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Molecular identification of *Stemphylium* spp. from leaf blight of onions in New Zealand

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CONTENTS

EXECUTIVE SUMMARY

Molecular identification of *Stemphylium* spp. from leaf blight of onions in New Zealand

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During the 2017-2018 onion growing season, significant outbreaks of leaf blight occurred in the Pukekohe, Hawkes Bay and Canterbury commercial onion fields. With the use of sequencing data and observation of spores, the causal organism responsible for the outbreak was identified to species level.

The results from the sequence data indicate that the outbreak of leaf blight observed in the 2017-2018 onion growing season was caused by *Stemphylium vesicarium*. Comparisons of the sequence data of these isolates with those of two New Zealand isolates from 1979 and 1983 revealed that the earlier isolates were also *S. vesicarium*.

These results show that the recent outbreak of leaf blight in onions in New Zealand was not the result of the introduction of a novel species of *Stemphylium*. Instead, the likely cause of the outbreak of *Stemphylium* leaf blight (SLB) was environmental. It is likely that the warm, wet, humid summer of 2017-18, in conjunction with several storms with high winds, resulted in unusually severe SLB.

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1 INTRODUCTION

During the 2017–18 onion growing season, significant outbreaks of leaf blight occurred in commercial fields of onion in New Zealand, including the Pukekohe, Hawke's Bay and Canterbury regions. In February 2018, a *Stemphylium* species was identified from all three regions based on conidial characteristics of spores taken from diseased onion leaves with leaf blight symptoms (Tyson, unpublished data).

Stemphylium leaf blight (SLB) in onions is caused by *Stemphylium vesicarium*. Symptoms of the disease are most apparent on the leaves, which begin as small, white, elongated lesions that turn purple over time and frequently coalesce into larger spotted lesions resulting in blighted leaves (Hausbeck 2010; Tesfaendrias et al. 2012). The disease progresses until infected leaves undergo necrosis causing premature defoliation of onions, ultimately compromising bulb quality and size. Onion fields severely affected by SLB can experience significant yield loss. Severe outbreaks of the disease have been reported to reduce yield by as much as 60% (Anon 2018). Disease control is by means of agrichemicals and agronomic methods.

SLB is generally considered a secondary infection, with *S. vesicarium* primarily attacking damaged tissues. In the absence of tissue damage caused by disease, insect pest feeding, or other sources of physical damage, the incidence of SLB is reduced (Gevens 2016). Infection from *S. vesicarium* also occurs via stomatal openings (Tayviah 2017). Infection of tissue and disease development of SLB is favoured by warm temperatures (between 18°C and 25°C), high humidity, and periods of leaf wetness lasting 8 hours or more (Tayviah 2017).

Searle & Wright (2018) suggested that the climate (in particular rainfall and temperature) may have influenced the recent high SLB incidence in Canterbury, Hawke's Bay and Pukekohe. It is also possible that the recent epidemic of SLB was the result of the emergence of a more aggressive disease than seen previously in New Zealand, posing an increased threat to onion production.

Historically, the *Stemphylium* species on onions and allied crops in New Zealand has been identified as *Pleospora herbarum* (*Stemphylium herbarum*) (Dingley 1969; Pennycook 1989). Singh (1977) however, found that on onions *S. vesicarium* was the only species present.

In order to identify the cause of the recent outbreak of SLB in New Zealand, DNA sequencing was undertaken for ten *Stemphylium* sp. isolates. In addition, two isolates of *Stemphylium* sp. collected in 1983 and 1979 were also identified.

2 MATERIALS AND METHODS

Leaves from onions with symptoms of SLB were collected from commercial onion fields in Te Kauwhata and Canterbury, and fungal isolations were made from lesions onto Potato Dextrose Agar (PDA) amended with antibiotics. Identification of *Stemphylium* to genus was by observation of spore formation and morphology under a light microscope.

Ten individual *Stemphylium* isolates were retained from infected onions from Te Kauwhata and Canterbury. In addition, two *Stemphylium* isolates from *Allium* sp. were obtained from the International Collection of Micro-organisms from Plants (ICMP), Landcare Research, Auckland (Table 1).

Two pairs of universal primers were obtained for molecular identification of each isolate (ITS1/ ITS2 targeting the internal transcribed spacer and GPD-F/GPD-R targeting the glyceraldehyde 3-phosphate dehydrogenase gene). DNA from the mycelium of each isolate was obtained using a QIAGEN DNeasy Plant Mini Kit, following the manufacturer's instructions. Polymerase chain reactions (PCR) were performed using both primer pairs with each isolate 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 for sequencing using Macrogen's sequencing services.

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

Isolate ID	Location	Year collected	Host
cc968	Canterbury, near Ashburton	2018	*Allium cepa*
cc969	Canterbury, near Ashburton	2018	*Allium cepa*
cc970	Canterbury, near Ashburton	2018	*Allium cepa*
cc971	Canterbury, near Ashburton	2018	*Allium cepa*
cc972	Canterbury, near Ashburton	2018	*Allium cepa*
cc973	Canterbury, near Ashburton	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	Hawkes Bay	1979	*Allium porrum*
ICMP8719	Pukekohe	1983	*Allium cepa*

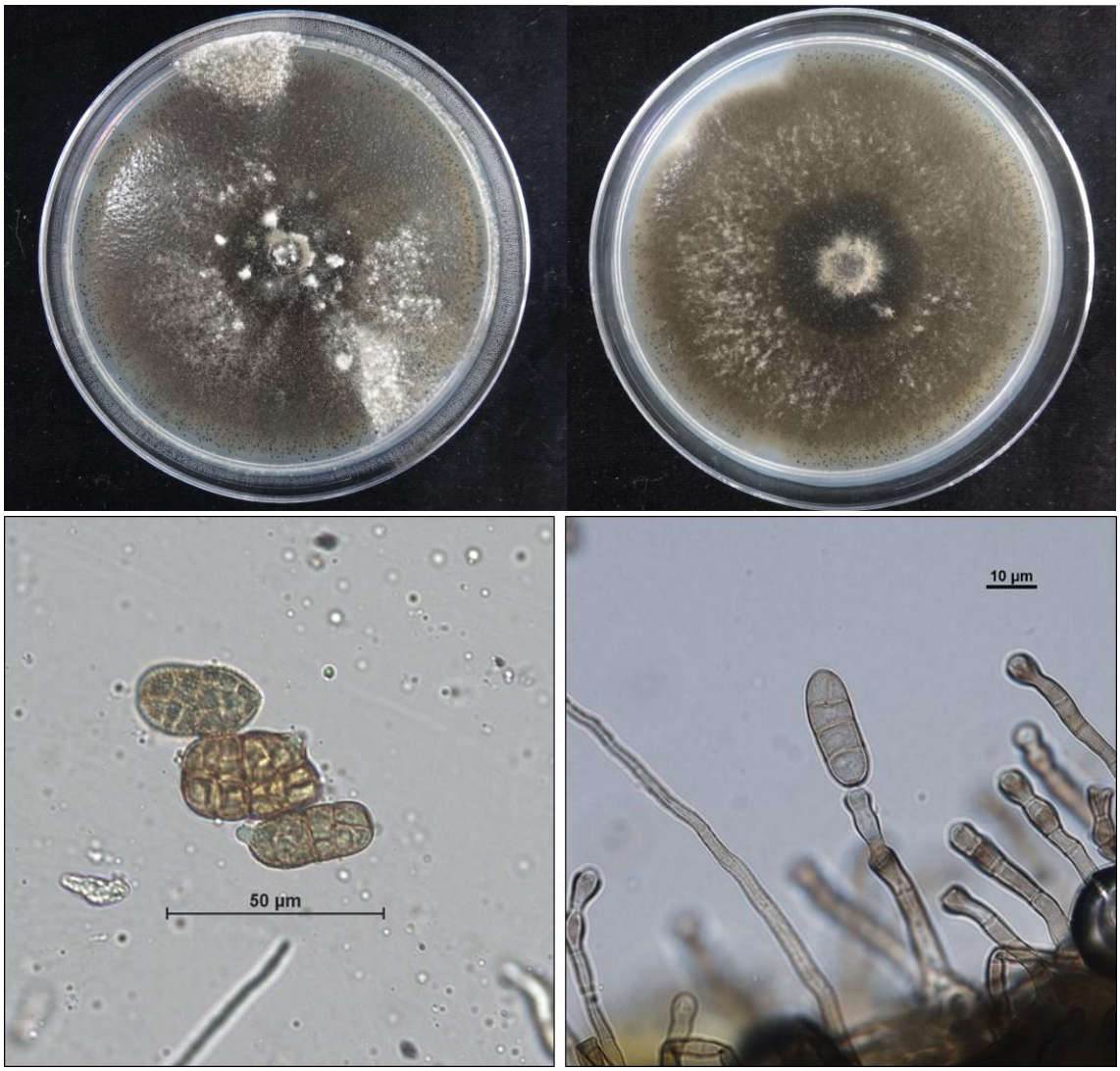

Figure 1. Top row: Isolates of *Stemphylium* sp. from the 2018 outbreak of Stemphylium leaf blight on onions. Isolates were grown on Potato Dextrose Agar (PDA). Bottom row: *Stemphylium* spores as seen under the light microscope.

3 RESULTS AND DISCUSSION

The identity of each *Stemphylium* isolate was determined using the Geneious BLAST function to compare the sequences generated by Macrogen with those stored in a nucleotide collection database.

Sequencing of ITS1/2 was found to be insufficient to confidently identify 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.

Results from the sequencing of the partial GPD gene allowed for confident identification of all collected isolates to species level. All collected isolates showed 100% similarity to sequences in the database identified as *S. vesicarium*. Other generated matches that were of a different species had lower percentages of similarity.

The results from the sequence data indicate that the outbreak of SLB observed in the 2017-2018 onion-growing season was caused by *S. vesicarium*. Together with results from the previous study by Wright and Searle analysing climate data from all onion growing seasons between 2014 and 2018 (Searle & Wright 2018), this suggests that the likely cause of the outbreak of SLB was environmental. It is likely that the warm, wet, humid summer of 2017-18, in conjunction with several storms with high winds, resulted in unusually severe SLB.

4 ACKNOWLEDGEMENTS

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