The current outbreak of stemphylium leaf blight of onion in New Zealand – identification of cause and review of possible risk factors associated with the disease

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Abstract During the 2017–18 growing season, significant outbreaks of leaf blight occurred in Pukekohe, Hawke's Bay and Canterbury commercial onion fields. It was unknown if the causal agent was *Stemphylium vesicarium*, a pathogen already present in New Zealand that causes stemphylium leaf blight (SLB), or a new introduction of another *Stemphylium* species. Morphological and molecular characterisation methods were used to identify the pathogen present on diseased onion leaves. The possibility that climate may have been a contributor to the outbreak was evaluated using hourly temperature and relative humidity data, and comparing the 2017–18 growing season with the previous four seasons in these regions when no disease was observed. Our research indicates that the recent leaf blight outbreak in New Zealand was caused by *S. vesicarium*, and not the introduction of a novel species of *Stemphylium*. The warm, and wet summer of 2017–18 possibly contributed to the SLB outbreak.

Keywords *Stemphylium vesicarium*, stemphylium leaf blight, onion, environmental factors, climate, management

INTRODUCTION

During the 2017–18 onion-growing season, significant outbreaks of leaf blight occurred in commercial fields of onion (*Allium cepa*) in New Zealand, including the Auckland, Waikato, Hawke's Bay and Canterbury regions. The disease, which in previous years occurred sporadically and at low levels, causes significant crop losses and yield reductions in 2017-18. In February 2018, a *Stemphylium* species was identified from all four regions based on conidial characteristics of spores taken from diseased onion leaves showing leaf blight symptoms (Tyson, unpublished data).

Stemphylium leaf blight (SLB) in onions is caused by *Stemphylium vesicarium*. The disease can lead to premature senesce of onion leaves, which can compromise bulb quality. In addition, this disease can make the crop more susceptible to secondary diseases that also affect bulb quality (i.e. storage rots caused by bacterial pathogens) (Paibomesai & Celetti 2012). Significant yield loss can occur in onion fields severely affected by SLB – such outbreaks have been reported to reduce yield by as much as 60% (Plantwise 2018). In recent years, outbreaks of SLB have become more common and severe in the US states of New York, Michigan and Wisconsin, and the Canadian provinces of Ontario and Quebec than previously experienced. Several local onion experts have postulated that SLB has transformed from being a secondary disease of necrotic tissue to an aggressive onion disease that can cause excessive leaf dieback (Hoepting 2016).

Stemphylium vesicarium is a pathogen of a wide range of hosts including: asparagus (*Asparagus officinalis*); European pear (*Pyrus communis*); garlic (*Allium sativum*); lucerne (*Medicago sativa*); mango (*Mangifera indica*); onion (*Allium cepa*); and soyabean (*Glycine max*) (CABI 2017; Farr & Rossman 2018). Miller & Schwartz (2008) reported that SLB of onion occurs in many onion- and garlic-growing regions of the world. Prior to 1970, the traditionally held view in New Zealand was that that the populations of the genus Stemphylium in this country were *S. botryosum* (teleomorph *Pleospora tarda*) (Singh 1977). However, Singh (1977) determined that there are three species of *Stemphylium* in New Zealand: *S. vesicarium; S. botryosum*; and *S. globuliferum*. In 1973, Singh (1977) studied 166 *Stemphylium* spp. isolates from 12 host species in New Zealand and reported that *S. vesicarium* was the most common species encountered, and that it was the only species present on onion, tomato, pepper and chrysanthemum.

In onion and garlic, infection usually remains restricted to the leaves and inflorescences, and does not extend to bulb scales (Aveling & Snyman 1993). Although SLB can be seen when the onion crop is at the 3- to 4-leaf stage, the disease most commonly occurs at plant maturity and when leaves begin to senesce (Tayviah 2017). The initial symptoms on onion leaves consist of small yellow to tan, water-soaked spots. These small spots/lesions develop into elongated (boat-shaped) light brown to tan leaf lesions, which turn dark olive brown when the pathogen begins to produce dense masses of spores (Basallote et al. 1993; Hausbeck 2010). Leaves may become completely blighted as the lesions coalesce (Tesfaendrias et al. 2012). As the disease progresses, infected leaves undergo rapid necrosis from the tip down, which can lead to desiccation of leaves and early death of the crop (Rao & Pavgi 1975). Sometimes, small, black, pin-like, raised fungal fruiting bodies called perithecia (pseudothecia) may appear in the blighted areas of the leaves and scape (Miller & Schwartz 2008). Typical SLB lesions are more commonly found in higher numbers on the side of leaves facing the prevailing wind (Miller et al. 1978). The symptoms of SLB can be confused with purple blotch, which is caused by *Alternaria porri* (Tesfaendrias et al. 2012). Both SLB and purple blotch lesions may occur on the same plant, and spores of each may occur on the same lesion (Abdel-Rahim et al. 2017; Hoepting 2016). The cause of symptoms cannot be determined easily until the spore phases of the two pathogens occur (Koike et al. 2006).

The disease cycle of SLB is characterised by sexual and asexual phases, and the SLB fungal spores are blown into onion fields from nearby plants (Nischwitz 2016). Ascospores are released from pseudothecia on dead plant tissues from the previous season, and conidia (asexual spores) are released from leaf infections of nearby onions (Basallote-Ureba et al. 1999). Infection of onion leaves by conidia can occur by various mechanisms such as through stomatal openings (Tayviah 2017), wounds caused by other diseases, insect pest feeding, or physical damage such as wind, hail and herbicide damage (Nischwitz 2016; du Toit 2017), and direct infection of dead or dying leaves (Behera et al. 2013; Hoepting 2016). du Toit (2017) stated that onion plants subjected to heat stress were more susceptible to SLB.

Stemphylium leaf blight infection and disease development are favoured by temperatures between 15 and 25°C, humid conditions, and long periods of leaf wetness lasting 8 h or more (Suheri & Price 2000a; Tayviah 2017). Miller & Schwartz (2008) stated that *S. vesicarium* spore populations on leaf surfaces may exceed 200 conidia per square centimetre under favourable conditions, i.e. temperatures above 18°C, high airborne conidia concentrations, and 24 hours of continuous wet weather. Sporulation usually occurs at the site of the initial lesions and is observed 6–14 days after lesion development (Basallote-Ureba et al. 1999).

Stemphylium vesicarium is generally regarded as a secondary pathogen that attacks previously damaged tissue; therefore, prevention of the primary infection is the most effective strategy to control the disease (Miller & Schwartz 2008). Disease incidence and severity depend on several factors including the presence of the pathogen and the number of spores, environmental conditions (especially rainfall, humidity and temperature), and the onion cultivar and health of the crop. No single tactic will effectively manage SLB but integrating several control practices can help onion growers to maintain crop quality while minimising economic losses because of the disease.

Currently, there are no commercially available onion cultivars that are resistant to SLB. Tolerance to SLB has been identified, but complete resistance has not been found in onion or other *Allium* species (Singh et al. 2018). Pathak et al. (2001) reported that onion cultivars screened in Taiwan were all susceptible to infection by *S. vesicarium*, but the degree of susceptibility differed among cultivars. Observations of onion-cultivar trials in Ontario also indicated differences in the susceptibility of various cultivars to SLB (Tayviah et al. 2015).

Cultural methods for management of fungal diseases such as SLB aim to reduce the pathogen inoculum load and to create environmental conditions that are less favourable for infection. Burying crop residues at the end of the onion growing season by deep tillage reduces SLB by facilitating plant decomposition, increasing the action of pathogen antagonists, and preventing aerial release of ascospores after pseudothecia mature (Basallote-Ureba et al. 1998; Paibomesai & Celetti 2012). Culls and plant debris can be a substrate for both pathogens and insects that cause wounding on onion plants so culls and volunteer plants should be removed from the field (Hausbeck 2010; Nischwitz 2016). A 3- to 4-year crop rotation can help reduce the amount of *S. vesicarium* inoculum in the soil, thereby reducing SLB incidence (Hausbeck 2010; Paibomesai & Celetti 2012; Nischwitz 2016). Careful use of fertilisers is recommended on onion crops as excessive nitrogen applications can increase SLB severity (Mishra et al. 2014; Nischwitz 2016).

Methods to reduce leaf wetness duration to reduce the incidence and severity of SLB include increasing plant spacing in seedbeds to facilitate air movement and rapid drying of the foliage (Paibomesai & Celetti 2012), aligning rows of plants to follow the direction of the prevailing wind (Plantwise 2018), and irrigating crops during the late morning or early afternoon to allow leaf surfaces to dry rapidly, thereby reducing the potential of infection (Hausbeck 2010). du Toit (2017) stated that furrow and drip irrigation controls SLB by reducing foliage wetness duration.

Various disease-forecasting systems have been used or developed for diseases caused by *S. vesicarium*, with the goal of reducing the number of fungicide applications required (Tayviah 2017). Such models use the relationship between the duration of wetness or humidity and temperature to determine disease infection periods and to predict disease risk. Two forecasting systems (STREP and FAST) developed for *Alternaria solani* on tomato were used to predict SLB incidence (Tayviah 2017). Disease-forecasting systems have not yet been specifically developed for SLB of onion but the success of this technology for other plant diseases caused by *S. vesicarium* highlights the potential of disease forecasting as a useful SLB disease control tool. Such a tool could reduce the number of fungicide sprays required to keep disease levels below economic thresholds compared with calendar-based applications (Tayviah 2017).

Biological control agents, including *Bacillus subtilis, Saccharomyces cerevisiae, Pseudomonas fluorescens* and *Trichoderma* spp., have been shown to reduce the severity of SLB in onion under controlled conditions. However, products incorporating these microbes often do not provide effective management of SLB under field conditions when used as the sole management strategy (Meena & Verma 2017; Tayviah et al. 2017).

Because *S. vesicarium* readily invades onions that have been physically damaged or infected by other diseases, it is important to maintain healthy plant stands and control other common foliar diseases such as purple blotch, downy mildew and *Botrytis* (Paibomesai & Celetti 2012), insect pests such as thrips (Nainwal & Vishunavat 2016), and to avoid injuring bulbs during production (Koike et al. 2006).

No fungicide is currently registered for control of SLB of onion in New Zealand (Agrimedia 2018), and fungicides that control downy mildew do not always control SLB (e.g. mancozeb) (Hoepting 2017; Suheri & Price 2000b). Fungicides currently registered in New Zealand for stemphylium leaf spot of asparagus caused by *S. vesicarium* are chlorothalonil (currently registered in New Zealand for the control of onion downy mildew as Cobra™ (Lonza NZ Limited), in a mixture with dimethomorph), copper (registered in New Zealand for onion downy mildew and bacterial blight), procymidone (registered in New Zealand for onion white rot), difenoconazole (not currently registered in New Zealand for any onion disease), and iprodione (not currently registered in New Zealand for any onion disease) (Agrimedia 2018).

Overseas, the fungicides reported as being the most effective for managing SLB of onion belong to Fungicide Resistance Action Committee (FRAC) groups 3 and 7 (Hausbeck 2010; Hoepting 2016; Tesfaendrias et al. 2012; Yadav et al. 2017). These groups of fungicides are often used in product mixtures with other fungicides from the same or other FRAC group (FRAC group in brackets), e.g. difenoconazole (3), propiconazole (3), tebuconazole (3), boscalid (7), fluopyram (7), and fluxapyroxad (7), which is currently registered in New Zealand for white rot of onions. Fungicides in FRAC group 2 that are used for control of SLB on onion overseas include procymidone and iprodione (Aveling & Snyman 1993; Basallote-Ureba et al. 1998).

The fungicides in FRAC group 11 that are used for SLB control overseas include azoxystrobin and pyraclostrobin (Tesfaendrias et al. 2012; Hoepting 2016). However, these chemicals appear to be becoming less effective in the US states of New York, Michigan and Wisconsin and the Canadian provinces of Ontario and Quebec (Hoepting 2016; du Toit 2017; Hay 2018). Hoepting (2016) reported that fungicide trials conducted in onions between 2013 and 2015 showed that protectant fungicides including chlorothalonil (FRAC code M5) and mancozeb (FRAC code M3) had very little activity against SLB, and fungicides belonging to FRAC groups 9 (pyrimethanil), 20 (pencycuron), 12 (fludioxonil) and 2 (iprodione) had poor activity against SLB.

The aims of this study were to identify the cause of the recent outbreak of SLB of onion in New Zealand and examine the relationship between prevailing climate conditions and SLB development.

METHODS

Identification of the causal organism of the recent leaf blight outbreak

Leaves from 20-30 onions with symptoms of SLB were collected in February 2018 from a commercial onion field in Te Kauwhata (Waikato) and from two fields near Ashburton in Canterbury. Small pieces of leaf with visible lesions were placed onto Difco Potato Dextrose Agar (PDA) amended with ampicillin and streptomycin and incubated at c. 22°C for two weeks under ambient laboratory lighting. The resulting colonies were identified as *Stemphylium* spp. by observation of spore formation and morphology under a light microscope.

Ten *Stemphylium* spp. isolates were obtained from SLB lesions from different onions from Te Kauwhata and Canterbury fields. In addition, two *Stemphylium* spp. isolates from *Allium* spp. were obtained from the International Collection of Micro-organisms from Plants (ICMP), Manaaki Whenua – Landcare Research, Auckland (Table 1).

Two pairs of universal primers were used for molecular identification of each isolate, ITS1/ITS2 targeting the internal transcribed spacer and GPD-F/GPD-R targeting the glyceraldehyde 3-phosphate dehydrogenase (GPD) gene. DNA from the mycelium of each isolate was obtained using a DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. Polymerase chain reactions (PCR) were performed using both primer pairs using the conditions described in Camara et al. (2002). PCR products were visualised on a 0.7% agarose gel and then purified using a QIAGEN QIAquick PCR Purification Kit before being sent to a commercial provider (Macrogen, Seoul, Korea) for sequencing. The identity of each *Stemphylium* isolate was determined using the Geneious BLAST function to compare the sequences generated by Macrogen with those stored in the National Center for Biotechnology Information (NCBI) database.

Climate conditions and stemphylium leaf blight development

Hourly minimum and maximum temperatures and relative humidity (RH) were obtained from the National Institute of Water and Atmospheric Research Limited (NIWA) climate database for Pukekohe (station 2006), Hawke's Bay (station 15876) and Canterbury (station 17603) as best representative climate stations for the sampled fields, so the Pukekohe climate station was used as representative of the fields sampled in Te Kauwhata. Missing data were added using a spline regression of daily pattern changes in the data. The dates used for climate assessment were from 1 August to 28 February for 5 years between 2013 and 2018. This period captured the bulk of the normal onion-growing season, and when SLB first appeared in late December 2017 to early January 2018.

Conditions ideal for SLB infection are when temperatures are above 15°C and leaves are wet (Suheri & Price 2000a). No hourly leaf wetness measurements exist for the Pukekohe, Hawke's Bay and Canterbury regions. Hence, an assumption was made that an ideal infection condition occurred when the minimum temperature was above 15°C and RH was above 88% for any given hour. These temperature and RH conditions should occur for 16 h for optimal infection; however, Suheri & Price (2000a) previously found that interruption of these conditions by a dry period of up to 24 h had very little effect on reducing SLB in onions.

The number of days with likely infection potential were calculated to evaluate possible environmental effects on SLB, and the accumulated days of infection conditions were estimated to compare seasons. The number of hours with the ideal temperature and RH conditions were summed for each day. These were then expressed as a fraction of a day (24 h)

Table 1 Source location and year of collection of each *Stemphylium* isolate used for identification.

Isolate number	Location	Year collected	Host
cc968	Canterbury – field 1	2018	*Allium cepa*
cc969	Canterbury – field 1	2018	*Allium cepa*
cc970	Canterbury – field 1	2018	*Allium cepa*
cc971	Canterbury – field 1	2018	*Allium cepa*
cc972	Canterbury – field 2	2018	*Allium cepa*
cc973	Canterbury – field 2	2018	*Allium cepa*
cc974	Te Kauwhata	2018	*Allium cepa*
cc975	Te Kauwhata	2018	*Allium cepa*
cc976	Te Kauwhata	2018	*Allium cepa*
cc977	Te Kauwhata	2018	*Allium cepa*
ICMP11210	Hawke's Bay	1979	*Allium porrum*
ICMP8719	Pukekohe, Auckland	1983	*Allium cepa*

and accumulated over the growing season from 1 August to the end of February.

Water stress may favour SLB infection but it was not possible to identify possible stress events as individual farmer records of irrigation were not available. Also, water stress is more likely to be local rather than regional in effect and SLB appears at a regional scale. Onions are affected by heat stress above 27°C (Coolong & Randle 2003) and SLB often infects weakened and heat-stressed plants (du Toit 2017) so the amount of heat stress experienced by crops in each region in the different seasons was evaluated by estimating the proportion of each day where temperatures were above 27°C. The heat stress experienced each day was compared with the infection period and overlapping periods were identified, which could likely result in plants that were more susceptible to infection.

RESULTS AND DISCUSSION

Identification of the causal organism of the recent leaf blight outbreak

The conidia produced by each of the ten isolated *Stemphylium* sp. (Fig. 1) on PDA conformed to the description of *Stemphylium vesicarium* given in Shishkoff and Lorbeer (1989). The perfect stage did not mature in the c. 2-months the cultures were observed and no ascospores were seen.

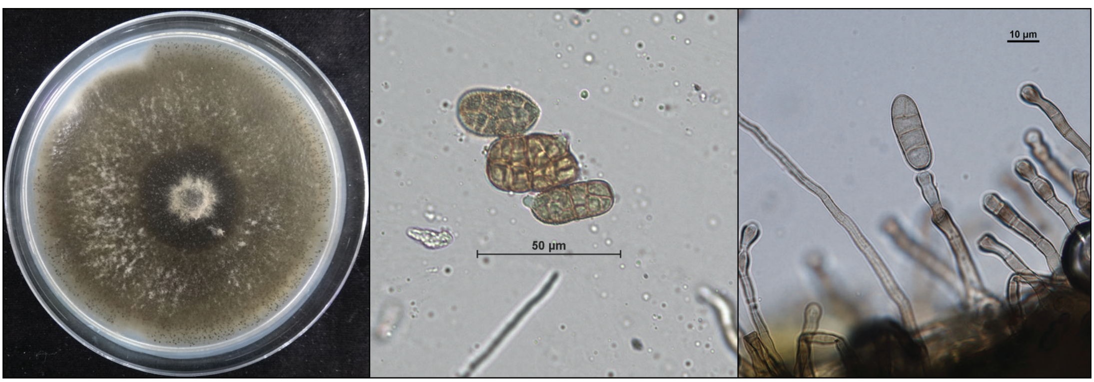
Insufficient data were obtained from DNA sequencing of the ITS1/2 region to confidently identify any of the collected isolates to species level. The majority of identical matches using ITS1/2 sequences were found to be *S. vesicarium*; however, various other species also appeared with a 100% identical sequence. This was the case for all collected isolates.

Sequencing of the partial GPD gene provided sufficient data for all collected isolates to be identified to species level. All collected isolates showed 100% similarity to sequences in the database identified as *S. vesicarium*. Lower percentages of similarity were obtained for other generated matches that were of different species.

Climate conditions and stemphylium leaf blight development

Overall, there was a greater number of infection days in Pukekohe than in Hawke's Bay or Canterbury across all five growing seasons examined (Fig. 2) Infection days started earlier in Pukekohe at 100 days after 1 August (i.e. 9 November) compared with 120 days after 1 August (i.e. 29 November) for either Hawke's Bay or Canterbury, which indicated that there was/is the potential for higher risk of infection in the Pukekohe region compared with the other two (Fig. 2). In 2017–18, there were a higher number of infection days than the previous growing seasons in all regions, with up to 35 days in Pukekohe, 25 days in Hawke's Bay and 20 days in Canterbury. In the previous seasons, the next highest number of infection days were 27 days in Pukekohe for the 2015–16 season, 14 days in

Figure 1 Left: Isolates of *Stemphylium vesicarium* grown on Potato Dextrose Agar (PDA). Centre and right: Spores as seen under a light microscope.



Hawke's Bay in the 2013–14 season, and 11 in Canterbury for the 2014–15 season. The higher number of infection days in the 2017–18 growing season could have provided the conditions necessary for the outbreak of SLB to occur as the pathogen was known to be present in New Zealand before then but had not been a serious problem.

In the Canterbury region, the highest number of infection days by 150 days after 1 August (i.e. in late December) occurred in the 2017–18 growing season. There was also a sharp increase in the number of infection days during the January–February period of the 2017–18 season, with a total of 11 infection days during this period (Fig. 2). This was most likely a key infection period in the Canterbury region for that season. In the 2013–14 growing season, number of infection days by late December was similar to the 2017–18 growing season but there was only slight increase over January and February. The total number of accumulated infection days was lower in the other seasons studied.

In Hawke's Bay, five infection days had accumulated by 120 days after 1 August in the 2013–14 growing season but not until 150+ days in the subsequent four growing seasons.

For Pukekohe, the accumulation of infection days did not vary much between growing seasons in contrast to Canterbury and Hawke's Bay (Fig. 2). In most growing seasons, the accumulated infection days in Pukekohe were similar up to late December (at 150 days after 1 August). The total accumulated infection days in the 2017–18 season in Pukekohe was only higher than other seasons from around 175 days after 1 August, equating to 23 January. Most crops in Pukekohe had observable symptoms by this stage, which suggested that it was not just the accumulated infection periods that were related to the observed symptoms – at least in Pukekohe.

The heat-stress results for Pukekohe, Hawke's Bay and Canterbury are shown in Figure 3. There tended to be more days with heat stress in Canterbury and Hawke's Bay than Pukekohe. However, there were more days with ideal fungal infection conditions infection periods in Pukekohe for the 2017-18 season than in Canterbury and Hawke's Bay. The actual number of days that reached the threshold of heat stress within an infection period was calculated, and these data are summarised in Figure 4. These data show that a greater number of days where heat stress and infection periods co-occurred existed in all regions in the 2017–18 season had compared with previous seasons. This finding suggests that there may have been higher risk for SLB in the 2017–18 season than in previous

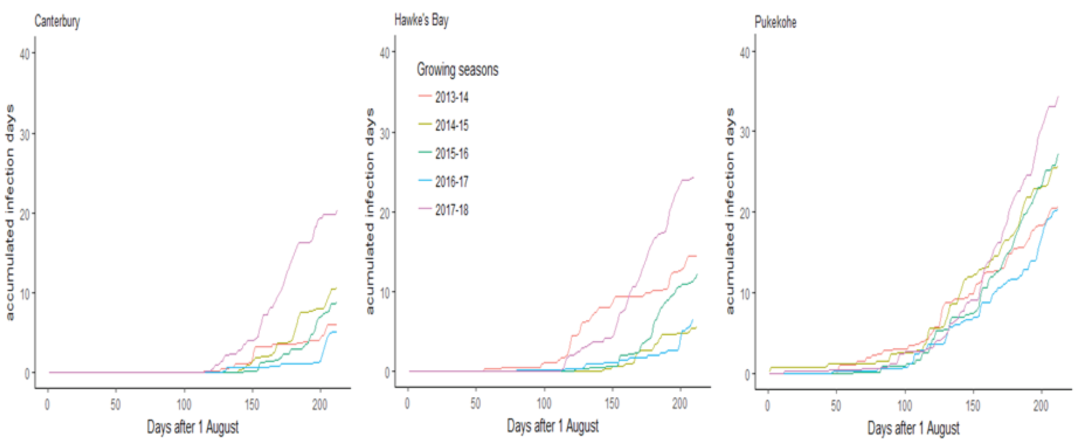


Figure 2 Accumulated infection days for stemphylium leaf blight after 1 August for the last five growing seasons in Canterbury, Hawke's Bay and Pukekohe.

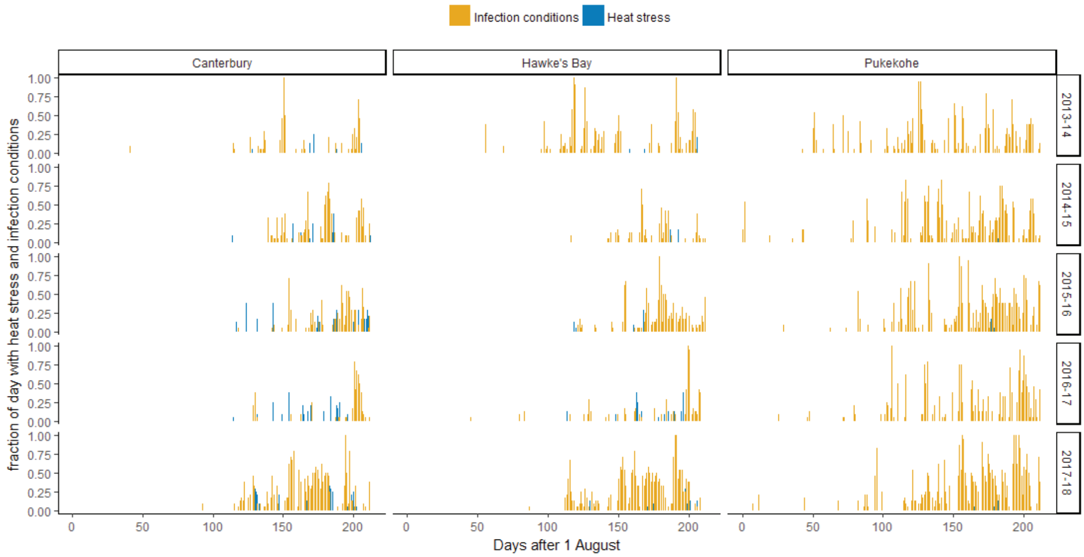


Figure 3 Fraction of day with ideal fungal infection conditions (orange lines) and heat stress (blue lines) for different growing seasons in Canterbury, Hawke's Bay and Pukekohe.

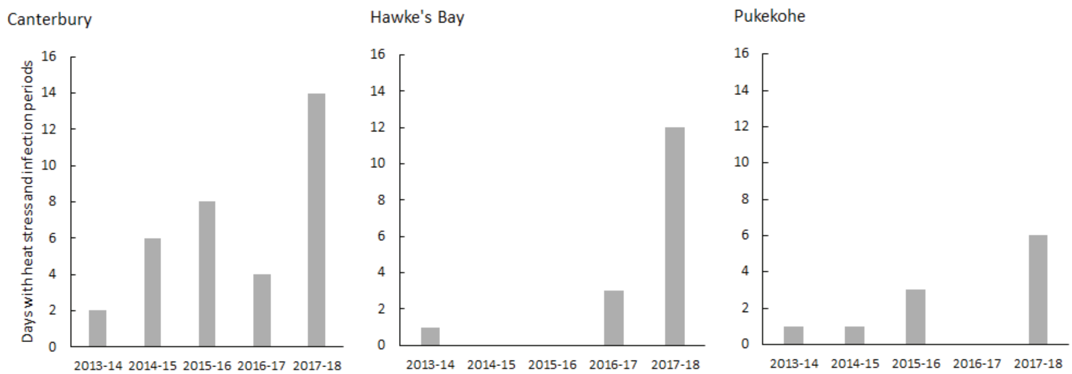


Figure 4 Days with combined heat stress and infection periods for stemphylium leaf blight for different seasons in Canterbury, Hawke's Bay and Pukekohe.

seasons. The number of combined heat stress and infection periods was highest for Canterbury and lowest for Pukekohe, which suggests that SLB is more likely to occur in Canterbury assuming all other possible factors were equal.

It is possible that the number of heat-stress days will increase in the future due to climate change, which may increase the likelihood of SLB occurring. Changes in rainfall patterns are also likely to occur as a result of climate change, which could increase or decrease the number of humid days in each region. The results obtained here indicate that the development of a disease-forecasting system for SLB of onion based on climate data would be beneficial in managing this disease.

CONCLUSIONS

Results from the partial GPD gene sequencing data indicate that the outbreak of SLB observed in the 2017–18 onion growing season was caused by *S. vesicarium*. This finding confirmed the data obtained by Singh (1977) who reported that *S. vesicarium* was the only *Stemphylium* species isolated from onions in New Zealand.

For Pukekohe, Hawke's Bay and Canterbury, the number of infection and heat stress days were higher in the 2017–18 growing season compared with the previous four growing seasons, suggesting there may have been some climate effect on disease development for the 2017–18 season. In Canterbury and Hawke's Bay, the number of infection days were much higher over the 2017–18 season, and coincided with heat stress, which may also have been a contributing factor for SLB in these regions. Although the number of combined heat stress and infection periods for the Pukekohe region were not as pronounced as in Hawke's Bay and Canterbury in 2017–18, the greater number of days that combined heat stress and infection periods compared with previous years may also contribute to the development of SLB in the Pukekohe region.

The number of accumulated infection days was much higher in Pukekohe than in other regions for the 2017–18 season, although the number of infection days in previous seasons were similar to the extreme values encountered in Canterbury and Hawke's Bay only in the 2017–18 season. These results suggest that there was a climate effect on the disease appearance in the 2017–18 season but that there may be other factors affecting disease development that were not studied here.

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