections remain latent on green foliage before harvest and the onion plants remain symptomless (Maude 1990; Maude and Presly 1977; Hafez et al. 2013). The latent nature of *B. allii* infections is one of the biggest roadblocks to the control of neck rot disease in prairie onion crops (Petropoulos et al. 2017). The post-harvest losses are detrimental to the commercial vegetable industry, as onions need to withstand long storage periods to provide a steady supply to consumers year-round (Statistics Canada 2019). Therefore, to keep onions in marketable condition throughout storage, the

development of onion production systems that reduce the incidence of neck rot in commercially produced onions is extremely important to the onion industry in Canada, and around the world.

Detecting diseased bulbs before storage has been attempted in the past. The methods that have been developed to detect latent *B. allii* in onion bulbs include culturing samples on semi-selective media, enzyme-linked immunosorbent assay (ELISA), and conventional Polymerase Chain Reaction (PCR) method (Kritzman and Netzer 1978; Linfield et al. 1995; Nielsen et al. 2002). The PCR protocol developed by Nielsen et al. (2002) stated that *Botrytis* species specific primer pair BA2f/ BA1r amplified all neck rot associated *Botrytis* isolates and a restriction digest of amplified product with ApoI enzyme separates the *Botrytis* spp. into five groups i.e. *Botrytis aclada*, *B. allii*, *B. cinerea*, *B. squamosa* and *B. byssoidea*.

Systematic work has not been done towards managing this disease effectively in Manitoba and right now, our only option is to rely on information based on work done elsewhere where conditions are not like Manitoba. Until now, there is no information available as to when this disease appears in Canadian prairie fields. The objective of the current study is to find out the right timing of the appearance of *Botrytis* neck rot latent infection in the onion plants using the conventional PCR method to predict the likelihood of neck rot development in stored onion bulbs. We intend to provide Manitoba onion producers valuable knowledge on the timing of neck rot disease to help them develop pre-harvest crop management strategies to reduce post-harvest losses.

Materials and Methods

Onion plant sample collection

Samples of onion cv. Redwing were collected from Mayfair farms (49°56'59" N, 98°17'35" W) located at Portage La Prairie, which is one of the main onion production regions of Manitoba. Onions were planted on April 24 in 2019 and April 27 in 2020, harvested on August 20 in 2019

and August 24 in 2020, field cured for 15 days after harvesting, and moved to storage on September 15, 2019, and on September 17, 2020. Thirty plant samples were collected every 10 days (dates were adjusted based on weather conditions) from May to September; a total of 360 samples were collected on 12 sampling dates during each year. To conduct uniform and representative sampling, the long rectangular field (1 acre-4047 m²) was divided into 3 grids of approximately similar size, and the 10 random plant samples were collected in a 'W' shaped path covering the entire area of each grid starting from the left-hand corner of the bottom of the field. The 'W' pattern ensured a representative number of sample units were taken throughout the sample area and crop edges were not given priority but were included in the sampling area (Barnett 2002). Where possible, the sampling site was located close to the previous year's cull pile hypothesizing it as a likely source of inoculum of *B. allii* for the current season's crop. Whole plants were carefully dug from the ground (not pulled out) so the root systems remain relatively intact. Plants were shaken to shed the soil and individual plants were placed in plastic bags, secured, and placed in a 4°C refrigerator until processing in the lab.

A similar sampling pattern and method was used for collecting plants from Assiniboine Community College's (ACC) research grow plot/farm (49°50'48" N, 99°57'11" W) measuring 3.2 acres (12945 m²) for three successive years. The size of the onion plot was 500 m². Twelve plant samples were randomly collected at 10 days intervals from 2 cultivars, viz. Redwing (red) and Pocono (yellow) on each of the sampling dates from May to September 2018-2020. The seedlings were transplanted on April 25, bulbs were lifted on August 19, and moved to cold storage after 21 days of bulb lifting for all three years. The plants were scouted for visible symptoms of neck rot disease in the field. The small size of the research plot made it possible to scout each plant for symptoms on every sampling date. However, due to the large field size of the commercial farm,

300 plants were randomly selected for monitoring within the sampling area on every sampling date, and the percentage of symptomatic plants was calculated. The disease symptoms included leaf distortion, stunted plant growth, splitting of leaves around the neck area, and occurrence of a grayish fungus near the neck area of plants.

Isolation of *B. allii* from onion tissue

Onion bulbs with apparent infection of neck rot were incubated in a plastic container for 72 hours and allowed to induce *B. allii* sporulation. Spores were then transferred onto a few healthy onion slices cut from surface-sterilized onion bulbs and incubated in a petri plate. Infection was noticed in 2-3 days, and 4-5 days later sporulation was seen on these slices. Using an inoculation needle, a few spores were spread onto petri-plates with PDA+ (potato dextrose agar amended with antibiotics, Streptomycin and Chloramphenicol). Using a stereoscope, hyphal tips were marked and transferred to new PDA+ plates. The cultures were maintained in PDA+ test tubes and used for PCR testing and identification.

DNA extraction from fungal tissue

Mycelium from *B. allii* sub-cultured on PDA for 10 days at 23 °C was scraped from the media surface and ground in liquid nitrogen using mortar and pestle. DNA was extracted using plant/fungal DNA isolation kit (Norgen Biotek Corp, Thorold, Ontario, Canada) following the steps described by the manufacturer. The purified total DNA was eluted with the 50 µl of elution buffer and stored at -20 °C until further use.

DNA extraction from plant tissue

The most basal oldest green leaf excluding dead dry leaves on each sample plant was used for extracting DNA. This was selected because of the assumption that the older leaves were more likely to harbour *B. allii* infection (Alderman and Lacy 1984). Furthermore, the oldest leaves

would have been exposed to potential infections for a longer period than younger leaves. Tissue (100 mg) from the neck and leaf region of onion plants were ground using liquid nitrogen in mortar and pestle. DNA was extracted using PureLink™ plant total DNA purification kit (Invitrogen, Massachusetts, United States) using the protocol described by the manufacturer. The purified DNA was stored at -20 °C until further use. The quality of the DNA was checked on 0.8 % agarose gel (Invitrogen) in 1×TBE buffer (Invitrogen). DNA (5 µl) was prepared with 4 µl of Gel Red (Biotium, Fremont, California, USA) and 2 µl of 5X DNA loading dye (Bio-rad Laboratories Canada Ltd., Mississauga, Ontario, Canada) to help visualize the band using molecular iImager Gel Doc XR+ (Bio-rad Laboratories Canada Ltd.).

PCR amplification

PCR amplification was performed in a reaction volume of 25 µl. The following compounds were added, with final volume indicated: 0.3 µl DreamTaq DNA Polymerase (500 U, 5 U/µl, Thermo Fisher Scientific, Waltham, Massachusetts, USA), 2.5 µl 10x DreamTaq Buffer (Thermo Fisher Scientific), 1µl 10 mM dNTP mix (Invitrogen), 1-1 µl of BA2f, and BA1r (50 pmol/µl) (Thermo Fisher Scientific), 18.2 µl sterile water, and 1 µl DNA. Duplicates of each sample were made. A PTC-100 Programmable Thermal Controller (MJ Research Inc. Quebec, Canada) thermocycler device was used with the following program: 94 °C for 5 minutes as an initial denaturation cycle, 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing of primer at 60 °C for 30 seconds and extension for 1 minute at 72 °C, and one cycle of final extension at 72 °C for 10 minutes. The PCR product was separated on 1.5 % agarose (Thermo Fisher Scientific) gels prepared in 1×TBE buffer (Thermo Fisher Scientific). PCR product (10 µl) was loaded into the gel which consists of 5 µl of amplified PCR product, 3 µl of Gel Red (1:10000) (Biotium), and 2 µl of 5X DNA Loading Dye (Bio-rad Laboratories Canada Ltd.). The bands were

visualized using the gel documentation system Gel Doc XR+ (Bio-rad Laboratories Canada Ltd.). Sequence-characterized amplified region primers, BA2f (nucleotide sequence 5'-3' GTGGGGGTAGGATGAGATGATG) and BA1r (nucleotide sequence 5'-3' TGAGTGCTGGCGGAAACAAA) were obtained based on a previous study by Nielsen et al. 2002; these primers were reported for use in amplification of DNA fragment for direct amplification of isolates of *Botrytis* spp. associated with neck rot of onions.

Restriction digestion:

BA2f/ BA1r amplified products (413 bp) from positive plant samples were further digested by the ApoI (New England Biolabs Inc., Beverly, Massachusetts, USA) restriction enzyme in a total volume of 10µl with 2µl of amplified product according to the protocol described by Neilson et al. (2002). This digested product (5µl) was separated on 2% agarose gel (Invitrogen), stained with Gel Red (1:10000) (Biotium) and visualized under UV light using Gel Doc XR+ gel documentation system (Bio-rad Laboratories Canada Ltd.).

Testing PCR specificity

The selection of the primer set based on a previous study necessitated the testing for the specificity of the primers BA2f/BA1r on artificially inoculated onion seedlings with *B. allii* before processing samples collected from the field. We tested eleven cultivars of onions viz. Pocono (yellow), Milestone (yellow), Red Nugent (red), SV4643NT (red), Catskill (yellow), Trailblazer (yellow), Powell (yellow), Trekker (yellow) [Stokes seeds Ltd, Thorold, Ontario, Canada], Red Sky (red), Gunnison (white), and Redwing (red) [Seedway LLC., Weiser, Idaho, USA], that are commercially planted by most vegetable growers of Manitoba. The seeds were planted in sterilized soilless media (mix of peat, perlite, and vermiculite) on February 7, 2019, at the greenhouse at the

Assiniboine Community College, Brandon, MB, and were kept in the growth chamber at 25 ± 2 °C under 14:10 light:dark cycles. The germinated seedlings at the flag leaf stage were sprayed with *B. allii* conidial suspension containing 10^6 conidia/ml twice at weekly intervals. The leaves were cut off from plants at 1-2 true leaf stage after 45 days of sowing and taken to the lab for DNA extraction and PCR amplification. DNA extracted from single spore culture of *B. allii* was used as positive control and Invitrogen TrackIt™ 100 bp DNA Ladder (Thermo Fisher Scientific) with band size of 0-2000 bp was taken as a reference marker for the experiment. The primers successfully amplified fungal strain yielding a product of approximately 413 bp and seedlings of all cultivars tested positive for the *B. allii* infection (Fig. 2). Therefore these primers were considered promising for their further use on field samples.

Weather data acquisition

Data on average daily temperature and humidity were recorded using Titan WS2 weather station configured with Argus Titan system version 718 (Argus Control Systems Ltd., Canada) installed at ACC's greenhouse/grow plot complex. Data on total precipitation for Brandon was acquired from Brandon Meteorological Station ID: CA005010490 (Environment Canada, 2020). Data on all environmental parameters for Portage La Prairie was obtained from Portage Southport Meteorological Station ID: CA005012324 (Environment Canada, 2020).

Results

The onion plant samples collected from the commercial farm on May 17, May 27, and June 3 tested negative for *B. allii* infection. The first occurrence of *B. allii* on plants was detected on the samples collected on June 20 in 2019 (Figs. 1a and 3), and on June 29 in 2020 (Fig. 1b) with more than 90% of samples testing positive for the infection at 5-7 leaf stage. All the 30 samples collected on July 13 of 2019 and 2020 tested positive for *B. allii* (Figs. 1a and 1b). Onion plants at

that time were 77-78 days old, had 8-10 leaves, and were at the bulb development stage. Starting from the end of June, throughout the growing season each year, only one or two DNA samples did not amplify for *B. allii* and tested negative while the rest tested positive for infection (Fig. 1). In total, 95 out of 360 samples in 2019, and 99 out of 360 samples in 2020 tested negative for the infection.

The positive leaf samples from June and July of each year from both locations were digested using *ApoI* restriction enzyme as described by Neilson et al. (2002) to confirm the *Botrytis* species. This was important to rule out the presence of *B. squamosa*, a common foliar pathogen of onion, which also gets amplified by the primers BA2f/BA1r used in the current study. The digestion yielded three fragments of 413 bp, 300 bp and 100 bp (Fig. 4) which were specific to *B. aclada*-Type II (reported as synonymous to *B. allii* by Neilson et al., 2002). All samples tested from both locations were found negative for *B. squamosa*.

The results showed that onion plants contained disease inoculum from the 5-7 leaf stage of plants until harvest. However, the plants looked healthy and remained visibly symptomless throughout the growing period with only 0.1 to 2.40 % of plants showing some disease symptoms towards the end of the cropping cycle (Figs. 1a and 1b). We stored the harvested bulbs in the commercial cold storage at 1.1 °C, and 65 % relative humidity, and inspected bulb lots for visible signs of *Botrytis* neck rot infection after 100 days. The bulbs of cv. Redwing from commercial farm recorded 34 % (2019), and 29 % (2020) neck rot disease and showed symptoms like the softness of neck, discoloration of scales near neck region, and appearance of grayish fungus on scales.

The processing of 2018 samples collected one week after transplanting from the ACC research farm revealed that the first *B. allii* positive samples were detected in cv. Redwing on June

25 (61 days after planting) when plants were at 6-7 leaf stage, and that in cv. Pocono was observed on July 1 at 67 days of crop age, and 8-12 leaf stage of onions (Fig. 1c). The number of positive samples in both cultivars remained low until August 5, after which the numbers consistently rose with more than 90% samples of both the cultivars testing positive after August 12 (Fig. 1c) except on September 2 when 67 % samples of cv. Redwing and 75% samples of cv. Pocono were found positive (Figs. 1c and 3b).. The crop was ready to be harvested at this stage with bulbs fully enlarged. We started seeing symptoms of the disease on plants towards the end of the season on August 26 when bulbs were lifted off the ground. The disease was more visible on cv. Redwing with 1.67-5.00 % plants with neck rot symptoms as compared with cv. Pocono where 1.00-2.34 % symptomatic plants were recorded (Fig. 1c). Infected plants exhibited leaf distortion and splitting of leaves around the neck area. A grayish fungus was also observed between leaf scales near the neck area in some plants.

In 2019, the first samples that tested positive for *B. allii* from ACC research farm were collected on June 11 when the plants were at the 4-6 leaf stage, and the crop age was 47 days (Fig. 1d). More than 80 % samples of cv. Redwing tested positive on July 29, when the crop was 95 days old, and bulbs are attaining large size (Figs. 1d and 3c). The trend remained the same until bulb harvesting with a majority of samples testing positive with the progression of the season. There were not many genetic differences observed as both the cultivars resulted in a high number of *B. allii* positive samples from August 12 onwards. However, the number of plants of cv. Redwing with visible symptoms was lower (1.00-2.50%) in 2019 than in 2018 (1.67-5.00%) (Fig. 1d).

More onion plants were found positive for *B. allii* infection earlier in the 2020 season as compared to previous years. The first positive samples were obtained as early as June 4 when

plants were 40 days old and at the 3-4 leaf stage (Fig. 1e). The sample positivity rates increased steadily in the season until early July after which it escalated fast with the majority of samples testing positive later in the season. We observed the first plants with disease symptoms on July 1 in cv. Pocono when plants were 67 days old, and at the bulb initiation stage. The number of plants with visible disease symptoms was low initially, but later at the time of bulb harvesting (September 16 sampling), 15.33 % of plants of cv. Redwing) and 8.85 % plants of cv. Pocono were found with greyish fungus between the scales around the neck area (Fig. 1e). The post-storage data of onions from the ACC research farm revealed that bulbs with neck rot symptoms were 20 % in cv. Redwing, 7 % in cv. Pocono in 2018; 25 % in cv. Redwing, 13 % in cv. Pocono in 2019; and 30 % in cv. Redwing, and 17 % in cv. Pocono in 2020.

Discussion

Our study confirmed that the neck rot disease started occurring in the onion fields as early as June when plants were at a very young age. The comparison of three years' data from research farm at Brandon showed that the onset of disease in the field was earlier with successive years of onion planting. None of the *Allium* species were planted at the ACC research farm and at neighboring farms before 2018, which means the plot did not have any potential source of *B. allii* inoculum in the soil. Although we rotated the crop every year and refrained from using the same area for the subsequent onion crops, the overall small size of the research plot means that inoculum from the previous year's plot might have already been present in 2019 and 2020.

The situation at the commercial farm was different. Although a 3year crop rotation was followed, and the farmer tried best to distance his crop from neighbouring onion farms, the field was located in the main vegetable growing area of the province and was surrounded by many vegetable farms where onions were planted in the past. There was a high chance of the presence

of inoculum in the surrounding area which could explain why the infection was detected earlier on onion plants during both sampling years. Other studies also confirmed that the conidia produced during the previous season can infect plants at the seedling stage (Maude and Presly 1977; Maude et al. 1982; du Toit et al. 2004), resulting in asymptomatic plants, which can develop symptoms of neck rot during storage (Maude 1990). Despite the seasonal variations in the temperature, relative humidity, and amount of precipitation that the Portage La Prairie area received during 2019 and 2020 (Figs. 5a and 5b), the disease appeared in the field at almost the same time both years (Figs. 1a and 1b). However, more bulbs were detected with neck rot disease after storage in 2019 compared to 2020. This could be attributed to heavy precipitation in September 2019 (Fig. 5a) that might have led to improper drying of the bulb necks during field curing, making the conditions ideal for the development of neck rot disease in storage. Delahaut and Stevenson (2004) reported that the neck rot infection mostly enters the onion bulb through the succulent onion necks at the time of harvest. Curing conditions and duration can affect bulb quality and yield losses, as they are closely related to the frequency of storage diseases of onions (Schroeder et al. 2012; Bansal et al. 2015).

Year-wise varietal variations were noted among bulbs grown at the ACC research farm with regards to post-storage development of disease symptoms. In Brandon, the precipitation during August of 2019 (Fig. 5d) also did not allow the crop to cure properly in the field, resulting in the storage of onion bulbs with incompletely dried necks. Similarly, heavy precipitation during July of 2020 (Fig. 5e) resulted in flooding of the ACC fields for several days. The onion research plot remained completely submerged in water for 3-4 days; these conditions might have contributed to the dispersal of inoculum from diseased to healthy plants resulting in high neck rot in storage during 2020 as compared to other years.

Delahaut and Stevenson (2004) reported that the *B.allii* infections on onions occurred during moist cool weather at temperatures between 10-24°C. Alderman and Lacy (1984) found that the mycelial growth of *B. allii* in onion tissue as well as sporulation on agar medium was optimal at 20°C. Maude and Presly (1977) reported that the incidence of onion bulbs with neck rot was greater in a wet growing season. Humid weather with the temperature range ideal for *B. allii* growth persisted in Manitoba during most of the onion growing season during all three years (Figs. 5a-e), explaining the high incidence of neck rot disease.

Genetics of onion cultivars also play an important role in the severity of the disease occurrence (Kritzman, 1983; Alderman and Lacy 1984; Bertolini and Tian 1997). Both the cultivars, Redwing and Pocono, had more than 90 % *B. allii* positive onion plants at harvest, but cv. Redwing resulted in more bulbs with severe disease symptoms after storage. Owen et al. (1950) noted that strongly pungent onion cultivars containing higher amounts of chemical, allyl-propyl-disulfide, had consistently lower neck rot indices than the mild cultivars tested. In our case, the yellow onion cv. Pocono was slightly more pungent than red cv. Redwing, but the differences were not very prominent.

Disease development is also influenced by other factors like the size of bulbs. Ward (1979) reported that as onion bulb size increases, so does the proportion of bulbs that develop neck rot in storage. The higher number of bulbs rotting in storage for cv. Redwing might be attributed to the bigger bulb size of this cultivar as compared to cv. Pocono.

Our results from both locations demonstrated that latent *B. allii* infections occurred early in the growing season when onion plants were at the 5-7 leaf stage. This is contrary to the common assumption by growers that bulb infection mainly occurs at harvest time due to defoliation. Our results are in close conformity with a previous study by Maude (1990) who reported that although

the open wound resulting from the removal of foliage at harvest was an important site for bulb infection, many plants were infected early in the growing season. In a previous study by Bisht et al. (2013), neck rot infection was detected in an onion field of Manitoba in mid-June. The disease incidence, however, was not confirmed using molecular methods.

We inspected the crop for visible signs or symptoms of infection on the plants during our sampling visits. The number of onion plants with visible disease symptoms (especially in the commercial farm) was low; we only started seeing symptoms towards the end of the season a few days before bulb lifting, but more visibly at the curing stage of bulbs (Figs. 1a-1e). These results were in confirmation with other studies (Maude 1990; Maude and Presly 1977; Hafez et al. 2013) reporting that with a latent period of 8-10 weeks, not just plants, but also the infected bulbs may also appear asymptomatic at harvest, only to develop symptoms of the neck rot during storage.

Our study confirmed that *B. allii* infections in the field could yield a significantly high percentage of infected bulbs. Future studies that examine the relationship between field infection and storage rot would be beneficial. It is also important to determine an infection threshold in onions at the time of harvest, as some onions may contain heavy amounts of inoculum, but still go without noticeable bulb rot in storage.

Our research provides baseline information and opens new vistas for researchers to conduct advanced studies on developing a real-time PCR assay that could be useful to determine the threshold of *B. allii* inoculum in onion bulbs at harvest. The ability to quantify the inoculum in bulb tissues will be of great importance because it is likely that the amount of inoculum in a bulb at harvest is related to bulb rot after storage. Previous studies have found that symptom development on onion bulb scales by *B. allii* was dependent on inoculum concentration, with a minimum of 10^4 conidia per ml required for the production of lesions (Stewart and Mansfield,

1984). The information generated could further help growers to decide to sell infected bulbs with a high risk for rot development first while storing the lowest risk bulbs for a comparatively longer time. Results of our current study may help to design future research trials to determine the time of application and efficacy of fungicides against neck rot in the field and their effect on storability of onion bulbs in Canada and elsewhere.

Acknowledgments

The authors thank Mr. Todd Griffin, owner of Mayfair farms, for allowing us to collect the onion samples from the farm and for valuable discussions on neck rot. We are also thankful to Dr. Manika Pradhan, Crop Diagnostic Specialist in the Manitoba Agriculture and Resource Development department for providing the *Botrytis allii* cultures. This work was supported by the 'Vegetable Growers of Manitoba' association. The funding for the project was provided by Agriculture and Agri-Food Canada and Manitoba Agriculture through the Canadian Agricultural Partnership Program.

References

- Alberta Agriculture and Rural Development. 2014. Commercial Vegetable Production on the Prairies. [Online]. Available from [https://www1.agric.gov.ab.ca/\\$Department/deptdocs.nsf/all/agdex15123/\\$FILE/250_13-1_web.pdf](https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex15123/$FILE/250_13-1_web.pdf) [15 Feb. 2021].
- Alderman, S.C., and Lacy, M.L. 1984. Influence of temperature and water potential on growth of *Botrytis allii*. Can. J. Bot. **62**:1567-1570. doi: <https://doi.org/10.1139/b84-209>

- Bansal, M.K., Boyhan, G.E., and MacLean, D.D. 2015. Effects of postharvest curing, ozone, sulfur dioxide, or low oxygen/high carbon dioxide storage atmospheres on quality of short-day onions. *Horttechnology*. **25**:639-644. doi: <https://doi.org/10.21273/HORTTECH.25.5.639>
- Barnett, V. 2002. *Sample Survey: Principles and Methods*. Chichester UK: John Wiley and Sons
- Bertolini, P., and Tian, S.P. 1997. Effect of temperature of production of *Botrytis allii* conidia on their pathogenicity to harvested white onion bulbs. *Plant Path.* **46**:432-438. doi: <https://doi.org/10.1046/j.1365-3059.1997.d01-24.x>
- Bisht, V., Gonsalves, T., and Desjardins, M. 2013, February 21-22. Onion neck rot control fungicide trials 2011 and 2012. Vegetable Growers of Manitoba Annual General Meeting Report, Manitoba, Canada.
- Delahaut, K., and Stevenson, W. 2004. Onion disorders: Botrytis leaf blight, leaf fleck and neck rot. A3803 University of Wisconsin Extension publication. [Online]. Available from <http://learningstore.uwex.edu/assets/pdfs/A3803.pdf> [Feb. 20 2021].
- du Toit, L., and Derie, M.L. 2004. Prevalence of *Botrytis* spp. in onion seed crops in the Columbia basin of Washington. *Plant Dis.* **88**:1061-1068. doi: <https://doi.org/10.1094/PDIS.2004.88.10.1061>
- Government of Canada. 2018. Statistical overview of the Canadian vegetable industry 2017. [Online]. Available from <https://www.agr.gc.ca/eng/horticulture/horticulture-sector-reports/statistical-overview-of-the-canadian-vegetable-industry-2017/?id=1541522324172-a1.3> [31 Jan. 2021].

- Hafez, E.E., Wasfy, E.H., Ramadan, A.H.M., and Abdel-Gayed, M.A. 2013. Molecular Identification of four *Botrytis* species, three of them associated with neck rot and blasting diseases of onion with special reference of two, *aclada* and *byssoidea*. J. Pure Appl. Microbio. **7**:79-91.
- Kaulbars, C., Harper, L., and Serafinchon J.A. 2014. Commercial vegetable production on the prairies. Alberta Agriculture and Rural Development. [Online]. Available from [https://www1.agric.gov.ab.ca/\\$Department/deptdocs.nsf/all/agdex15123/\\$FILE/250_13-1_web.pdf](https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex15123/$FILE/250_13-1_web.pdf) [Jan. 4 2021].
- Kritzman, G., and Netzer, D. 1978. A selective medium for isolation and identification of *Botrytis* spp. from soil and onion seed. Phytoparasitica. **6**:3-7. doi: <https://doi.org/10.1007/BF02981180>
- Kritzman, G. 1983. Identification of latent *Botrytis allii* Munn. in onion bulbs. Crop Prot. **2**:243-246. doi: [https://doi.org/10.1016/0261-2194\(83\)90051-0](https://doi.org/10.1016/0261-2194(83)90051-0)
- Linfield, C., Kenny, S.R., and Lyons, N.R. 1995. A serological test for detecting *Botrytis allii*, the cause of neck rot of onion bulbs. Ann. Appl. Biol. **126**:259-268. doi: <https://doi.org/10.1111/j.1744-7348.1995.tb05364.x>
- Maude, R.B., and Presly, A.H. 1977. Neck rot (*Botrytis allii*) of bulb onions. Seed-borne infection in relation to the disease in store and the effect of seed treatment. Ann. Appl. Biol. **86**:181-188. doi: <https://doi.org/10.1111/j.1744-7348.1977.tb01830.x>
- Maude, R.B., Bambridge, J.M., and Presly, A.H. 1982. The persistence of *Botrytis allii* in field soil. Plant Path. **31**:247–252. doi: <https://doi.org/10.1111/j.1365-3059.1982.tb01275.x>

- Maude, R.B. 1990. Storage diseases of onions. In: Rabinowitch HD, Brewster JL, editors. Onions and allied crops volume II: Agronomy, biotic interactions, pathology and crop protection, Boca Raton (FL): CRC Press; p. 273-296.
- Nielsen, K., Yohalem, D.S., and Jensen, D.F. 2002. PCR detection and RFLP differentiation of *Botrytis* species associated with neck rot of onion. Plant Dis. **86**: 682-686.
<https://doi.org/10.1094/PDIS.2002.86.6.682>
- Owen, J. H., Walker, J. C., and Stahmann, M. A. 1950. Variability in onion neck-rot fungi. Phytopathology. **40**: 749-768.
- Petropoulos, S.A., Ntatsi, G., and Ferreira, I.C.F.R. 2017. Long-term storage of onion and the factors that affect its quality. A critical review. Food Rev. Int. **33**:62–83. doi: <https://doi.org/10.1080/87559129.2015.1137312>
- Schroeder, B., Humann, J., and Du Toit, L. 2012. Effects of postharvest onion curing parameters on the development of sour skin and slippery skin in storage. Pl. Dis. **96**: 1548–1555. doi: <https://doi.org/10.1094/PDIS-02-12-0117-RE>
- Statistics Canada. 2019. Area, production and farm gate value of vegetables. [Online]. Available from <https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210036501andpickMembers%5B0%5D=1.1andpickMembers%5B1%5D=3.11> [Feb. 5 2021].
- Stewart, A., and Mansfield, J.W. 1984. Fungal development and plant response in detached onion, onion bulb scales and leaves inoculated with *Botrytis allii*, *B.cinerea*, *B. fabae* and *B. squamosa*. Plant Path. **33**:401-409. doi: <https://doi.org/10.1111/j.1365-3059.1984.tb01335.x>

- Tietjen, W.H., and Ceponis, M.J. 1981. Dry onion storage losses in New York. *Phytopathology*. **71**: 261.
- Walcott, R.R., Gitaitis, R.D., and Langston, D.B.J. 2004. Detection of *Botrytis aclada* in onion seed using magnetic capture hybridization and the polymerase chain reaction. *Seed Sci. Technol.* **32**:425-438. doi: <https://doi.org/10.15258/sst.2004.32.2.14>
- Ward, C.M. 1979. The effect of bulb size on the storage-life of onions. *Ann. Appl. Biol.* **91**: 113-117.
- Woodward, J.L.W. 2001. Georgia plant disease loss estimates. University of Georgia Cooperative Extension Service. [Online]. Available from <https://extension.uga.edu/publications/detail.html?number=SB41-04&title=2001%20Georgia%20Plant%20Disease%20Loss%20Estimates> [Jan. 15 2021].
- Yohalem, D.S., Nielsen, K., and Nicholaisen, M. 2003. Taxonomic and nomenclatural clarification of the onion neck rotting *Botrytis* species. *Mycotaxon*. **85**:175-182.

Fig. 1. Percentage of *B. allii* positive samples and symptomatic plants of onions from a) Mayfair farms, 2019, b) Mayfair farms, 2020, c) ACC research farm, 2018, d) ACC research farm, 2019, e) ACC research farm, 2020. Thirty samples were collected from Mayfair farms and 12 plants (per cultivar) were collected from ACC farm on each sampling date. The crop growth stages corresponding to sample collection dates are written using abbreviations: L-leaves, BI-bulb initiation, BD-bulb development, BE-bulb enlargement, TD-tops down, BL-bulb lifting; FC-field curing, H-harvesting.

a)

b)

c)

d)

e)

Fig. 2: Electrophoretic pattern of PCR product amplified by primers BA2f/BA1r. Lanes M: Invitrogen TrackIt™ 100 bp DNA Ladder with maximum band size of 2000 bp, Lane 1: *B. allii* positive control, Lanes 2-13: *B. allii* inoculated seedlings of onion cultivars: Lane 2: Pocono, Lane 3: Milestone, Lane 4: Red Nugent, Lane 5: SV4643NT, Lane 6: Catskill, Lane 7: Trailblazer, Lane 8: Trekker, Lane 9: Red Sky, Lane 10: Gunnison, Lane 11: Trekker (pelleted seeds) Lane 12: Redwing, Lane 13: Powell. Occurrence of bands in onion cultivars corresponding to similarly located bands of *B. allii* at 413 bp explains that primer amplified the *B.allii* successfully and the inoculated seedlings of all cultivars tested positive for the infection.

Fig. 3. PCR amplification with primers BA2f/BA1r on onion plant samples. M: Invitrogen TrackIt™ 100 bp DNA Ladder with a maximum band size of 2000 bp, Lane 1: *B. allii* positive control. Lanes 2-13: onion plant samples a) cv. Redwing collected from Mayfair farms on June 20, 2019; b) cv. Pocono collected from ACC research farm on September 2, 2018; c) cv. Redwing collected from ACC research farm on July 29, 2019. Lanes showing bands at the 413 bp mark corresponding to *B. allii* bands indicate that samples collected from plants were positive for infection.

a)

b)

c)

Fig. 4. Restriction digestion of amplified PCR product with BA2f.BA1r. M: Invitrogen TrackIt™ 100 bp DNA Ladder with a maximum band size of 2000 bp. Lane 1: *B. allii* positive control. Lanes 2-8: *B. allii* positive onion samples from Mayfair farms, July 13, 2019; Lanes 9-13: *B. allii* positive onion samples from ACC research farm, June 18, 2020. The digestion yielded three fragments of □413 bp, □300 bp and □100 bp (Fig 4) which were specific to *B. allii*.

Fig. 5. The average daily temperature (°C) average daily humidity (%), and total precipitation (mm) received between two sampling dates during onion growing season of a) 2019, and b), 2020 at Portage La Prairie, Manitoba; c) 2018, d) 2019, and e) 2020 at Brandon, Manitoba.

a)

b)

c)

d)

e)