Epidemiology and Management of Stemphylium Leaf Blight on Onion (*Allium Cepa* L.) in The Holland Marsh, Ontario.

By

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ABSTRACT

Epidemiology and management of stemphylium leaf blight on onion (*Allium cepa* L.) in the Holland Marsh, Ontario.

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Stemphylium leaf blight (SLB), caused by *Stemphylium vesicarium* Wallr (Simmons) (teleomorph: *Pleospora allii*. (Rabenh.) Ces. & De Not), is a destructive disease of onion. The objective of this study was to assess the epidemiology of SLB in Ontario and evaluate management strategies. Stemphylium leaf blight first appeared at the end of June to mid-July in 2015 and 2016, coinciding with air-borne conidia of *S. vesicarium* (based on spore trapping), frequent rainfall, and air temperatures ≥ 15 °C. Onion cultivars Pontiac, Milestone and Hendrix were slightly less susceptible to SLB compared to the other commercial cultivars in the study. A protective application of fluopyram plus pyrimethanil at the 3 - 4 leaf stage reduced SLB incidence, but later applications had little impact. Aerial infrared photography demonstrated the potential to identify differences in onion plots, but these differences were not related to SLB. Additional research is needed to evaluate more fungicides and spray-timings for SLB management.

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CHAPTER ONE

LITERATURE REVIEW

1.1 Onion

1.1.1 Taxonomy and description

Onions (*Allium cepa* L.) are monocots in the sub-family Alliodaea (Alliceae) and family Amarallidaceae (Angiosperm Phenology Group III. 2003; Chase et al. 2009). Onions are biennials but are often cultivated as annuals grown for bulbs. The Alliodaea sub-family contains about 780 cultivated species (Fritsch and Friesen 2002). Other commonly cultivated crops in this sub-family are garlic (*A. sativum*), Welsh onion (*A. fistulosum*), leeks (*A. ampeloprasum*), chives (*A. schoenoprasum*), and Chinese chives (*A. tuberosum*) (Brewster 2008).

The shoot portion of the onion consists of photosynthetic leaf blades with non-photosynthetic leaf bases (scales). The leaf blades grow alternately in a flattened, fan-shaped band. They are fleshy, hollow, cylindrical and flat on one side (Fritsch and Friesen 2002). The crop accumulates and stores food as it approaches maturity by forming several bladeless scales at the base of the plant (Heath and Hollies 1965; Fritsch and Friesen 2002). These scales swell up to form the bulb. At maturity the bulb size can range from 5–100 mm in size (Lancaster et al. 1996).

Onion bulbs overwinter if not harvested at the end of the first season. The crop develops a new shoot system consisting of a scape and inflorescence in the spring after a cold winter through vernalisation (Fig. 1.1) (Brewster 2008; Lee et al. 2013). The inflorescence is a round umbel with many white flowers. These flowers are insect

pollinated and each flower develops a single black seed (Pathak et al. 2001; Brewster 2008).

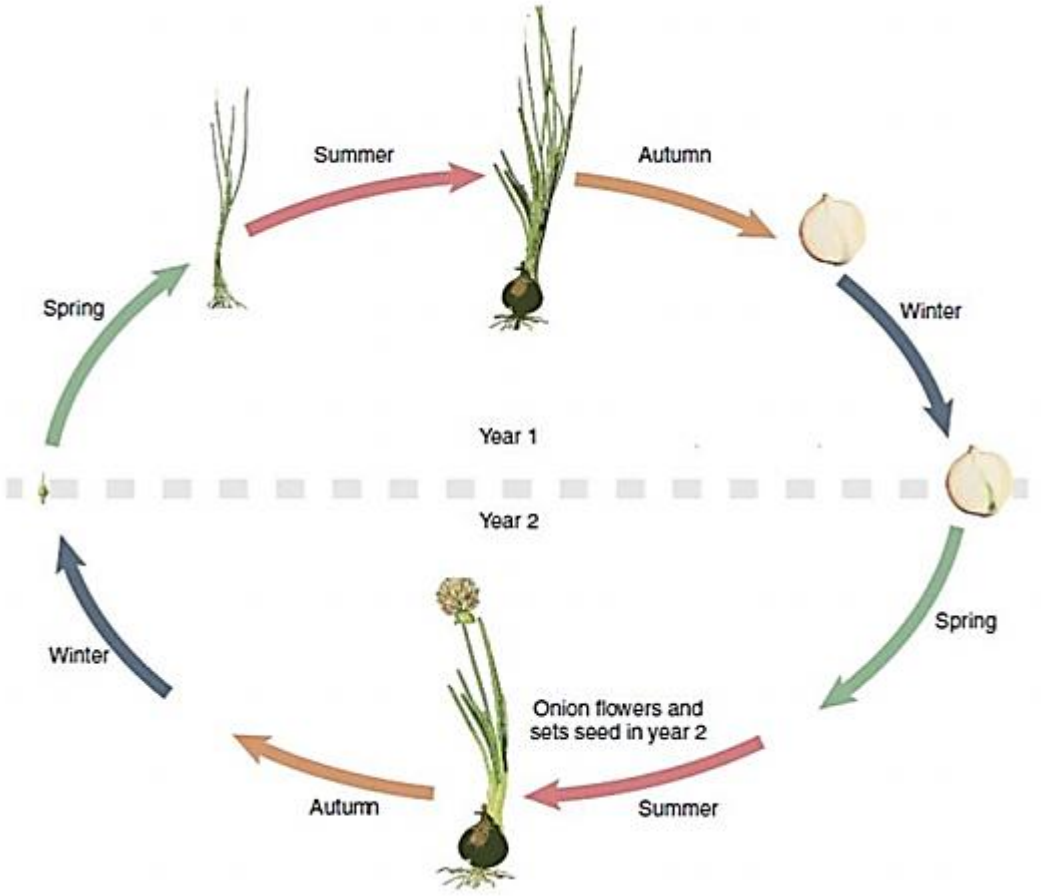

Figure 1.1 Onion development cycle (from Lee et al. 2013).

1.1.2 Origin, distribution and types

The origin of onions remains an enigma, but they are believed to have originated from Central Asia (Vavilov 1951). However, the highest onion diversity is observed in the eastern Mediterranean region and through Afghanistan, Tajikstan, Pakistan and India (Brewster 2008; Kik 2008). The crop is cultivated worldwide on a range of soils and climatic conditions. There are several groupings of onion based on variation in bulb

characteristics, response to photoperiod length, and storage qualities (Lancaster et al. 1996; Rabinowitch and Currah 2002).

Horticulturally, onions are grouped into two categories: the bulb group (*A. cepa* var *cepa*) and the aggregate group (*A. cepa* var *aggregatum*). The bulb group, also referred to as the common onion, produces one bulb per plant, whereas the plants in the aggregate group produce a bunch or cluster of small bulbs. The bulb group is usually commercially propagated from seed and the aggregate group is propagated vegetatively (Hanelt 1990; Rabinowitch and Currah 2002). Shallots (*A. cepa* var *aggregatum*) are the most common of the aggregate group (Brewster 2008).

Cultivation and distribution of onion is influenced by photoperiod length, light quality and light interception (Garner and Allard 1920; Bertaud 1986; Brewster 2008). Long-day (LD) cultivars require ≥ 16 h photoperiod to form bulbs, whereas intermediate-day (ID) cultivars bulb at photoperiods between 14–16 h and short day (SD) cultivars require a maximum of a 12 h photoperiod to form bulbs (Rabinowitch and Currah 2002; Brewster 2008).

Onion bulb formation at a particular photoperiod is influenced by temperature (Fritsch and Friesen 2002). Long-day and ID cultivars are suitable for cultivation in the temperate and semi-arid regions of the world, and SD cultivars are most suitable for warm tropical and sub-tropical climates (Uzo and Currah 1990; Brewster 2008). Long-day cultivars grown under SD conditions will not bulb and SD cultivars grown under LD conditions start to bulb upon emergence.

1.1.3 Onion production

Onions are the second most cultivated vegetable crop worldwide after tomatoes (FAOSTATS 2015). In 2012, the area of onion cultivated worldwide was approximately 4.3 million hectares in over 170 countries, generating $4.0 billion in revenue. China, India, and the USA are the three highest onion producers. These countries produce more than 50% of the world's onions (FAOSTATS 2015). The average yield is 17 tonnes per hectare. However, Canada, South Korea, Japan and USA have the highest yield per hectare at 40 - 60 t/ha (YARA 2015; Mailvaganam 2016).

Onions are produced and supplied all year round worldwide due to diverse growing conditions and improved long-term storage options. The Netherlands generates approximately $504 million from onion exports, making it the largest onion exporter worldwide. Canada is the eleventh highest exporter of onion globally, exporting $4.2 million, and is the fastest growing exporter (FAOSTATS 2015; Workman 2016).

Onion is a major vegetable consumed in multi-cultural Canada with a consumption of 8.5 kg per person per year. Annually, onion is cultivated on 4740 ha in Canada. This production yields approximately 200,000 mt per year with a farm gate value of $74 million (Agriculture and Agri-food Canada 2014). Ontario is the highest onion producing province (Table 1.1). In Ontario, 2460 ha of onion is cultivated with an annual farm gate value of $34 million (Agriculture and Agri-food Canada 2014; Mailvaganam 2016). The majority of this production is on muck soil at the Holland Marsh ($44^{o}5$'N and $79^{o}35$'W) in the York Region of the province.

Table 1.1 Acreage of onion production in Canada, by province, in 2012 (Statistics Canada, 2013)

Province	Area cultivated (ha)
Ontario	2460
Quebec	1938
Nova Scotia	253
Manitoba	209
British Columbia	101
Total	5436

1.1.4 Onion cultivation

In Canada, the main types of onion produced are white, red and yellow globe onions. In Ontario, the majority of onions cultivated are the yellow globe cultivars (Valk 1988). The common commercial hybrids mature 85–115 days after seeding (McDonald et al. 2015). Onion plants progress through 10 vegetative development stages, similar to other *Allium* crops, from seeding to harvest (Fig 1.2) (Rey et al. 1974).

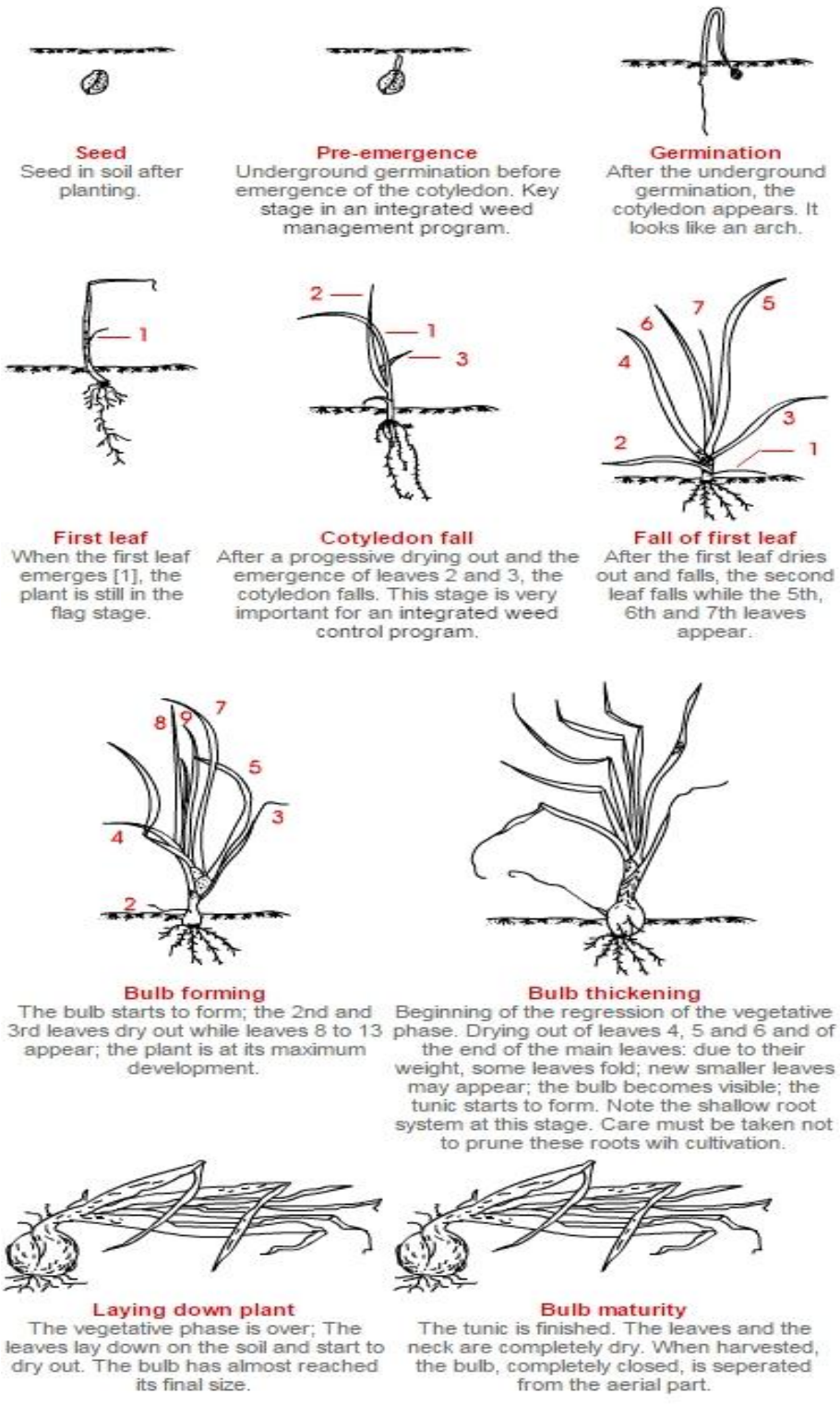

Figure 1.2 Stages of vegetative growth of Allium crops (Rey et al. 1974)

Direct seeding is the most common method for commercial onion production worldwide. However, onions can be grown from transplants or dry sets (Currah and Proctor 1990). In Ontario, the majority of onion crops are direct seeded in late April to mid-May (Valk 1988). Soil temperature and the depth of sowing seed are critical to the emergence of seeds. The optimum depth for direct seeding is about 1 - 2 cm below the soil surface. Onions are shallow rooted, with almost all of the roots within 40 cm of the soil surface (Bosch-Serra and Currah 2002).

Onions are cultivated on a range of soil types, but the ideal is sandy loam with a pH of 6 - 7 (Currah and Proctor 1990). Optimum moisture and nutrition during the early stages of crop establishment and at bulbing are critical for optimum yield (De Melo 2003). Fertilizers are applied during land preparation before sowing the seed (Bosch-Serra and Currah 2002).

Weed management is also crucial in onion production. Weeds are controlled with combination of pre-emergence and post emergence herbicides (Menges 1987). Insects, nematodes and occasionally mites are the main pests of onions worldwide (Lorbeer et al. 2002). The most destructive onion insect pests worldwide are onion thrips (*Thrips tabaci* L.) and onion maggot (*Delia antiqua* Meigen). Insect pests are managed using integrated pest management (IPM) methods (Bosch-Serra and Currah 2002).

1.2 Foliar diseases of onion

Onions are affected by a variety of foliar diseases (Maude 1990). Globally, xanthomonas blight caused by *Xanthomonas campestris* Pammel (Dowson) is the most common bacterial disease of onion (Alvarez 1978; Isakeit et al. 2000; Schwartz and

Mohan 2008). Onion yellow spot virus, Irish yellow spot virus, and leek yellow strip virus are the most common foliar viral diseases (Salomon 2002; Brewster 2008).

There are several fungal and fungal-like diseases affecting onion foliage worldwide. In Ontario, the most destructive foliar diseases are purple blotch caused by *Alternaria porri* (Ellis) Cif., botrytis leaf blight caused by *Botrytis squamosa* Walker, and onion downy mildew caused by the oomycete *Peronospora destructor* (Berk) (Chaput 1995). A new fungal blight, stemphylium leaf blight (SLB), has recently been reported in the Holland Marsh (Paibomesai et al. 2012).

1.3 Stemphylium leaf blight

1.3.1 Causal agent and symptoms

Stemphylium leaf blight of onion is caused by *Stemphylium vesicarium* Wallr (Simmons) (teleomorph: *Pleospora allii*. (Rabenh.) Ces. & De Not). It is an Ascomycete belonging to the family Pleosporaceae and the order Moniliales. The pathogen is closely related to *A.* porri, (Simmons 1967; Bessey 1968). Diseases caused by these two fungi are sometimes misdiagnosed because the initial symptoms are similar (Simmons 1967; Jakhar et al. 1996; Suheri and Price 2001). In Ontario, *S. vesicarium* was reported on onion in 2008, following reports on asparagus (*Asparagus officinalis* L) (Roddy 2011).

The initial symptoms of SLB on onion include small, yellowish brown to tan, water-soaked lesions (Rao and Pavgi 1975). However, the initial symptoms of purple blotch are small, sunken, whitish lesions with purple centers (Everts 1990). Stemphylium leaf blight is restricted to onion leaves and inflorescences (Rao and Pavgi 1975; Aveling and Snyman 1993).

In the Holland Marsh, SLB is first observed when the onion crop is at the 3- to 4-leaf stage, reaching maximum severity when leaves begin to senescence. As SLB progresses, extensive necrosis of infected leaves develop back from the tip. This necrosis is associated with host-specific toxins produced by *S. vesicarium* after infection (Singh et al. 2000; Wolpert et al. 2002). The disease results in desiccation and premature lodging of onion, which can lead to a severe reduction in bulb size. Severely infected plants produce small bulbs that are unmarketable or sold at a discount (Rao and Pavgi 1975; Miller et al. 1978; Lorbeer 1993).

1.3.2 Pathogenicity and host

Stemphylium vesicarium has been detected on a wide range of crops as both a pathogen and saprophyte. In addition to onion, *S. vesicarium* is pathogenic on garlic (Basallote 1993), leek (Suheri and Price 2001), Welsh onion (Misawa and Yasuoka 2012), asparagus (Falloon 1987), and European pear (*Pyrus communis* L.) (Llorente and Montesinos 2006). Also, in addition to known hosts, the pathogen can cause asymptomatic infections and develop as endophytes in the living tissues of various plants (Köhl et al. 2009b; Misawa and Yasuoka 2012).

Host-specific toxins are involved in the pathogenicity and aggressiveness of isolates (Singh et al. 1999; Wolpert et al. 2002). Isolates that are pathogenic on European pear cultivars produce two toxins, SV-toxin I and SV-toxin II (Singh et al. 1999) that are not pathogenic to asparagus or onion (Pattori et al. 2006; Köhl et al. 2009a). The toxicity and concentration of the toxins is correlated with SLB severity (Singh et al. 1999, 2000). Isolates of *S. vesicarium* from asparagus, onion, and garlic were reported to be

pathogenic to all three crops (Basallote-Ureba et al. 1999). Isolates from parsley were pathogenic to members of the Apiaceae family but not to Allium crops (Koike et al. 2013).

1.3.3 Losses and distribution

Stemphylium leaf blight is an important disease of onion in both tropical and temperate countries (FAO/IPGRI 1997). The disease caused 90% yield losses on onion in Texas and New York State in 1978 (Miller et al. 1978; Lorbeer 1993), 80 85% yield loss in Portugal (Tomaz and Lima 1988), and large losses in Egypt (Hassan et al. 2007), India (Rao and Pavgi 1975), Japan (Misawa and Yasuoka 2012), New Zealand (Suheri and Price 2001), South Africa (Aveling 1992) and Spain (Basallote 1993). On asparagus, it can result in 100% loss of spears (Falloon 1987; Hausbeck 2009). Brown spot disease on pear, caused by *S. vesicarium*, resulted in 60 - 90% loss of yield (Montesinos and Vilardell 1992; Montesinos et al. 1995b; Llorente and Montesinos 2002).

1.4 Disease cycle

1.4.1 Sexual and asexual spores

The disease cycle of SLB is characterised by sexual and asexual phases (Prados-Ligero et al. 1998; Basallote-Ureba et al. 1999). In the sexual phase, pseudothecia develop on both diseased and symptomless tissues of host and non-host plants (Rossi et al. 2008; Misawa and Yasuoka 2012). On onion, however, pseudothecia development is restricted to the inflorescence (Rao and Pavgi 1975). The development of pseudothecia in temperate regions occurs in the winter at temperatures between 5 - 15 °C and high

relative humidity (Prados-Ligero et al. 2003; Llorente and Montesinos 2004). At these low temperatures, ascospores maturation takes 1 - 6 months (Simmons 1969; Prados-Ligero et al. 1998). The pseudothecia are black and bear several cylindrical asci (Fig 1.3) (Simmons 1969). Pseudothecia release ascospores in the spring, coinciding with rainfall events.

Ascospores are yellowish brown and ellipsoidal, with the upper half narrowly tapered (Fig 1.3). Matured ascospores have 5 - 7 complete transverse septa and zero to several incomplete longitudinal septa. The average size of a mature ascospore is about 18 × 38 µm. Ascospores of *P. allii* are indistinguishable from ascospores of *P. herbarum* (Fries) Rabenhorst (anamorph: *S. botryosum* Wallroth), but the asci of *P. herbanum* are smaller (Simmons 1969, 1985). Ascospores can infect onion plants under laboratory conditions (Prados-Ligero et al. 1998), but their role in the epidemiology of SLB is unclear.

In Allium crops, primary infection in the field is associated with asexual conidia (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). Conidia are olive-brown, oval to ovoid, and are borne on conidiophores that are pale to brown with dark edges and bands. The conidia have 1 - 5 transverse septa and are constricted at 1 - 3 transverse septa. The conidia also have 1 - 2 complete longitudinal septa (Fig. 1.3) (Simmons 1969; Ellis 1971). Conidia of *S. vesicarium* are twice as long as wide (mean 18 × 34 µm), whereas those of *S. botryosum* have roughly equal length and width (25 × 28 µm, (Simmons 1969).

Molecular procedures can be used to accurately identify *Stemphylium* species, based on specific primers in polymerase chain reaction (PCR) of the internal transcribe

spacer (ITS) region or DNA sequencing of the glyceraldehyde-3-phosphate dehydrogenase (*gpd)*gene sequence (Câmara et al. 2002; Köhl et al. 2009b).

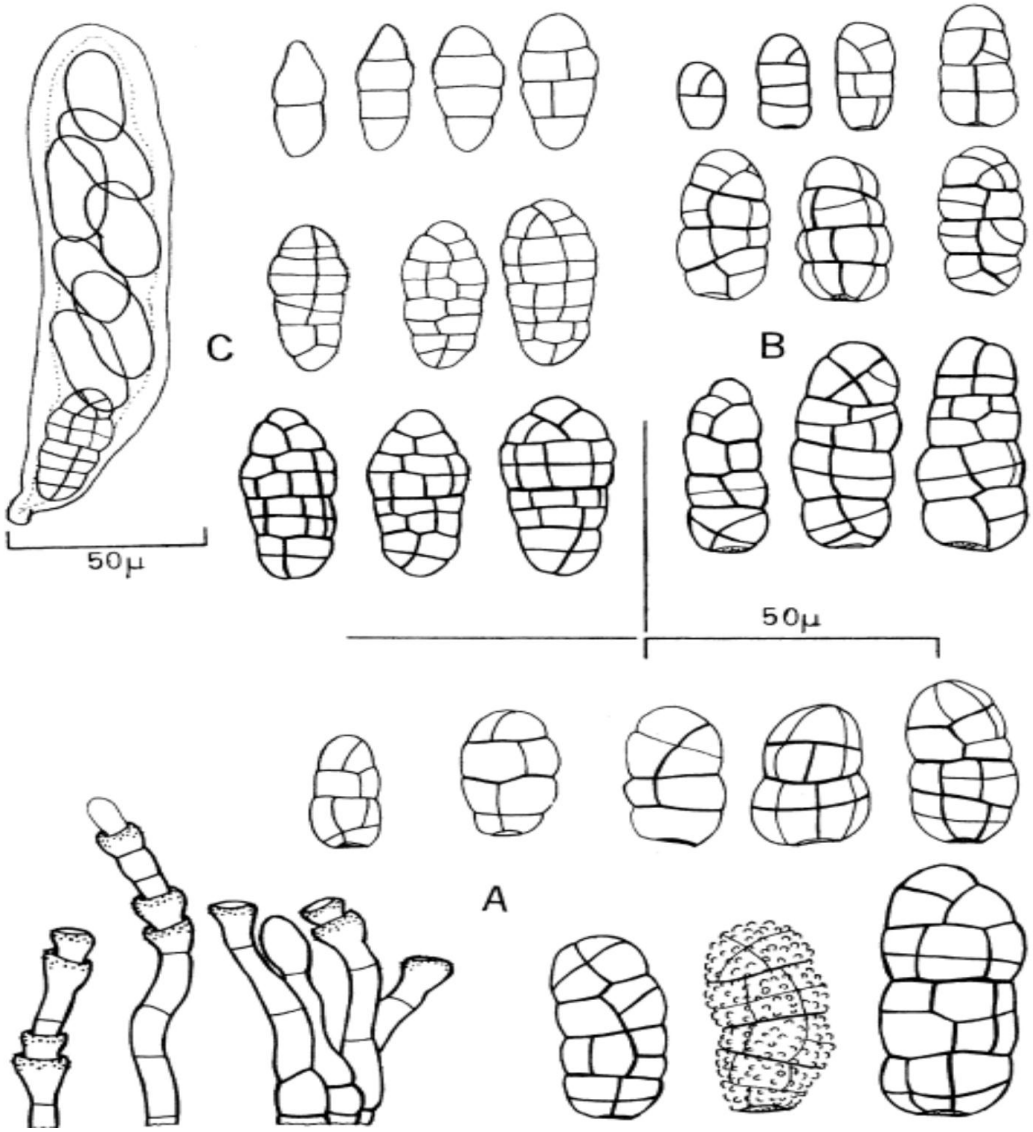

Figure 1.3 *Stemphylium vesicarium* (teleomorph: *Pleospora allii*): (A) Conidiophores and natural conidia; (B) Conidia from culture; (C) Ascus and ascospore from culture (Simmons 1969)

1.4.2 Inoculum dispersal and distribution

Information on inoculum availability and distribution for SLB on onion is not available. In pear and garlic crops in Spain (Prados-Ligero et al. 2003; Rossi et al. 2008), Welsh onion in Japan (Misawa and Yasuoka 2012) and asparagus in Michigan, USA (Granke and Hausbeck 2010), both ascospores and conidia are present during the cropping season. Ascospores are captured in the early part of the cropping season and conidia are captured later, during disease development.

In Allium crops, there was a time lag between when ascospores are first detected and first incidence of SLB (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). In pear orchards, it is postulated that ascospores colonise plant debris on the orchard floor and produce abundant conidia, which then infection pear (Llorente and Montesinos, 2006; Rossi et al. 2008; Rossi et al. 2005). In asparagus, both ascospores and conidia infect spears in the field (Granke and Hausbeck 2010).

Both ascospores and conidia are air-borne. The daily release and volume of ascospores and conidia are influenced by precipitation, temperature, relative humidity, vapour pressure deficit, wind speed, and solar radiation (Granke and Hausbeck 2010; Misawa and Yasuoka 2012; Prados-Ligero et al. 2003; Rossi et al. 2005). Daily release of airborne ascospores and conidia show a diurnal pattern, as with many other Ascomycetes (Meredith 1966; Prados-Ligero et al. 2003).

1.4.3 Sporulation and infection

Stemphylium vesicarium sporulates on onion, garlic and asparagus, producing abundant conidia (Falloon 1987; Basallote-Ureba et al. 1999; Suheri and Price 2000,

2001). Sporulation usually occurs at the site of initial lesions. On onion and garlic leaves, sporulation is observed 6 days after the development of initial lesions (Basallote-Ureba et al. 1999).

Stemphylium species infect their host mainly through stomata openings and wounds (Bradley et al. 2003). Older onion leaves are more susceptible to infection than young leaves. Germination of conidia on onion leaves occurs at 4–35 °C (Srivastava et al. 1996; Suheri and Price 2000). The optimal conditions for conidial infection are temperatures > 18 °C, leaf wetness duration > 6 h, and high relative humidity (Shishkoff and Lorbeer 1989; Suheri and Price 2000; Prados-Ligero et al. 2003).

The pathogen starts producing host-specific toxins immediately after infection. These toxins increase the severity of infection by inducing electrolyte losses from host tissues, causing ultra-structural changes in cells that lead to extensive veinal necrosis (Singh et al. 1999, 2000). Typical symptoms of leaf spot and apical necrosis on onion and garlic occur 6–14 days after inoculation (Shishkoff and Lorbeer 1989; Basallote-Ureba et al. 1999; Suheri and Price 2000; Misawa and Yasuoka 2012).

1.5 Factors influencing infection and development

1.5.1 Temperature

Temperature is a critical factor in overwintering of *S. vesicarium*, release of air-borne spores, and infection of Allium crops (Shishkoff and Lorbeer 1989; Basallote-Ureba et al. 1999). The optimum temperature for development and maturation of pseudothecia on garlic and pear debris in Spain in winter is 5–15 °C (Prados-Ligero et al. 1998; Llorente and Montesinos 2006). Ascospore release in garlic crops is highest at

cooler temperatures (10–21 °C) compared to conidia (15 - 32 °C) (Prados-Ligero et al. 2003). The optimum temperature for the development of infection on onion under controlled conditions is 10–25 °C (Suheri and Price 2000). In Ontario, the average winter temperatures are below 0 °C and it often snows, whereas daily mean summer temperatures range from 10 - 27 °C (Environment Canada 2016). Information is lacking on the temperature requirements for formation and maturation of pseudothecia on onion residue in Ontario.

1.5.2 Moisture

Stemphylium vesicarium requires the presence of free water for infection (Llorente and Montesinos 2002; Prados-Ligero et al. 2003). A minimum leaf wetness period of 6 h is sufficient for infection at favourable temperatures on pear (Montesinos et al. 1995b). Conidial germination and infection on onion occurs at favourable temperatures with a leaf wetness period exceeding 8 h (Shishkoff and Lorbeer 1989; Suheri and Price 2000).

1.5.3 Solar radiation and vapour pressure deficit

Solar radiation has an indirect effect on infection via the effect on available moisture and air temperature. Vapour pressure deficit (VPD) is a measure of the difference between the amount of moisture the air can hold at a particular temperature and the actual amount of moisture it holds (Abtew and Melesse 2013) and is the true measure of dryness (Anderson 1936). Conidial availability increases with solar radiation, and the number of infections increase with VPD between 0.1 - 1.5 kPa (Prados-Ligero et

al. 2003; Granke and Hausbeck 2010). The highest ascospore concentrations in garlic are captured at low VPD, between 0.1 - 0.5 kPa (Prados-Ligero et al. 2003).

1.6 Management of SLB

1.6.1 Host resistance, biological and cultural

Use of host resistance would be the most efficient way to manage SLB on Allium crops (Pathak et al. 2001; Mishra et al. 2009). However, no strong source of resistance has been identified in common onion. Lines of onion screened in Taiwan were all susceptible to infection by *S. vesicarium*, but the degree of susceptibility differed among cultivars (Pathak et al. 2001). Five lines of Welsh onion and seven lines of garlic exhibited complete resistance to *S. vesicarium*, but transfer of that resistance into common onion using conventional plant breeding is not possible (Pathak et al. 2001; Mishra et al. 2009).

Biological control agents such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Pseudomonas fluorescens* and *Trichoderma* species reduced the severity of SLB in onion under controlled conditions (Kamal et al. 2008). However, these products do not provide effective management of SLB when used as the sole management strategy under field conditions (Wright et al. 2005). In pear orchards, sanitation combined with application of *Trichoderma* spp. reduced SLB incidence by 60% (Llorente et al. 2008).

Cultural methods for management of fungal diseases often aim to reduce the primary inoculum present at the start of the growing season, and to create conditions that are unfavourable for infection (Llorente et al. 2012). Removal of debris and general sanitation in pear orchards reduced both initial inoculum and SLB incidence (Llorente et

al. 2008). Cultural strategies recommended for managing SLB on onion include sanitation, crop rotation, reduced plant densities, and removal or burying of crop residue (Shanmugasundaram and Kalb 2001).

1.6.2 Fungicides

Repeated calendar-based application of preventative fungicides has been suggested as an effective way of managing SLB on onion, garlic, asparagus and pear (Basallote et al. 1993; Meyer et al. 2000; Gupta et al. 2010; Llorente et al. 2012). However, the pathogen has been reported to be insensitive to several fungicides (Alberoni et al. 2010; Hoepting 2015), which indicates that this approach may not be sustainable. In Spain, boscalid plus pyraclostrobin, iprodione (dicarboximide) and prochloraz fungicides supressed *S. vesicarium* growth on garlic *in vitro*, but these combinations were not assessed in the field (Gálvez et al. 2016). In Canada, fluopyram plus pyrimethanil is registered for the suppression of SLB on onion (Bayer CropScience Inc. Canada 2016), and ethylenebisdithiocarbamate (EBDC) fungicides are registered for management of *S. vesicarium* on asparagus in the USA (Meyer et al. 2000).

1.6.3 Forecasting models

Repeated calendar-based application of fungicides can result in unnecessary applications, e.g., when environmental factors would not support disease development or inoculum is not present (Montesinos and Vilardell 1992; Alberoni et al. 2010; Llorente et al. 2012). Also, unneeded applications increase the risk of development of fungicide insensitivity within pathogen populations (Alberoni et al. 2010). It is essential, therefore,

to understand the dynamics of SLB development and conditions that are conducive for disease increase. Forecasting models for *S. vesicarium* could reduce the number of fungicide sprays required to keep disease levels below economic thresholds compared to calendar-based application (Montesinos et al. 1995a; Meyer et al. 2000).

The epidemiology of brown spot disease of pear has been studied extensively, and integrated management programs effectively reduce disease severity (Llorente et al. 2012). Disease forecasting models have been developed and tested for pear production in Europe. The two initial models, STREP and FAST (Forecast System for *Alternaria solani* on Tomato), were about 71% accurate in predicting SLB incidence (Montesinos and Vilardell 1992). These have mostly been replaced by PAMCAST and brown spot of pear forecast (BSPCAST) (Llorente et al. 2012). PAMCAST uses temperature and relative humidity during the winter to estimate the amount of mature pseudothecia in pear orchards at the start of the season (Llorente and Montesinos 2004). This estimate is used in initiating cultural management and the application of biological control agents, which reduce primary infection of pear leaves arising from colonized debris (Llorente and Montesinos 2004; Llorente et al. 2012). The model has been used in pear orchards and has been validated over several years. BSPCAST uses leaf wetness duration and temperature during the wetness period to predict the risk of infection in pear orchards. It is used to recommend the start of fungicide sprays (Montesinos et al. 1995b; Llorente et al. 2011). The model has been studied and validated in pear orchards in Spain and Italy. BSPCAST suppresses disease similar to calendar applications, while reducing fungicide application by 60% (Montesinos et al. 1995b; Llorente et al. 2000b, 2011).

Purple spot on asparagus has been managed using TOMCAST and PASO forecasting models (Meyer et al. 2000; Eichhorn et al. 2009). TOMCAST was developed in Ontario to manage foliar diseases on tomato (Poysal et al. 1993). It uses leaf wetness duration and the average temperature during the wet period to calculate disease severity values (DSV) (Table 1.2) (Pitblado 1992a; Poysal et al. 1993). Use of the model can reduce spray application by 60% without compromising the quality of the asparagus spears (Meyer et al. 2000; Hausbeck 2005). PASO is used in forecasting infection risk in asparagus in Germany. There is little information available on the details of this model, except that it uses models of dew and rain to recommend initiation of fungicide sprays. The model is reported to be less efficient than TOMCAST (Eichhorn et al. 2009).

Table 1.2 Relationship between temperature and leaf wetness used in calculating DSV in TOMCAST (from Madden, 1978)

Mean temp. (°C)	Leaf wetness duration (h) required to produce DSV of				
	0	**1**	**2**	**3**	**4**
13–17	0–6	7–15	16–20	21+	
18–20	0–3	4–8	9–15	16–22	23+
21–25	0–2	3–5	6–12	13–20	21+
26–29	0–3	4–8	9–15	16–22	23+

A critical consideration for fungicide application for management of SLB is to apply fungicides before the conidia germinate. This is because the pathogen quickly produces host-specific toxins that reduce the post-curative potential of fungicides (Llorente et al. 2000a; Puig et al. 2014). The conditions required for release of

S. vesicarium conidia are similar those for *B. squomosa* (Lacy and Pontius 1983; Prados-Ligero et al. 2003).

BOTCAST (Botrytis Forecaster) is a forecasting model that uses a relationship between weather and *B. squomosa* infection to recommend spray thresholds for botrytis leaf blight of onion (Fig. 1.4). The lower threshold (Threshold I) is called a warning threshold and fungicides need not be applied unless rainfall is forecasted. At the higher threshold (Threshold II), however, the risk of disease is high and the recommendation is that fungicides be initiated promptly (Sutton et al. 1986).

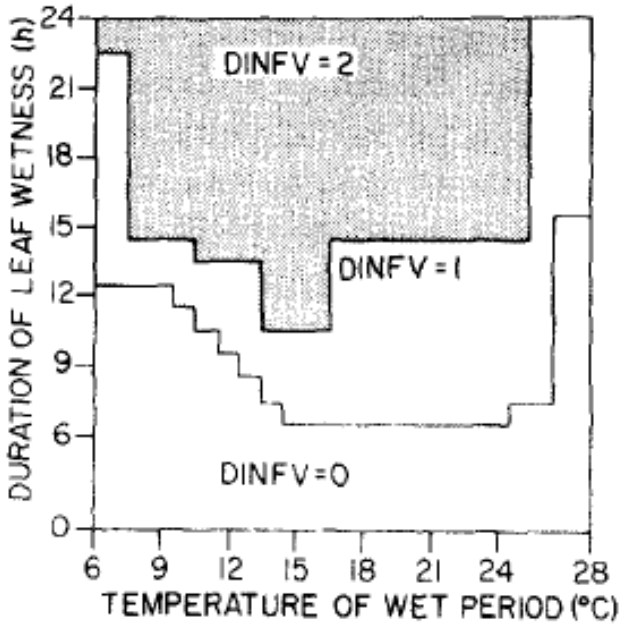

Figure 1.4 Chart for determining daily infection values (DINFV) based on duration and mean hourly temperature of the leaf wetness period in BOTCAST (Sutton et al. 1986)

1.7 Disease detection

1.7.1 Visual detection

The appearance of visual symptoms is the oldest and one of the most reliable means of SLB detection. On pear fruit, *S. vesicarium* produces 1 - 2 mm circular, brown necrotic lesions (Llorente and Montesinos 2006). On asparagus, leek, and garlic crops, purple water-soaked lesions develop (Falloon 1987; Basallote 1993; Suheri and Price 2000). Welsh onion leaf blight and SLB on onion are characterized by yellowish brown to tan water-soaked lesions (Basallote-Ureba et al. 1999; Misawa and Yasuoka 2012). The disadvantage of visual detection is that it is highly subjective (Bock et al. 2010). Also, this method is ineffective when the disease has a latent period after infection (Martinelli et al. 2014).

1.7.2 Molecular methods for detection

Molecular methods provide greater objectivity in the detection of disease (Martinelli et al. 2014). The presence of *S. vesicarium* on pear was detected using molecular techniques such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) (Köhl et al. 2009a, 2009b). Molecular techniques are sensitive, accurate, and effective for confirming visual assessments. However, molecular methods can be a difficult to use in the field to detect disease before the appearance of symptoms (Martinelli et al. 2014) because they require detailed sampling procedures, expensive infrastructure, and can misrepresent the level of spread of infections. Furthermore, these methods are destructive and can only be used practically on small number of plants (Sankaran et al. 2010; Martinelli et al. 2014).

1.8 Remote sensing

1.8.1 Overview

Early detection of diseases caused by *S. vesicarium* is important to reduce disease spread and damage to crops (Llorente et al. 2012; Yang et al. 2013). Advancements in technology such as remote sensing to monitor crop characteristics have been used to detect disease stress. Remote sensing is a relatively new approach to disease detection, but it has the potential to detect diseases more quickly and with higher accuracy and precision using spectral changes in crop canopy (West et al. 2003; Sankaran et al. 2010).

Remote sensing is being used as an indirect method of collecting data on vegetation without physical contact, by measuring the electromagnetic energy reflected or emitted for a particular tissue (Jensen 2000; De Jong and van der Meer 2006). Remote sensing potentially provides a non-destructive means of plant disease detection, identification and quantification (Mahlein et al. 2012). For example, it was used in assessing the relationship between disease severity and the spectral reflectance of tomato (*Lycopersicon esculentum* Mill.) (Fig. 1.5) (Zhang et al. 2003).

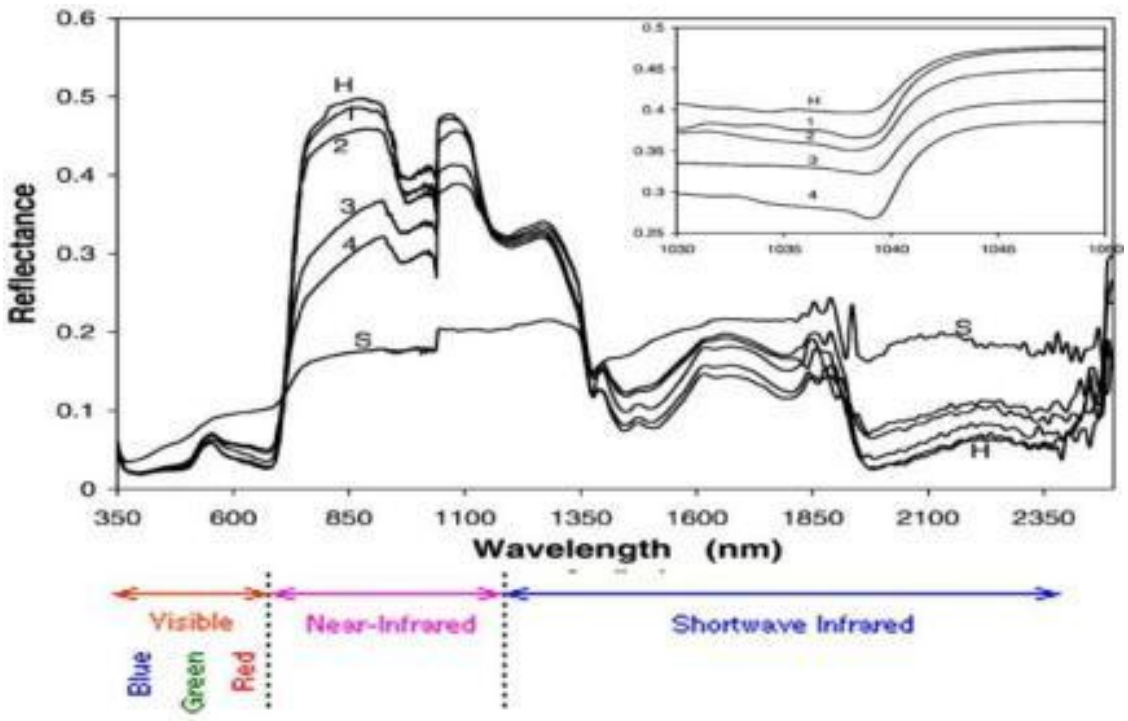

Figure 1.5 Field spectral reflectance. Curve H: the average spectra of healthy tomato plants. Curve 1: the average spectra of infected plants at stage 1 and curves 2, 3 and 4: at stages 2, 3 and 4, respectively. Curve S: the average spectra of the soil. (Zhang et al. 2003)

Plants that are being attacked by plant pathogens activate complex molecular defense mechanisms (Rejeb et al. 2014). This leads to changes in physiological functions such as a reduction in photosynthesis. These changes influence the absorption and reflectance of portions of the electromagnetic spectrum (West et al. 2003; Bravo et al. 2004). Also these changes induce changes in variables such as leaf area index, chlorophyll content and surface temperature of the foliage (Delalieux et al. 2009). Remote sensing with the appropriate sensors has the potential to collect data on these

changes, which can be used to produce spectral signatures that differentiate between healthy and disease plants even before development of symptoms (Meroni et al. 2010).

The high cost of spectra data collection equipment (hyperspectral sensors, cameras, etc.) is an important challenge to the use of remote sensing as a disease detection method. Another limitation is that remote sensing requires specialized experience with data collection and analysis. Most of the current protocols available are not applicable to all crops, and most of them are concentrated on field crops with little attention to horticultural crops and diseases detection (Moshou et al. 2004; Sankaran et al. 2010; Martinelli et al. 2014).

1.8.2 Disease detection with remote sensing

Remote sensing methods for plant disease detection are grouped into imaging and non-imaging sensor-based methods. The imaging sensor methods include the use of visible and infrared cameras (Bock et al. 2010), multispectral (broadband) sensors, hypersectral (narrowband) sensors (Sasaki et al. 1998; Aleixos et al. 2002; Shafri and Hamdan 2009), thermal infrared sensors, and fluorescence imaging sensors (Delalieux et al. 2009). The non-imaging sensor methods include the use of radiometers-spectroradiometers and fluorescence radiometers (Wu et al. 2008; Yang et al. 2013).

These sensors are differentiated into active and passive sensors. Active sensors emit artificial radiation and measure the energy reflected or backscattered. Radar (radio detection and ranging) and Lidar (light detection and ranging) are the most common active sensor remote sensing instruments (McGill 2004). Passive sensor equipment measures the reflected solar radiation or emitted thermal radiation. Hyperspectral,

multispectral, and simple cameras (infrared and visible) are the most common passive sensor equipment (Qin et al. 2009; Shafri and Hamdan 2009; Bock et al. 2010).

A measurement of the reflectance from Debney's tobacco (*Nicotiana debneyi* L.) using multispectral cameras showed a reduction in reflectance after infection with tomato mosaic tobamovirus. This reduction was attributed to an overall reduction in photosynthesis during the early stages of infection (Polischuk et al. 1997). A similar decrease in reflectance were observed in bailey (*Acacia baileyana* F. Muelle) plants infected with powdery mildew (Lorenzen and Jensen 1991). These changes indicate a deviation from normality such as infection and disease (Martinelli et al. 2014), but are not explicitly indicative of disease or specific stresses (West et al. 2003; Moshou et al. 2004). Notwithstanding,

1.8.3 Image analysis and vegetative indices

Imaging passive sensor tools measure reflected solar radiation in the visible (VIS wavelength = 400 - 700 nm), near-infrared (NIR wavelength = 700 - 1100 nm), and short-wave infrared (SWIR wavelength = 1100 - 2500 nm) (Sankaran et al. 2010; Garcia-Ruiz et al. 2013) spectrum. Reflectance data needs to be processed to extract the effective data. The biggest challenge with extracting data from images is selection of the most appropriate processing software and techniques (Martinelli et al. 2014).

Remote sensed images for disease detection can be analysed using specific spectral vegetative indices, which are sensitive to disease presence (Mahlein et al. 2012; Keremane et al. 2015). Other methods of analyzing these images are i) conducting correlation or regression analysis of the presence of disease with changes in specific band

wavelengths (Yang et al. 2013); ii) applying data mining algorithms to spectral data (Zhang et al. 2003; Delalieux et al. 2009); and iii) using machine learning and classification techniques to distinguish presence or absence of disease (Aleixos et al. 2002; Moshou et al. 2004).

Generally stressed plants show a greater increase in reflectance in the visible region (blue, green, red) than in the rest of the spectrum, compared to healthy plants (Carter 1993). Vegetative indices, therefore, use the reflectance figures from two or more regions (Table 1.3) to compute values specific to plant conditions (Hatfield and Prueger 2010). Changes in the reflectance in different regions are strongly linked to chlorophyll absorption and in the physical structure of the leaf (Kumar and Silva 1973). The portion of the reflectance spectrum that is most sensitive to changes in a leaf is the red edge band (Baranoski and Rokne 2005).

Table 1.3 The regions of the reflectance spectrum used in calculating vegetative indices (Hatfield and Prueger 2010)

Region	Wavelength (nm)
Blue	400–510
Green	520–590
Red	630–685
Red-edge	690–730
Near-infrared	760–850

The most common vegetative index is the normalised difference vegetative index (NDVI) (Rouse et al. 1973). Differences in NDVI have been used to detect Huanglongbing-infected citrus trees (Garcia-Ruiz et al. 2013), ganoderma stem base rot

of oil palm trees (Shafri and Hamdan 2009), and anthracnose in citrus (Aleixos et al. 2002). Disease index (Moshou et al. 2004) and green NDVI (Contreras-Medina et al. 2012) are other vegetative indices that can be used to detect changes in plant leaves relating to disease.

The main limitation in using vegetative indices for disease detection is the selection of disease-specific spectral bands. Also, selection of classification algorithms for the spectral bands is a challenge. This is dependent on the imaging device and environmental conditions under which the images are taken (Sankaran et al. 2010).

1.8.4 Airborne platforms and unmanned aerial vehicles

The increasing availability of smaller, light-weight, less expensive and high resolution imaging sensor tools has significantly propelled the use of remote sensing for disease management (Sankaran et al. 2010; Martinelli et al. 2014). These tools are built so that they can be mounted on relatively small airborne devices such as unmanned aerial vehicles (UAVs). Unmanned aerial vehicles are reported to be more efficient in collecting real-time aerial images with better spatial resolution with easy adjustment of flying altitudes compared to manned aircrafts (Garcia-Ruiz et al. 2013).

1.9 Summary and objective

Stemphylium leaf blight is a destructive but poorly understood disease of onion in Ontario. Since it was first reported in 2008, the incidence and severity of SLB have increased within the Holland Marsh. Fungicides applied in combination with IPM strategies have not reduced SLB levels to acceptable levels. Improved understanding of

the biology of the pathogen (overwintering, inoculum dispersal, alternative hosts) and the conditions that favour disease development in the Holland Marsh are needed to improve disease management.

The original source of the pathogen on onion in the Holland Marsh is not known. However, the pathogen was previously reported on asparagus in Ontario. Investigations into the pathogenicity and aggressiveness of isolates of *S. vesicarium* from other hosts and locations may help to identify possible sources of the pathogen. This in turn may indicate if it is possible to transfer successful management techniques from other hosts to onion.

At present, the options for effective management of SLB on onion are limited. There are no cultivars with strong resistance but cultivars differ in susceptibility to SLB, so evaluation of the reaction of locally grown cultivars could identify those with lower susceptibility. *Stemphylium vesicarium* has been shown to develop insensitivity to fungicides over very short periods of time, so application of fungicides should be recommended only when needed, to minimize the development of insensitivity and to minimize the cost of SLB management. Early detection may prove useful in managing SLB, so use of aerial infrared imagery should be assessed to determine its potential to detect early disease stress in the onion crop.

In the course of these studies, the following hypotheses were made:

1. The incidence of SLB on onion in the Holland Marsh coincides with frequent rainfall events, temperature exceeding 18 °C, leaf wetness duration exceeding 6 h, vapour pressure deficit less than 1.5kPa, and abundance of airborne conidia,

2. Ascospores and conidia are available at different times during the growing season in the Holland Marsh,
3. Pseudothecia overwinter on infected onion residue in Ontario,
4. Commercial onion cultivars differ in susceptibility to SLB, but none are resistant,
5. Isolates of *S. vesicarium* from different hosts and locations in Canada are pathogenic on onion, but differ in aggressiveness,
6. Spray timing programs can reduce the number of fungicide applications required to keep SLB below an economic threshold on onion, and

Aerial infrared photography can be used to detect differences between healthy onions and those infected with SLB.

The overall objective of this research was to improve the management of SLB on onion in the Holland Marsh of Ontario. The specific objectives tested were to:

1. Investigate the availability and seasonal distribution of airborne inoculum for SLB during the growing season,
2. Investigate the relationship between rainfall, temperature, vapour pressure deficit, and leaf wetness duration and airborne spore concentration and SLB on onion,
3. Investigate the overwintering of *S. vesicarium* on onion crop residue,
4. Evaluate the susceptibility of commercially grown onion cultivars to SLB under controlled conditions and in the field,
5. Evaluate the pathogenicity and aggressiveness on onion of selected isolates of *S. vesicarium* from other hosts and locations in Canada,
6. Evaluate selected spray timing programs for management of SLB on onion, and

7. Investigate the use of aerial infrared photography to detect SLB on onion in the Holland Marsh.

In the course of these studies, the following hypotheses were tested:

7. The incidence of SLB on onion in the Holland Marsh coincides with frequent rainfall events, temperature exceeding 18 °C, leaf wetness duration exceeding 6 h, vapour pressure deficit less than 1.5kPa, and abundance of airborne conidia,
8. Ascospores and conidia are available at different times during the growing season in the Holland Marsh,
9. Pseudothecia overwinter on infected onion residue in Ontario,
10. Commercial onion cultivars differ in susceptibility to SLB, but none are resistant,
11. Isolates of *S. vesicarium* from different hosts and locations in Canada are pathogenic on onion, but differ in aggressiveness,
12. Spray timing programs can reduce the number of fungicide applications required to keep SLB below an economic threshold on onion, and
13. Aerial infrared photography can be used to detect differences between healthy onions and those infected with SLB.

CHAPTER TW0

RELATIONSHIP AMONG WEATHER VARIABLES, CONCENTRATION OF AIRBORNE SPORES AND DEVELOPMENT OF STEMPHYLIUM LEAF BLIGHT

2.1 Introduction

The Holland Marsh is the single largest production area for onion in Canada. Stemphylium leaf blight (SLB) was observed in the Holland Marsh in 2008 and incidence has since increased drastically (Paibomesai et al. 2012; McDonald et al. 2015). The disease caused yield losses of up to 90% on onion at various locations worldwide (Rao and Pavgi 1975; Miller et al. 1978; Lorbeer 1993; FAO/IPGRI 1997)

Initially, SLB on onion is characterized by small, yellow to tan, water-soaked lesions that turn dark brown when the pathogen sporulates (Rao and Pavgi 1975). The pathogen starts producing host-specific toxin immediately after conidial germination (Singh et al. 2000; Wolpert et al. 2002). The toxins cause extensive necrosis of the infected leaves from the tip, resulting in desiccation and premature lodging of the crop (Rao and Pavgi 1975; Basallote-Ureba et al. 1999). Stemphylium leaf blight affects all foliar parts of the onion crop and severely infected crops develop small to no bulbs (Rao and Pavgi 1975).

Stemphylium vesicarium produce ascospores and conidia, which infect the various hosts (Simmons 1969). The epidemiology of *S. vesicarium* has been studied extensively on pear (Llorente et al. 2012), and garlic (Prados-Ligero et al. 2003) in Spain, and on Welsh onion in Japan (Misawa and Yasuoka 2012). However, studies of the pathogen on common onion are limited. On garlic tissues, pseudothecia production and ascospore

maturation are highly favoured by temperatures between 4.5 - 10.5 °C and relative humidity exceeding 98%. Maturation of ascospores take 1-4 months (Prados-Ligero et al. 1998).

Ascospore release in garlic coincides with frequent precipitation, temperatures between 10 - 21 °C and low vapour pressure deficit (VPD) of 0.1 - 0.5 kPa (Prados-Ligero et al. 2003). In pear orchards, ascospores are postulated to first colonise debris and dried grasses in the orchard. This initial colonisation leads to the abundant production of conidia later in the season, which causes infections on pear fruit and young twigs (Rossi et al. 2005, 2008).. The role of ascospores in the epidemiology of SLB on Allium crops is not known (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012).

On Allium crops, conidia are abundant during primary infection (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). Release of conidia is favoured by frequent rainfall, temperatures of 15 - 32 °C and VPD of 0.1 - 1.5 kPa (Prados-Ligero et al. 2003). Maximum germination of conidia and infection on onion leaves occurs between 10 - 25 °C with leaf wetness duration exceeding 8 h (Suheri and Price 2000).

The daily concentration of airborne ascospores and conidia show a diurnal pattern in asparagus, leek, garlic and pear (Granke and Hausbeck 2010; Prados-Ligero et al. 2003; Rossi et al. 2005; Suheri and Price 2001). In asparagus fields in Michigan, the highest concentrations of airborne conidia were captured between 0700 - 1300 h (Granke and Hausbeck 2010). In garlic crops in Spain, the highest concentrations of airborne ascospores and conidia were captured between the 00 – 0600 and 1200 – 1800 respectively (Prados-Ligero et al. 2003). Weather conditions recorded in the 10 days

before spore capture influenced the concentration of airborne spores and subsequent disease development (Prados-Ligero et al. 2003).

Initial symptom development on garlic and Welsh onion crops coincided with high airborne conidia concentration, rainfall, and temperatures above 18 °C (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). In asparagus, purple spot symptoms coincided with both high ascospore and conidia concentrations and prolonged wetness periods (Granke and Hausbeck 2010). There is little information on the nature and availability of airborne inoculum of SLB on onion, or on the weather conditions that favour SLB development.

Understanding the epidemiology of SLB on onion is essential for development of effective management strategies. The objectives of this research, therefore, were to i) investigate the concentration and distribution of airborne spores during the growing season, ii) evaluate the relationship between airborne spore concentration and weather variables, iii) evaluate the relationship between airborne spore concentration and SLB incidence, and iv) investigate overwintering of pseudothecia in the Holland Marsh.

2.2 Materials and Methods

2.2.1 Trapping field

Onion cv. La Salle (Stokes Seeds, Thorold, ON), previously shown to be susceptible to SLB, was assessed at two sites that has been planted to onion the previous year and with a history of SLB. In 2015, plugs of onion (2-3 seedlings per plug) were transplanted on 25 May using a mechanical transplanter. The trial was conducted on an organic soil (organic matter ≈ 62%, pH ≈ 7.2) at the Jane Street research site near the

Muck Crop Research Station (MCRS), Holland Marsh. In 2016, the crop was direct seeded at about 35 seeds m^{-1} per row on 4 May using a Stanhay Precision seeder into organic soil (organic matter ≈ 71%, pH ≈ 5.7) at the MCRS. The seeds germinated 10-12 days after planting (DAP). In both years, the plot used for spore trapping was 15 × 24 m in size (Fig. 2.1A).

In 2015, flumioxazin herbicide (Chateau®, Valent Corporation, Guelph, ON) was applied following label recommendations to control weeds at 45 days after transplanting (DAT). In 2016, bromoxynil herbicide (Pardner®, Bayer Crop Science Inc., Mississauga, ON) was applied at 39 DAP and repeated at 56 DAP to control weeds. Weeds were removed by hand throughout the remainder of the growing season until maturity. In 2015, seedlings were drenched 45 days after seeding with chlorpyrifos insecticide (Pyrinex™, Adama Agricultural Solutions Canada Ltd., Winnipeg, MB) for the management of onion maggot. The spore trap plot did not receive applications of fungicide so as to provide a natural representation of inoculum levels. Within the trapping plot, four beds, each consisting of four rows, 42 cm apart and 5 m in length, were marked out for disease assessment.

Figure 2.1 Aerial view of the spore trap plot planted at the MCRS in 2016, and (B) Burkard 7-day volumetric sampler used to sample air-borne spores at the site.

2.2.2 Spore trapping

The concentration of ascospores and conidia was estimated using a 7-day Burkard volumetric sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) (Fig. 2.1B). Airborne spores were trapped from 20 May - 16 September in 2015 (120 days), and from 21 April - 6 September in 2016 (139 days). The suction orifice of the sampler was set at about 0.7 m above the soil, facing the direction of the prevailing wind. The sampling airflow rate was set at 10 L min^{-1}. Airborne spores were collected onto a clear Melinex (cellophane) tape coated with an adhesive mixture consisting of 50 mL petroleum jelly, 6 g paraffin, and 0.6 g phenol and mounted on a metal drum.

The Melinex tape was placed on a clean metal drum (Fig. 2.2) using a mounting stand and secured with a piece of double-sided tape placed between the green line and the top black line on the drum. A small amount of adhesive was applied uniformly to the tape using a toothbrush. This produced a thin and even layer of adhesive over the tape and excess adhesive was removed. The prepared drum was then mounted unto the clock head

(Fig. 2.2), which was part of the lid of the trap. The clock was fully wound anti-clockwise on the trap. The red line on the drum was lined up with the metal arrow on the clock, and then firmly secured with a bolt. The drum was changed weekly and cut into seven 48-mm-long pieces, with each piece carrying the spores trapped on one of the previous 7 days. The daily tape segments were fixed onto microscope slides that had been marked to 0.33 mm across to represent every 2-hr interval on the tape. The prepared slides were scanned with a compound microscope at 400× magnification. Ascospores and conidia were identified based on their morphological characteristics, as described by Simmons (1969).

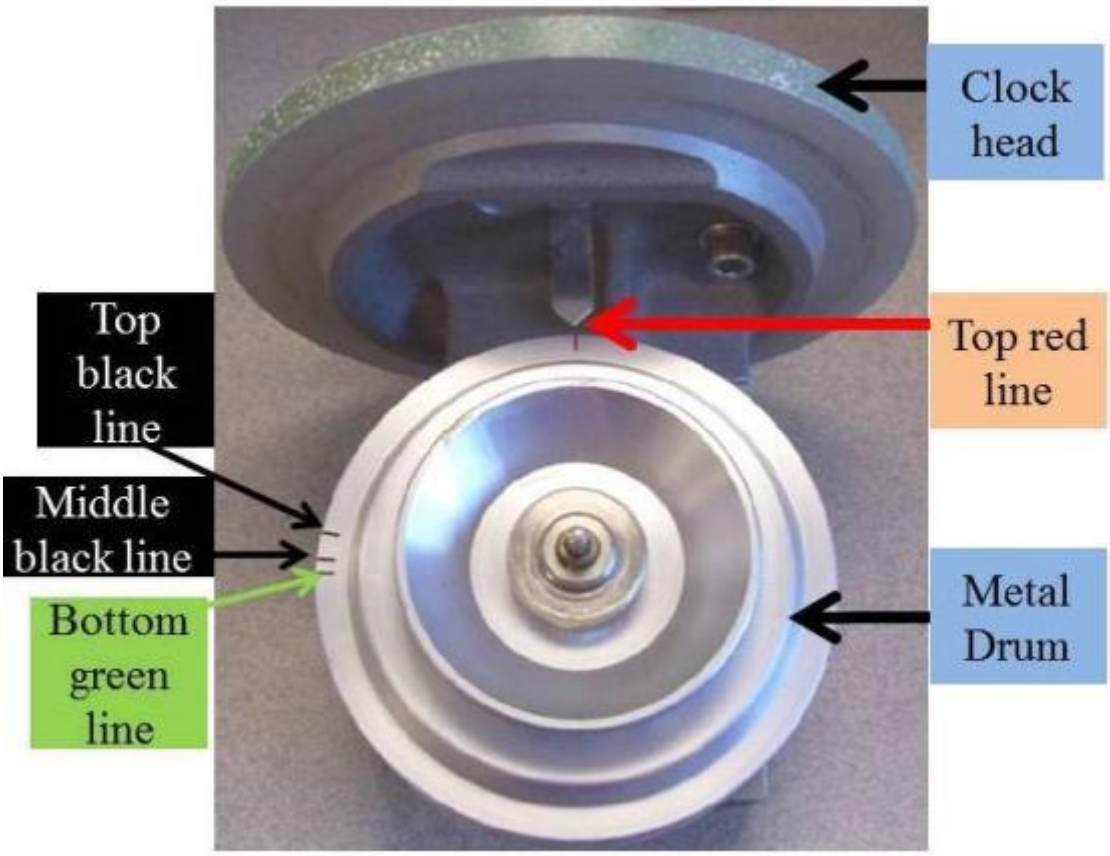

Figure 2.2 Burkard 7-day volumetric spore sampler clock head mounted with prepared drum for monitoring of airborne spore in the Holland Marsh, 2016.

2.2.3 Weather data

Hourly meteorological data of temperature (°C), relative humidity (%), rainfall (mm) and leaf wetness (%) were measured and recorded by an Onset® automatic weather station (Onset Corporation, Bourne, MA) placed in the center of the trapping plot. Rainfall was measured with a rain gauge smart sensor (Model: S-RGB-M002) installed with a plug-in modular connector that allowed it to communicate with a data logger and store data. Two leaf wetness smart sensors (Model: S-LWAM003) were used to measure leaf wetness. The leaf wetness smart sensors were placed at in an upright position (almost

90 °) to simulate the angle of an onion leaf; the sensors did not require painting or coating following the manufacturers' instructions. Routine visual checks for moisture on onion leaves were compared to the values from the two leaf wetness sensors. The data from the sensor identified as more sensitive and accurate were used to calculate daily leaf wetness duration (LWD). Air temperature and relative humidity were measured with a temperature/RH sensor (Model: S-THB-M00x). The daily average temperature, average relative humidity, total daily rainfall and daily number of hours with temperature exceeding 15 °C were computed. Vapour pressure deficit (VPD) (kPa) was calculated using average daily relative humidity and temperature (Abtew and Melesse 2013) as follows:

$$e_s = 0.611 \exp\left(\frac{17.27\,T}{T + 237.3}\right)$$

$$VPD = e_s\left(1 - \frac{RH}{100}\right)$$

Where e_s is saturation vapor pressure in kPa and T (°C) is 24 h average air temperature.

2.2.4 Disease assessment

The disease assessment plots were observed weekly for the onset of SLB lesions. In 2015, the number of lesions was assessed on one transplant plug (consisting of 2 - 3 seedlings) at each of eight places along the middle two rows of each bed, for a total of 16 - 24 plants per plot. Plugs were selected at roughly 1-m intervals along each row, starting 1 m from the end of the row. The first and second fully developed outer leaves on the two largest seedlings per plug were assessed for lesions. Overall, 32 leaves were

assessed and the mean number of lesions per leaf was calculated. In 2016, lesions were counted on 10 consecutive plants in each of the two middle rows as described above (total = 40 leaves).

SLB incidence in 2015 was assessed by counting the total number of transplant plugs per plot and the number with SLB symptoms. In 2016, 100 plants in the middle two rows of each plot were assessed. A method similar to that for lesion assessment was used in both years to select onion plants for assessment. However, the plants assessed for lesions were not the same as assessed for leaf dieback. The total length and the length of the dieback on the fifth and sixth fully developed true leaves of each plant were measured with a 60-cm clear plastic ruler. The length of leaf dieback relative to the total leaf length for each leaf was assessed and the average percentage leaf dieback for each bed for each cultivar was calculated.

Onion plants in the trapping plot were pulled up on 10 September in 2015 and 6 September in 2016. Yield was not assessed in this trial.

2.2.5 Overwintering

Onion plants in the disease assessment and trapping plots in both years were visually assessed at the end of the planting season for the formation of pseudothecia. In 2015, 64 symptomatic leaves and 32 bulbs of diseased plants were collected and placed in light polyester mesh bags that allowed exchange of gases and moisture. An experimental unit consisted of a bag with eight leaves or four bulbs per bag. Four bags of leaves and four bags of bulbs were buried 10-cm deep in muck soil in separate 2 L pots, and the remaining bags were placed on the soil surface of the same pots. Thus each pot

had two bags of leaves or bulbs, one buried and the other on the surface. The pots were then placed outside on 31 October. Two bags of each tissue (one from the surface and one from 10-cm depth) were sampled on 30 December, 30 January, 29 February and 30 March and observed visually and microscopically for formation of pseudothecia.

2.2.6 Data analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute, 2015). Visual and statistical analyses were used to determine the relationship between the weather data and type and number of spores trapped. First, the data were plotted to visually compare the timing of release of ascospores and conidia with both weather data and subsequent SLB incidence and leaf dieback. PROC GLM was used for analysis of variance of the concentration of ascospores and conidia. Outliers were assessed using Lund's test and no outliers were identified. The normality of the data was tested using the PROC UNIVARIANT function. This showed that the data were not normally distributed. Therefore, the association between the daily concentration of airborne spores and the weather variables listed in Table 2.1 was calculated using Spearman's rank correlation in PROC CORR at $P < 0.05$. A nonparametric test was chosen because the data were not normally distributed and most of the weather variables were measured on an ordinal scale. Multiple stepwise regressions of spore concentrations with weather variables were conducted using PROG REG.

Table 2.1 Weather variables assessed and tested for correlation with conidia and ascospores of *Stemphylium vesicarium* recorded in onion at the Holland Marsh, ON.

Temp	Average daily temperature (˚C)
NTemp	Number of hours daily with temperature ≥ 15 ˚C (h)
DTemp*	Number of days with average temperature ≥ 15 ˚C (days)
WTemp	Average temperature during leaf wetness period (˚C)
LWD	Daily leaf wetness duration (h)
DLWD*	Number of days with LWD ≥ 6 h (days)
VPD	Vapour pressure deficit (kPa)
NVPD	Number of hours daily with VPD ≤ 0.5 kPa (h)
DVPD*	Number of days with VPD ≤ 0.5 kPa (days)
Rain	Total daily rainfall (mm)
Train*	Cumulative total rainfall (mm)
NRain*	Number of days with rainfall ≥ 2mm (days)

*Calculated for cumulative periods up to 10 days prior to spore trapping

2.3 Results

2.3.1 Daily airborne spore concentration

The daily concentration of airborne conidia and ascospores were higher in 2015 compared to 2016. However, the daily pattern of spore capture was similar in the two years. The number of airborne spores captured varied throughout the day, but the concentration was highest in the early morning. In 2015, 49% of ascospores and 56% of conidia were captured from 0600–1200 h, and 73% of ascospores and 60% of conidia in 2016 (Table 2.2). Few or no spores were captured during rainy periods and after dark.

2.3.2 Seasonal pattern of airborne spores

Conidia were captured throughout the entire season in both years, but the concentrations differed substantially. The average daily conidia concentration was 33 conidia m^{-3} air in 2015 and 7 conidia m^{-3} air in 2016. In 2015, the highest daily conidia concentration was 97 conidia m^{-3} air captured on 11 July, compared with 29 conidia m^{-3} air captured on 29 July and 10 August in 2016 (Fig. 2.3).

The daily concentrations of ascospores declined as the season progressed, and ascospores became more difficult to identify as their concentration dropped and the concentration of conidia increased. Ascospore were last captured on 6 July 2015 and 7 June 2016. The average daily ascospores concentration was 12 ascospores m^{-3} air in 2015 and 4 ascospores m^{-3} air in 2016. The highest daily concentration of ascospores in 2015 was 54 ascospores m^{-3} air counted on 2 June, compared with 6 ascospores m^{-3} air counted on 23 and 26 April, 2016. Furthermore, ascospores were captured on only 4 days in 2016 (Fig. 2.4).

Table 2.2 Percentage of airborne ascospores and conidia captured at 2-hr intervals each day during the growing season in onion plots at the Holland Marsh, ON in 2015 and 2016.

Spore type	Hours of the day											
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24
2015												
Ascospores	2	3	16	23	20	6	5	6	7	6	4	3
Conidia	1	4	14	22	22	12	7	6	5	5	2	1
2016												
Ascospores	0	15	4	31	31	12	0	0	0	0	0	0
Conidia	1	3	6	20	21	18	12	7	4	4	3	1

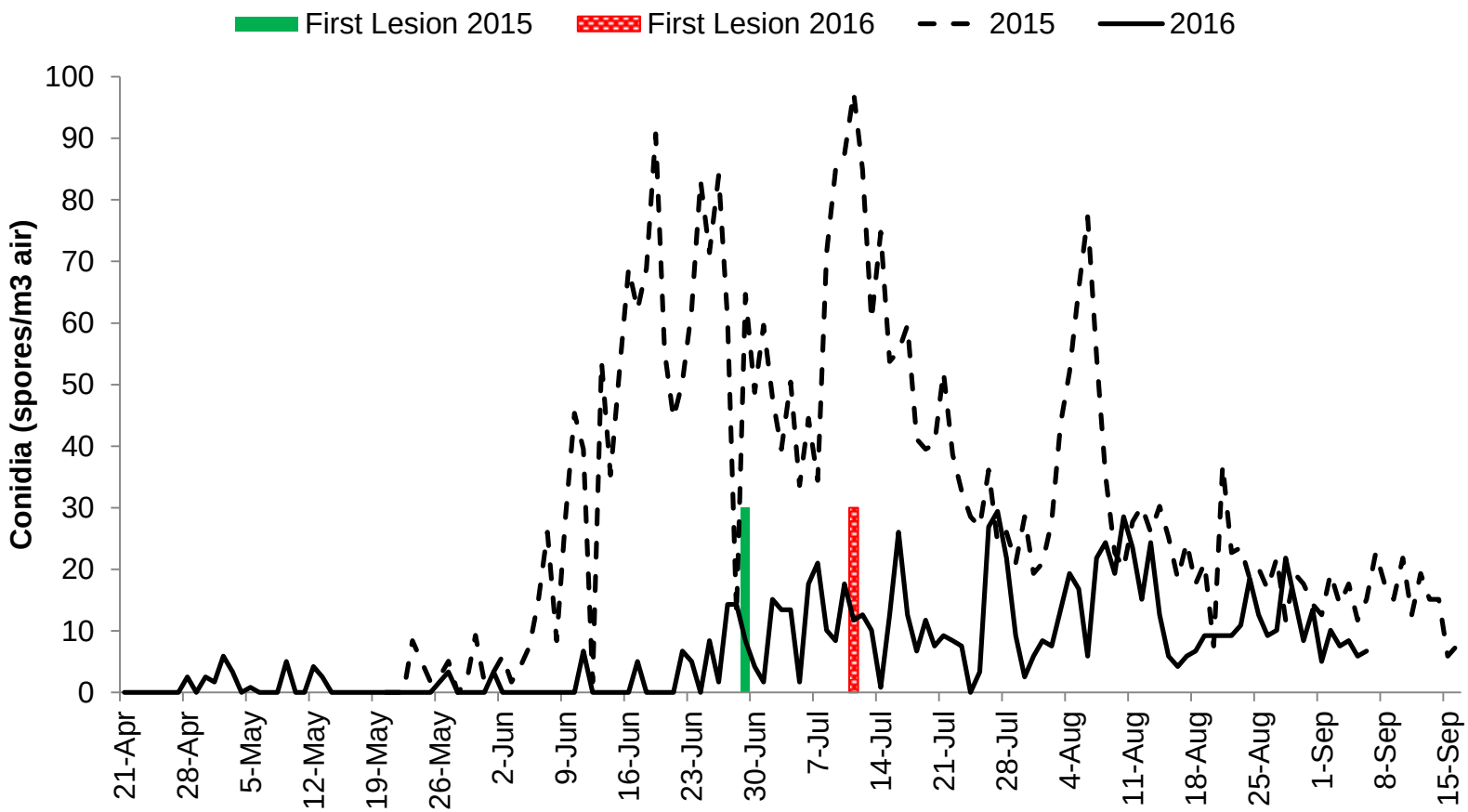

Figure 2.3 Seasonal patterns of conidial capture and first observation of stemphylium leaf blight lesions on onion at the Holland Marsh, ON in 2015 and 2016.

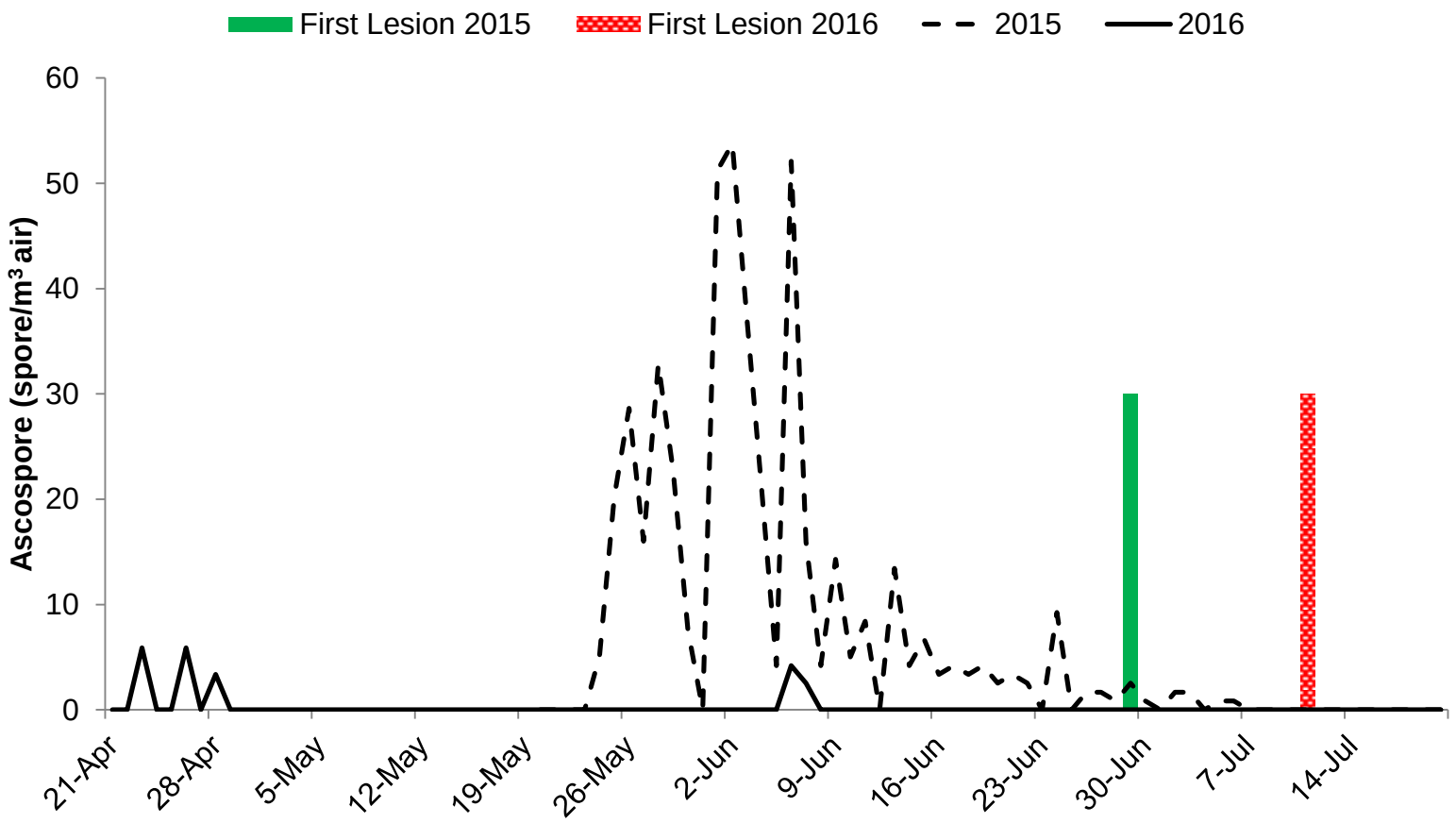

Figure 2.4 Seasonal pattern of ascospore capture and the first observation of stemphylium leaf blight lesions on onion at the Holland Marsh, ON in 2015 and 2016.

2.3.3 Weather variables and airborne spore concentration

In 2015, approximately 340 mm of precipitation fell over the course of 28 days, and 245 mm fell over 22 days in 2016 (Table 2.3). Daily conidial concentration was correlated with the total rainfall volume (r = 0.49, $P < 0.0001$) and the number of rainy days (r = 0.23, $P = 0.01$) in the 10 days prior to capture (Table 2.4). From observations in 2015, conidia concentration increased substantially 2–72 h after rainfall event. However, few or no spores were trapped during a rainfall event (Figs. 2.5 and 2.6).

The mean daily temperature during the trapping period was about 19 °C and 18 °C in 2015 and 2016 respectively (Table 2.3). Average daily temperatures exceeding 15 °C were recorded on 91% of the trapping days in 2015 and 77% of the trapping days in 2016 (Table 2.3). The average temperature during LWD was 19 °C in 2015 and 18 °C in 2016 (Table 2.3). The daily conidial concentration was positively correlated with the number of days with temperatures exceeding 15 °C in the 10 days prior to capture in both 2015 (r = 0.49, $P < 0.0001$) and 2016 (r = 0.68, $P < 0.0001$) seasons (Table 2.4). In 2016, the daily conidia concentration was correlated with the average daily temperature (r = 0.56, $P < 0.0001$) and the average temperature during leaf wetness periods (r = 0.53, $P < 0.0001$) (Table 2.4). The daily concentration of ascospores captured decreased with daily average temperatures exceeding 15 °C, whereas conidia concentration increased as the season progressed and then declined towards the end of the season (Figs. 2.7 and 2.8).

In 2015, 87% of the days assessed had daily LWD exceeding 6 h, and 96% of days in 2016 (Table 2.3). In 2016, the daily LWD correlated with daily ascospore (r = 0.31, $P = 0.03$) and conidial (r = 0.41, $P < 0.0001$) concentration. In 2015, daily ascospore concentration was correlated with the number of day with LWD exceeding 6 h

in the 10 days prior to capture, but not in 2016. In 2016, daily conidial concentration was correlated ($r = 0.63$, $P < 0.0001$) with the number of days with LWD exceeding 6 h in the 10 days prior to capture (Table 2.4).

The proportion of days in the trapping period with VPD less than 0.5 kPa was 57% in 2015 and 36% in 2016 (Table 2.3). Daily ascospore concentration was correlated with the number of hours in a day when VPD was less than 0.5 kPa in 2016 ($r = 0.31$, $P = 0.04$). In 2016, the daily conidial concentration was also correlated with daily VPD ($r = 0.20$, $P = 0.02$) and the number of days with VPD less than 0.5 kPa in the 10 days prior to capture ($r = -0.34$, $P < 0.0001$) (Table 2.4).

Table 2.3 Weather conditions recorded during the period when airborne spores were trapped, cumulative number of days with favourable weather conditions for stemphylium leaf blight on onion in the Holland Marsh, ON in 2015 and 2016.

Year	Total spore kind [a]		Weather variables [b]								
			Total	Seasonal daily average of:				Seasonal number of days with:			
	Ascospores	Conidia	Rainfall (mm)	Temp. (˚C)	Temp. during LWD	VPD (kPa)	LWD (h)	Temp. ≥ 15 ˚C	Daily VPD ≤ 0.5 kPa	Rainfall (≥ 2 mm)	LWD ≥ 6 h
2015	470	3903	340	19	19	0.5	10	109	68	28	104
2016	22	933	245	18	18	0.6	10	107	50	22	133

[a] Ascospores were captured from 20 May–6 July in 2015 and 21 April–7 June in 2016, conidia were captured 20 May–16 September in 2015 and 21 April–6 September in 2016.

[b] Weather variables recorded from 20 May–16 September in 2015 and 21 April–6 September in 2016.

VPD = vapour pressure deficit. LWD = leaf wetness duration

Table 2.4 Spearman's correlation coefficients between weather variables and daily airborne spore captured from onion plots in the Holland Marsh, ON.

Year	Spore type [a]	Daily weather variables [b]						Weather variables recorded ten day before capture [c]					
		Rain (mm)	Temp. (˚C)	WTemp. (˚C)	VPD (kPa)	LWD (h)	NTemp. (h)	NVPD (h)	DTemp(days)	DVPD (days)	DLWD (days)	NRain (days)	TRain (mm)
2015	Ascospores	-0.06	0.23	-0.11	0.27	-0.17	-0.12	0.17	-0.27	-0.08	**-0.31***	-0.08	-0.22
	Conidia	-0.06	0.07	0.06	-0.01	-0.06	0.11	-0.01	**0.49***	0.12	0.14	**0.23***	**0.49***
2016	Ascospores	0.12	-0.24	-0.21	-0.23	**0.31***	-0.20	**-0.30***	-0.04	-0.11	-0.10	-0.04	-0.13
	Conidia	-0.16	**0.56***	**0.53***	**0.20***	**0.41***	**0.56***	0.13	**0.68***	**-0.34***	**0.63***	0.05	0.10

[a] Ascospores were captured from 20 May-– 6 July in 2015 and 21 April-– 7 June in 2016, conidia were captured 20 May-– 16 September in 2015 and 21 April-– 6 September in 2016.

[b] Daily weather variable recorded during the trapping period: Rain = total daily rainfall, Temp = average daily temperature, WTemp. = average temperature during leaf wetness period, VPD = vapour pressure deficit, LWD = leaf wetness duration, and NTemp = Number of hours with temperature ≥ 15 ˚C.

[c] Weather variables recorded 10 days prior to capture: NVPV = number of hours daily with VPD ≤ 0.5 kPa, DTemp = number of days with average temperature ≥ 15 ˚C, DVPD = number of days with VPD ≤ 0.5 kPa, DLWD = number of days with LWD ≥ 6 h, NRain = Number of days with rainfall ≥ 2mm, and TRain = Cumulative total rainfall.

* Significant at $P < 0.05$.

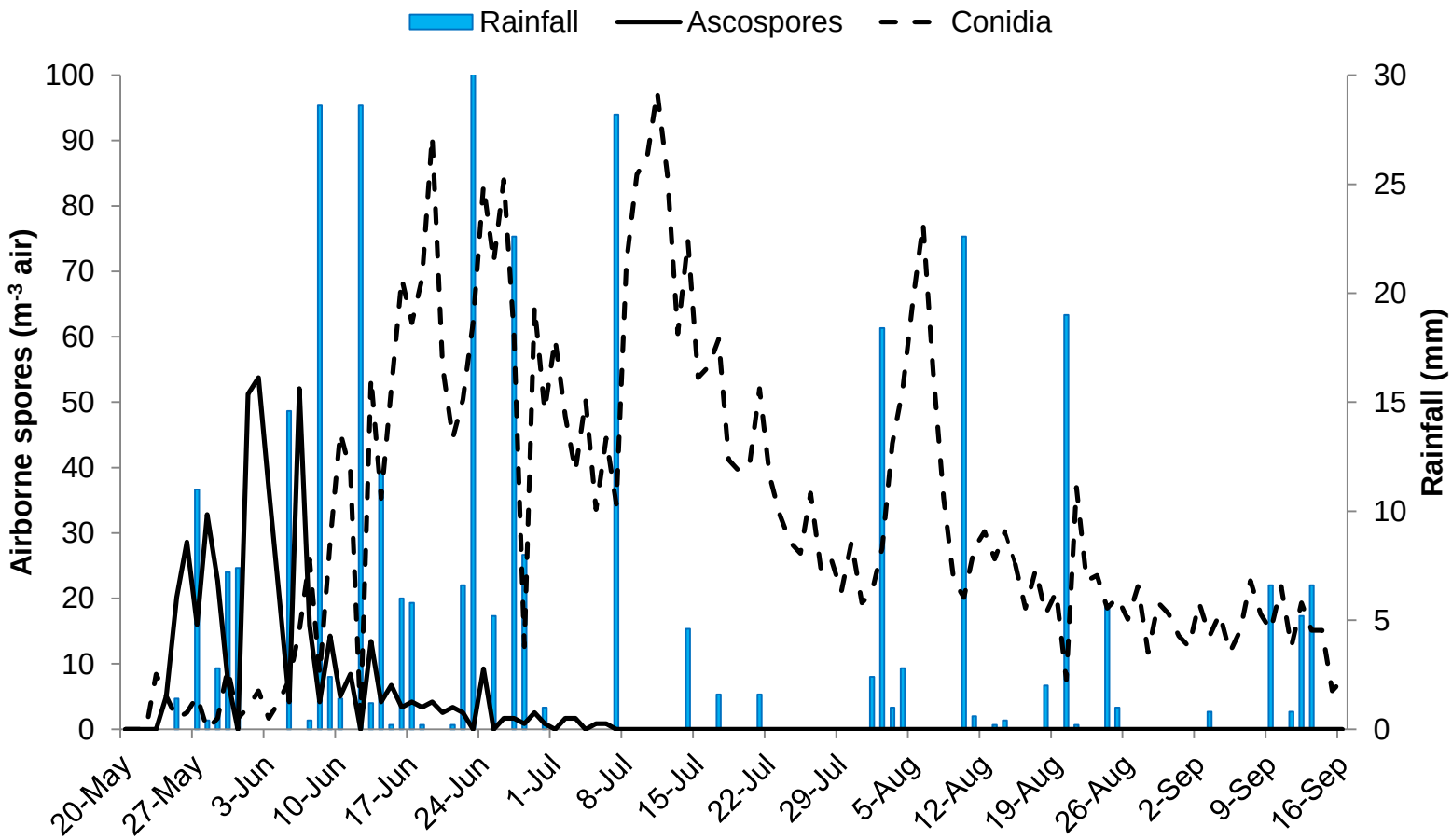

Figure 2.5 Seasonal distribution of airborne ascospores and conidia in relation to daily rainfall recorded in an onion plot at the Holland Marsh, ON, 2015.

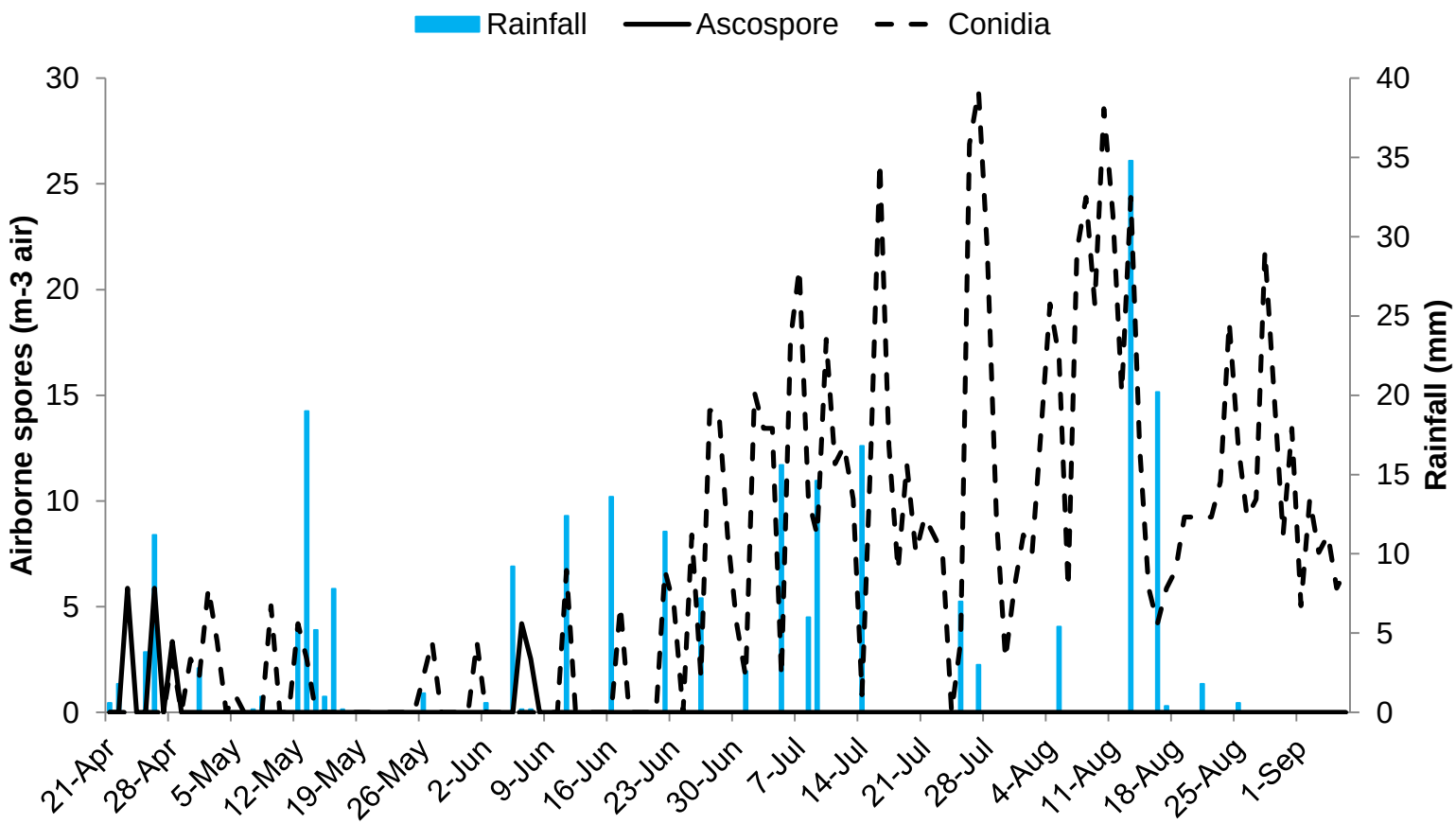

Figure 2.6 Seasonal distribution of airborne ascospores and conidia of *S. vesicarium* in relation to total daily rainfall recorded in onion plots grown in the Holland Marsh, ON, 2016.

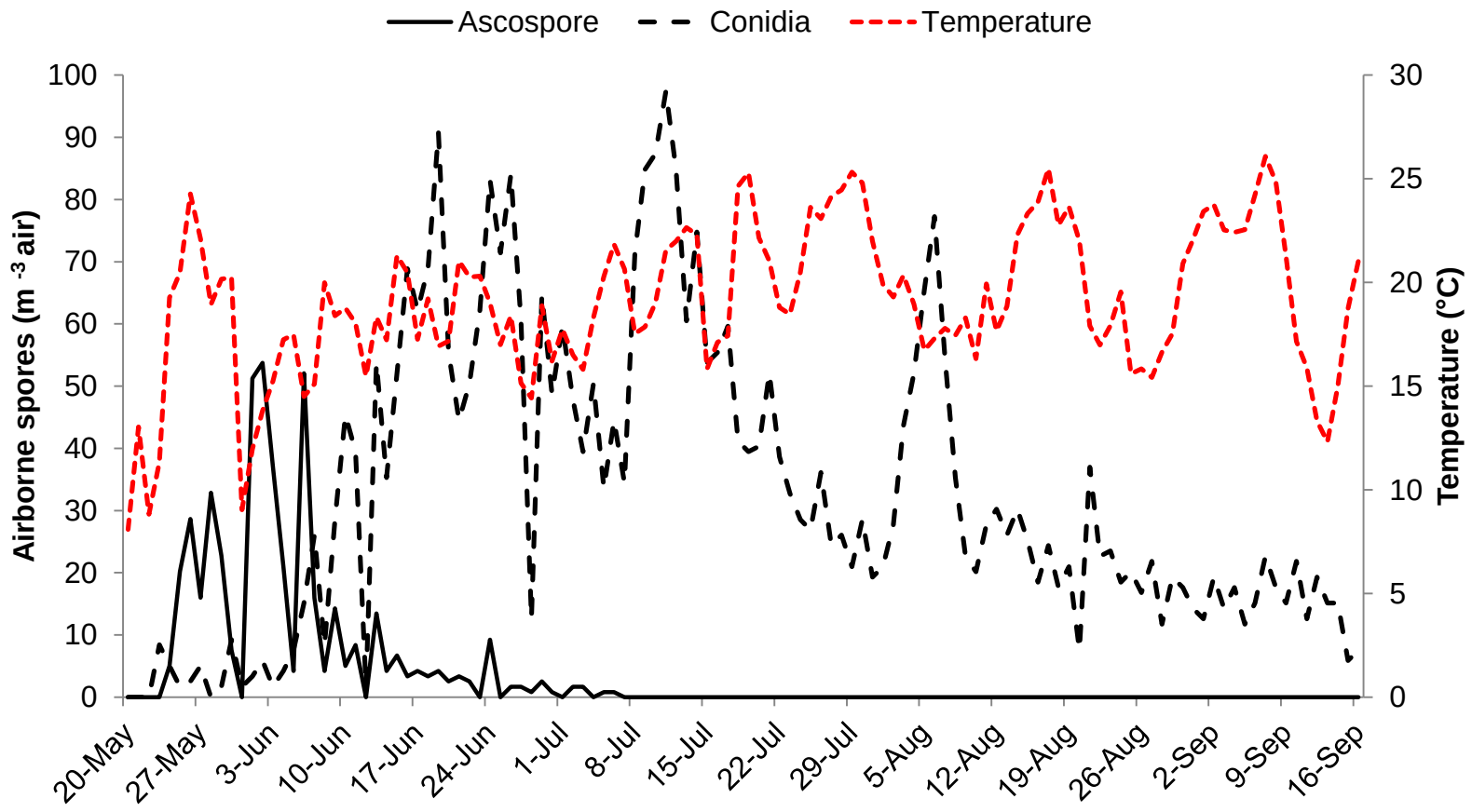

Figure 2.7 Seasonal distribution of airborne ascospores and conidia of *S. vesicarium* in relation to average daily temperature recorded in onion plots grown in the Holland Marsh, ON, 2015.

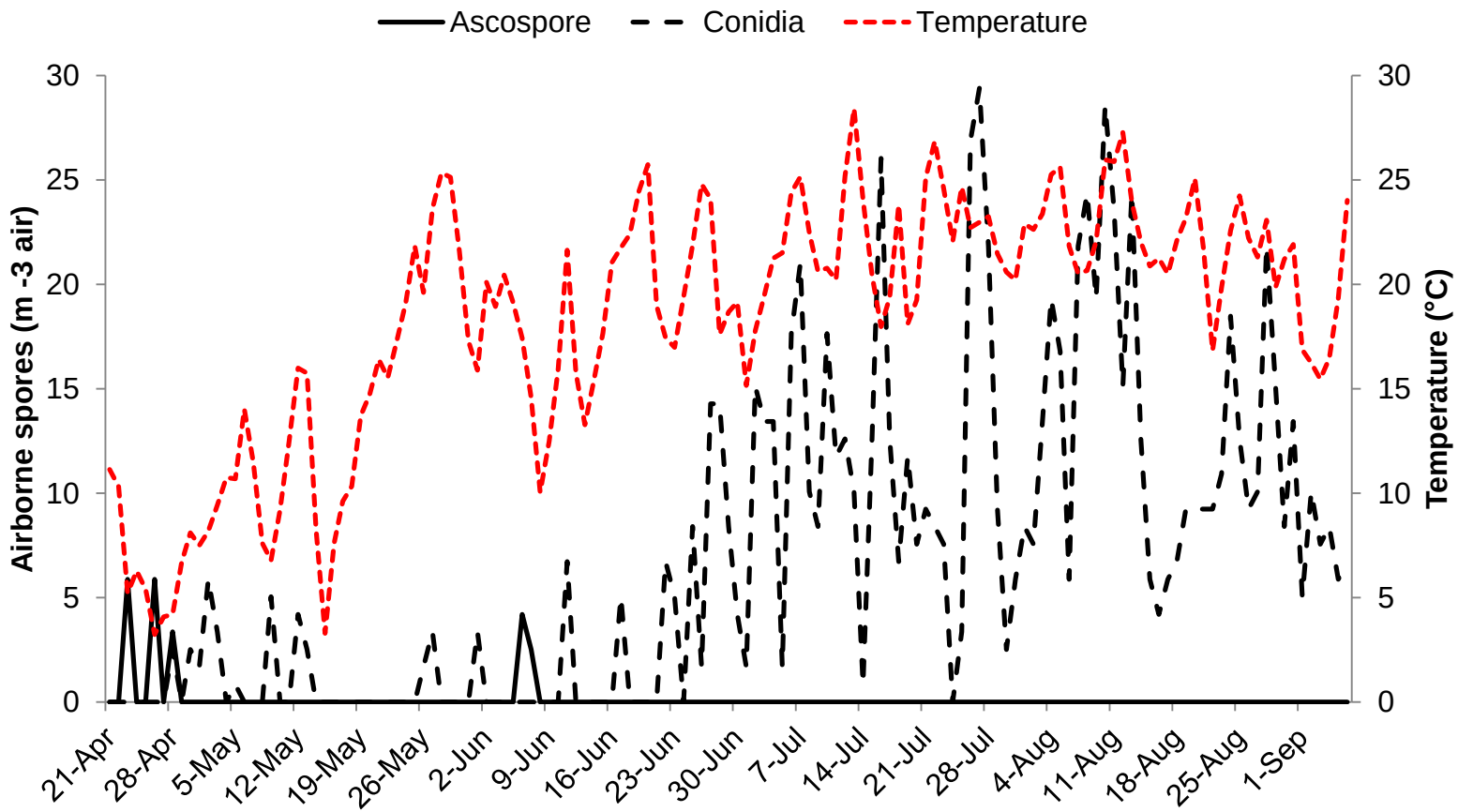

Figure 2.8 Seasonal distribution of airborne ascospores and conidia of *S. vesicarium* in relation to average daily temperature recorded in onion plots grown in the Holland Marsh, Ontario, 2016.

The models derived from multiple stepwise regressions between airborne conidia concentration and weather variables were not consistent across the two years (Table 2.5). The daily concentration of airborne conidia in 2015 was influenced by the cumulative total rainfall in the 10 days prior, the daily number of hours with VPD less than 0.5 kPa, the number of days with temperature exceeding 15 °C in the 10 days prior, and the number of days with VPD less than 0.5 kPa in the 10 days prior. The number of days with temperature exceeding 15 °C had the greatest influence on daily conidia concentration in 2015 (Fig. 2.9A).

Table 2.5 Stepwise regressions of the daily concentration of conidia versus weather parameters at the Holland Marsh, ON in 2015 and 2016.

Weather variable[a]	Partial R-squared	Model R-squared	F Value	Pr > F
2015				
DTemp	0.22	0.22	32.64	<0.0001
TRain	0.34	0.36	25.26	<0.0001
NVPD	0.09	0.44	18.38	<0.0001
NRain	0.03	0.47	5.72	0.02
DVPD	0.03	0.50	6.91	0.01
Rain	0.03	0.54	8.34	0.005
2016				
DLWD	0.26	0.26	48.92	<0.0001
DVPD	0.14	0.40	31.49	<0.0001
LWD	0.02	0.42	4.14	0.04

[a] Weather variables recorded during the trapping period: Rain=total daily rainfall, LWD = leaf wetness duration, NVPV = number of hours daily with VPD ≤ 0.5 kPa, DTemp .= number of days with average temperature ≥ 15 ˚C recorded 10 days prior to capture, DVPD = number of days with VPD ≤ 0.5 kPa recorded 10 days prior to capture, DLWD = number of days with LWD ≥ 6 h recorded 10 days prior to capture, NRain = Number of days with rainfall recorded 10 days prior to capture, and TRain = cumulative total rainfall recorded 10 days prior to capture.

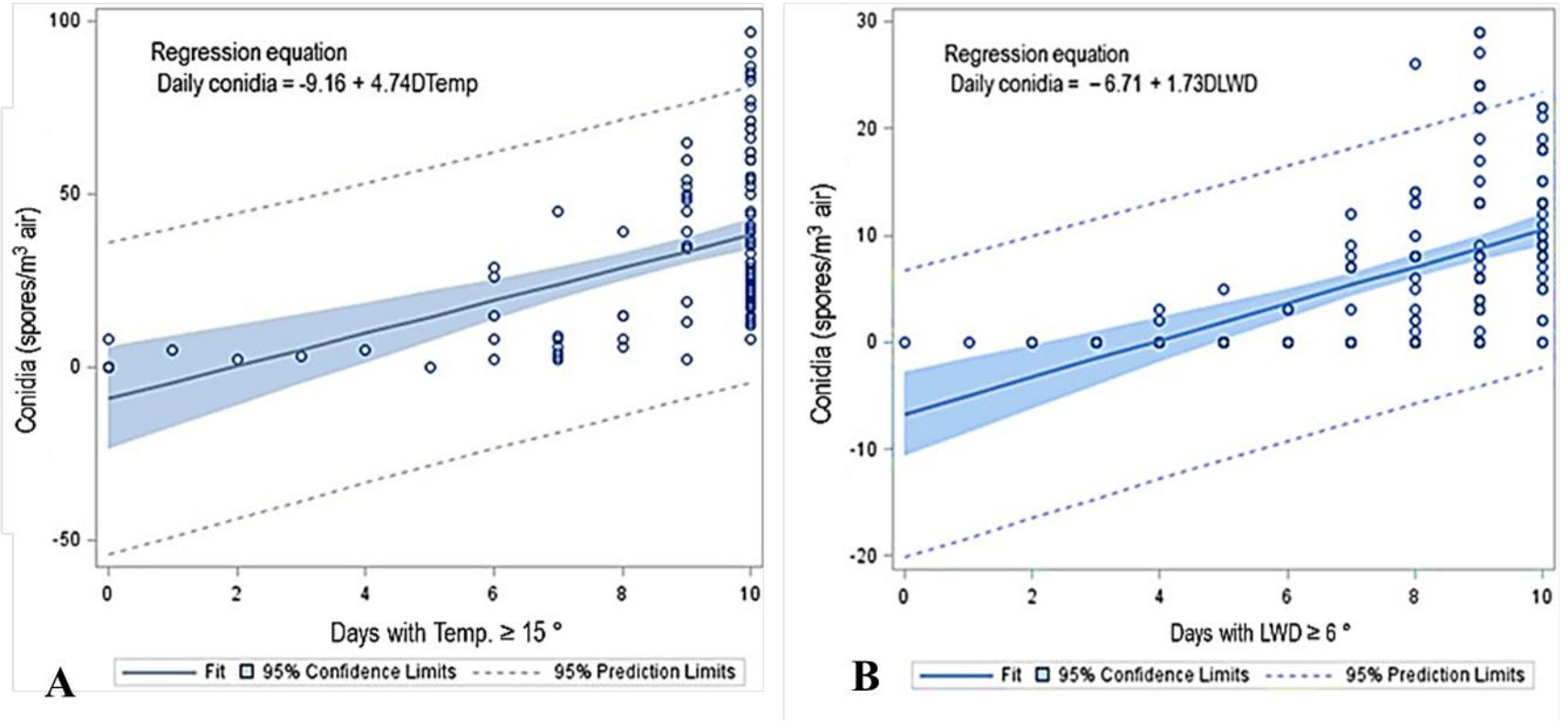

Figure 2.9 Regression (± 95% confidence interval) for daily conidia concentration versus number of days with (A) average temperature exceeding 15 °C in an onion plot in 2015 (R^2 = 0.22, P < 0.0001) and (B) number of days with leaf wetness duration exceeding 6 h (R^2 = 0.26, P < 0.0001) in onion plots at the Holland Marsh, ON in 2016.

In 2016, the concentration of conidia was correlated with the number of days with temperature exceeding 15 °C, VPD less than 0.5 kPa, the number of days with LWD exceeding 6 h in the 10 days prior, and daily LWD (Table 2.5). The number of days with LWD exceeding 6 h had the greatest influence on daily conidia concentration (Fig. 2.9B).

The models derived from stepwise regression between airborne ascospore concentration and selected weather variables were also not consistent across the two years (Table 2.6). In 2015, total rainfall in the 10 days prior to capture had the greatest influence on daily ascospore concentration (Fig 2.10A). In 2016, the daily LWD had the greatest influence on daily ascospore concentration (Fig. 2.10B). The other variables had no impact on ascospore concentration.

Table 2.6 Stepwise regression of the daily concentration of ascospores versus weather parameters at the Holland Marsh, ON in 2015 and 2016.

Weather variable [a]	Partial R^2	Model R^2	F Value	Pr > F
2015				
TRain	0.11	0.11	5.39	0.003
NRain	0.15	0.25	8.72	0.005
2016				
LWD	0.11	0.11	5.59	0.02

[a] Weather variables recorded during the trapping period: LWD=leaf wetness duration, NRain = Number of days with rainfall recorded 10 days prior to capture, and TRain = cumulative total rainfall recorded 10 days prior to capture.

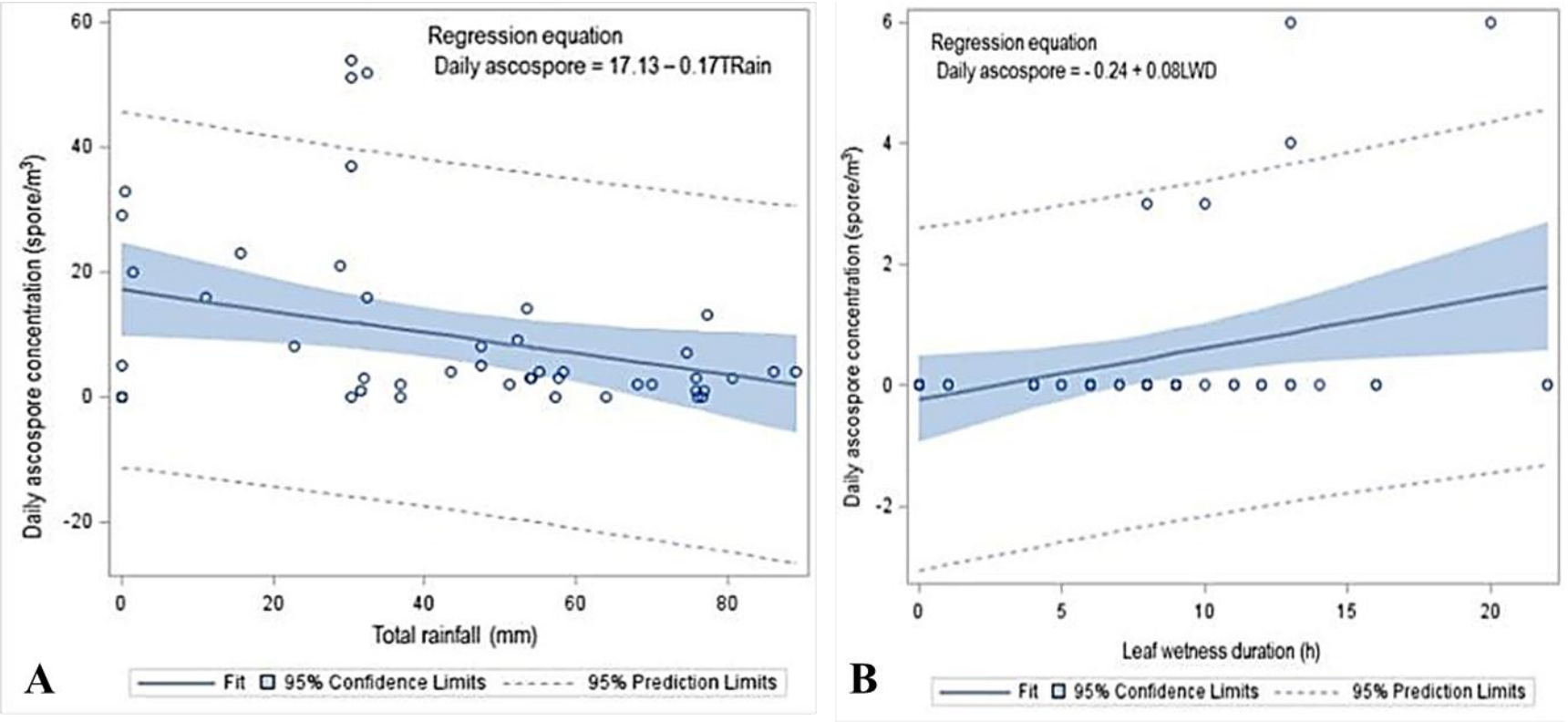

Figure 2.10 Regression (+ 95% confidence interval) of daily ascospore concentration versus (A) rainfall in the 10 days prior to capture in 2015 ($R^2 = 0.11$, $P = 0.02$) and (B) daily leaf wetness duration in 2016 ($R^2 = 0.11$, $P = 0.02$) in the Holland Marsh, ON.

2.3.4 Disease assessment

The small, yellow to light brown lesions (Fig. 2.11A) of SLB were first observed in the plots on 29 June 2015 and 07 July 2016. After 10–18 days, the onion leaves showed signs of leaf dieback and the initial lesions turned dark brown, which indicated the presence of sporulation. In both years, lesions were first observed when plants were at the 4–6 leaf stage. Leaf dieback (Fig. 2.11B) was observed 10–14 days after initial lesions were observed.

The mean SLB incidence scores at the final assessment date were 97% in 2015 and 25% in 2016. In both years, the first observation of SLB coincided with the abundance of conidia and few to no ascospores in the 10 days before the observation of the first SLB incidence (Table 2.7). In 2015, there was a minimum of 5 days with rainfall events in the 10 days before the observation of the first SLB incidence and in 2016, there was 3 rainy days. In both years, the average daily temperatures in the 10 days before the observation of the first SLB incidence exceeded 15 °C. The minimum LWD in the 10 days prior to the observation of first SLB incidence was 5 h in 2015 and 9 h in 2016. The highest VPD in the 10 days prior to the observation of first SLB incidence was 0.6 kPa in 2015 and 0.03 in 2016 (Table 2.7). There was an overall 225% and 285.7% increment in leaf dieback in 2015 and 2016 (Table 2.7). The percentage increase of leaf dieback per day was approximately 9% in 2015 and 12% in 2016.

Figure 2.11 Onion cv. La Salle showing (A) a young lesion, and (B) leaf dieback and sporulation after infection with stemphylium leaf blight at the Holland Marsh, 2015

Table 2.7 Influence of weather variables on accumulated numbers of airborne spores and stemphylium leaf blight levels on onion at the Holland Marsh, Ontario in 2015 and 2016.

Date	Lesions per leaf	Incidence (%)	Leaf dieback (%)	Spores (m^{-3} air)[a]		Weather variables [b]				
				Asco-spores	Conidia	TRain (mm)	NRain (days)	VPD (kPa)	LWD (h)	T (˚C)
2015										
29 June	5	34	0	24	589	75.8	5	0.1–0.6	5–20	15–21
06 July	8	45	0	11	462	31.6	2	0.1–0.6	8–20	15–21
13 July	.	55	0	20	649	28.2	1	0.3–0.6	6–18	18–23
20 July	.	82	20	0	607	6.2	1	0.3–0.7	5–13	16–25
27 July	.	.	32	0	360	1.6	0	0.5–0.9	0–13	19–25
04 August	.	.	41	0	300	24.6	3	0.4–1.0	2–15	19–25
14 August	.	97	65	0	390	23.8	1	0.2–0.5	9–18	16–22
2016										
07 July	3	12	.	0	130	36.2	3	0.3–0.9	9–24	18–15
19 July	5	15	7	0	123	16.8	1	0.5–1.3	0 -15	18–28
25 July	.	17	14	0	93	7.0	1	0.5–1.1	4–22	18 - 27
03 August	.	22	19	0	129	10.0	2	0.4–0.9	0–22	20–25
11 August	.	25	27	0	181	5.4	1	0.7–1.0	5 - 24	21 - 26

[a] Accumulated numbers of spores captured during the 10 days prior to assessment.

[b] Weather data for the 10 days prior to capture: TRain = cumulative total rainfall, NRain = number of day with rainfall ≥ 2 mm,VPD = range of daily vapour pressure deficit, LWD = range of daily leaf wetness duration and T = range of daily average temperature.

2.3.4 Overwintering

All of the onion leaves that had been buried were completely degraded after 4 months of incubation outdoors overwinter (assessed on 29 February) and 81% of leaves left on the surface were totally degraded after 5 months (30 March). Approximately 90% of the onion bulbs buried remained intact, but the bulbs left on the surface of the soil were partially decayed after 5 months. No pseudothecia were found on onion leaves or bulbs after 5 months of incubation.

2.4 Discussion

The current research study represents the first assessment of the seasonal pattern of airborne inoculum for SLB on onion and the relationships among inoculum concentration, SLB incidence and weather. Conidia were trapped throughout the season and conidial concentration increased as the season progressed. In contrast, ascospore concentrations which decreased as the season progressed and temperature exceeded 15 °C. Frequent rainfalls up to 10 days before capture increased the concentration of conidia but decreased the concentration of ascospores. Release of conidia and ascospores exhibited a diurnal pattern with the highest concentrations captured from 0600-1200 h. The first incidence of SLB on onion in the Holland Marsh was observed when the crop was at the 4 - 6 leaf stage. From observation, the first SLB symptoms on onion coincided with abundant conidia, frequent rainfall, warm temperature ($\geq$ 15 °C), minimum LWD of 5 h, and VPD < 0.9 kPa.

The observation of initial lesions of SLB at the end of June in 2015 and early July in 2016 was in agreement with earlier reports in the Holland Marsh (Paibomesai et al.

2012). Regardless of planting method, onions were at about the 4 - 6 leaf stage by the end of June. Availability of susceptible host tissue, release of conidia, and favourable environmental conditions explains the consistent observation of symptoms at this particular time. Initial lesions occurred predominantly on older leaves, which supports previous reports that older onion leaves are more susceptible to SLB than young leaves (Shishkoff and Lorbeer 1989).

Disease incidence increased through the season, likely associated with increased conidial concentration from sporulation on initial lesions (Basallote-Ureba et al. 1999), aging of leaves (Shishkoff and Lorbeer 1989), and prolonged exposure of onion leaves to inoculum and conducive environment for infection (Suheri and Price 2000). SLB incidence was higher in 2015 than in 2016. This can be attributed to the higher conidia concentrations in 2015 and of more days with favourable conditions for SLB infection, such as warm temperatures (≥ 18 °C) and prolonged wet periods (LWD ≥ 8 h) (Basallote-Ureba et al. 1999; Suheri and Price 2000).

Leaf dieback was likely associated with toxins produced by *S. vesicarium* (Singh et al. 2000). The rate of increase in the percentage leaf dieback per day was similar in both years. However, the final mean leaf dieback in 2015 was higher compared to 2016. This may be attributed to the earlier and higher number lesions observed at the start of the epidemic, and differences in the weather between the two seasons. In addition to the abundance of inoculum, a minimum of 57% of the entire onion growing season had favourable conditions (temperature ≥ 15 °C, LWD ≥ 6 h, and VPD ≤ 0.5) for SLB development in 2015 compared to 36% in 2016 (Prados-Ligero et al. 2003).

The difference between spore concentrations in the two years was ascribed to differences in weather conditions. Ascospore release from pseudothecia is triggered by moderate rainfall and cool temperatures (10 - 21 °C) (Prados-Ligero et al. 2003), which were common in late spring of 2015. The spring of 2016 was warmer and drier compared to 2015. This may have resulted in the quicker degeneration of pseudothecia, and hence reduced ascospore concentrations in 2016. Pseudothecia degenerates with temperatures exceeding 15 °C (Prados-Ligero et al. 1998; Llorente et al. 2006). Additionally, the more conducive infection conditions in 2015 resulted in early infection, which led to the production of large numbers of conidia throughout the season.

In addition to the differences in the weather conditions between the two seasons, the difference in ascospore and conidia concentration was attributed to the location of trapping plots. In 2015, the plot was located on the Jane Street research site, bordered to the east by a ditch with bolting onion plants from the previous season and weeds. In 2016, the trapping field was located at the MCRS and was surrounded by weed-free onions (established at the same time) and carrots. Pseudothecia may have overwintered on these onion inflorescence stalks (Rao and Pavgi 1975) and weeds (Misawa and Yasuoka 2012) in 2015, resulting in higher ascospore concentration at the start of the season. Furthermore, initial conidia may have infected the bolting onions, resulting in increased conidial production later in the season.

In previous studies on Welsh onion in Japan, airborne ascospores were detected in April, prior to crop establishment (Misawa and Yasuoka 2012). Also, studies on garlic in southern Spain reported that airborne ascospore concentrations peaked in February and March, before the first incidence of disease (Prados-Ligero et al. 2003). In 2016, when

spore trapping was initiated prior to planting, a few ascospores and conidia were trapped. However, ascospore concentrations peaked before the first observation of SLB in the Holland Marsh in both years.

On other hosts and in other locations, pseudothecia form on crop debris at the end of the season and release ascospores in spring (Falloon 1987; Basallote-Ureba et al. 1999; Misawa and Yasuoka 2012). The pseudothecia often take between 1–4 months to mature (Prados-Ligero et al. 1998). In contrast, no pseudothecia were observed on diseased onion tissues after 5 months outdoors at the Holland Marsh. Over 80% of all onion leaves were completely or partially degraded at 5 months after harvest. These results indicated that onion leaves may not be suitable materials for pseudothecia attachment and development in this region. These results support a previous report that *S. vesicarium* rarely overwinters on onion leaves (Rao and Pavgi 1975). In contrast. pseudothecia were occasionally observed on the stalks of onion inflorescences in India (Rao and Pavgi 1975) and were common on dried asparagus spears (Falloon 1987).

The presence of ascospores in early spring at the Holland Marsh demonstrates that the pathogen overwinters close to this site. In onion crops in the Holland Marsh, the pathogen may be overwintering on alternative crops such as carrot or on weeds. Earlier reports noted overwintering of *S. vesicarium* on both diseased and symptomless leaves of Welsh onion and other crops (Misawa and Yasuoka 2012).

The infection potential of ascospores on onion was not investigated in this study. However, the presence of few or no ascospores when SLB was first observed and the ten day prior to this observation indicates that ascospores are likely not the primary inoculum for SLB on onion at the Holland Marsh. This conflicts with earlier reports that suggested

that ascospores are the primary inoculum for SLB on Allium crops (Basallote-Ureba et al. 1999). Ascospore can infect onion and garlic tissues under controlled environmental conditions (Prados-Ligero et al. 1998), but their role under field conditions is still not understood.

In both years, airborne conidia were present before the first symptoms of SLB were observed. The abundance of conidia prior to the observation of first SLB incidence was similar to previous observations in pear (Rossi et al. 2008), garlic (Prados-Ligero et al. 2003), leek (Suheri and Price 2001) and Welsh onion (Misawa and Yasuoka 2012). The concentration of conidia peaked in July in both years. The high concentration of conidia in July likely resulted from sporulation on initial lesions. The abundance of conidia during SLB development on onion indicated that conidia were the most important inoculum type on onion at the Holland Marsh.

Symptoms of *S. vesicarium* infection on Allium crops become visible 7 - 14 days after inoculation (Shishkoff and Lorbeer 1989; Basallote-Ureba et al. 1999; Misawa and Yasuoka 2012). However, there was a time lag (more than 30 days) between when the first conidium was trapped and the first SLB symptom was observed on onion crops in the Holland Marsh. These initial conidia may be causing symptomless infections on onion or other crops and weeds growing around onion fields. A similar time lag has been reported in Welsh onion, with latent development of *S. vesicarium* on several crops (Misawa and Yasuoka 2012). Also, DNA of *S. vesicarium* has been reported from symptomless pear leaves and grasses (Köhl et al. 2009). Latent development of *S. vesicarium* was not investigated in the current research project.

Ascospore concentration declined with prolonged, heavy rainfall events. On leek (Suheri and Price 2001) and garlic (Prados-Ligero et al. 2003), ascospore concentration increased 24–36 h after short periods of rainfall. However, large volumes of rainfall in the Holland Marsh earlier in 2015 may have flooded pseudothecia and inhibited the release of ascospores. In the absence of persistent rain events in 2016, prolonged LWD with saturated air (VPD $\leq$ 0.5 kPa) may have influenced airborne ascospore concentration. These results support the importance of precipitation on daily ascospore concentration (Prados-Ligero et al. 2003).

Rainfall in the 10 days prior to spore capture increased the daily number of conidia captured. Also, the concentration of conidia increased substantially 2 - 72 h after rainfall. The influence of rainfall on conidial concentration had been reported previously (Suheri and Price 2001; Prados-Ligero et al. 2003; Granke and Hausbeck 2010). Few to no conidia were captured during rain events. This can be attributed to rains washing conidia from infected leaves, and also to the formation of a water layer on leaf surfaces that prevented the dispersal of conidia (Meredith 1966; van der Werff 1967).

Daily conidia concentrations were positively correlated with the number of days with temperatures exceeding 15 °C. A similar relationship has been reported in garlic and leek, where the highest concentrations of conidia coincided with temperature ranging from 15–32 °C in the days preceding capture (Suheri and Price 2001; Prados-Ligero et al. 2003). However, ascospore concentration declined as temperatures increased. These results indicated that the increase in temperature that generally occurs from late spring into summer influenced the type of airborne spore captured and the spore concentration in the Holland Marsh.

Approximately 60% of conidia and 50–70% of ascospores were captured between 0600–1200 h at the Holland Marsh. The lowest concentrations of spores were captured after dark. This diurnal pattern of ascospore and conidial capture was similar to that reported on garlic in southern Spain (Prados-Ligero et al. 2003), on leek in Australia (Suheri and Price 2001) and on asparagus in Michigan (Granke and Hausbeck 2010).

Alternaria species, which are closely related to *Stemphylium* species (Simmons 1967), exhibit a similar pattern of diurnal periodicity (Meredith 1966; Strandberg 1977). Sporulation is favoured by high relative humidity and dew, conditions that are frequently present during the night (Cohen and Rotem 1987). However, very few spores are released at night due to the generally low wind speed and the surface-tension effect of dew (Meredith 1966). It has been postulated that the rapid reduction in relative humidity and evaporation of dew that generally occurs in the morning causes hygroscopic movements that weaken the points of spore attachment. As temperature and wind speed increase, mature spores are released (Meredith 1966). This explains the large number of airborne spores captured in the early hours of the day within the Holland Marsh.

In conclusion, SLB developed on onion at the Holland Marsh when abundant conidia coincided with warm temperatures (≥ 15 °C) and moderately wet conditions. The first symptoms were observed on onion at the 4–6 leaf stage. Small tan to light brown, oval to oblong lesions were observed first on the oldest leaves. As the disease progressed, the older leaves senesced and served as sources of secondary inoculum for subsequent infection.

CHAPTER THREE

SUSCEPTIBILTY OF ONION CULTIVARS TO ISOLATES OF STEMPHYLIUM VESICARIUM

3.1 Introduction

Stemphylium vesicarium, cause of stemphylium leaf blight (SLB), is a widely distributed fungal pathogen of Allium crops (Basallote-Ureba et al. 1999; Suheri and Price 2001; Misawa and Yasuoka 2012), asparagus (Falloon 1987) and pear (Montesinos and Vilardell 1992). SLB can cause up to 90% yield loss on onion (Tomaz and Lima 1988; Hassan et al. 2007), and is one of the most destructive diseases on onion crops in Ontario. On Allium crops, conidia are the primary inoculum, but the role of ascospores is unclear (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012).

Infection of onion leaves by *S. vesicarium* occurs mainly through stomatal openings and wounds. Infection is favoured by temperatures between 18–25 °C and leaf wetness lasting 6 h or more (Montesinos et al. 1995a; Prados-Ligero et al. 1998). Shortly after infection, the pathogen initiates production of toxins that cause ultra-structural changes in the cells of susceptible hosts. These changes weaken host tissues, resulting in extensive necrosis (Singh et al. 1999, 2000).

Latent (asymptomatic) infection by *S. vesicarium* can develop in susceptible host tissues and in several alternative hosts (Köhl et al. 2009b; Misawa and Yasuoka 2012). On Allium crops, visual symptoms of infections are usually observed 6–14 days after inoculation (Shishkoff and Lorbeer 1989; Prados-Ligero et al. 1998; Suheri and Price 2000; Misawa and Yasuoka 2012). The initial symptoms on onion leaves consist of small, yellow to tan, water-soaked lesions that turn dark brown as the pathogen begins to

sporulate (Basallote et al. 1993). As the disease progresses, infected leaves undergo rapid necrosis from the tip down. This leads to desiccation of leaves and early dying of the crop (Rao and Pavgi 1975).

Host resistance can provide effective management of *S. vesicarium.* For example, there are resistant lines of garlic (Mishra et al. 2009) and Welsh onion (Pathak et al. 2001). All common onion lines screened in a previous study were susceptible to SLB (Pathak et al. 2001). However, observations in onion cultivar trials at the Muck Crop Research Station (MCRS), Holland Marsh, Ontario indicated that there may be differences in the susceptibility of onion cultivars to SLB (McDonald and Vander Kooi 2014a). In pear, cultivars differed in susceptibility based on their reaction to toxins produced by the pathogen (Singh et al. 2000; Wolpert et al. 2002). Identifying cultivars with lower susceptibility to SLB would be useful for managing SLB.

In some cases, the toxins produced by *S. vesicarium* are highly specific. For example, toxins from isolates collected on European pear were not pathogenic on Japanese pear cultivars, onion or asparagus (Singh et al. 1999, 2000). In contrast, isolates from parsley were pathogenic on carrot, celery and wounded pear fruit (Koike et al. 2013) and isolates from onion, garlic and asparagus in Spain produced disease on all three hosts (Basallote-Ureba et al. 1999). The host specificity of *S. vesicarium* on onion in Canada is not known.

The objectives of this study were to confirm the pathogenicity of isolates of *S. vesicarium* from onion and asparagus on onion, and to assess the susceptibility of commercial onion cultivars to selected isolates of *S. vesicarium*.

3.2 Materials and Methods

3.2.1 Plant material and experimental design

Overall, 13 commonly grown commercial onion cultivars (Table 3.1), selected to represent a range of maturity, were assessed in each trial.

Table 3.1 Onion cultivars screened for disease reaction to Stemphylium vesicarium in growth room studies and field trials at the Holland Marsh, ON.

Cultivar Name	† Days to Maturity	Source and location
Highlander	92	American Takii, CA, USA
Hendrix	101	Bayer seeds, BC, Canada
Hamlet	101	Stokes seeds, ON, Canada
Madras	103	Bejo seeds, CA, USA
Genesis	103	Crookham seeds, ID, USA
Trailblazer	106	America Takii, CA, USA
La Salle	106	Stokes seeds, ON, Canada
Patterson	107	Bejo Seeds, CA, USA
Milestone	110	American Takii, CA, USA
Braddock	111	Bejo seeds, CA, USA
Stanley	111	Solar seeds, ON, Canada
Pontiac	111	Crookham seeds, ID, USA
Prince	115	Bejo seeds, CA, USA

† Days from seeding until maturity (85% of tops down) when grown on muck soil (McDonald et al. 2015).

Controlled environment experiments were carried out in growth rooms in the Plant Agriculture Department, University of Guelph, Guelph, ON. Onion seedlings were started in plug trays filled with soil-less mix (Growers-mix, ABS Greenworld Inc., Mount

Elgin, ON). Seedlings of each of the cultivars emerged 10-14 days after planting (DAP). The leaves of the emerged onions were trimmed at 30 DAP, following commercial practice, to stimulate development of additional roots and a firmer pseudostem for ease of transplanting. The seedlings were transplanted (two plants per plug) at 44 DAP into 1.5-L pots filled with soil-less mix (Sunshine ProMix BX, Sun Gro Horticulture Canada Ltd, Agawam, MA.). Each experiment assessed the interaction of 12 onion cultivars and five isolates of *S. vesicarium* (Table 3.2), plus mock-inoculated onion plants as checks. Three isolates were from onion in Ontario, one from onion in Nova Scotia and one from asparagus in Ontario. Each experimental unit consisted of two onion plants, arranged in a randomized complete block factorial design with four blocks.

Table 3.2 Source of the isolates of *Stemphylium vesicarium* screened for pathogenicity and aggressiveness on onion under controlled conditions.

Name	Year	Collection location	Crop	Collector
OO55	2014	MCRS, Holland Marsh, ON	Onion	S. Tayviah
OO54	2014	Simcoe Research Station, Simcoe, ON	Onion	S. Tayviah
OO27	2013	Marshland Gardens, Holland Marsh, ON	Onion	J.M. Foster
NO36	2013	AAFC, NS	Onion	P. Hildebrand
OA46	2013	Millennium Seed Field, Dundas, ON	Asparagus	J.M. Foster

The isolates of *S. vesicarium* from Ontario were collected from naturally infected onion leaves and asparagus spears. Infected tissues were cut into small pieces and thoroughly washed with tap water, surface sterilized with 75% ethanol for 2 min and 2% sodium hypochlorite for 2 min, rinsed several times with sterile deionized water and dried between layers of sterile filter paper. The samples were placed onto potato dextrose agar

(PDA) (Difco, Becton Dickinson, and Co., Sparks, MO) and incubated on a laboratory bench at room temperature (20–23 °C) and ambient light intensity.

The resulting fungal colonies (Fig. 3.1) were purified using hyphal tip isolation. Identification of isolates of *S. vesicarium* was based on the morphological characteristics of conidia and conidiophores (Simmons 1969) and were confirmed by PCR and sequencing, as described below.

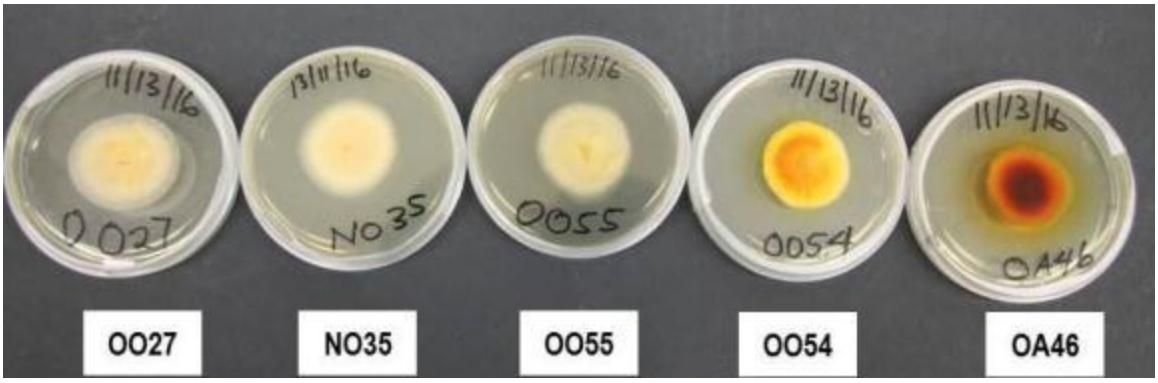

Figure 3.1 Cultures of the five isolates of *Stemphylium vesicarium* screened for pathogenicity and aggressiveness on onion in a growth room study.

Actively growing mycelia of the isolates were transferred onto V8-agar medium, consisting of 200 mL of V8 juice (Campbell Soup Co., Etobicoke, ON), 3 g calcium carbonate, 20 g agar, and 800 mL deionized water. After 4 to 6 days, when the colony reached about 5 cm in diameter, the mycelium was matted down by placing 10 drops of sterile water on the mycelia and flattening the aerial mycelium with a sterile glass rod. The treated colonies were incubated under UV light (Westinghouse Lamps, McNulty,

PA) for 12 hr at room temperature to stimulate production of conidiophores, followed by 12 hr darkness to induce conidia production.

Field experiments were conducted in 2015 and 2016. All field experiments were arranged as a randomized complete block design with four replicates. Each experimental unit (plot) consisted of a 1.6 × 5.0 m bed with four rows of onion and 0.4 m between rows.

In 2015, onion seedlings were started in plug trays with soil-less mix (Growers-mix, ABS Greenworld Inc., Canada) on 30 March. Seedlings emerged within 10-14 days DAP. The seedling leaves were trimmed at 25 DAP and 45 DAP. The seedlings (2-3 seedlings per transplant plug) were transplanted manually into organic soil (organic matter ≈ 62%, pH ≈ 7.2) at the Jane Street research site of the MCRS on 25 May (57 DAP). In 2016, the onion crop was direct seeded at about 35 seeds m^{-1} into organic soil (organic matter ≈ 71%, pH ≈ 5.7) on 4 May using a custom-built double-row precision seeder. The seeds emerged 10–14 DAP. Seed of cv. Madras was not available in 2016, so it was replaced with cv. Braddock.

In 2015, the herbicide flumioxazin (Chateau®, Valent Corporation, Guelph, ON.) was applied, following label recommendation, to control weeds at the 4-6 leaf stage of the onion crop, 45 days after transplanting (DAT). In 2016, bromoxynil (Pardner®, Bayer Crop Science Inc., Mississauga, ON.) was applied at 2-3 leaf stage at 39 DAP and repeated at the 5–6 leaf stage at 56 DAP to control weeds. Subsequent weed control was done by hand until maturity. In 2015, chlorpyrifos insecticide (Pyrinex™, Adama Agricultural Solutions Canada Ltd., Winnipeg, MB.) was applied at 45 days after seeding

to manage onion maggot as recommended by the IPM program. In 2016, there was no need to spray again onion maggot as per the IPM recommendations.

3.2.2 Molecular confirmation

The isolates of *S. vesicarium* were cultured on PDA for 3 days at room temperature and light intensity. Then a piece of the actively growing mycelium tip was transferred into a sterile flask containing 500 mL potato dextrose broth media (Difco, Becton Dickinson, and Co., Sparks, MO). After 24 h, the inoculated flask was transferred unto an orbital shaker (ThermoFisher Inc., Marietta, OH) at 90 rpm. After 7 days of continuous shaking, the mycelial mats formed were collected by filtering through sterile cheesecloth and transferred to sterile plastic tubes. The mycelial mats were flash frozen with liquid nitrogen and stored at -83 °C. DNA was extracted from the mycelia of each isolate using the PowerSoil® DNA isolation kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's protocol. DNA samples were stored at -20 °C.

The specific primers f5'-ATATCAAAGCTAACCGCGTCTCAC-3' and r5' GCAGAGATGACAACCTTCTTGG-3' were designed to amplify a region of the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene of *S. vesicarium*. The PCR reaction was performed in a total volume of 15 µL containing 1x PCR buffer (50 mM Tris-HCl, pH 8.5); 2.5 mM MgSO4; 0.2 mM dNTP; 0.5 µM of each primer separately; 0.6 U Tag DNA polymerase (Biobasic, Scarborough, ON); and 1 µL DNA template. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad Laboratories Inc., Mississauga, ON.). The PCR program consisted of an initial denaturation at 95 °C for 2

min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 58 °C for 40 sec and extension at 72 °C for 40 sec. The final extension was at 72 °C for 4 min.

Amplicons were resolved by horizontal gel electrophoresis in 1.5% agarose gels in 0.5 × Tris-borate-EDTA (TBE) buffers at 100 V cm^{-1} for 45 min. Gels were pre-stained with ethidium bromide (0.5 μg mL^{-1}), and digitally visualized and photographed on a Gel Doc-lt™ Imaging System (UVP-LLC, Upland, ON). DNA from a confirmed isolate of *S. vesicarium* was run as the positive control and DNA-free sterile water and DNA of *Alternaria porri* was used as negative controls. Pure cultures of the isolates were also submitted to the University of Guelph Agriculture and Food laboratory for sequencing.

3.2.3 Pathogenicity test and inoculation

The isolates were induced to sporulate on V8-agar media, as described above. The conidia were collected by flooding the colony with 10 mL sterile water and gently dislodging the conidia by scraping the colony surface with a sterile microscope slide. The suspension was filtered through layers of sterile cheesecloth to remove mycelial fragments. The concentration of conidia was determined using a hemocytometer and adjusted to ≈ 2×10^6 conidia mL^{-1}. A drop of the non-ionic surfactant Tween 20 (J.T Baker Inc., Philipsburg, NJ) was added to every 10 ml of conidial suspension to disperse the conidia within the suspension.

The leaves of onion plants at the 4-leaf stage (74 DAP) were rubbed gently with sterile cheesecloth to remove wax and create tiny wounds. The onion plants were inoculated with the conidial suspension by spraying to run-off with an Optimus® hand

atomizer (Home Depot, Guelph, ON.). Control plants were rubbed gently and sprayed with sterile water plus Tween 20 solution. After inoculation, the plants were placed in moist chambers for 72 h at high humidity (≥ 96%) to promote conidial germination and infection. The plants were misted with water every 12 h to increase the humidity and maintain leaf wetness. The temperature and relative humidity were recorded using an Enviro-Meter™ (Fisher Scientific, Austin, TX). The plants were then moved to a growth room at 60-70% RH, 18-23 °C and 16-hr photoperiod for disease development and assessment. After inoculation, the onion plants were irrigated from the bottom to avoid splashing of inoculum.

3.2.4 SLB assessment and yield

After inoculation, the onion plants in the growth room study were observed daily for SLB lesions. Lesions were first observed and assessed at 11 days post-inoculation (DPI) for the first repetition of the study and at 14 DPI for the second repetition. Lesions were assessed on the second and third fully developed leaves on each plant, and the mean number of lesions on the two plants of each experimental unit was calculated. Leaf dieback on these same two leaves per plant was calculated from measurement of the total length of the leaf and the length of the dieback. Leaf dieback was first observed and the assessment was initiated 10 days after initial lesions were recorded, and repeated every 7 days: at 21, 28, 35 and 42 DPI for the first repetition of the study and 24, 31, 38 and 45 DPI the second repetition.

In the field trials, each plot was rated weekly for *S. vesicarium* lesions. In 2015, the number of lesions was assessed on one transplant plug at each of eight sites at roughly

1-m intervals along the two middle rows, starting 1 m from the end of each row, as described in Chapter 2. The number of lesions on the first and second fully developed outer leaves of the two largest seedlings per plug was assessed. In total, 32 leaves per replicate plot were assessed and the average number of lesions per leaf was calculated. In 2016, lesions were counted on 10 consecutive plants in each of the two middle rows (total = 40 leaves) as described previously.

SLB incidence in 2015 was assessed by counting the total number of transplant plugs per plot and the number with blight symptoms. In 2016, 100 plants in the middle two rows of each plot were assessed.

Leaf dieback was assessed using a 60-cm clear plastic ruler as described previously. A method similar to the one used for lesion counts was used to identify plants to assess in both years. It is important to note, however, that the plants selected for leaf dieback assessments were not the same plants used for lesion counts. Leaf dieback was assessed on the fifth and sixth fully developed true leaves of each selected plant, and the mean percentage leaf dieback was calculated for each plot.

In field trials, the initial SLB lesions per leaf were assessed on 29 June in 2015 and 7 July in 2016. In 2015, leaf dieback was observed 21 days after the observation of initial symptoms. Leaf dieback for each cultivar was assessed on 20 July, 27 July, 04 August and 14 August. In 2016, leaf dieback was observed 12 days after lesions were first observed and assessed on 19 July, 25 July, 3 August and 11 August.

The onion plants in two 2.3-m-long sections of the middle two rows of each plot were pulled up by hand at harvest on 10 September, 2015 and 6 September 2016. They

were placed in windrows to cure for 2 weeks prior to yield assessment, then topped and stored at 20–23 °C until yield assessment.

Yield was assessed on 21 October 2015 and 22 September 2016. Onion bulbs were graded and weighed according to diameter: as jumbo (> 76 mm), medium (45–76 mm) and small (< 45 mm). Jumbo and medium onions were grouped as marketable yield. Small, damaged or sprouting bulbs were culled. The percentage of bulb size distribution and weight, the percentage marketable yield and the marketable yield (t ha^{-1}) were calculated. In 2016, disease and yield were not assessed on Pontiac due to poor seedling establishment.

3.2.5 Data analysis

All statistical analyses were conducted using SAS v.9.4 (SAS Institute Inc., Cary, NC). Mixed model analysis of variance was used to assess the disease and yield data (PROC GLM and PROC Mixed). No outliers were identified using Lund's test. The normality of each data set was assessed using PROC UNIVARIANT. A logarithm transformation was applied to the growth room data to improve the fit to a normal distribution, and back-transformed data are presented. Growth room data were pooled across repetitions when analysis showed no repetition x treatment interaction. The variance of the growth room data was partitioned into random effects (block and repetition) and fixed effects (cultivar, inoculum and cultivar × inoculum). Pearson's rank correlation in PROC CORR at $P < 0.05$ was used for correlations between the mean number of lesions per leaf and the mean percentage leaf dieback for the growth room study. Field data were analysed separately for the individual years. Field data were not

pooled across years as the analysis showed a repetition (year) x treatment interaction. Variance in the field trial data was partitioned into random effects (block) and fixed effects (cultivar). Means were separated using Tukey's multiple range test ($\alpha = 0.05$). Spearman's rank correlation in PROC CORR at $P < 0.05$ was used for field correlations. A nonparametric test was chosen because the field data were not normally distributed.

3.3 Results

3.3.1 Identification, sporulation and pathogenicity

All five isolates were identified as *S. vesicarium*, based on conidial morphology (Fig. 3.2). The conidia were oblong in shape, and ranged from 19-23 × 22-46 µm. The conidia of isolates OO27 and OA46 were slightly larger than the other isolates, based on visual observation but not specific measurement. Isolates OO46 sporulated on V8 media even without UV light stimulation. The mean number of spores collected per colony (Petri dish) was 1×10^4 conidia mL^{-1} for each isolate, except NO35, where spore recovery was only 1×10^2 conidia mL^{-1}. For inoculation, the spore suspension for each isolate was concentrated to $\approx 2 \times 10^6$ by washing off spores from more colonies.

Molecular analysis and DNA sequencing confirmed that each isolate was *S. vesicarium.* Each isolate reacted with species-specific primers in PCR (Fig. 3.3), and the similarity of each isolate was greater than 99.6% to a gene accession previously identified as *S. vesicarium* (Submission number: 16-091062) based on 16s rRNA sequences (BLAST, Genbank. National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast).

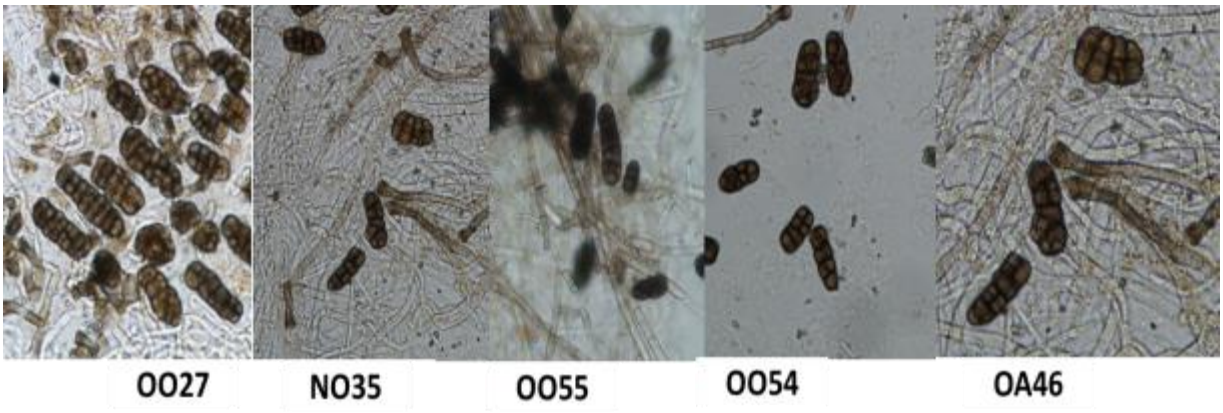

Figure 3.2 Conidia of the five *Stemphylium vesicarium* isolates assessed in a growth room study of pathogenicity and aggressiveness on onion.

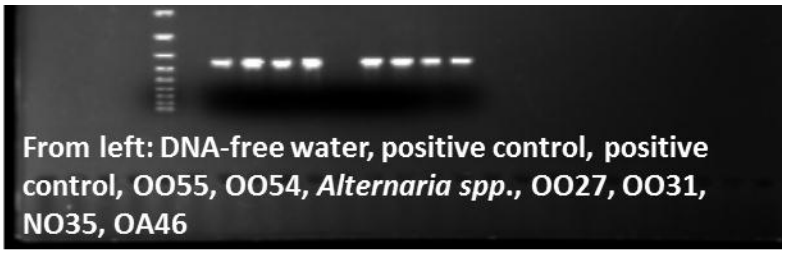

Figure 3.3 Image of gel of the reaction of PCR products with a DNA probe from different isolates of *Stemphylium vesicarium* (OO55, OO54, OO27, OO31, NO35 and OA45).

Each isolate produced lesions when inoculated onto onion plants and *S. vesicarium* was re-isolated from lesions caused by each isolate. The lesions caused by isolate OA46 were larger than those produced by the other isolates. No sporulation was observed on inoculated plants and there were no lesions on mock inoculated plants.

3.3.2 SLB assessment

In the controlled environment studies, the first lesions of *S. vesicarium* were observed on onion leaves at 9–14 DPI. There were differences among isolates in relation to the number of lesions per leaf. The lowest number of lesions per leaf was recorded on plants inoculated with isolates NO35 and OO54, and the highest numbers developed on plants inoculated with isolates OO27 and OA46 (Table 3.3). The mock-inoculated plants did not develop SLB symptoms in either repetition.

There were also differences among isolates in relation to leaf dieback. The lowest dieback was recorded on plants inoculated with isolates NO35 and OA46, and the highest with isolate OO27 (Table 3.3).

Table 3.3 Lesions per leaf and leaf dieback (%) assessed on onion inoculated with five isolates of Stemphylium vesicarium under controlled conditions.

Isolate	Lesion per leaf [1]	Leaf dieback (%)[1]
NO35	5 d [2]	20 c
OA46	13 ab	21 c
OO54	7 cd	28 b
OO55	9 bc	30 b
OO27	14 a	52 a

[1] Data were log transformed for analysis, and back-transformed for presentation.

[2] Means in a column followed by the same letter do not differ using Tukey's multiple range test at $P = 05$, based on pooled data from two repetitions.

There were no differences in the number of lesion per leaf assessed on all the onion cultivars assessed except of the number of lesion per leaf assessed on cv. Pontiac

compared to cv. Patterson (Table 3.4). Onion cv. Pontiac developed, numerically, the lowest number of lesions per leaf. However, this was not significantly different from the other cultivars except for Patterson, which numerically had the highest number. There were also differences in leaf dieback on the cultivars; Pontiac had the lowest dieback and Highlander had the highest (Table 3.4).

SLB incidence in the field trials was higher in 2015 compared to 2016 (Fig. 3.4). In 2015, Pontiac had the numerically lowest incidence (74%), but this was only significantly lower than Milestone (96%), La Salle (95%), and Trailblazer (93%) (Fig. 3.4). In 2016, Braddock replaced Madras because the seed of Madras was not available, and Pontiac was not assessed due to poor germination. In 2016, Hendrix (20%), Patterson (24%) and La Salle (25%) had the lowest incidence (numerical) but this was only significantly lower than Milestone (39%) (Fig. 3.4).

In the field trials, the number of lesions per leaf in 2015 was slightly higher compared to 2016 (Fig. 3.5). There were differences in the initial number of lesions per leaf for the cultivars between the two years. In 2015, Pontiac had the lowest number of lesions per leaf but this was only different from LaSalle and Milestone. In 2016, Hendrix, Highlander and Prince had the lowest number of lesions per leaf, lower than Milestone and Trailblazer (Fig. 3.5).

Table 3.4 Lesions per leaf and leaf dieback (%) assessed on onion cultivars inoculated with *Stemphylium vesicarium* under controlled conditions.

Cultivar	Lesion per leaf [1]	Leaf die back (%)[1]
Pontiac	7 b [2]	16 e
Hendrix	9 ab	23 d
Milestone	10 ab	23 d
LaSalle	8 ab	26 cd
Madras	10 ab	29 bcd
Stanley	7 ab	29 bcd
Genesis	9 ab	31 abcd
Prince	9 ab	31 abcd
Trailblazer	9 ab	32 abcd
Patterson	11 a	33 abc
Hamlet	8 ab	37 ab
Highlander	8 ab	40 a

[1] Data were log transformed for analysis and back-transformed for presentation.

[2] Means in a column followed by the same letter do not differ using Tukey's multiple range test at $P > 05$, based on pooled data from two repetitions.

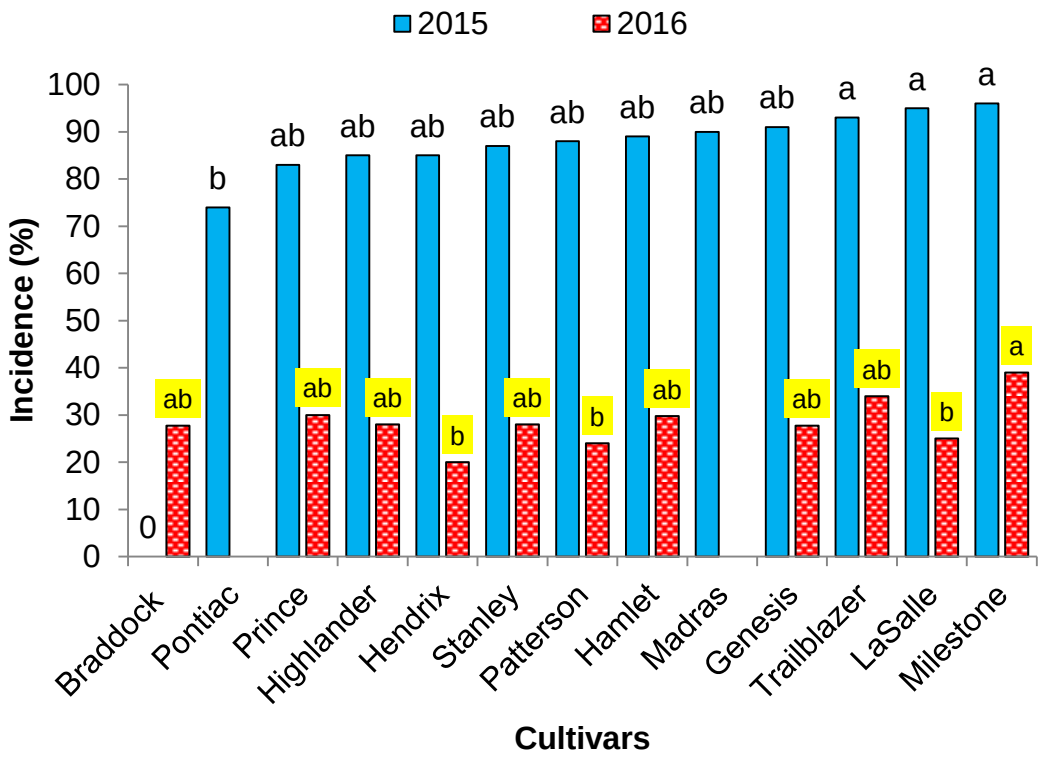

Figure 3.4 Mean incidence (final assessment) of stemphylium leaf blight on onion cultivars at the Holland Marsh, ON in 2015 and 2016.

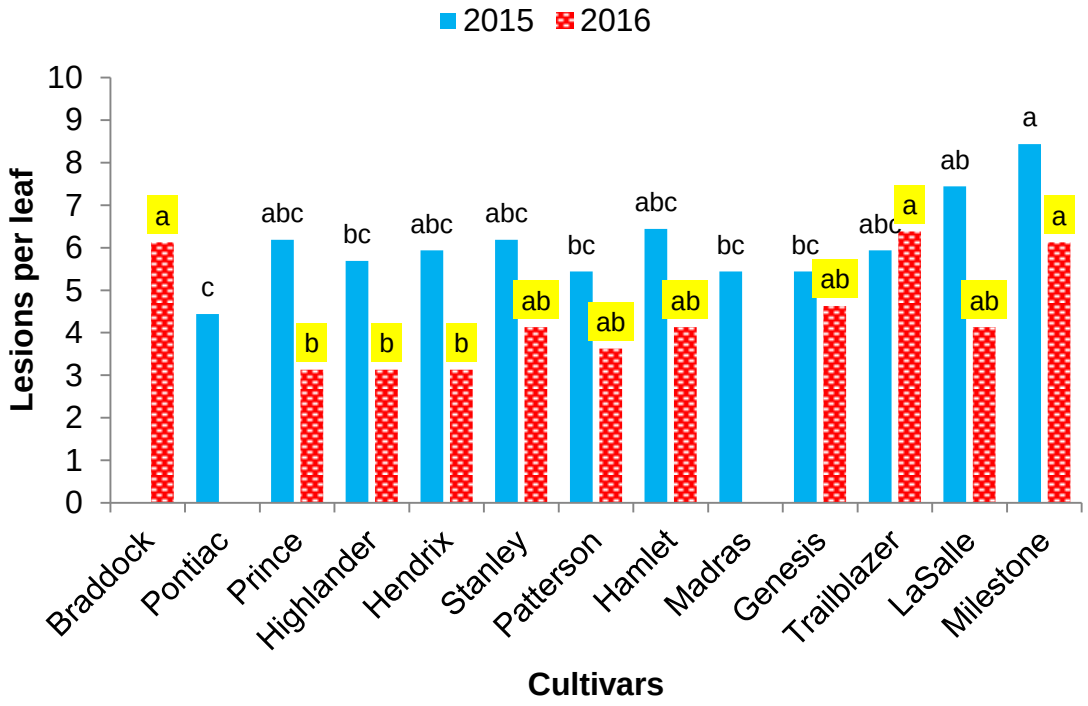

Figure 3.5 Lesions per leaf on onion cultivars screened for susceptibility to stemphylium leaf blight at the MCRS, Holland Marsh, ON in 2015 and 2016.

Leaf dieback was also higher in 2015 compared to 2016. In 2015, Highlander (83%) had the highest percentage leaf dieback of all the cultivars except LaSalle (65%). In 2015, Pontiac (54%) had the numerically lowest leaf dieback. In 2016, Highlander (32%) again had numerically the highest leaf dieback in comparison to the all cultivars but this was not significantly different except for Hendrix (17%), which had the numerically lowest leaf dieback (Fig. 3.6).

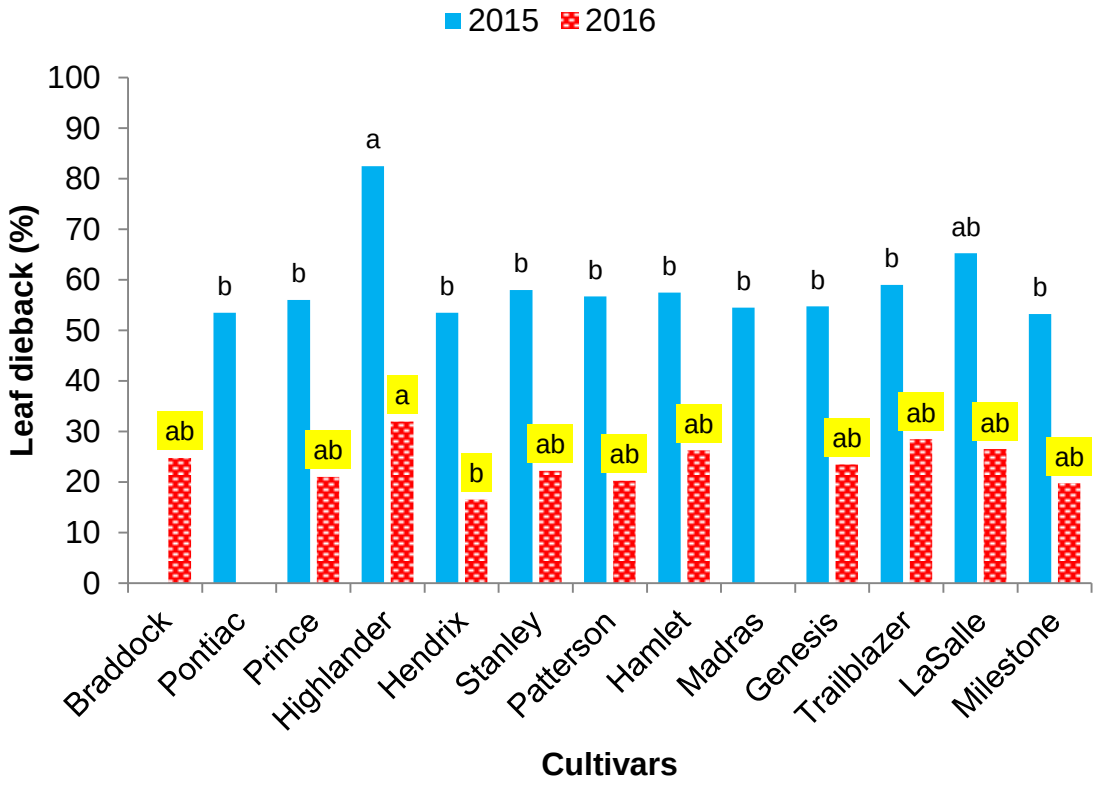

Figure 3.6 Leaf dieback on onion cultivars caused by stemphylium leaf blight at the MCRS, Holland Marsh, ON in 2015 and 2016.

In both growth room and field trials, the mean number of SLB lesions was not correlated with the overall leaf dieback per cultivar. Also, there was no correlation between leaf dieback and the days to maturity for each cultivar.

Similarly, there was no correlation between the mean number of lesion per leaf assessed in the growth room studies and in the field trials. However, there was a strong correlation between the mean percentage leaf dieback assessed in the growth room and in the field in 2015 ($r = 0.68$, $P = 0.02$). In both the growth room and field studies, Milestone showed high susceptibility to initial SLB lesions and Highlander showed high susceptibility to leaf dieback.

In 2015, there was spray-drift injury caused by flumioxazin herbicide at the 4–6 leaf stage (Fig. 3.7).

Figure 3.7 (A) Herbicide caused by spray-drift of flumioxazin herbicide and (B) the initial infection and sporulation of *Stemphylium vesicarium* on herbicide injured onion leaves in the Holland Marsh, ON, 2015.

3.3.3 Yield

There were no differences in marketable yield in either year, but yield in 2015 was higher compared to 2016. In 2015, cv. Patterson had the numerically highest marketable yield and Genesis had the lowest yield. There were no differences in the percentage of jumbo bulbs. Highlander had the numerically highest number of medium bulbs but this was not significantly different from other cultivars except for Hendrix,

which had the numerically lowest number of medium bulbs. Hendrix had a numerically higher percentage of culls compared to all other cultivars, but this was not different from other cultivars except for Patterson, Prince, Highlander and Pontiac (Table 3.5).

In 2016, cv. Braddock had the numerically lowest marketable yield and Milestone had the highest yield. Patterson, Prince, Trailblazer, Hamlet and Highlander had no jumbo-size bulbs. Hendrix had the numerically highest percentage of jumbo bulbs in 2016 but this was not different from Milestone, Genesis, La Salle, Stanley and Braddock. La Salle had the numerically highest percentage of medium bulbs and Braddock had the lowest but these were not significantly different. Patterson had the numerically highest percentage of culls but was not significantly different from the other cultivars except Hendrix (Table 3.6). There was no correlation between leaf dieback and yield in either year (Tables 3.5 and 3.6).

The average number of plants with 2.3 m row was about 46 plants in 2015 and 62 plants in 2016. However, the average number of bulbs harvested was about 43 bulbs per 2.3 m row in 2015 and 32 bulbs in 2016.

Table 3.5 Marketable yield and bulb size distribution of onion cultivars evaluated for stemphylium leaf blight at the MCRS, Holland Marsh, ON, 2015

Cultivar	Marketable yield (t $ha^{-1)}$	Bulb size distribution (%)		
		Jumbo (>76 mm)	Medium (45-76 mm)	Culls (<45 mm)
Genesis	46 ns	7 ns	79 ab	15 ab
LaSalle	53	6	79 ab	15 ab
Trailblazer	53	5	84 ab	11 ab
Hendrix	55	5	70 b	26 a
Pontiac	55	8	85 ab	7 b
Madras	56	5	86 ab	8 ab
Milestone	57	10	86 ab	4 b
Stanley	58	11	82 ab	7 b
Hamlet	60	4	88 ab	9 ab
Highlander	63	3	91 a	6 b
Prince	63	6	88 ab	6 b
Patterson	67	9	83 ab	7 b

Numbers in a column followed by the same letter do not differ based on Tukey's multiple range test at $P > 05$, ns = not significant.

Table 3.6 Marketable yield and bulb size distribution of onion cultivars evaluated for stemphylium leaf blight at the MCRS, Holland Marsh, ON, 2016

Cultivar	Marketable yield (tha^{-1})	Bulb size distribution (%)		
		Jumbo (>76 mm)	Medium (45-76 mm)	Culls (<45 mm)
Braddock	27 ns	4 ab	68 ns	28 ab
Patterson	28	0 b	71	29 a
Stanley	29	2 ab	74	24 ab
Prince	30	0 b	78	22 ab
Trailblazer	32	0 b	80	20 ab
Hamlet	34	0 b	78	22 ab
LaSalle	35	4 ab	81	15 ab
Highlander	36	0 b	79	21 ab
Hendrix	38	14 a	74	12 b
Genesis	41	7 ab	75	18 ab
Milestone	46	10 ab	73	17 ab

Numbers in a column followed by the same letter do not differ based on Tukey's multiple range test at $P > 05$, ns = not significant

4.4 Discussion

Previous surveys in the Holland Marsh first identified the presence of SLB on onion in 2008. All of the commercial onion cultivars assessed were susceptible to SLB, but there were differences in the degree of susceptibility. Studies of five isolates of *S. vesicarium* also identified variation in the aggressiveness of the isolates. Variation in the aggressiveness of isolates has been attributed to differences in the toxins they produce (Singh et al. 2000). Therefore, the limited variation in susceptibility of onion cultivars to

SLB observed in the field trials is likely related to differences in reaction to the toxins produced by the pathogen population at the Holland Marsh.

Four isolates from SLB lesions on onion and an isolate from asparagus in Ontario were identified as *S. vesicarium* based on the morphology of conidia and conidiophores (Simmons 1969, 1985). This identification was confirmed based on molecular similarity assessed using BLAST (Câmara et al. 2002; Wang et al. 2010).

Onion cultivars inoculated with *S. vesicarium* produced lesions after 9-14 days. In previous studies, lesions developed on Allium crops at 7–14 days after inoculation (Shishkoff and Lorbeer 1989; Basallote-Ureba et al. 1999; Suheri and Price 2000; Misawa and Yasuoka 2012). In contrast to earlier reports (Rao and Pavgi 1975; Shishkoff and Lorbeer 1989; Basallote-Ureba et al. 1999), the lesions in the current study rarely coalesced. Leaf dieback was observed 10-21 days after the development of initial lesions, which was similar to the timing of extensive necrosis on infected pear (Pattori et al. 2006).

Each of the five isolates of *S. vesicarium* was pathogenic on onion, but they differed in aggressiveness, measured as percentage leaf dieback. This indicated that *S. vesicarium* populations from asparagus and onion in Canada were not highly host specific. This result was similar to a previous study of isolates from garlic, onion and asparagus (Basallote-Ureba et al. 1999), but differed from some reports of isolates from European pear (Köhl et al. 2009a; Singh et al. 1999; Pattori et al. 2006). Isolate OO27 from onion was the most aggressive isolate on onion. Isolate OA46 from asparagus produced larger, more numerous lesions but less leaf dieback compared to isolates NO35, OO54 and OO55 from onion.

All 13 onion cultivars assessed were susceptible to SLB, but there was variation in the level of susceptibility. There was no correlation between the number of initial SLB lesions and leaf dieback among the cultivars. This indicates that the reaction of onion cultivars to infection by conidia might differ from the response to the toxins responsible for leaf dieback. This is similar to earlier reports on the susceptibility of pear, where the number of initial necrotic spots often differed from the subsequent rate of disease progress, which is caused by host-specific toxins (Montesinos et al. 1995; Pattori et al. 2006; Singh et al. 1999).

In the field, SLB incidence and leaf dieback were higher in 2015 compared to 2016. These differences can be attributed to the wet and warm weather conditions and higher density of airborne conidia in 2015 (see chapter 2). This observation supports previous reports that the numbers of both conidia and infections on Allium crops were higher in warm, humid seasons compared to dry seasons (Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). In 2015, spray-drift injury caused by flumioxazin herbicide may have also increased the number of initial infections.

Marketable yields of onion in both seasons were not correlated with leaf dieback. This was in contrast to previous reports (Miller et al. 1978; Lorbeer 1993) and a deviation from the expectation that increasing the level of leaf dieback would reduce yield. The differences in yield in this study were more likely associated with cultivar characteristics (Matimati et al. 2006). Cultivars with lower leaf dieback had a higher proportion of jumbo-sized onion bulbs. This is consistent with previous studies that increasing levels of SLB leaf dieback reduced bulb size (Rao and Pavgi 1975). However, the extent of leaf dieback may have a more substantial effect on yield than the number of initial lesions that

develop on a crop. Extensive leaf dieback can result in under-development of bulbs (Rao and Pavgi 1975). Also, severe desiccation of the green foliage may affect the activity of maleic hydrazide, which is applied a few weeks before lodging to prevent sprouting in storage (Brewster 2008).

Onion yield was higher in 2015 compared to 2016. This can be attributed, in part, to differences in planting methods. Use of a double-row precision seeding planter maximized the number of onion plants per unit area and contributed to the development of more uniform-sized medium bulbs (Valk 1988). The use of transplants in 2015 resulted in a lower density of plants per unit area and the development of more jumbo bulbs in comparison to 2016 (Stoffella 1996). However, the overall harvest in 2015 had more jumbo bulbs. Also, the average number of bulbs harvested per unit area was lower in 2016 compared to 2015. This can be attributed to poor emergence in certain cultivars.

To further understand the variation in susceptibility of onion cultivars and aggressiveness of onion isolates of *S. vesicarium* Canada, there is the need to conduct *in vitro* studies on the effects of toxins of *S. vesicarium* isolates on onion tissue.The toxins of *S. vesicarium* isolates from European pear did not cause necrosis on onion tissues or Japanese pear cultivars. Furthermore, the toxins from the different pear isolates varied in the extent of necrosis cause on the host tissues (Singh et al. 1999, 2000). These previous studies indicated that toxins produced by *S. vesicarium* may not only be host specific but location specific as well.

In conclusion, *S. vesicarium* was confirmed as the causal agent of SLB on onion in the Holland Marsh. All of the onion cultivars assessed were susceptible to isolates from onion, as well as an isolate from asparagus. The reaction of onion cultivars to

infection versus toxin production differed, as has been demonstrated in pear fruit. Pontiac, Hendrix and Milestone were relatively less susceptible to leaf dieback compared to Highlander, La Salle and Trailblazer.

CHAPTER FOUR

COMPARISON OF SPRAY TIMING PROGRAMS FOR MANAGEMENT OF STEMPHYLIUM LEAF BLIGHT OF ONION

4.1 Introduction

The total annual onion (*Allium cepa* L.) production in Canada is about 200,000 metric tonnes, with a farm-gate value of $74 million (Agriculture and Agri-food Canada 2014). The majority of onions are cultivated in Ontario, Quebec, Nova Scotia, Manitoba and British Columbia. Ontario ranks as the highest producer, producing approximately 92,000 Mt with a farm-gate value of $34 million (Mailvaganam 2015).

The main challenges with onion cultivation in Ontario are diseases, insects and adverse weather conditions (Chaput 1995). Stemphylium leaf blight (SLB), caused by *S. vesicarium*, is a relatively new disease on onion in Ontario (Paibomesai et al. 2012). The disease has been observed in onion fields from about the end of June until harvest. The pathogen also attacks other Allium crops (Basallote 1993; Suheri and Price 2001; Misawa and Yasuoka 2012), asparagus (Falloon 1987) and pear (Llorente and Montesinos 2006).

On onion, infection starts as small light yellow to tan water-soaked lesions (Rao and Pavgi 1975; Miller et al. 1978). The lesions turn brown to dark brown to black as the pathogen begins to sporulate. Infected leaves die back from the tip (Basallote-Ureba et al. 1999), caused by host-specific toxins produced by *S. vesicarium* (Singh et al. 2000). This leads to desiccation of the leaves and early lodging, resulting in underdevelopment of bulbs (Rao and Pavgi 1975). Infection is favoured by leaf wetness periods lasting 6 h or

more and warm temperatures (18–26 °C) (Montesinos et al. 1995b; Prados-Ligero et al. 1998).

The most effective way to manage SLB on onion is regular application of preventative fungicides (Gupta et al. 2010). However, very few fungicides have been reported to be effective in the management of SLB. High concentrations of mancozeb, azoxystrobin, propiconazole and propineb *in vitro* inhibited *S. vesicarium* growth (Mishra and Gupta 2012) but had little to no impact on SLB incidence in the field (Hoepting 2015). In Spain, tebuconazole or procymidone were used to reduce initial infection on garlic (Basallote-Ureba et al. 1998). Ethylenebisdithiocarbamate (EBDC) is registered to manage *S. vesicarium* on asparagus in the USA (Meyer et al. 2000). In Ontario, fluopyram plus pyrimethanil (group 7 + 9) was recently registered to manage SLB on onion (Bayer CropScience Inc. Canada 2016).

The detection of *S. vesicarium* conidia in pear orchards is used in recommending when to initiate fungicide application (Llorente et al. 2012). Currently, onion growers in the Holland Marsh apply fungicides when disease is reported in the local area. Subsequent sprays are applied according to a routine 7-14 day calendar application method. However, favourable conditions for SLB development are not always present in the field. Therefore, calendar application of fungicides may not always be economical or environmentally friendly (Montesinos et al. 1995b; Meyer et al. 2000). Use of spray timing programs can reduce the number of applications required to manage SLB while keeping disease levels below economic thresholds (Montesinos and Vilardell 1992; Meyer et al. 2000).

TOMCAST (a modification of FAST – Forecasting for *Alternaria solani* on tomato) is a spray timing program for management of septoria leaf spot and anthracnose on tomato (Pitblado 1992b). TOMCAST uses daily leaf wetness duration and the average temperature during the wet period to calculate disease severity values (DSV) (Madden 1978). At predetermined DSVs, fungicides are applied and the program is reset (see chapter 1). The use of TOMCAST DSV 15 for management of purple spot of asparagus reduced fungicide applications by 60% (Meyer et al. 2000).

BOTCAST (Botrytis forecaster) was developed for management of botrytis leaf blight on onion. It uses similar weather parameters as TOMCAST but employs different combinations of temperature and leaf wetness to estimate inoculum availability and conditions for infection (Sutton et al. 1986). A combination of daily inoculum value and daily infection value is used in calculating cumulative disease severity values (CDSI). Fungicide applications are initiated at one of two thresholds; medium risk at 21–30 CDSI, and high risk of disease at 31–40 CDSI (see chapter 1). In preliminary trials at the Muck Crop Research Station (MCRS), BOTCAST showed potential to reduce the number of spray applications for managing SLB (McDonald and Vander Kooi 2014b).

The objective of this research was to evaluate selected spray timing programs for use in the integrated pest management program for SLB on onion in the Holland Marsh. The effectiveness of the spray timing programs in reducing SLB severity and increasing yield was assessed. Also, the costs associated with each spray timing program were calculated and compared.

4.2 Materials and Methods

4.2.1 Plant material and plot layout

The trial was carried out at two sites with a history of SLB. In 2015, the trial was conducted at the Jane Street research site of the MCRS on an organic soil (organic matter ≈ 62%, pH 7.2). In 2016, the trial was conducted at the MCRS on an organic soil (organic matter ≈ 71%, pH 5.7). In both years, onion cv. LaSalle (Stokes Seeds, Thorold, ON) was used because it was shown to be susceptible to SLB in preliminary trials.

In 2015, onion transplants (3 seeds per plug) were transplanted on 25 May, using a mechanical transplanter. There were four rows per bed. The beds were 42 cm apart, rows were 40 cm apart and the plugs were 10 cm apart within the row. In 2016, the crop was direct seeded (≈ 35 seeds m^{-1}) on 4 May using a Stanhay precision seeder. There were four double rows per bed, with 40 cm between rows, and the plants were approximately 7.5 cm apart within the row. The seedlings emerged 10-12 days after planting (DAP).

Flumioxazin (Chateau®, Valent Corporation, Guelph, ON.) was applied following label recommendations to control weeds at the 4-6 onion leaf stage, 45 days after transplanting (DAT) in 2015. Onion maggots were managed using chlorpyrifos (Pyrinex™, Adama Agricultural Solutions Canada Ltd., Winnipeg, MB.) 45 days after seeding following label recommendation. In 2016, bromoxynil (Pardner®, Bayer Crop Science Inc., Mississauga, ON.) was applied at the 2-3 leaf stage at 39 DAP and repeated at the 5–6 leaf stage at 56 DAP to control weeds. Weeds were removed by hand throughout the rest of the growing season until maturity.

4.2.2 Experimental design and treatments

The experiment was designed as a randomized complete block design with four replicates. Each experimental unit (plot) consisted of two beds of onion, each 1.6 × 5.0 m. There were six treatments, including the non-sprayed check, which are described in Table 4.1. Two treatments were consistent in the two years, BOTCAST, and TOMCAST with a DSV of 15. Some fungicide treatments in 2016 were modified based on the results of the 2015 trials.

In 2015, fluopyram plus pyrimethanil (Luna Tranquility®, Bayer Crop Science Inc. Calgary, AB) was applied for each spray. This product showed the greatest SLB reduction in preliminary studies (McDonald and Vander Kooi 2014b). In 2016, mancozeb (Dithane™, Dow Agro Sciences, Calgary, AB) was applied as an initial protective spray in all spray timing treatments, followed by subsequent applications of fluopyram plus pyrimethanil. Fungicides were applied with a custom-built, tractor-mounted sprayer, fitted with air induction, flat spray tips (model: AI9503 EVS, AI TeeJet, IL) at 275 kPa calibrated to deliver 500 L/ha. Fluopyram plus pyrimethanil was applied at a rate of 0.15 kg fluopyram plus 0.45 kg pyrimethanil ha^{-1} and mancozeb at 2.44 kg ha^{-1}.

Table 4. 1 Fungicide spray-timing programs tested for management of stemphylium leaf blight on onion cv. La Salle grown at MCRS, Holland Marsh, ON in 2015 and 2016.

Treatment	Description
Both 2015 and 2016	
BOTCAST	The first fungicide application was made when the program accumulated 21 CDSI (lower threshold). Subsequent applications made every 7-14 days (Sutton et al. 1986).
TOMCAST 15	The first application at 15 DSV. Subsequent applications every 15 DSV accumulations (Pitblado 1992a).
Control (No sprays)	No fungicide application.
2015 only	
STEMCAST	A modification of BOTCAST with conditions favourable for the *S. vesicarium* infections. The first spray applied at 21 CDSI. Subsequent sprays applied every 7–14 days.
Conidia plus phenology (CP1)	The first spray was applied when conidia were present and onions had fully developed enough foliage (≈3 true leaves) to receive fungicide application. Subsequent sprays applied every 7-14 days.
Local disease report	Routine 7–14 calendar application, with the first spray applied after the first report of SLB in the Holland Marsh.
2016 only	
TOMCAST 15/25	Initial spray was applied at 15 DSV. Subsequent sprays were applied after every 25 DSV accumulations.
TOMCAST 15/R	Initial spray was applied at 15 DSV. Subsequent sprays were applied at every 15 DSV accumulation if there was rainfall predicted in the next 24 h.
Conidia plus phenology (CP2)	First spray applied when conidia were detected and onions had fully developed 4–6 true leaves. Subsequent sprays applied every 7-14 days.

4.2.3 Weather data

Hourly meteorological data on temperature (°C), leaf wetness duration (h) and rainfall (mm) were collected throughout the growing season. In 2015, data were collected from 25 May (transplanting date) to 15 August. In 2016, data were collected from 14 May (emergence) to 20 August. All weather variables were recorded using an Onset® HOBO® automatic weather station (Onset Corporation, Bourne, MA). Hourly data on temperature were recorded with a temperature/RH sensor (Model: S-THB-M00x). Two leaf-wetness sensors (Model: S-LWAM003) were installed in the plot to measure leaf wetness. These sensors did not require any painting or coating. Each sensor was placed upright to simulate the orientation of an onion leaf. Regular visual checks for moisture on onion leaves were compared to the values from the two sensors. The values from the sensor identified as more sensitive and accurate were used to calculate leaf wetness duration (LWD). A tipping gauge rain gauge smart sensor (Model: S-RGB-M002) was used to record hourly rainfall.

4.2.4 SLB assessment

Plots were observed weekly for SLB lesions, as described previously. Initially, the number of lesions was counted and SLB incidence was recorded. Later, percentage leaf dieback was measured as the percentage of necrotic or chlorotic length of the leaf compared to the total leaf length. Lesions were counted on the first and second fully developed true leaves in both years as described previously (see chapter 3). In 2015, 32 leaves per bed (four leaves per plug) were assessed and 40 leaves per bed were assessed

in 2016. Lesions were first observed and assessed on 2 July in 2015 and 11 July in 2016. In 2015, all of the plugs in each bed were assessed to determine SLB incidence. In 2016, 100 plants in the center of each bed were assessed. In 2015, SLB incidence was assessed on 2, 9, 16 and 31 July. In 2016, incidence was assessed on 11, 19, 25 July and 9 August. Leaf dieback was measured on the fifth and sixth fully developed true leaves on 32 leaves in 2015 and 40 leaves in 2016, as described previously (see chapter 3). In 2015, leaf dieback was measured on 23, 31 July, 6 August and 13 August. The area under the disease progress curve (AUDPC) was calculated for disease incidence and leaf dieback as follows:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where *Yi* and Y_n are assessments at the first and last observations, respectively, and *t1, t2, t*$_{n-1}$, and t_n are the times of the first, second, penultimate, and last observations, D is the number of days of total observation and n is the number of observations. AUDPC was standardized to sAUDPC (standard AUDPC per day) (Simko and Piepho 2012).

$$sAUDPC = \frac{\text{AUDPC}}{\text{D}}$$

4.2.5 Yield assessment

The plots were harvested on 10 September, 2015 and 6 September, 2016. All of the plants from two 2.3-m lengths of two rows from each replicate were pulled, windrowed and cured for 1 to 2 weeks, then topped and stored at 20–23 °C until the yield assessments were complete. Yield assessment was done on 21 October, 2015 and 20

September, 2016. The onion bulbs were graded according to size (diameter) as jumbo (> 76 mm), medium (45–64 mm) and small (< 45 mm). Jumbo and medium onions were considered as marketable yield. Small onions, damaged bulbs and sprouting onions were considered culls. The percentage of bulb size distribution and weight, the percentage marketable yield, and the marketable yield (t/ha) were calculated.

4.2.6 Data analysis

Statistical analyses were conducted using SAS v.9.4 (SAS Institute Inc., Cary, NC). Disease and yield assessment were analyzed using a mixed model analysis of variance (ANOVA) in PROC MIXED. The Shapiro-Wilk test was used to test the normality of residuals and distribution of error was assessed using residual plots. The data were normally distributed. The data were checked for outliers using Lund's test and no outliers were identified. Variance was partitioned into random (block) effects and fixed (treatment) effects and mean separation was conducted using Tukey's multiple range test ($\alpha = 0.05$). Spearman's correlations were conducted between leaf dieback and yield using PROC CORR.

4.3 Results

4.3.1 Weather assessment

There were 25 days with rainfall in 120 seasonal days 2015 compared to 20 in 139 seasonal days 2016. June was the wettest month in 2015, whereas July was wettest in 2016. Overall, higher temperatures were recorded in 2016. In both years, the highest

temperature ranges were recorded in July. Monthly LWD ranges were similar in both years (Table 4.2). On rainy days, LWD could last up to 24 h.

Table 4.2 Monthly weather variables recorded during the onion production season at the Holland Marsh, ON in 2015 and 2016.

Weather variable	May	June	July	August
2015				
Rainy days (days) [a]	4	12	2	7
Daily temp (˚C)	8–24	12–21	16–25	15–25
Daily LWD (h)	0–18	1–20	0–20	5–18
2016				
Rainy days [a]	5	5	7	3
Daily temp. (˚C)	3–25	10–26	15–28	19–27
Daily LWD (h)	0–22	0–22	0 -24	524

[a] Number of days with total rainfall ≥ 2 mm

4.3.2 SLB assessment

In 2015, the numbers of lesions per leaf in the CP1 and TOMCAST 15 treatments were slightly lower than in the other treatments. In 2016, there were no differences in the number of lesions per leaf (Table 4.3). SLB incidence was higher in 2015 in comparison to 2016 (Table 4.3). In 2015, the lowest AUDPC for incidence occurred in the CP1 and TOMCAST 15 treatments. The highest overall incidence occurred in the unsprayed control, which did not differ from the sprayed treatments. In 2016, the AUDPC for incidence in the unsprayed control was higher than in any of the treatments that received fungicides, except for TOMCAST 15/R, which was intermediate (Table 4.3).

In 2015, AUDPC for leaf dieback was higher in the unsprayed control than in any of the treatments that receive fungicides. In 2016, there were no differences in the AUDPC for leaf dieback. There was no correlation between the number of SLB lesions and AUDPC for leaf dieback in 2015 ($r = 0.27$, $P > 0.19$) or 2016 ($r = 0.39$, $P > 0.06$).

Table 4.3 Lesion counts, overall incidence and overall severity of SLB on onion sprayed following selected spray-timing programs at the Holland Marsh, ON in 2015 and 2016.

Timing program	Incidence (AUDPC)	Lesions per leaf	Leaf dieback (AUDPC)
2015			
CP1	63 c	4 a	26 b
TOMCAST 15	68 bc	4 a	25 b
BOTCAST	79 ab	7 b	26 b
STEMCAST	80 ab	7 b	31 b
Local disease report	86 a	7 b	29 b
Control	92 a	7 b	47 a
2016			
CP2	10 b	3 ns	17 ns
TOMCAST 15	6 b	1	15
TOMCAST 15/25	9 b	1	14
TOMCAST 15/R	14 ab	3	16
BOTCAST	10 b	1	16
Control	22 a	4	26

[1]Numbers in a column followed by the same letter do not differ based on Tukey's multiple range test at $P = 05$. ns = not significant.

4.3.3 Fungicide Applications

Airborne conidia were trapped prior to crop establishment in both years, with the first capture on 23 May in 2015 and 28 April in 2016. In 2015, the CP1 treatment recommended sprays starting at 19 DAT (2-3 leaf stage). In 2016, the CP2 treatment recommended sprays starting at 54 DAP (4-6 leaf stage). In 2015, CP1 received 10 fungicide applications, but CP2 received only four applications in 2016, (Table 4.4).

In 2015, TOMCAST 15 also recommended early fungicide application. The model recommended six fungicide applications in 2015, starting at 19 DAT. In 2016, TOMCAST 15, TOMCAST 15/25 and TOMCAST 15/R recommended the first fungicide application at 56 DAP. TOMCAST 15 recommended five applications and TOMCAST 15/25 and TOMCAST 15/R recommended three applications (Table 4.4).

1n 2015, BOTCAST recommended the first fungicide application at 40 DAT. STEMCAST recommended the first fungicide application at 41 DAT, coinciding with first local report of SLB in commercial fields. In 2015, BOTCAST, STEMCAST and local disease report treatments recommended eight sprays each. In 2016, BOTCAST recommended only three applications (Table 4.4).

Table 4.4 Application dates, number of applications and cost of fungicide based on spray timing treatments to manage SLB on onion in the Holland Marsh, ON in 2015 and 2016

Timing program	Spray dates	No. sprays	Cost($)
2015			
CP1	13, 19, 28 June; 8, 15, 22, 29 July; 5, 12, 19 August	10	$1300
BOTCAST	28 June; 8, 15, 22, 29 July; 5, 12, 19 August.	8	$1064
STEMCAST	29 June, 8, 15, 22, 29 July; 5, 12, 19 August	8	$1064
Local disease report	29 June; 8, 15, 22, 29 July; 5, 12, 19 August	8	$1064
TOMCAST 15	13, 30 June; 14, 26 July; 4, 12 August.	6	$798
Control	Not sprayed	0	0
2016			
TOMCAST 15	26 June; 8, 21, 28 July; 6 August	5	$740
CP2	24 June; 8, 21 July; 4 August	4	$592
BOTCAST	5, 21 July; 4 August.	3	$444
TOMCAST 15/25	26 June; 19 July; 4 August.	3	$444
TOMCAST 15/R	26 June; 8, 24 July	3	$444
Control	Not sprayed	0	0

4.3.4 Yield

There were no differences in the overall yield or bulb-size distribution in either year. Yield was substantially lower in 2016 compared to 2015 (Table 4.5). There was no correlation between yield and tip dieback in 2015 ($r = 0.14$, $P > 0.50$) or 2016 ($r = 0.004$, $P > 0.90$).

Table 4.5 Marketable yield and bulb size distribution of onion cv. La Salle following selected spray-timing programs at the Holland Marsh, ON in 2015 and 2016.

Spray timing	Marketable yield (t/ha)	Bulb size distribution (%)		
		Jumbo	Medium	Cull
2015				
STEMCAST	46 ns	28 ns	67 ns	5 ns
BOTCAST	45	27	66	7
CP1	45	32	60	8
TOMCAST 15	44	25	66	9
Control	42	23	74	3
Local disease report	40	23	70	7
2016				
TOMCAST15R	26 ns	1 ns	62 ns	37 ns
CP2	25	1	68	31
TOMCAST 15	25	1	57	42
BOTCAST	25	0	69	31
TOMCAST 1525	22	1	61	38
Control	20	1	64	35

[1]Means in a column followed by the same letter do not differ based on Tukey's multiple range test at $P = 0.05$. ns = not significant.

4.4 Discussion

Disease pressure was higher in 2015 compared to 2016. In both years, there were very little to no differences in SLB incidence and severity among sprayed and unsprayed onion, so the unsprayed control provided the highest economic returns. It can be concluded that fungicide applications after initial infection had occurred may have been unnecessary. Also, it is unclear whether the fungicides used in this study are effective in

controlling SLB on onion. Under high disease pressure, TOMCAST 15 provided a reduction in SLB incidence and severity and fungicide cost saving when compared to other spray timing programs in this study. Under low disease pressure, BOTCAST, STEMCAST, and LDR recommended the lowest number of fungicide application.

The difference in SLB incidence and severity between the two years can be attributed to the differences in weather. The weather in 2015 was warm with frequent rainfall events, and 2016 was also warm, but with longer intervals between rainfall events. The longer periods of dry weather in 2016 appear to be unfavourable for development of SLB, which was in agreement with previous reports (Basallote-Ureba et al. 1999; Prados-Ligero et al. 2003). Also, the warm and wet weather in 2015 was favourable for abundant inoculum production and infection, as discussed earlier (see chapter 2).

Regardless of the planting methods, initial SLB lesions were observed at the end of June to the middle of July. This was in agreement with an earlier disease survey conducted in the Holland Marsh (Paibomesai et al. 2012). The onion crops are usually at the 4-6 leaf stage at this time, which provides an abundance of susceptible host tissues and coincides with the abundance of airborne conidia, as earlier discussed (see chapter 2).

There was no correlation between the number of SLB lesions observed and subsequent percentage leaf dieback. This was similar to the results presented in Chapter 3. These results indicate that leaf dieback on onion may be due to the activity of host-specific toxins produced by *S. vesicarium* after infection rather than the initial number of lesions (Singh et al. 1999; Wolpert et al. 2002). In contrast to previous reports (Shishkoff

and Lorbeer 1989; Basallote-Ureba et al. 1999), initial lesions observed on onion leaves rarely coalesced.

All of the sprayed treatments had an overall lower SLB incidence compared to the unsprayed control, but they did not reduce SLB severity below economic levels in comparison to the control. Effective spray timing for the management of *S. vesicarium* should recommend protective fungicide applications before infection takes place (Llorente et al. 2000a; Gupta et al. 2010). Fungicides are less effective post-infection because the severity of diseases caused by *S. vesicarium* is related to toxins produced after infection (Singh et al. 1999, 2000; Llorente et al. 2000a).

Initial lesions of *S. vesicarium* on Allium crops develop 7-14 days after infection (Shishkoff and Lorbeer 1989; Prados-Ligero et al. 1998; Suheri and Price 2000; Misawa and Yasuoka 2012). In 2015, TOMCAST 15 and CP1 recommended a fungicide application 13-15 days prior to the observation of the first SLB symptoms, which should have provided protection from this initial infection. Therefore, the lower number of initial lesions and SLB incidence in the TOMCAST and CP1 treatments can be attributed to the application of protective fungicide at this time. In 2016, all spray timing programs recommended the first fungicide application 6-15 days before the observation of initial lesions. However, disease pressure in 2016 was very low and there were no difference in the incidence between sprayed treatments and the unsprayed control. These results indicate that fungicide application when disease pressure was low did not result in significant reduction in disease.

Similarly, there were no differences in yield between the sprayed treatments and the unsprayed control in either year, so the unsprayed control provided the highest

economic return. This result was similar to an earlier report on garlic (Basallote-Ureba et al. 1998). It is, therefore, unclear if fungicide applications were effective or even necessary in managing SLB at this site. Also, yield in both years was not correlated with SLB levels. There are many other factors that affect onion yield, such as nutrition and weed control (Brewster 2008), which were not investigated in this study. For example, the trial in 2016 was very weedy during the bulbing stage (8-9 leaf stage), which may account for the lower yields in 2016 compared to 2015.

Previous studies showed that mancozeb (Mishra and Gupta 2012) and fluopyram plus pyrimethanil (Tesfaendrias et al. 2014) provided some reduction of SLB on onion. In the current study, fungicide applications only provided an overall reduction of SLB incidence of 16-29%, and severity by 12-21%. Recent studies in the USA (Hoepting 2015) and Europe (Alberoni et al. 2010) reported that *S. vesicarium* isolates from onions and pear were insensitive to several fungicides, so it is possible that the pathogen population at this site is insensitive to these fungicides.

Future research is needed to screen more fungicides for SLB management in the Holland Marsh. Also, it will be important to investigate the sensitivity of local *S. vesicarium* isolates to fungicides. Furthermore, spray timing programs should be modified to include precipitation, as it was an important weather variable that affected when disease was initiated (see Chapter 2). Growers should be advised to apply a protective fungicide when the onion crop has developed enough foliage (≈ 3 leaf stages) to receive it, since inoculum is available even before planting. This application may be redundant in warm and dry years. However, the initial application of fungicides before

infection and development of symptom in both seasons of this trial reduced SLB incidence.

CHAPTER FIVE

DETECTION OF STEMPHYLIUM LEAF BLIGHT ON ONION USING AERIAL PHOTOGRAPHY

5.1 Introduction

Onion (*Allium cepa* L.) is grown on about 2460 ha in Ontario, which represents 45% of the fresh market production in Canada (Statistics Canada 2013). About 90% of this production is located in the Holland Marsh (Valk 1988), where it contributes $33 million to the provincial economy (Mailvaganam 2016). Environmental conditions in the Holland Marsh favour vegetable production and also favour several fungal diseases, including stemphylium leaf blight (SLB) on onion.

Stemphylium leaf blight was observed for the first time in Ontario in 2008 (Paibomesai et al. 2012) after earlier reports of the pathogen on asparagus (Roddy 2011). The disease affects only the foliage of onion, and high disease pressure results in the reduction of bulb development (Rao and Pavgi 1975). Initial visual symptoms include small, yellow to tan, water-soaked lesions, followed by coalescing of lesions and extensive blighting of the leaf blade, starting at the tip (Shishkoff and Lorbeer 1989). This results in early drying up of leaves and lodging of the crop (Rao and Pavgi 1975; Basallote-Ureba et al. 1999).

One approach to management of SLB on Allium crops might be to use resistant cultivars (Pathak et al. 2001; Mishra et al. 2009). However, no onion lines have strong resistance (Pathak et al. 2001), so application of foliar fungicides (Gupta et al. 2010) is often required to maintain yield and quality. *Stemphylium vesicarium* produces host-specific toxins after infection that are associated with extensive tissue necrosis (Singh et

al. 1999, 2000). These toxins reduce the efficacy of fungicides applied after infection (Alberoni et al. 2010; Puig et al. 2014). In pear, fungicide applications initiated after visual detection of necrotic spots of *S. vesicarium* resulted in at least 10% loss (Puig et al. 2014). Therefore, early detection of SLB is important to minimize yield losses (Köhl et al. 2009b; Llorente et al. 2012).

The presence of *S. vesicarium* in pear can be detected prior to symptom development using molecular techniques (Köhl et al. 2009b). Molecular detection is sensitive but not effective for use in commercial situations because it is destructive and only a few plants can be sampled (Martinelli et al. 2014). Remote sensing has potential for rapid detection of crop diseases with minimal disturbance to the crop (Bock et al. 2010; Martinelli et al. 2014). Disease detection involving the use of passive imaging sensors that measure the radiation from the crop canopy can be precise and objective compared to visual assessments (Zhang et al. 2005; Bock et al. 2010; Martinelli et al. 2014).

Remote sensing is defined as collection of data about an object without physical contact (De Jong and van der Meer 2006). Remote sensing using passive imaging sensors measures and captures the amount of reflected radiation from a crop canopy (Lorenzen and Jensen 1991), while active sensors emit artificial radiation and measure the energy reflected or backscattered (McGill 2004). Passive sensor equipment include digital (visible and near-infrared), multispectral, and hyperspectral cameras (Sankaran et al. 2010; Garcia-Ruiz et al. 2013). Multispectral cameras have sensors that capture images with several specific bands of the electromagnetic spectrum. Multispectral sensors usually have between 3 and 10 band measurements for each pixel captured.

Hyperspectral cameras have sensors that capture images with narrower and more numerous bands than multispectral sensors. Hyperspectral images can contain as many as 200 or more contiguous spectral bands (Adam et al. 2010).

Specialized cameras can capture images containing reflectance data in the visible (400-685 nm), red-edge (690-730 nm), near-infrared (NIR, 730-850 nm), and shortwave-infrared (SWIR, 850-2500 nm) spectra (Baranoski and Rokne 2005; Sankaran et al. 2010; Garcia-Ruiz et al. 2013). These images can be analysed and specific vegetative indices that are sensitive to disease presence can be calculated (Mahlein et al. 2012). Vegetative indices compare the relative reflectance in two or more spectral regions (Hatfield and Prueger 2010).

There are several vegetative indices that detect changes in the chlorophyll content and leaf reflectance (Kumar and Silva, 1973; Hatfield and Prueger, 2010). For example, the normalised difference vegetative index (NDVI) has been used to detect citrus trees infected with Huanglongbing disease (Garcia-Ruiz et al. 2013) and oil palm trees infected with ganoderma stem base rot (Shafri and Hamdan 2009). However, the major limitation of vegetative indices for disease detection is that changes in chlorophyll content are indicative of many kinds of stress, of which plant disease is only one (Bravo et al. 2004).

Advances in the technology and availability of unmanned aerial vehicles (UAVs) have increased the potential for use of remote sensing in disease detection. Also, recent improvements in passive imaging sensors have substantially reduced their size and weight, so that they can be mounted unto relatively inexpensive UAVs (Sankaran et al. 2010; Martinelli et al. 2014). Combining UAVs with multispectral or hyperspectral sensors has provided aerial images that have a high resolution compared to manned

aircraft and so can used to distinguish between diseased and healthy vegetation (Garcia-Ruiz et al. 2013).

The overall goal of this study was to investigate the potential of incorporating UAVs mounted with a relatively simple camera (red, green, infrared filters) into the integrated pest management (IPM) program for onion at the Holland Marsh. The potential of aerial infrared images in detecting SLB on onion and the relationship between four selected vegetative indices and SLB incidence were investigated. The best height and format to acquire aerial images for SLB detection on onion was also assessed.

5.2 Materials and Method

5.2.1 Aerial imaging

The acquisition of aerial images (visible and near-infrared) was conducted by High Eye Imaging Inc. (Wasaga, ON). Aerial images of the research trials at the Jane Street research site and Muck Crop Research Station were captured at about 2-week intervals in 2015 and 2016 using a Xnite-Canon SX230NDVI (Canon USA Inc., Melville, NY) mounted on a modified Cine Star – 8 MK Heavy Lift RTF octocopter UAV (The Quadrocopter Company, Columbia Falls, MT) (Fig. 5.1). Ground reflectance measurements were not taken in this study.

Figure 5.1 A modified Cine Star – 8 MK Heavy Lift RTF octocopter mounted with a camera for aerial image collection at the Holland Marsh, ON. 2015

5.2.2 Regions of interest

In 2015, SLB levels on two trials, the onion cultivar screening trial (Fig. 5.2a) and the onion spray timing trial (Fig. 5.2b), were selected as two regions of interest (ROI). In 2016, only the spray timing trial was considered as the ROI due to poor emergence in the cultivar trial. There were 12 onion cultivars (Table 5.1) grown in the cultivar trials each year. In the spray timing trial, the effect of fungicide application timing treatments was assessed on onion cv. La Salle (Table 5.2). In 2016, white stakes (60 cm) with 15 × 15 cm red-painted plywood pieces nailed to the top were used as ground control points for the ROI.

In both years, the trials were arranged in a randomized complete block design with four replicates. Each plot was 1.6 × 5 m, with four rows per plot and 2 m between

plots. In 2015, onion plugs in both ROIs were transplanted (2-3 seedlings per plug) using a mechanical planter on 25 May. The spacing was 40 cm between rows and 10 cm between plugs within-row. In 2016, the crop was direct seeded using a double row Stanhay Precision Seeder at about 35 seeds m^{-1} on 4 May, with four double rows per plot. In 2015, 12 onion cultivars were assessed for the susceptibility to stemphylium leaf blight. These were Stanley (1), Prince (2), Highlander (3), Hendrix (4), Hamlet (5), Trailblazer (6), Madras (7), Patterson (8), Milestone (9), Genesis (10), La Salle (11) and Pontiac (12) (Fig. 5.2A).

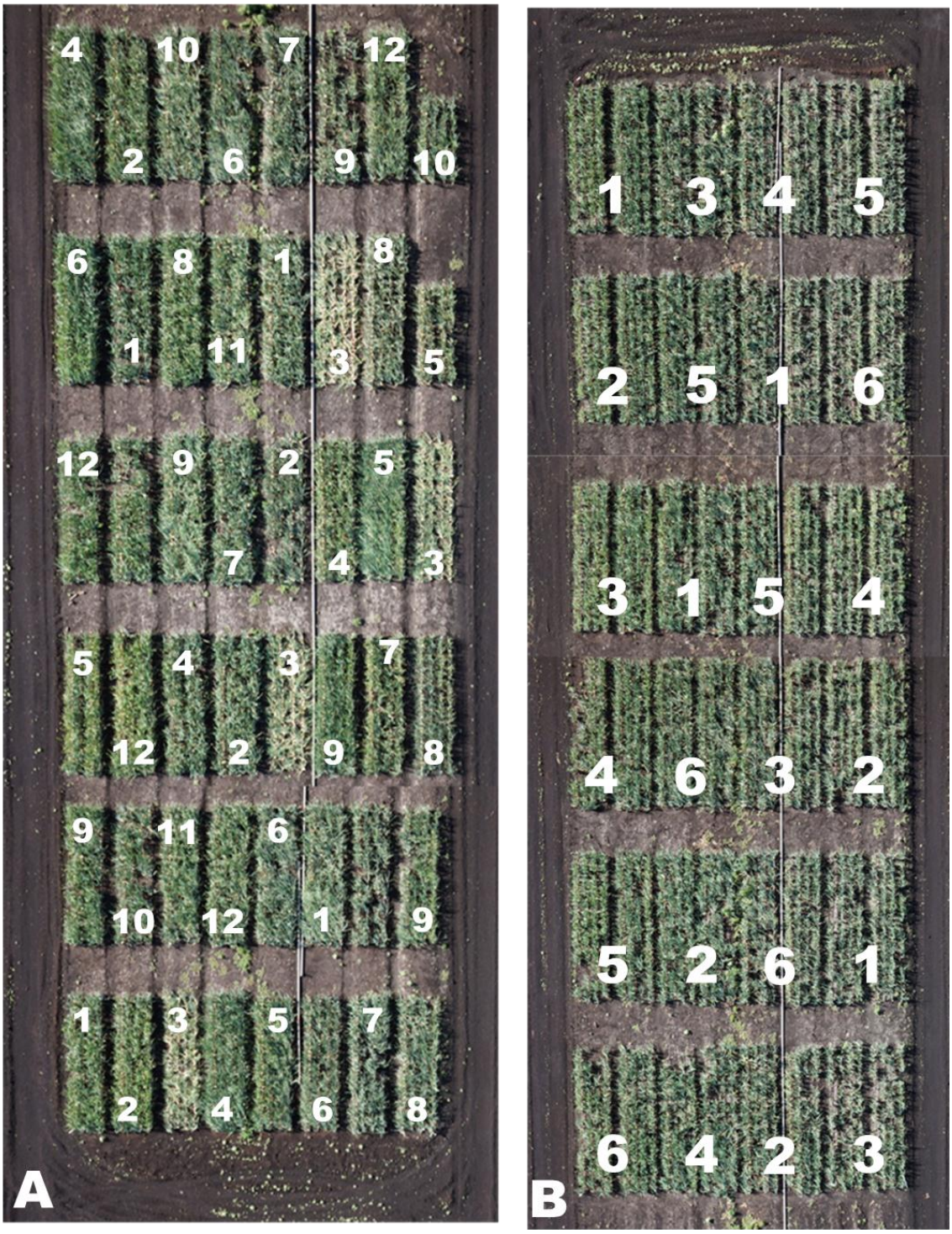

Figure 5.2 Study area for (A) the onion cultivar trial and (B) fungicide spray-timing trial in the Holland Marsh, ON in 2015.

Table 5. 1 Fungicide spray-timing programs evaluated on onion cv. La Salle in the spray timing trials at Holland Marsh, ON in 2015 and 2016.

Number	Treatment name [1]	Reference
Both 2015 and 2016		
1	BOTCAST	Sutton et al. 1986
2	TOMCAST DSV 15	Pitblado 1992
6	Unsprayed control	
2015 only		
3	STEMCAST	Chapter 3
4	Conidia plus phenology (CP1)	Chapter 3
5	Local disease report	Chapter 3
2016 only		
3	TOMCAST 15/25	Chapter 3
4	TOMCAST 15/R	Chapter 3
5	Conidia plus phenology (CP2)	Chapter 3

[1] Details on treatment, refer to chapter four

5.2.3 Data collection

In 2015, aerial images for SLB detection were taken in the ROIs on 29 June, 13 July, 27 July and 4 August. In 2016, aerial images were captured on 27 June, 11 July, 25 July and 2 August. The corresponding ground visual assessments of SLB incidence and leaf dieback in each plot were collected the same day as the aerial images. In 2015, images were captured with a camera that had the red-region filter replaced with a near-infrared filter, and the images were saved in JPEG (joint photographic experts group)

format. The images were taken from 15–20 m and 25–30 m above ground. In 2016, the images were taken from 15–20 m above ground with a camera in which the blue-region filter was replaced with a near infrared filter, and the images were saved in TIFF format (tagged image file format). Visual disease ratings were collected in each plot as previously described (Chapter 3 and 4).

5.2.4 Image analysis and vegetative indices

All of the images from the ROI were analysed using Whitebox Geospatial Analysis Tools (University of Guelph, Guelph, ON). Scripts written using Python language were used to calculate four selected vegetative indices (Table 5.3) from the selected set of images. Only the images acquired from 15-20 m above ground level were processed. These images covered an area of 15 × 30 m per ROI, with 24 beds each as described in Fig.5.2. The spatial resolution of the images was calculated using a field of view (FOV) chart for the camera used. The image resolution yielded a pixel size of about 3.1×10^{-3} m per pixel.

The vegetative indices (Table 5.2) for each bed were calculated for 0.4 × 3 m (129 × 968 pixels) portions for each bed. In 2015, images taken on 29 June could not be assessed effectively because the small seedlings could not be separated from the intense background of the black muck soil. Also, the images taken on 27 July could not be assessed effectively because they were taken at an angle instead of vertically. There was no red band in the images taken in 2015, so the assessment program replaced the red values with reflectance from the blue region. In 2016, images taken on 27 June and 11 July could not be assessed effectively because of the intensity of the background soil.

Table 5. 2 Vegetative indices calculated from aerial images of onion trials at the Holland Marsh, ON in 2015 and 2016.

Index	Wavebands	Crop parameter	Reference
Normalised vegetative indices (NDVI)			
Red NDVI	$\mathrm{NDVI} = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}}$	Intercepted photosynthetically active radiation (PAR)/ Biomass	(Deering 1978; Govaerts and Verhulst 2010)
Green NDVI	$\mathrm{GNDVI} = \frac{R_{NIR} - R_{GREEN}}{R_{NIR} + R_{GREEN}}$	PAR/Biomass	(Gitelson et al. 2003; Govaerts and Verhulst 2010)
Chlorophyll indices (CI)			
Green CI	$\mathrm{CI} = (R_{NIR}/R_{GREEN}) - 1$	Chlorophyll	(Gitelson et al. 2003)
Plant senescence reflective index (PSRI)			
PSRI	$\mathrm{PSRI} = \frac{(R_{RED} - R_{GREEN})}{R_{NIR}}$	Plant senescence	(Lee et al. 2008)

5.2.5 Statistical analysis

PROC GLM in SAS was used for analysis of variance of the visual assessments of SLB incidence and the calculated vegetative indices. No outliers were identified using Lund's test. The data were not normally distributed when assessed using PROC UNIVARIANT. The relationship between SLB incidence and vegetative indices was assessed using Spearman's rank correlation (nonparametric) at $P < 0.05$ in PROC CORR.

5.3 Results

5.3.1 Image analysis

The NIR images acquired at 15-20 m (Fig. 5.3A) had an average ground sampling distance (GSD) of 0.5 cm/pixel and an average FOV of 15 × 20 m. These images had higher resolution compared to images taken from 25-30 m (Fig. 5.3B). The images taken from 25-30 m above ground had an average GSD of 0.75 cm per pixel with a wider average FOV of 24 × 31 m.

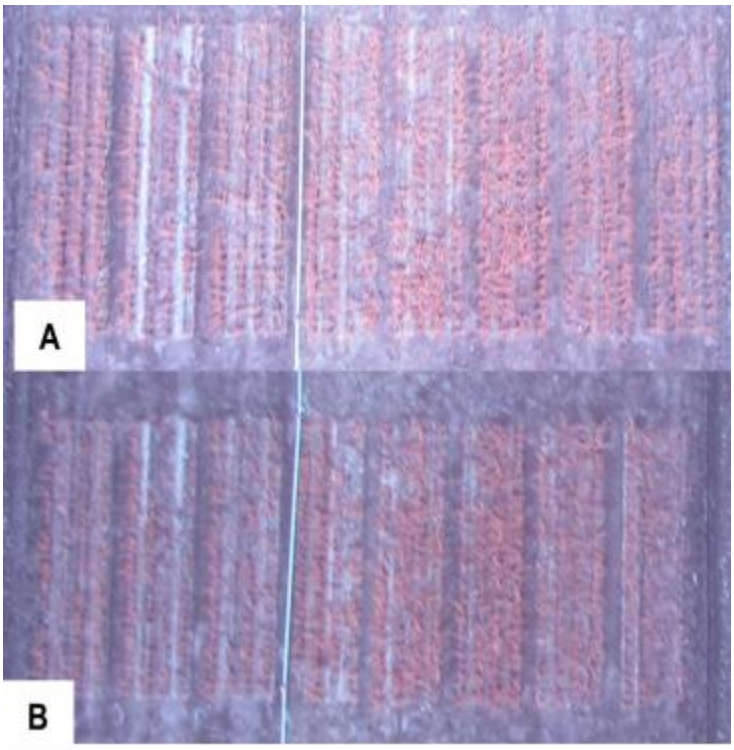

Figure 5.3 An aerial near-infrared image of onion plots taken on 13 July at (A) 15-20 m and (B) 25-30 m above ground at the MCRS, Holland Marsh, ON, 2015.

5.3.2 Disease and vegetative indices

Stemphylium leaf blight incidence and leaf dieback cultivar and spray-timing trials were higher in 2015 than in 2016. Lesions were first detected on 29 June, 2015 and 11 July, 2016. Images taken on the earliest dates could not be analysed due to the high intensity of the soil background.

There were significant difference in the incidence and percentage leaf dieback differed among cultivars and spray-timing treatments (Table 5.3, 5.4 and 5.5). Incidence and leaf dieback were higher in August (4 August, 2015 and 2 August, 2016) compared to July (13 July, 3015 and 25 July, 2016) in both years. There were significant differences in the vegetative indices measured on the different cultivars and different spray-timing treatments. However, the differences in incidence and severity among the onion cultivars and spray-timing treatment were not correlated with any of the vegetative indices.

Onion cv. Prince and Stanley had the lowest NDVI value and Pontiac had the highest NDVI on 13 July 2015. Whereas on 4 August 2015, Madras had the lowest NDVI and Pontiac had highest NDVI. Onion cv. Hamlet had a significantly lower GNDVI compared to other cultivars on 13 July 2015. However, on 4 August 2015, Hendrix, Pontiac and Highlander had the lowest GNDVI and Prince, Stanley, and Madras had highest GNDVI. Generally, GNDVI numbers were lower compared to NDVI (Table 5.3). In contrast to 13 July measurements, NDVI and GNDVI values on 4 August were negatively correlated (r= -0.87, P=0.0002).

On 13 July 2015, lowest CI value was recorded on onion cv. Genesis and Prince had the highest CI value. Whereas, on 4 August 2015, Hendrix had the lowest CI value

and Madras had the highest (Table 5.3). On 13 July, the CI values were correlated with GNDVI (r=0.98, *P*<0.0001), and PSRI (r=0.72, *P*=0.0082). The CI values measured on 4 August were correlated with NDVI (r= -0.86, *P*=0.0003), GNDVI (r=0.99, *P*<0.0001) and PSRI (r=0.71, *P*=0.0099).

Plant senescence reflective index (PSRI) was higher compared to the other vegetative indices. Similar to incidence and leaf dieback, PSRI was higher on in August compared to July. On 13 July, Genesis had the lowest PSRI and Madras had the highest PSRI. On 4 August, Hamlet had the lowest PSRI, and Prince and Stanley had the highest PSRI (Table 5.3). The PSRI values were correlated to GNDVI (r=0.64, *P*=0.0082) and CI (r=0.72, *P*=0.0082) on 13 July. However, on 4 August, PSRI was correlated with NDVI (r= -0.71, *P*=0.0093), GNDVI (r=0.72, *P*=0.0080) and CI (r=0.71, *P*=0.0099).

Table 5.3 Stemphylium leaf blight incidence, severity and vegetative indices calculated for onion cultivars screened for the susceptibility to SLB at the MCRS, Holland Marsh, ON, 2015.

Cultivar	Incidence [1]	Severity	Vegetative indices [2]			
			NDVI	GNDVI	CI	PSRI
13 July						
Pontiac	51 c	nd	0.22 a	0.06 bcd	0.08 cd	0.37 cd
Highlander	55 bc	nd	0.13 ab	0.04 cd	0.05 cd	0.37 cd
LaSalle	55 bc	nd	0.21 a	0.06 bcd	0.10 bcd	0.39 bcd
Prince	55 bc	nd	0.09 b	0.20 a	0.35 a	0.49 abc
Stanley	55 bc	nd	0.09 b	0.20 a	0.34 a	0.43 bcd
Hendrix	58 bc	nd	0.16 ab	0.03 cd	0.03 cd	0.39 bcd
Patterson	59 abc	nd	0.19 a	0.14 abc	0.23 abc	0.51 ab
Madras	59 abc	nd	0.17 ab	0.17 ab	0.30 ab	0.59 a
Genesis	61 abc	nd	0.21 a	0.02 cd	0.01 d	0.34 d
Trailblazer	63 ab	nd	0.17 ab	0.10 abcd	0.16 abcd	0.47 abcd
Hamlet	66 ab	nd	0.18 ab	0.01 d	0.03 cd	0.45 bcd
Milestone	70 a	nd	0.20 a	0.09 abcd	0.13 abcd	0.39 bcd
4 August						
Pontiac	68 b	35 b	0.21 ab	0.02 c	0.04 c	0.46 cd
Highlander	79 ab	62 a	0.23 a	0.04 c	0.09 bc	0.47 bcd
LaSalle	89 a	41 b	0.13 abcd	0.08 bc	0.13 bc	0.45 cd
Prince	78 ab	43 b	0.08 cd	0.16 ab	0.28 ab	0.62 a
Stanley	81 ab	38 b	0.12 bcd	0.11 bc	0.18 bc	0.62 a
Hendrix	80 ab	37 b	0.21 ab	0.01 c	0.02 c	0.44 cd
Patterson	82 ab	42 b	0.13 abcd	0.13 abc	0.21 abc	0.56 abc
Madras	85 ab	46 b	0.02 d	0.25 a	0.42 a	0.60 ab
Genesis	85 ab	42 b	0.23 ab	0.04 bc	0.09 bc	0.44 cd
Trailblazer	87 a	42 b	0.16 abc	0.09 bc	0.14 bc	0.53 abcd
Hamlet	83 ab	46 b	0.17 abc	0.07 bc	0.11 bc	0.40 d

Milestone	90 a	43 b	0.19 abc	0.03 c	0.03 c	0.50 abcd

[1] Means in a column followed by the same letter do not differ based on Tukey's multiple range test at $P > 05$, ns = not significant, nd = no data.

[2] Vegetative indices calculated: NDVI = normalized difference vegetative index, GNDVI = green NDVI (GNDVI), CI = chlorophyll index and PSRI = plant senescence reflectance index.

In the spray-timing trial, the unsprayed treatment had the highest SLB incidence on in 2015 and CP1 had the lowest. Leaf dieback was significantly higher in the unsprayed plots compared to other treatments (Table 5.4). There were no differences in NDVI values on 13 July 2015. The unsprayed control and BOTCAST treatment had the lowest NDVI on 4 August and STEMCAST had the highest NDVI (Table 5.4). Also, there were significant differences in the GNDVI on 13 July but no difference on 4 August. On 13 July, BOTCAST and STEMCAST had the lowest GNDVI and LDR the highest. The NDVI and GNDVI values measure on 13 July were correlated (r= -0.89, *P*=0.0175).

There were significant differences in the CI calculated on 13 July and 4 August. On 13 July, LDR and CP1 had the lowest CI and BOTCAST had the highest. On 4 August, CI was lowest in STEMCAST and CP1 and highest in the control (Table 5.4). The CI values measured on 13 July were correlated with NDVI (r= -0.02, *P*=0.0120) and GNDVI (r= -0.92, *P*=0.0081). The CI values measured on 4 August were only correlated with NDVI (r= -91, *P*=0.0120).

There were significant differences in the PSRI calculated on 13 July and 4 August. On 13 July, PSRI was lowest in STEMCAST, and highest in the control treatment. On 4 August, PSRI was highest in STEMCAST and lowest in BOTCAST (Table 5.4). The PSRI and NDVI values measure on 4 August were correlated (r=0.88, *P*=0.0198).

In 2016, the unsprayed treatment again had a significantly higher SLB incidence compared to the other treatments. There were no differences in leaf dieback among the sprayed treatment. However, there were differences in the vegetative indices measured for each treatment (Table 5.5).

On 25 July, NDVI was lowest in TOMCAST 15R and highest in the control but on 2 August, the unsprayed treatment had the lowest NDVI and TOMCAST 1525 had the highest NDVI. There were no differences in the GNDVI in 2016 (Table 5.5). The NDVI and GNDVI values measured on 25 July were correlated (r= -0.89, *P*=0.0175).

There were differences in the CI values. On 25 July, TOMCAST 15R had the lowest CI and BOTCAST had the highest whereas, on 2 August, TOMCAST 1525 and TOMCAST 15R had the lowest CI and the unsprayed had the highest (Table 5.5). The CI values measured on 25 July correlated with NDVI (r=0.88, *P*=0.0219) and GNDVI (r= -0.93, *P*=0.0081). However, on 2 August, CI values only correlated with NDVI (r= -0.85, *P*=0.0266).

Similar to 2015, PSRI in 2016 was higher in August compared to July. On 25 July, there were significant differences in the PSRI calculated. On 25 July, BOTCAST had the lowest PSRI and TOMCAST 1525 had the highest PSRI. However, there were no

differences in the PSRI measured on 2 August (Table 5.5). The PSRI and NDVI values measured on 2 August were correlated (r=0.79, *P*=0.0321).

Table 5.4 Stemphylium leaf blight incidence, leaf dieback, and vegetative indices measured on onions in a trial sprayed following selected fungicide application programs at the MCRS, Holland Marsh, 2015.

Treatment [1]	Incidence [2]	Leaf dieback	Vegetative indices [3]			
			NDVI	GNDVI	CI	PSRI
13 July						
CP1	59 c	nd	0.08 ns	0.16 ab	0.03 c	0.30 b
TOMCAST 15	65bc	nd	0.10	0.07 b	0.15 ab	0.31 b
BOTCAST	73 ab	nd	0.09	0.07 b	0.15 a	0.34 b
STEMCAST	74 ab	nd	0.09	0.13 ab	0.08 bc	0.29 b
LDR	79 a	nd	0.08	0.21 a	0.04 c	0.39 ab
Unsprayed	85 a	nd	0.09	0.13 ab	0.12 ab	0.47 a
4 August						
CP1	74 c	29 b	0.09 ab	0.06 ns	0.05 c	0.57 ab
TOMCAST	79 bc	29 b	0.09 ab	0.05	0.08 bc	0.58 ab
BOTCAST	85 b	30 b	0.06 b	0.04	0.12 ab	0.46 a
STEMCAST	86 b	36 b	0.10 a	0.05	0.05 c	0.63 b
LDR	90 ab	32 b	0.07 ab	0.05	0.12 ab	0.50 ab
Unsprayed	98 a	49 a	0.06 b	0.04	0.16 a	0.51 ab

[1] Details of treatment in Table 5.3 and chapter 4.

[2] Means in a column followed by the same letter do not differ based on Tukey's multiple range test at $P > 05$, ns = not significant, nd = not data.

[3] Vegetative indices calculated: NDVI = normalized difference vegetative index, GNDVI = green NDVI (GNDVI), CI = chlorophyll index and PSRI = plant senescence reflectance index.

Table 5.5 Stemphylium leaf blight incidence, leaf dieback, and vegetative indices measured on onions in a trial sprayed following selected fungicide application programs at the MCRS, Holland Marsh, 2015.

Treatment [1]	Incidence [2]	Leaf dieback	Vegetative indices [3]			
			NDVI	GNDVI	CI	PSRI
25 July						
TOMCAST 15	7 b	4 ns	0.18 ab	0.27 ns	0.33 a	0.19 ab
TOMCAST 15/25	11 b	7	0.17 b	0.22	0.19 b	0.21 a
CP2	11 b	1	0.18 ab	0.24	0.09 b	0.17 ab
BOTCAST	12 b	6	0.20 ab	0.35	0.34 a	0.15 b
TOMCAST 15R	15 ab	5	0.16 b	0.21	0.08 b	0.19 ab
Control	25 a	10	0.28 a	0.41	0.20 ab	0.17 ab
2 August						
TOMCAST 15	8 b	8 ns	0.21 ab	0.10 ns	0.03 bc	0.62 ns
TOMCAST 1525	12 b	8	0.23 a	0.10	0.02 c	0.64
CP2	12 b	10	0.15 bc	0.12	0.04 ab	0.76
BOTCAST	13 b	9	0.15 bc	0.11	0.04 ab	0.66
TOMCAST 15R	17 ab	12	0.22 ab	0.13	0.02 c	0.77
Control	27 a	17	0.11 c	0.09	0.05 a	0.74

[1] Details of treatment in Table 5.3 and chapter 4

[2] Means in a column followed by the same letter do not differ based on Tukey's multiple range test at $P > 05$, ns = not significant, nm=not measured

[3] Vegetative indices calculated: NDVI = normalized difference vegetative index, GNDVI = green NDVI (GNDVI), CI = chlorophyll index and PSRI = plant senescence reflectance index

There were no differences in the vegetative indices calculated for JPEG and TIFF images of the same area. The vegetative indices calculated for images taken with red filter replaced (Fig. 5.4A) were not different from those with the blue filter replaced (Fig. 5.4B).

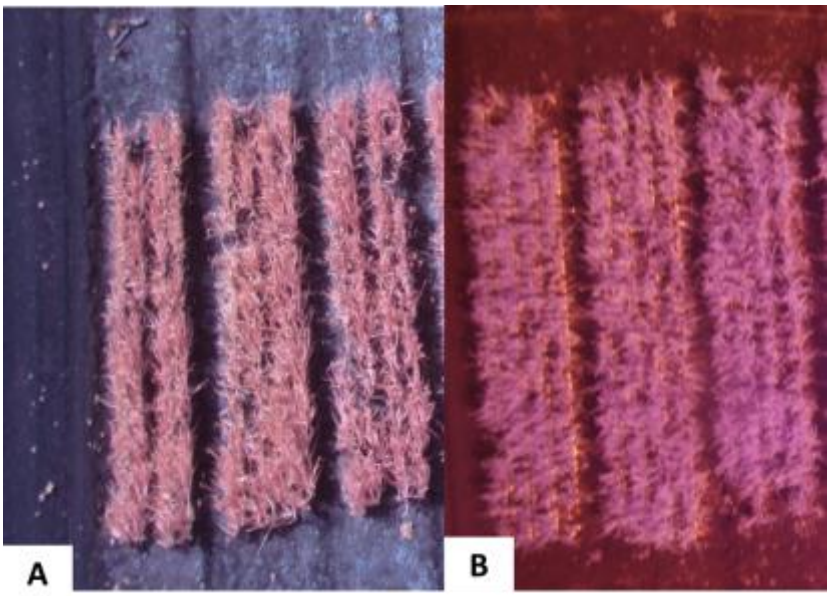

Figure 5.4 Aerial infrared image of onion trials taken on 4 August with a Xnite-Canon SX230NDVI camera with the red-filter replaced (A) and blue-filter with a near-infrared filter at the MCRS, Holland Marsh, 2016.

5.4 Discussion

The current study was the first to assess the potential for using remote sensing to detect SLB incidence on onion. It was expected that onion crops with higher SLB incidence would have lower NDVI, GNDVI, and CI values and higher PSRI values compared to healthy onion or onion crops with lower incidence. However, this trend was

not observed. There were differences in the vegetative indices among the treatments in several of the trials, but there was no significant relationship to levels of SLB. PSRI was expected to be higher for older vegetation than for younger crops (Peñuelas et al. 1994). This trend was observed in the current study at the Holland Marsh.

The incidence of SLB was higher in 2015 compared to 2016. The difference can be attributed to differences in the weather conditions in 2015 and 2016 (Chapters 2 and 3). Incidence differed among onion cultivars (Chapter 3) and the spray-timing programs (Chapter 4), but these differences were not captured by the assessments of the aerial photographs. There were differences, however, in the vegetative indices for some treatments. This variation was similar to that reported from Huanglongbing-infected citrus trees (Li et al. 2012; Garcia-Ruiz et al. 2013). However, in contrast to these earlier studies, the calculated vegetative indices showed no clear relationship with levels of SLB. The observed trends might be associated with other physiological conditions, such as foliage colour, maturation date (Hatfield and Prueger 2010), reflectance from foliage that had received fungicide spray, and even how recently the fungicide had been applied.

Vegetative indices are influenced by crop senescence (Lee et al. 2008; Hatfield and Prueger 2010). Severe SLB on onion causes extensive necrosis (Rao and Pavgi 1975), which increases the rate of senescence. Plant senescence reflectance index is the index most sensitive to plant canopy senescence (Peñuelas et al. 1994). Although there was no relationship between PSRI and SLB incidence, PSRI values in August were consistently higher than in July. This observation was in agreement with previous phenological studies in corn (Hatfield and Prueger 2010) and sunflower (Peñuelas et al.

1994). Differences in PSRI in this study likely were associated with difference in senescence resulting from differences in days to maturity among the cultivars.

In the images taken by the commercial company and provided for use in this study, reflectance information was compressed in a way that could not be split into individual bands (green, red and NIR). In 2015, the images collected by the commercial company were saved in JPEG format. Such images are small and easy to work with, but have a lossy (irreversible) compression that results in loss of data. In 2016, the images were saved in TIFF format, which are larger but retain more data compared to JPEG, but can have both lossy and lossless (reversible) compression formats (Patel and Patel 2014). However, there were no differences between the vegetative indices calculated from JPEG or TIFF images, likely because of lossy compression of the TIFF images.

Stemphylium leaf blight produced profound physiological changes in onion leaves. These changes, together with other factors such as phenology and fungicide application, may have produced the variation in the vegetative indices observed among the treatments. PSRI values were consistently higher in older onion beds. This indicated that changes in the crop canopy could potentially be measured using remote sensing.

Canopy reflectance changes with changes in chlorophyll content (Kumar and Silva 1973). Disease stress leads to a reduction in chlorophyll content, which results in an increase in reflectance in the visible portion of the spectrum and a reduction in reflectance in the NIR portion (Carter 1993; West et al. 2003). However, changes in chlorophyll content are not related exclusively to disease stress (Moshou et al. 2004). The current study indicated that physiological changes in the onion crop caused by SLB can potentially be identified using aerial infrared images. However, early detection of

diseases may be difficult because of the shape of the onion leaves and the dark background of the muck soil. Onion leaf blades are upright (Fritsch and Friesen 2002) and do not form the thick, uniform canopy that might produce more consistent readings from these indices.

Combining UAVs with an effective imaging platform has potential for use in collecting objective data on onion crops. Additional research is needed on use of multispectral cameras to measure the reflectance in the visible region, red-edge and NIR regions of the spectrum. Specific vegetative indices might be developed for SLB using reflectance from two or more spectra (Bock et al. 2010). Reflectance in individual regions might also be more informative for early detection of *S. vesicarium*.

In addition to multispectral cameras, sensors to measure environmental conditions such as solar radiation and cloud cover should be installed on the image platform (camera) to aid in image resolution and interpretation. Another critical recommendation for future studies is the selection of the right image data processing software prior to image acquisition. This will aid in the selection of the right image capturing device and storage formats that will conserve the data.

CHAPTER SIX

GENERAL DISCUSSION

Stemphylium vesicarium, cause of SLB, is a destructive fungal pathogen on Allium crops worldwide. Under high disease pressure, onion crops develop small to no bulbs and yield losses can be up to 90% (Rao and Pavgi 1975; Miller et al. 1978). The disease was first reported on onion in Ontario in 2008, after an earlier report on asparagus (Roddy 2011).

The current research is the first to investigate the epidemiology of SLB on onion in Canada. The seasonal distribution of airborne inoculum was investigated and the relationship between local weather conditions, inoculum availability and SLB incidence. Ascospores and conidia were captured with a volumetric sampler in the Holland Marsh. The concentrations of ascospores were highest at the start of the growing season (late spring) and decreased with increasing temperatures. Conidia were present throughout the growing season. The pseudothecia, which bear ascospores, have been reported to degenerate with increasing temperatures above 15°C and with frequent rainfall (Prados-Ligero et al. 2003). The daily distribution of both conidia and ascospores showed a diurnal pattern, with the highest concentration of spores trapped between 0600-1200 h.

High concentrations of conidia (≈10 conidia m^{-3} air day^{-1}) coincided with the first SLB incidence in both 2015 and 2016. Similar results had been reported on other Allium crops (Suheri and Price 2001; Prados-Ligero et al. 2003; Misawa and Yasuoka 2012). Conidial concentration peaked after SLB symptoms were observed in the field. This was likely because the initial lesions on onion sporulated and produced conidia.

Airborne concentrations of both conidia and ascospores were higher in 2015 compared to 2016. This can be attributed to the warm and wet conditions in 2015. The weather in 2016 was also warm, but with less frequent rainfall events. The incidence and severity of SLB was much higher in 2015 than in 2016, as the moisture created favourable environment for infection and production of conidia.

The presence of ascospores confirmed that the pathogen overwinters in or around the Holland Marsh. No pseudothecia were found on infected onion tissues in this study. Further research is needed to investigate the possible overwintering of the pathogen on other crops and weeds close to onion fields as previous reported for Welsh onion (Misawa and Yasuoka 2012).

Stemphylium vesicarium is reported to have a wide host range with the production host specific toxins during infection (Köhl et al. 2009a; Koike et al. 2013). This study investigated the pathogenicity and aggressiveness of isolates of *S. vesicarium* from asparagus and onion on 12 onion cultivars at the Holland Marsh. Each isolate was confirmed as *S. vesicarium* based on conidial characteristics (Simmons 1969), and primer-specific PCR and DNA sequencing (Câmara et al. 2002). In contrast to previous studies (Singh et al. 2000), the isolates assessed in the current study did not exhibit host or location specificity.

Stemphylium leaf blight causes extensive leaf dieback on onion. The extent of the necrosis is related to host-specific toxins produced by the pathogen (Singh et al. 2000; Wolpert et al. 2002). Isolates from onion in Ontario caused more dieback than isolates from onion in Nova Scotia or isolates from asparagus. These differences among isolates

may be associated with differences in aggressiveness of toxins from the different isolates (Singh et al. 2000).

There was variation in the degree of susceptibility among onion cultivars. The number of initial lesions and percent leaf dieback differed among cultivars in both growth room and field trials. The cultivars were grouped based on SLB severity as low, moderate and high susceptibility based on growth room and field trials. Growth room and field experiment in 2015 were strongly correlated ($r = 0.68$, $P = 0.02$). Pontiac and Hendrix exhibited low susceptibility, Patterson, Prince, Hamlet, Stanley, Genesis, and Braddock were moderately susceptible, and Highlander, Trailblazer, and La Salle were highly susceptible. There was no interaction between cultivar and isolate in the current study, and there was no correlation between the number of initial lesions per leaf and final percentage leaf dieback. A similar lack of correlation between the number of necrotic spots and the final extent of tissue necrosis has been reported on pear (Montesinos et al. 1995a; Pattori et al. 2006).

Although commercial onion cultivars screened in this study were susceptible to SLB, cvs. Highlander and La Salle were the most severely affected and Pontiac, Hendrix and Milestone the least. At present, planting cultivars with lower susceptibility would help in the management of the disease while other options are being explored.

Currently, the strategy for managing SLB on onion is routine application of preventative fungicide. However, development of insensitivity has been reported in *S. vesicarium* following routine applications of certain fungicide groups in Europe and the USA. The effectiveness of selected spray-timing programs in reducing SLB incidence, improving yield, and reducing cost were evaluated. Three spray timing

programs; BOTCAST, TOMCAST (DSV 15, DSV 15/25, DSV 15/Rain), and a modification of existing programs especially for SLB (STEMCAST), were assessed, as well as application based on the first local disease report (LDR), and spray application based on conidia concentration plus crop phenology (CP1 and CP2) were evaluated in 2015 and 2016.

Symptoms of *S. vesicarium* diseases on Allium crops develop 7-14 days after infection (Shishkoff and Lorbeer 1989; Prados-Ligero et al. 1998; Misawa and Yasuoka 2012). In 2015, the first SLB incidence was recorded on 29 June. The earliest fungicide was recommended on 13 June by CP1 and TOMCAST 15. Fungicide application based on recommendations from the other spray-timing treatments started on 29 June, after symptoms were observed, and hence after infection had occurred. In 2016, the first SLB incidence was recorded on 11 July. All of the spray-timing treatments, with the exception of BOTCAST, recommended a fungicide application before initial infection occurred.

In 2015, SLB incidence and the number of lesions per leaf were lower in CP1 and TOMCAST 15 compared to all of the other treatments. This was attributed to the early initiation of fungicide application, which served as a protectant against initial infection. Early application has previously been reported to be critical for management of SLB on onion (Gupta et al. 2010). Under low disease pressure in 2016, there were no differences in incidence or number of lesions between sprayed and unsprayed treatments.

At the end of the season in 2015, severity was only 18-21% lower in treatments that received fungicide compared to the control. In 2016, there were no differences in severity between sprayed and unsprayed treatments. Also, there was no correlation between severity and initial number of lesions observed in either year. These

observations support the previous conclusion that SLB severity was related to toxins produced by the pathogen and not the number of initial lesions. Post-infection application of fungicides has also been reported to have little or no effect on reducing the severity of SLB (Llorente et al. 2000a; Puig et al. 2014).

In New York, isolates of *S. vesicarium* from onion are insensitive to several fungicides (Hoepting 2015). This may explain the low level of SLB suppression observed among sprayed and unsprayed treatments in the current study. There is the need to assess the fungicide sensitivity of *S. vesicarium* present in the Holland Marsh.

The final component of the current study assessed the potential for use of aerial infrared photography to support the IPM program in detecting SLB on onion. Currently, identification of SLB is based on visual assessment, which can be time-consuming and laborious. Recent advancements in the use of unmanned aerial vehicles (UAVs) with imaging platforms have increased the potential of for use in disease detection (Garcia-Ruiz et al. 2013). The appropriate height for acquiring images, the various filters bands, and the appropriate software for image analysis were investigated. Also, four vegetative indices [normalised difference vegetative index (NDVI), green normalised difference vegetative index (GNDVI), chlorophyll index (CI), and plant senescence reflectance index (PSRI)] were computed from acquired images to assess their relationship with the ground visual assessment.

In 2015 and 2016, four sets of images of onion trials at the MCRS were acquired during the growing season and compared to ground visual assessments conducted the same day. Images acquired from lower altitudes (15-20 m) were used for further analysis. There were differences among treatments in the indices, but there was no correlation

between SLB levels and vegetative indices. The differences in the vegetative indices were likely due to differences in canopy characteristics among cultivars and the physiological changes (Hatfield and Prueger 2010) in onion canopy that had received fungicides and those that had not received any fungicides.

Future research should be targeted at monitoring total daily counts of inoculum rather than hourly to set an the conidia threshold that would warrant the application of fungicides. This can be done with a rotorod spore trap or the Burkard spore sampler. Since conidia are available throughout the season, these daily totals would be used to set thresholds for the initiation of fungicide application. Also, the role of overwintering pseudothecia and ascospores in the epidemiology of SLB on onion in the Holland Marsh were not elucidated in the current study. Investigating the source of overwintering pseudothecia and the role of ascospores in the infection on onion might help reduce the overall inoculum level and the secondary production of conidia. The effects of the toxins produced by different isolates of *S. vesicarium* on onion cultivars should also be investigated. This could be used to screen onions for resistance.

There is the need to screen more fungicides for efficacy against SLB. Future research is also needed to assess the fungicide sensitivity of *S. vesicarium* in the Holland Marsh. Also, important weather variables such as rainfall and temperature exceeding 15 °C and LWD exceeding 5 h, which were observed as important for SLB infection on onion in the Holland Marsh should be used to improve upon spray-timing programs for SLB on onion.

Selection of appropriate cameras for aerial imaging and the appropriate software should be considered in future research. Stemphylium leaf blight may be related to the

reflectance in a particular region of the spectrum, so the use of a multispectral camera might increase the potential for early identification of SLB infection.

None of the management strategies evaluated in this study effectively reduced SLB on onion. To minimise the losses caused by SLB, growers are advised to grow cultivars with lower susceptibility, such as Pontiac and Hendrix, where possible. Also, growers should apply fluopyram plus pyrimethanil when onions are at the 3-5 leaf stage to reduce initial infection. Finally, fungicides with different modes of actions should be alternated to reduce the possibility of *S. vesicarium* in the Holland Marsh becoming insensitive to one or more fungicide groups.

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APPENDIX 1: SUPPLEMENTARY TABLES FOR CHAPTER TWO

Table A1. 1 Hourly total ascospores and conidia captured in onion plots at the MCRS, Holland Marsh, ON in 2015

Hour	Total ascospore (spores/m^3 air)	Total conidia (spores/m^3 air)
00-0200	10	21
0200-0400	13	137
0400-0600	74	559
0600-0800	108	849
0800-1000	95	848
1000-1200	26	468
1200-1400	23	276
1400-1600	27	233
1600-1800	32	210
1800-2000	29	199
2000-2200	18	69
2200-2400	15	35
Total	470	3903

Table A1. 2 Total hourly ascospores and conidia captured in onion plots at the MCRS, Holland Marsh, ON in 2016

Hour	Total ascospore (spores/m^3 air)	Total conidia (spores/m^3 air)
00-0200	0	13
0200-0400	3	24
0400-0600	1	60
0600-0800	7	192
0800-1000	7	197
1000-1200	3	172
1200-1400	2	111
1400-1600	0	61
1600-1800	0	36
1800-2000	0	39
2000-2200	0	26
2200-2400	0	5
Total	22	937

Table A1. 3 Seasonal total ascospores and conidia captured in onion plots at the MCRS, Holland Marsh, ON in 2015

Date	Total ascospore (spores/m^3 air)	Total conidia (spores/m^3 air)
20-May	0	0
21-May	0	0
22-May	0	0
23-May	0	8
24-May	5	5
25-May	20	2
26-May	29	3
27-May	16	5
28-May	33	0
29-May	23	2
30-May	8	9
31-May	0	2
1-Jun	51	3
2-Jun	54	6
3-Jun	37	2
4-Jun	21	4
5-Jun	4	8
6-Jun	52	15
7-Jun	16	26
8-Jun	4	8
9-Jun	14	29
10-Jun	5	45
11-Jun	8	39
12-Jun	0	2
13-Jun	13	54
14-Jun	4	35
15-Jun	7	52

16-Jun	3	69
17-Jun	4	62
18-Jun	3	69
19-Jun	4	91
20-Jun	3	55
21-Jun	3	45
22-Jun	3	50
23-Jun	0	62
24-Jun	9	83
25-Jun	0	71
26-Jun	2	84
27-Jun	2	60
28-Jun	1	13
29-Jun	3	65
30-Jun	1	49
1-Jul	0	60
2-Jul	2	48
3-Jul	2	39
4-Jul	0	50
5-Jul	1	34
6-Jul	1	45
7-Jul	0	34
8-Jul	0	71
9-Jul	0	85
10-Jul	0	87
11-Jul	0	97
12-Jul	0	85
13-Jul	0	60
14-Jul	0	75
15-Jul	0	54
16-Jul	0	55

17-Jul	0	60
18-Jul	0	41
19-Jul	0	39
20-Jul	0	40
21-Jul	0	52
22-Jul	0	39
23-Jul	0	33
24-Jul	0	29
25-Jul	0	27
26-Jul	0	36
27-Jul	0	24
28-Jul	0	26
29-Jul	0	21
30-Jul	0	29
31-Jul	0	19
1-Aug	0	21
2-Aug	0	28
3-Aug	0	44
4-Aug	0	52
5-Aug	0	66
6-Aug	0	77
7-Aug	0	55
8-Aug	0	35
9-Aug	0	23
10-Aug	0	20
11-Aug	0	28
12-Aug	0	30
13-Aug	0	26
14-Aug	0	30
15-Aug	0	25
16-Aug	0	18

17-Aug	0	24
18-Aug	0	18
19-Aug	0	21
20-Aug	0	8
21-Aug	0	37
22-Aug	0	23
23-Aug	0	24
24-Aug	0	18
25-Aug	0	20
26-Aug	0	17
27-Aug	0	22
28-Aug	0	12
29-Aug	0	19
30-Aug	0	18
31-Aug	0	14
1-Sep	0	13
2-Sep	0	19
3-Sep	0	14
4-Sep	0	18
5-Sep	0	12
6-Sep	0	15
7-Sep	0	23
8-Sep	0	18
9-Sep	0	15
10-Sep	0	22
11-Sep	0	13
12-Sep	0	19
13-Sep	0	15
14-Sep	0	15
15-Sep	0	6
16-Sep	0	8

Total	470	3903

Table A1. 4 Seasonal total ascospores and conidia captured in onion plots at the MCRS, Holland Marsh, ON in 2016

Date	Total ascospore (spores/m^3 air)	Total conidia (spores/m^3 air)
22-Apr	0	0
23-Apr	6	0
24-Apr	0	0
25-Apr	0	0
26-Apr	6	0
27-Apr	0	0
28-Apr	3	3
29-Apr	0	0
30-Apr	0	3
1-May	0	2
2-May	0	6
3-May	0	3
4-May	0	0
5-May	0	1
6-May	0	0
7-May	0	0
8-May	0	0
9-May	0	5
10-May	0	0
11-May	0	0
12-May	0	4
13-May	0	3
14-May	0	0
15-May	0	0
16-May	0	0
17-May	0	0
18-May	0	0

19-May	0	0
20-May	0	0
21-May	0	0
22-May	0	0
23-May	0	0
24-May	0	0
25-May	0	0
26-May	0	2
27-May	0	3
28-May	0	0
29-May	0	0
30-May	0	0
31-May	0	0
1-Jun	0	3
2-Jun	0	0
3-Jun	0	0
4-Jun	0	0
5-Jun	0	0
6-Jun	4	0
7-Jun	3	0
8-Jun	0	0
9-Jun	0	0
10-Jun	0	0
11-Jun	0	7
12-Jun	0	0
13-Jun	0	0
14-Jun	0	0
15-Jun	0	0
16-Jun	0	0
17-Jun	0	5
18-Jun	0	0

19-Jun	0	0
20-Jun	0	0
21-Jun	0	0
22-Jun	0	7
23-Jun	0	5
24-Jun	0	0
25-Jun	0	8
26-Jun	0	2
27-Jun	0	14
28-Jun	0	14
29-Jun	0	8
30-Jun	0	4
1-Jul	0	2
2-Jul	0	15
3-Jul	0	13
4-Jul	0	13
5-Jul	0	2
6-Jul	0	18
7-Jul	0	21
8-Jul	0	10
9-Jul	0	8
10-Jul	0	18
11-Jul	0	12
12-Jul	0	13
13-Jul	0	10
14-Jul	0	1
15-Jul	0	13
16-Jul	0	26
17-Jul	0	13
18-Jul	0	7
19-Jul	0	12

20-Jul	0	8
21-Jul	0	9
22-Jul	0	8
23-Jul	0	8
24-Jul	0	0
25-Jul	0	3
26-Jul	0	27
27-Jul	0	29
28-Jul	0	22
29-Jul	0	9
30-Jul	0	3
31-Jul	0	6
1-Aug	0	8
2-Aug	0	8
3-Aug	0	13
4-Aug	0	19
5-Aug	0	17
6-Aug	0	6
7-Aug	0	22
8-Aug	0	24
9-Aug	0	19
10-Aug	0	29
11-Aug	0	24
12-Aug	0	15
13-Aug	0	24
14-Aug	0	13
15-Aug	0	6
16-Aug	0	4
17-Aug	0	6
18-Aug	0	7
19-Aug	0	9

20-Aug	0	9
21-Aug	0	9
22-Aug	0	9
23-Aug	0	11
24-Aug	0	18
25-Aug	0	13
26-Aug	0	9
27-Aug	0	10
28-Aug	0	22
29-Aug	0	15
30-Aug	0	8
31-Aug	0	13
1-Sep	0	5
2-Sep	0	10
3-Sep	0	8
4-Sep	0	8
5-Sep	0	6
6-Sep	0	7
Total	22	933

Table A1. 5 Seasonal weather data recorded in onion plots at the MCRS, Holland Marsh, ON in 2015

Date	Temp. (°C)	VPD (Kpa)	LWD (h)	Rain (m)	NVPD (h)	NTemp. (h)	WTemp. (°C)	DTemp. (days)	DVPD (days)	DLWD (days)	TRain (mm)	NRain (days)
20-May	8	0	2	0	11	1	5	0	1	0	0	0
21-May	13	1	0	0	15	10	11	0	1	0	0	0
22-May	9	0	2	0	13	0	13	0	2	0	0	0
23-May	11	1	8	0	15	10	6	0	2	1	0	0
24-May	19	1	0	0	21	16	17	1	2	1	0	0
25-May	20	1	8	1	16	19	21	2	2	2	1	1
26-May	24	1	8	0	15	24	23	3	2	3	0	1
27-May	22	1	3	11	11	24	25	4	2	3	11	2
28-May	19	1	9	0	11	18	19	5	2	4	0	2
29-May	20	1	9	3	15	18	19	6	2	5	16	2
30-May	20	0	2	7	11	20	23	7	2	5	23	3
31-May	9	0	18	7	0	0	13	7	3	6	30	4
1-Jun	12	0	12	0	9	9	9	7	3	7	30	4
2-Jun	14	0	9	0	11	12	13	7	4	7	30	4
3-Jun	15	1	2	0	14	12	15	7	4	7	30	4
4-Jun	17	0	5	0	12	15	16	7	5	6	29	4

5-Jun	17	0	10	15	1	19	19	7	6	6	43	5
6-Jun	14	1	2	0	11	11	15	6	6	6	32	4
7-Jun	15	0	6	0	11	15	14	6	7	6	32	4
8-Jun	20	0	13	29	5	24	19	6	8	6	58	4
9-Jun	18	0	10	2	9	22	19	6	8	7	53	4
10-Jun	19	0	11	1	11	17	18	7	8	7	47	3
11-Jun	18	1	2	0	10	16	19	8	7	6	47	3
12-Jun	15	0	11	29	0	17	18	9	7	6	76	4
13-Jun	18	0	22	1	9	17	16	9	8	7	77	4
14-Jun	17	0	10	12	2	20	19	9	8	8	89	5
15-Jun	21	0	20	0	3	24	19	9	8	8	75	4
16-Jun	20	0	14	6	10	23	22	10	9	9	81	5
17-Jun	17	1	6	6	15	17	18	10	8	9	86	6
18-Jun	19	1	1	0	12	16	18	10	7	8	58	5
19-Jun	17	1	6	0	11	19	19	10	6	8	55	4
20-Jun	17	1	7	0	15	16	15	10	5	8	54	4
21-Jun	21	1	7	0	11	24	21	10	5	9	54	4
22-Jun	20	1	8	7	13	18	19	10	4	9	32	4
23-	20	0	10	33	9	24	23	10	4	9	64	5

Jun 24-Jun	19	1	5	0	12	16	17	10	3	8	52	4
Jun 25-Jun	17	0	8	5	3	18	20	10	3	8	57	5
Jun 26-Jun	18	0	17	0	12	17	17	10	3	8	51	4
Jun 27-Jun	15	0	10	23	3	13	18	10	4	8	68	4
Jun 28-Jun	14	0	20	8	0	9	15	9	5	9	76	5
Jun 29-Jun	19	0	14	0	10	17	16	9	6	9	76	5
Jun 30-Jun	16	0	12	1	0	16	19	9	7	9	77	5
1-Jul	18	0	20	0	3	23	17	9	8	9	77	5
2-Jul	17	1	9	0	12	14	16	9	8	9	70	4
3-Jul	16	0	8	0	11	14	15	9	8	9	37	3
4-Jul	18	1	8	0	12	15	17	9	8	10	37	3
5-Jul	20	0	9	0	13	17	20	9	8	10	32	2
6-Jul	22	1	10	0	12	22	22	9	7	10	32	2
7-Jul	21	0	6	28	4	24	23	9	7	10	37	2
8-Jul	18	0	18	0	10	18	18	10	7	10	29	1
9-Jul	18	0	11	0	10	14	17	10	7	10	29	1
10-Jul	19	1	10	0	13	16	18	10	6	10	28	1
11-Jul	22	1	10	0	13	17	21	10	5	10	28	1
12-Jul	22	1	9	0	14	20	22	10	5	10	28	1
13-Jul	23	1	8	0	14	22	23	10	4	10	28	1
14-Jul	22	0	5	5	8	24	23	10	5	9	33	2
15-Jul	16	0	7	0	10	13	18	10	5	9	33	2
16-Jul	17	0	9	0	12	15	16	10	6	9	33	2
17-Jul	17	0	9	2	0	20	18	10	6	9	6	1

18-Jul	25	0	13	0	12	24	20	10	6	9	6	1
19-Jul	25	1	8	0	12	24	26	10	5	9	6	1
20-Jul	22	1	8	0	15	20	22	10	5	9	6	1
21-Jul	21	1	9	2	13	24	23	10	5	9	8	1
22-Jul	19	1	0	0	13	17	19	10	5	8	8	1
23-Jul	18	1	7	0	14	15	18	10	5	8	8	1
24-Jul	20	1	9	0	14	16	19	10	4	9	3	0
25-Jul	24	1	8	0	12	24	24	10	3	9	3	0
26-Jul	23	1	10	0	14	21	23	10	2	9	3	0
27-Jul	24	1	8	0	13	23	24	10	1	9	2	0
28-Jul	24	1	10	0	13	22	24	10	0	9	2	0
29-Jul	25	1	10	0	14	24	25	10	0	9	2	0
30-Jul	25	1	2	0	21	24	26	10	0	8	2	0
31-Jul	22	1	6	0	16	20	22	10	0	8	0	0
1-Aug	20	1	5	2	13	21	21	10	0	8	2	1
2-Aug	19	0	11	18	8	16	19	10	1	8	21	2
3-Aug	20	1	15	1	11	24	20	10	1	8	22	2
4-Aug	19	0	6	3	8	23	20	10	2	8	25	3
5-Aug	17	0	11	0	10	14	17	10	3	8	25	3
6-Aug	17	0	9	0	11	14	16	10	4	8	25	3
7-Aug	18	0	10	0	11	14	18	10	5	8	25	3
8-Aug	17	0	11	0	9	15	18	10	6	8	25	3
9-Aug	18	1	9	0	12	15	17	10	6	9	25	3
10-Aug	16	0	11	23	4	15	18	10	7	9	47	4
11-Aug	20	0	18	1	9	24	19	10	8	10	45	3
12-Aug	18	0	9	0	10	19	19	10	8	10	27	2
13-Aug	19	0	9	0	11	16	16	10	9	10	26	2
14-	22	0	15	0	4	24	22	10	9	10	24	1

Aug 15-												
Aug	23	1	14	0	10	24	22	10	8	10	24	1
16-												
Aug	24	1	10	0	13	24	23	10	7	10	24	1
17-												
Aug	25	1	8	0	13	24	25	10	6	10	24	1
18-												
Aug	23	0	11	2	6	24	25	10	6	10	26	2
19-												
Aug	24	0	12	0	9	24	23	10	7	10	26	2
20-												
Aug	22	0	13	19	6	24	24	10	7	10	22	2
21-												
Aug	18	0	6	0	9	20	19	10	7	10	22	2
22-												
Aug	17	0	11	0	10	14	16	10	7	10	22	2
23-												
Aug	18	0	11	0	9	15	17	10	7	10	22	2
24-												
Aug	20	0	6	6	12	24	21	10	7	10	27	3
25-												
Aug	16	0	10	1	5	13	17	10	8	10	28	3
26-												
Aug	16	0	7	0	2	13	16	10	9	10	28	3
27-												
Aug	15	0	14	0	5	10	15	10	10	10	28	3
28-												
Aug	17	0	12	0	10	14	15	10	10	10	26	2
29-												
Aug	18	0	11	0	5	16	18	10	10	10	26	2
30-	21	0	14	0	10	24	19	10	10	10	7	1

Aug 31-Aug	22	0	9	0	9	23	21	10	10	10	7	1
1-Sep	23	0	14	0	9	24	23	10	10	10	7	1
2-Sep	24	0	12	0	10	24	23	10	10	10	7	1
3-Sep	23	0	16	1	5	24	24	10	10	10	2	0
4-Sep	22	1	14	0	13	24	22	10	9	10	1	0
5-Sep	23	1	8	0	13	20	21	10	8	10	1	0
6-Sep	24	1	11	0	11	24	24	10	7	10	1	0
7-Sep	26	1	12	0	13	24	25	10	6	10	1	0
8-Sep	25	1	12	0	11	24	26	10	5	10	1	0
9-Sep	21	0	8	7	12	23	25	10	5	10	7	1
10-Sep	17	1	9	0	13	14	17	10	4	10	7	1
11-Sep	16	0	10	1	6	14	17	10	4	10	8	1
12-Sep	13	0	13	5	0	1	15	9	4	10	13	2
13-Sep	12	0	20	7	0	0	12	8	4	10	19	3
14-Sep	15	0	7	0	10	12	13	8	5	10	19	3
15-Sep	19	1	11	0	11	15	16	8	5	10	19	3
16-Sep	21	1	7	0	13	17	21	8	5	10	19	3

Table A1. 6 Seasonal weather data recorded in onion plots at the MCRS, Holland Marsh, ON in 2016

Date	Temp. (°C)	VPD (Kpa)	LWD (h)	Rain (m)	NVPD (h)	NTemp. (h)	WTemp. (°C)	DTemp. (days)	DVPD (days)	DLWD (days)	TRain (mm)	NRain (days)
21-Apr	11	0	1	1	7	2	8	0	1	0	1	0
22-Apr	10	0	22	2	0	0	13	0	2	1	2	0
23-Apr	5	0	13	0	8	0	6	0	3	2	0	0
24-Apr	6	0	0	0	10	0	5	0	4	2	0	0
25-Apr	5	0	6	4	0	0	7	0	5	3	4	1
26-Apr	3	0	20	11	0	0	4	0	6	4	11	2
27-Apr	4	0	16	0	7	0	3	0	7	5	0	2
28-Apr	4	0	8	0	1	0	4	0	8	6	0	2
29-Apr	7	0	4	0	8	0	6	0	9	6	0	2
30-Apr	8	0	9	0	14	0	6	0	10	7	17	2
1-May	8	0	9	3	2	0	10	0	10	8	20	3
2-May	8	0	22	0	4	0	7	0	10	8	18	3
3-May	9	0	11	0	9	0	9	0	10	8	18	3
4-May	11	0	8	0	10	0	10	0	10	9	18	3
5-May	11	0	9	0	8	0	11	0	10	9	14	2
6-May	14	1	6	0	13	9	12	0	9	9	3	1
7-May	11	0	7	0	9	2	14	0	9	9	3	1
8-May	8	0	13	1	2	0	9	0	9	9	4	1

9-May	7	0	12	0	9	0	7	0	9	10	4	1
10-May	9	0	8	0	13	1	7	0	9	10	4	1
11-May	12	1	6	0	13	10	11	0	8	10	1	0
12-May	16	1	5	5	15	12	14	1	7	9	6	1
13-May	16	0	11	19	10	5	18	2	7	9	25	2
14-May	8	0	16	5	0	0	13	2	7	9	31	3
15-May	3	0	1	1	0	0	4	2	7	8	32	3
16-May	8	0	0	8	6	0	5	2	8	7	39	4
17-May	10	0	13	0	9	0	9	2	8	7	39	4
18-May	10	0	4	0	9	0	10	2	8	6	38	4
19-May	14	1	0	0	12	7	13	2	7	5	38	4
20-May	15	1	7	0	13	11	13	2	6	5	38	4
21-May	16	1	4	0	15	10	16	3	6	4	38	4
22-May	16	1	0	0	15	10	17	3	6	4	33	3
23-May	17	1	0	0	15	12	15	3	5	3	14	2
24-May	19	1	6	0	15	13	18	4	4	3	9	1
25-	22	1	0	0	20	16	22	5	3	3	8	1

26-May	20	0	9	1	9	18	21	6	3	4	1	0
27-May	24	1	14	0	14	23	22	7	2	4	1	0
28-May	25	1	9	0	16	20	24	8	1	5	1	0
29-May	25	1	5	0	19	24	26	9	1	5	1	0
30-May	22	1	4	0	19	18	23	10	1	4	1	0
31-May	17	1	8	0	13	12	19	10	1	5	1	0
1-Jun	16	1	8	0	12	10	15	10	1	6	1	0
2-Jun	20	1	9	1	12	13	19	10	1	7	2	0
3-Jun	19	1	10	0	14	13	19	10	1	7	2	0
4-Jun	20	1	8	0	15	16	19	10	1	8	2	0
5-Jun	19	0	12	9	6	12	21	10	1	8	10	1
6-Jun	17	0	13	0	9	8	18	10	2	8	10	1
7-Jun	15	0	10	0	8	2	16	9	3	8	10	1
8-Jun	10	0	0	0	2	0	12	8	4	8	10	1
9-Jun	12	1	1	0	12	5	10	7	4	8	10	1
10-Jun	16	1	4	0	16	12	13	7	4	7	10	1
11-Jun	22	1	6	12	18	16	19	7	4	7	23	2
12-Jun	16	1	0	0	19	3	20	7	4	6	22	2
13-Jun	13	0	0	0	10	0	14	6	5	5	22	2
14-Jun	15	1	8	0	13	11	13	6	5	5	22	2
15-Jun	18	1	7	0	13	12	17	6	4	5	13	1
16-Jun	21	1	16	14	14	14	20	6	3	5	26	2
17-Jun	22	1	11	0	16	16	21	7	2	5	26	2
18-Jun	22	1	8	0	14	15	22	8	1	6	26	2
19-Jun	24	1	10	0	16	17	24	9	1	7	26	2

20-Jun	26	1	0	0	24	24	26	9	1	7	26	2
21-Jun	19	1	0	0	19	14	22	9	1	6	14	1
22-Jun	17	1	13	11	11	9	18	9	1	7	25	2
23-Jun	17	1	10	0	15	13	17	10	0	8	25	2
24-Jun	19	1	16	0	15	14	18	10	0	8	25	2
25-Jun	22	1	11	0	16	17	21	10	0	8	25	2
26-Jun	25	1	7	7	17	19	24	10	0	8	19	2
27-Jun	24	1	14	0	16	24	26	10	0	8	19	2
28-Jun	18	0	11	0	5	13	21	10	1	8	19	2
29-Jun	19	1	6	0	14	14	17	10	1	8	19	2
30-Jun	19	1	8	0	16	15	18	10	1	9	19	2
1-Jul	15	0	10	3	3	7	19	10	2	10	21	3
2-Jul	18	1	20	0	14	12	16	10	2	10	10	2
3-Jul	19	1	9	0	14	15	18	10	2	10	10	2
4-Jul	21	1	10	0	16	15	20	10	2	10	10	2
5-Jul	22	1	12	16	13	16	21	10	2	10	25	3
6-Jul	24	1	23	0	16	18	23	10	2	10	18	2
7-Jul	25	1	24	0	13	24	26	10	2	10	18	2
8-Jul	22	0	17	6	8	23	24	10	2	10	24	3
9-Jul	21	0	13	15	7	24	22	10	3	10	39	4
10-Jul	21	1	15	0	13	15	20	10	3	10	39	4
11-Jul	20	1	12	0	15	16	20	10	2	10	36	3
12-Jul	25	1	4	0	21	24	23	10	2	9	36	3
13-Jul	28	1	0	0	24	24	27	10	2	8	36	3
14-Jul	24	1	13	17	12	24	26	10	2	8	53	4
15-Jul	20	0	8	0	13	16	23	10	3	8	37	3
16-Jul	18	1	10	0	13	12	18	10	3	8	37	3
17-Jul	19	1	8	0	15	16	18	10	3	8	37	3
18-Jul	24	1	4	0	20	21	24	10	2	7	31	2
19-Jul	18	1	7	0	14	13	19	10	1	7	17	1
20-Jul	19	1	10	0	13	13	17	10	1	7	17	1
21-Jul	25	1	11	0	16	17	23	10	1	7	17	1

22-Jul	27	1	10	0	18	24	27	10	1	8	17	1
23-Jul	25	1	18	0	18	21	25	10	1	9	17	1
24-Jul	22	1	17	0	16	17	23	10	1	9	0	0
25-Jul	25	1	22	7	11	24	24	10	0	9	7	1
26-Jul	23	1	12	0	12	19	23	10	0	9	7	1
27-Jul	23	1	13	3	13	17	23	10	0	9	10	2
28-Jul	23	1	16	0	13	22	23	10	0	10	10	2
29-Jul	21	1	9	0	15	20	22	10	0	10	10	2
30-Jul	21	1	0	0	13	16	21	10	0	9	10	2
31-Jul	20	0	10	0	11	17	21	10	1	9	10	2
1-Aug	23	1	17	0	12	24	21	10	1	9	10	2
2-Aug	23	1	11	0	14	16	22	10	1	9	10	2
3-Aug	23	1	9	0	15	17	23	10	1	9	10	2
4-Aug	25	1	9	0	16	18	24	10	1	9	3	1
5-Aug	26	1	9	5	12	24	27	10	1	9	8	2
6-Aug	22	1	13	0	12	17	23	10	1	9	5	1
7-Aug	21	1	24	0	13	14	21	10	1	9	5	1
8-Aug	21	1	11	0	14	14	19	10	1	9	5	1
9-Aug	22	1	9	0	15	16	21	10	1	10	5	1
10-Aug	26	1	5	0	16	23	25	10	0	9	5	1
11-Aug	26	1	11	0	13	20	26	10	0	9	5	1
12-Aug	27	1	13	0	11	24	28	10	0	9	5	1
13-Aug	24	0	17	35	0	24	26	10	1	9	40	2
14-Aug	22	0	20	0	9	23	23	10	2	9	40	2
15-Aug	21	0	11	0	12	17	20	10	3	9	35	1
16-	21	0	15	20	0	23	22	10	4	9	55	2

17-Aug	21	0	24	0	7	16	20	10	5	9	55	2
18-Aug	22	0	24	0	9	18	21	10	6	9	55	2
19-Aug	23	1	24	0	13	17	23	10	6	9	55	2
20-Aug	25	1	24	0	10	24	25	10	6	10	55	2
21-Aug	22	1	19	2	13	20	25	10	6	10	57	2
22-Aug	17	0	20	0	10	10	17	10	7	10	57	2
23-Aug	20	1	15	0	12	16	17	10	6	10	22	1
24-Aug	23	1	14	0	15	17	21	10	5	10	22	1
25-Aug	24	0	11	1	8	24	25	10	5	10	23	1
26-Aug	22	1	14	0	11	17	23	10	4	10	3	0
27-Aug	21	1	11	0	15	16	21	10	3	10	2	0
28-Aug	23	1	8	0	12	22	23	10	2	10	2	0
29-Aug	20	0	13	0	11	14	21	10	3	10	2	0
30-Aug	21	1	11	0	10	16	20	10	3	10	2	0
31-Aug	22	1	12	0	11	21	24	10	3	10	1	0
1-Sep	17	0	9	0	8	11	18	10	3	10	1	0

2-Sep	16	0	7	0	10	9	17	10	4	10	1	0
3-Sep	15	0	10	0	10	10	15	10	5	10	1	0
4-Sep	16	0	10	0	10	10	16	10	5	10	0	0
5-Sep	19	1	11	0	12	14	17	10	5	10	0	0
6-Sep	24	1	8	0	14	17	23	10	5	10	0	0

Table A1. 7 Analysis of variance- Stepwise regression for conidia and weather, 2015

Source	*df*	Mean square	F Value	Pr > F
Model	6	6180.28	21.65	<.0001
Error	113	285.4		
Variable	Estimate	Standard error	F Value	Pr > F
Intercept	-14.64	9.76	2.25	0.1366
Rain	-0.79	0.27	8.34	0.0046
NVPD	0.87	0.45	3.63	0.0592
DTemp	4.53	0.71	40.74	<.0001
DVPD	-2.34	0.70	11.02	0.0012
TRain	1.01	0.15	48.16	<.0001
NRain	-6.25	2.02	9.54	0.0025

Table A1. 8 Analysis of variance -stepwise regression for conidia and weather, 2016

Source	*df*	Mean square	F Value	Pr > F
Model	3	1100.09	32.82	<.0001
Error	135	33.52		
Variable	Estimate	Standard error	F Value	Pr > F
Intercept	-3.76	1.91	3.88	0.0509
LWD	0.18	0.09	4.28	0.0405
DVPD	-0.96	0.17	31.21	<.0001
DLWD	1.52	0.24	41.27	<.0001

Table A1. 9 Analysis of variance -stepwise regression for ascospores and weather, 2015

Source	*df*	Mean square	F Value	Pr > F
Model	2	1631.25	11.57	<.0001
Error	45	141		
Variable	Estimate	Standard error	F Value	Pr > F
Intercept	6.74	4.14	2.64	0.1111
TRain	-0.59	0.12	23.1	<.0001
NRain	8.35	2.09	15.99	0.0002

Table A1. 10 Analysis of variance -stepwise regression for ascospores and weather, 2016

Source	*df*	Mean square	F Value	Pr > F
Model	1	10.40	5.59	0.0223
Error	46	1.86		
Variable	Estimate	Standard error	F Value	Pr > F
Intercept	-0.24	0.36	0.46	0.4995
LWD	0.08	0.04	5.59	0.0223

Table A1. 11 Lesion per leaf recorded on onions in spore trapping plots in 2015

Date	Block	Incidence (%)
29-Jun	1	7
29-Jun	2	6
29-Jun	3	4
29-Jun	4	4
6-Jul	1	9
6-Jul	2	7
6-Jul	3	7
6-Jul	4	9

Table A1. 12 Disease incidence on onions in spore trapping plots in 2015

Date	Block	Incidence (%)
29-Jun	1	26
29-Jun	2	37
29-Jun	3	39
29-Jun	4	35
6-Jul	1	39
6-Jul	2	44
6-Jul	3	45
6-Jul	4	51
13-Jul	1	53
13-Jul	2	53
13-Jul	3	55
13-Jul	4	59
20-Jul	1	76
20-Jul	2	86
20-Jul	3	78
20-Jul	4	86
14-Aug	1	93
14-Aug	2	100
14-Aug	3	94

14-Aug	4	100

Table A1. 13 Leaf dieback on onion in spore trapping plots in 2015

Date	Block	Leaf dieback (%)
20-Jul	1	27
20-Jul	2	20
20-Jul	3	22
20-Jul	4	11
27-Jul	1	38
27-Jul	2	31
27-Jul	3	35
27-Jul	4	26
4-Aug	1	42
4-Aug	2	43
4-Aug	3	44
4-Aug	4	34
14-Aug	1	70
14-Aug	2	68
14-Aug	3	70
14-Aug	4	53

Table A1. 14 Lesion per leaf recorded on onions in spore trapping plots in 2016

Date	Block	Incidence (%)
7-Jul	1	2
7-Jul	2	4
7-Jul	3	0
7-Jul	4	2
11-Jul	1	2
11-Jul	2	5
11-Jul	3	1
11-Jul	4	5
19-Jul	1	3
19-Jul	2	5
19-Jul	3	7
19-Jul	4	5

Table A1. 15 Disease incidence on onions in spore trapping plots in 2016

Date	Block	Incidence (%)
11-Jul	1	12
11-Jul	2	13
11-Jul	3	11
11-Jul	4	11
19-Jul	1	17
19-Jul	2	13
19-Jul	3	14
19-Jul	4	15
25-Jul	1	17
25-Jul	2	14
25-Jul	3	17
25-Jul	4	18
3-Aug	1	23
3-Aug	2	16
3-Aug	3	28
3-Aug	4	19
11-Aug	1	26
11-Aug	2	16
11-Aug	3	31
11-Aug	4	25

Table A1. 16 Leaf dieback on onion in spore trapping plots in 2016

Date	Block	Leaf dieback(%)
19-Jul	1	9
19-Jul	2	5
19-Jul	3	0
19-Jul	4	15
25-Jul	1	14
25-Jul	2	12
25-Jul	3	4
25-Jul	4	24
3-Aug	1	14
3-Aug	2	19
3-Aug	3	13
3-Aug	4	29
11-Aug	1	23
11-Aug	2	31
11-Aug	3	18
11-Aug	4	34

APPENDIX 2: SUPPLEMENTARY TABLES FOR CHAPTER THREE

Table A2. 1 Controlled environment study- Pooled data: the number of lesion per leaf - onion cultivars inoculated with different S. vesicarium isolates. (Data log transformed)

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0.01	0.01	0.69	0.2446
Residual	0.07	0.01	13.27	<.0001
Fixed effects	Num df	Den df	F	Pr > F
Cultivar	12	352	2.19	0.0117
Isolate	4	352	32.9	<.0001
Cultivar*isolate	48	352	1.03	0.4302
Repetition	1	352	0.13	0.7209
Cultivar*repetition	11	352	0.21	0.9971
Inoculum*repetition	4	352	0.09	0.9845
Cultivar*isolate*repetition	44	352	0.15	1.000

Table A2. 2 Controlled environment study- Pooled data: the percentage leaf dieback- onion cultivars inoculated with different *S. vesicarium* isolates. (Data log transformed)

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0.00	0.00	0.67	0.2505
Residual	0.04	0.00	13.29	<.0001
Fixed effects	Num df	Den df	F	Pr > F
Cultivar	12	353	10.45	<.0001
Isolate	4	353	57.07	<.0001
Cultivar*isolate	48	353	1.18	0.207
Repetition	1	353	1.74	0.1883
Cultivar*repetition	11	353	0.36	0.9716
Inoculum*repetition	4	353	0.03	0.9987
Cultivar*isolate*repetition	44	353	0.03	1.000

Table A2. 3 Raw data for controlled environment study - onion cultivars screened against *S. vesicarium* isolates (first repetition)

			Days after inoculation				
			11	21	28	35	42
Cultivar	Inoculum	Block	Lesion	%LDb1	%LDb2	%LDb3	%LDb4
Stanley	OO55	1	8	11	19	42	54
Stanley	OO54	1	4	8	13	29	38
Stanley	OA46	1	9	9	15	33	42
Stanley	OO27	1	17	23	38	86	100
Stanley	NO35	1	6	5	9	19	25
Stanley	Mock	1	0	0	0	0	0
Prince	OO55	1	10	5	9	21	27
Prince	OO54	1	5	12	21	46	60
Prince	OA46	1	12	6	11	25	32
Prince	OO27	1	15	16	28	63	81
Prince	NO35	1	4	7	12	28	36
Prince	Mock	1	0	0	0	0	0
Highlander	OO55	1	11	11	19	43	55
Highlander	OO54	1	5	12	21	47	61
Highlander	OA46	1	9	5	8	19	30
Highlander	OO27	1	7	22	38	86	100
Highlander	NO35	1	2	6	11	13	20
Highlander	Mock	1	0	0	0	0	0
Hendrix	OO55	1	8	7	12	14	22
Hendrix	OO54	1	9	4	7	9	14
Hendrix	OA46	1	14	3	5	6	9
Hendrix	OO27	1	14	13	21	27	41
Hendrix	NO35	1	4	4	6	8	12
Hendrix	Mock	1	0	0	0	0	0
Hamlet	OO55	1	9	13	22	27	42
Hamlet	OO54	1	5	7	12	15	24
Hamlet	OA46	1	14	7	11	14	22
Hamlet	OO27	1	8	13	21	27	41
Hamlet	NO35	1	4	5	9	11	17
Hamlet	Mock	1	0	0	0	0	0

Trailblazer	OO55	1	15	11	21	27	41
Trailblazer	OO54	1	10	8	15	18	39
Trailblazer	OA46	1	15	10	20	25	54
Trailblazer	OO27	1	17	12	24	29	63
Trailblazer	NO35	1	6	8	16	20	44
Trailblazer	Mock	1	0	0	0	0	0
Madras	OO55	1	5	7	14	18	38
Madras	OO54	1	13	10	19	24	51
Madras	OA46	1	10	8	15	34	73
Madras	OO27	1	8	9	18	40	46
Madras	NO35	1	7	4	7	17	19
Madras	Mock	1	0	0	0	0	0
Patterson	OO55	1	13	10	19	43	50
Patterson	OO54	1	15	15	28	64	73
Patterson	OA46	1	10	11	22	50	58
Patterson	OO27	1	16	30	58	67	86
Patterson	NO35	1	3	12	24	54	62
Patterson	Mock	1	0	0	0	0	0
Milestone	OO55	1	18	7	13	29	34
Milestone	OO54	1	8	8	15	33	38
Milestone	OA46	1	12	4	9	19	22
Milestone	OO27	1	11	8	15	35	40
Milestone	NO35	1	6	6	12	26	30
Milestone	Mock	1	0	0	0	0	0
Genesis	OO55	1	7	12	23	51	59
Genesis	OO54	1	9	7	13	29	34
Genesis	OA46	1	10	7	14	26	30
Genesis	OO27	1	6	17	32	60	69
Genesis	NO35	1	5	8	14	27	31
Genesis	Mock	1	0	0	0	0	0
LaSalle	OO55	1	8	6	10	19	21
LaSalle	OO54	1	5	6	10	19	21
LaSalle	OA46	1	6	13	23	43	49
LaSalle	OO27	1	10	15	25	47	54
LaSalle	NO35	1	4	7	11	21	24
LaSalle	Mock	1	0	0	0	0	0
Pontiac	OO55	1	8	3	5	9	10

Pontiac	OO54	1	3	4	7	12	14
Pontiac	OA46	1	12	2	3	6	7
Pontiac	OO27	1	8	12	21	39	45
Pontiac	NO35	1	5	2	3	6	7
Pontiac	Mock	1	0	0	0	0	0
Stanley	OO55	2	4	15	26	48	55
Stanley	OO54	2	2	9	16	29	45
Stanley	OA46	2	4	8	13	25	39
Stanley	OO27	2	8	12	22	40	63
Stanley	NO35	2	3	4	8	14	22
Stanley	Mock	2	0	0	0	0	0
Prince	OO55	2	5	8	14	25	39
Prince	OO54	2	2	6	11	20	31
Prince	OA46	2	7	4	7	13	21
Prince	OO27	2	5	12	21	39	61
Prince	NO35	2	3	4	7	12	19
Prince	Mock	2	0	0	0	0	0
Highlander	OO55	2	3	15	26	48	74
Highlander	OO54	2	4	11	20	37	57
Highlander	OA46	2	8	2	2	5	7
Highlander	OO27	2	8	24	37	70	88
Highlander	NO35	2	1	4	6	11	17
Highlander	Mock	2	0	0	0	0	0
Hendrix	OO55	2	3	9	14	26	40
Hendrix	OO54	2	2	11	18	33	51
Hendrix	OA46	2	7	9	13	25	38
Hendrix	OO27	2	8	5	8	14	22
Hendrix	NO35	2	4	5	8	13	21
Hendrix	Mock	2	0	0	0	0	0
Hendrix	Mock	2	0	0	0	0	0
Hamlet	OO55	2	3	13	20	34	52
Hamlet	OO54	2	2	8	12	21	32
Hamlet	OA46	2	8	7	11	18	28
Hamlet	OO27	2	7	19	29	50	77
Hamlet	NO35	2	1	6	9	16	24
Hamlet	Mock	2	0	0	0	0	0
Trailblazer	OO55	2	2	13	19	33	51

Trailblazer	OO54	2	3	7	10	18	27
Trailblazer	OA46	2	9	10	15	26	40
Trailblazer	OO27	2	8	23	35	59	91
Trailblazer	NO35	2	2	6	9	14	22
Trailblazer	Mock	2	0	0	0	0	0
Madras	OO55	2	3	7	10	18	28
Madras	OO54	2	6	8	12	20	31
Madras	OA46	2	8	3	5	9	14
Madras	OO27	2	9	15	23	39	62
Madras	NO35	2	3	2	4	6	10
Madras	Mock	2	0	0	0	0	0
Patterson	OO55	2	8	7	10	17	27
Patterson	OO54	2	4	9	13	22	36
Patterson	OA46	2	6	4	6	10	16
Patterson	OO27	2	13	18	27	46	73
Patterson	NO35	2	2	4	6	10	16
Patterson	Mock	2	0	0	0	0	0
Milestone	OO55	2	4	7	10	19	31
Milestone	OO54	2	5	6	9	17	28
Milestone	OA46	2	7	3	4	7	12
Milestone	OO27	2	8	17	26	49	79
Milestone	NO35	2	3	4	6	11	18
Milestone	Mock	2	0	0	0	0	0
Genesis	OO55	2	5	6	9	16	26
Genesis	OO54	2	2	9	13	26	41
Genesis	OA46	2	12	2	3	6	9
Genesis	OO27	2	11	12	17	33	53
Genesis	NO35	2	4	4	6	11	18
Genesis	Mock	2	0	0	0	0	0
LaSalle	OO55	2	3	5	7	13	21
LaSalle	OO54	2	4	9	13	26	41
LaSalle	OA46	2	11	3	5	10	16
LaSalle	OO27	2	7	15	22	42	67
LaSalle	NO35	2	3	6	9	18	28
LaSalle	Mock	2	0	0	0	0	0
Pontiac	OO55	2	2	2	4	7	11
Pontiac	OO54	2	2	6	9	16	26

Pontiac	OA46	2	5	4	6	11	18
Pontiac	OO27	2	5	9	14	27	43
Pontiac	NO35	2	1	9	14	26	42
Pontiac	Mock	2	0	0	0	0	0
Stanley	OO55	3	10	4	7	13	20
Stanley	OO54	3	8	2	4	7	11
Stanley	OA46	3	6	3	5	9	14
Stanley	OO27	3	13	9	13	25	40
Stanley	NO35	3	8	4	6	12	20
Stanley	Mock	3	0	0	0	0	0
Prince	OO55	3	8	6	8	16	25
Prince	OO54	3	6	12	18	34	54
Prince	OA46	3	11	4	6	12	18
Prince	OO27	3	16	13	18	35	56
Prince	NO35	3	2	3	4	7	11
Prince	NO35	3	0	0	0	0	0
Highlander	OO55	3	8	9	13	25	40
Highlander	OO54	3	7	7	10	20	40
Highlander	OA46	3	8	9	13	24	48
Highlander	OO27	3	15	19	28	53	97
Highlander	NO35	3	4	6	8	16	28
Highlander	Mock	3	0	0	0	0	0
Hendrix	OO55	3	9	3	4	8	14
Hendrix	OO54	3	5	4	6	15	27
Hendrix	OA46	3	10	3	4	10	18
Hendrix	OO27	3	11	10	14	34	44
Hendrix	NO35	3	2	2	3	7	10
Hendrix	Mock	3	0	0	0	0	0
Hamlet	OO55	3	10	12	17	40	52
Hamlet	OO54	3	9	11	16	37	48
Hamlet	OA46	3	14	6	8	20	25
Hamlet	OO27	3	9	19	28	65	84
Hamlet	NO35	3	7	7	10	23	29
Hamlet	Mock	3	0	0	0	0	0
Trailblazer	OO55	3	11	9	13	29	38
Trailblazer	OO54	3	11	7	11	25	33
Trailblazer	OA46	3	12	6	8	19	25

Trailblazer	OO27	3	7	18	26	61	79
Trailblazer	NO35	3	8	10	15	34	45
Trailblazer	Mock	3	0	0	0	0	0
Madras	OO55	3	11	7	11	25	32
Madras	OO54	3	12	5	7	17	22
Madras	OA46	3	6	5	8	18	24
Madras	OO27	3	8	12	17	40	54
Madras	NO35	3	7	4	6	15	20
Madras	Mock	3	0	0	0	0	0
Patterson	OO55	3	10	7	11	25	35
Patterson	OO54	3	13	4	7	17	24
Patterson	OA46	3	5	5	10	22	30
Patterson	OO27	3	15	26	48	62	85
Patterson	NO35	3	5	8	16	37	47
Patterson	Mock	3	0	0	0	0	0
Milestone	OO55	3	13	6	12	27	35
Milestone	OO54	3	6	4	7	17	21
Milestone	OA46	3	9	6	11	25	33
Milestone	OO27	3	11	12	23	53	68
Milestone	NO35	3	8	3	6	14	18
Milestone	Mock	3	0	0	0	0	0
Genesis	OO55	3	6	7	13	31	40
Genesis	OO54	3	4	10	18	41	53
Genesis	OA46	3	8	7	14	33	42
Genesis	OO27	3	6	18	34	79	100
Genesis	NO35	3	7	8	15	35	45
Genesis	Mock	3	0	0	0	0	0
LaSalle	OO55	3	4	11	21	31	40
LaSalle	OO54	3	10	10	19	28	36
LaSalle	OA46	3	13	12	21	32	41
LaSalle	OO27	3	16	15	27	40	52
LaSalle	NO35	3	7	7	12	18	23
LaSalle	Mock	3	0	0	0	0	0
Pontiac	OO55	3	7	3	5	7	9
Pontiac	OO54	3	8	6	11	16	21
Pontiac	OA46	3	13	6	11	16	21
Pontiac	OO27	3	11	11	20	29	38

Pontiac	NO35	3	4	4	7	11	14
Pontiac	Mock	3	0	0	0	0	0
Stanley	OO55	4	4	15	27	40	49
Stanley	OO54	4	1	11	20	30	36
Stanley	OA46	4	3	7	14	20	24
Stanley	OO27	4	5	20	37	56	67
Stanley	NO35	4	1	15	32	48	57
Stanley	Mock	4	0	0	0	0	0
Prince	OO55	4	4	28	60	89	100
Prince	OO54	4	4	23	50	75	90
Prince	OA46	4	3	6	14	20	25
Prince	OO27	4	6	19	41	61	74
Prince	NO35	4	4	13	28	41	49
Prince	Mock	4	0	0	0	0	0
Highlander	OO55	4	3	30	64	76	85
Highlander	OO54	4	4	36	78	86	100
Highlander	OA46	4	8	17	37	55	65
Highlander	OO27	4	5	36	78	88	98
Highlander	NO35	4	4	19	41	61	74
Highlander	Mock	4	0	0	0	0	0
Hendrix	OO55	4	4	11	23	34	41
Hendrix	OO54	4	5	15	32	48	57
Hendrix	OA46	4	8	8	18	27	33
Hendrix	OO27	4	7	32	69	82	100
Hendrix	NO35	4	1	12	25	38	45
Hendrix	Mock	4	0	0	0	0	0
Hamlet	OO55	4	4	28	60	89	100
Hamlet	OO54	4	3	23	50	75	90
Hamlet	OA46	4	4	13	28	41	49
Hamlet	OO27	4	6	30	64	86	100
Hamlet	NO35	4	2	21	46	68	82
Hamlet	Mock	4	0	0	0	0	0
Trailblazer	OO55	4	3	11	23	34	41
Trailblazer	OO54	4	1	15	32	48	57
Trailblazer	OA46	4	7	8	18	27	33
Trailblazer	OO27	4	9	19	41	61	74
Trailblazer	NO35	4	2	2	5	7	8

Trailblazer	Mock	4	0	0	0	0	0
Madras	OO55	4	5	17	37	55	65
Madras	OO54	4	6	6	14	20	25
Madras	OA46	4	7	15	32	58	69
Madras	OO27	4	7	32	69	84	100
Madras	NO35	4	3	8	18	33	40
Madras	Mock	4	0	0	0	0	0
Patterson	OO55	4	4	19	41	74	89
Patterson	OO54	4	3	8	18	33	40
Patterson	OA46	4	9	8	18	33	40
Patterson	OO27	4	6	23	50	71	89
Patterson	NO35	4	4	4	9	17	20
Patterson	Mock	4	0	0	0	0	0
Milestone	OO55	4	1	15	32	58	69
Milestone	OO54	4	3	4	9	17	20
Milestone	OA46	4	10	4	9	17	20
Milestone	OO27	4	8	21	46	83	99
Milestone	NO35	4	2	4	9	17	20
Milestone	Mock	4	0	0	0	0	0
Genesis	OO55	4	7	17	37	66	79
Genesis	OO54	4	2	19	41	74	89
Genesis	OA46	4	6	7	16	29	35
Genesis	OO27	4	6	14	30	54	64
Genesis	NO35	4	5	7	15	26	32
Genesis	Mock	4	0	0	0	0	0
LaSalle	OO55	4	6	8	17	31	37
LaSalle	OO54	4	1	5	11	20	24
LaSalle	OA46	4	4	8	17	31	37
LaSalle	OO27	4	8	34	72	100	100
LaSalle	NO35	4	1	7	15	26	32
LaSalle	Mock	4	0	0	0	0	0
Pontiac	OO55	4	2	11	24	43	51
Pontiac	OO54	4	1	4	10	17	21
Pontiac	OA46	4	2	4	10	17	21
Pontiac	OO27	4	8	11	25	45	53
Pontiac	NO35	4	0	0	0	0	0
Pontiac	Mock	4	0	0	0	0	0

Table A2. 4 Raw data for controlled environment study - onion cultivars screened against S. vesicarium isolates (second repetition)

Days after inoculation			14	24	31	38	45
Cultivar	Inoculum	Block	Lesion	%LDb1	%LDb2	%LDb3	%LDb4
Stanley	OO55	1	5	9	16	36	46
Stanley	OO54	1	5	7	11	25	32
Stanley	OA46	1	12	7	12	28	36
Stanley	OO27	1	18	19	33	74	95
Stanley	NO35	1	5	4	7	17	21
Stanley	Mock	1	0	0	0	0	0
Prince	OO55	1	13	5	8	18	23
Prince	OO54	1	3	10	18	40	51
Prince	OA46	1	16	6	9	21	27
Prince	OO27	1	18	14	24	54	69
Prince	NO35	1	5	7	12	28	36
Prince	Mock	1	0	0	0	0	0
Highlander	OO55	1	17	11	19	44	56
Highlander	OO54	1	7	13	21	48	62
Highlander	OA46	1	12	5	9	19	30
Highlander	OO27	1	10	23	39	87	91
Highlander	NO35	1	1	6	11	13	21
Highlander	Mock	1	0	0	0	0	0
Hendrix	OO55	1	12	7	12	15	23
Hendrix	OO54	1	11	4	7	9	14
Hendrix	OA46	1	14	3	5	6	9
Hendrix	OO27	1	10	13	22	27	42
Hendrix	NO35	1	5	4	7	9	14
Hendrix	Mock	1	0	0	0	0	0
Hamlet	OO55	1	11	15	26	32	50
Hamlet	OO54	1	7	9	15	18	28
Hamlet	OA46	1	17	8	13	16	25
Hamlet	OO27	1	8	15	25	32	49
Hamlet	NO35	1	4	6	10	13	20
Hamlet	Mock	1	0	0	0	0	0
Trailblazer	OO55	1	16	11	22	27	42

Trailblazer	OO54	1	11	8	15	19	41
Trailblazer	OA46	1	16	11	21	26	55
Trailblazer	OO27	1	18	12	24	30	65
Trailblazer	NO35	1	8	9	17	21	45
Trailblazer	Mock	1	0	0	0	0	0
Madras	OO55	1	3	8	15	18	39
Madras	OO54	1	20	10	19	24	52
Madras	OA46	1	8	8	15	35	75
Madras	OO27	1	5	9	18	41	47
Madras	NO35	1	6	2	5	11	12
Madras	Mock	1	0	0	0	0	0
Patterson	OO55	1	17	6	12	28	32
Patterson	OO54	1	19	9	18	41	47
Patterson	OA46	1	9	7	14	32	37
Patterson	OO27	1	19	19	37	84	97
Patterson	NO35	1	5	8	15	35	40
Patterson	Mock	1	0	0	0	0	0
Milestone	OO55	1	23	4	8	19	22
Milestone	OO54	1	9	5	10	21	25
Milestone	OA46	1	11	3	6	13	14
Milestone	OO27	1	14	5	10	22	26
Milestone	NO35	1	7	4	8	17	20
Milestone	Mock	1	0	0	0	0	0
Genesis	OO55	1	6	8	15	33	38
Genesis	OO54	1	8	4	8	19	22
Genesis	OA46	1	10	5	9	17	20
Genesis	OO27	1	8	11	21	38	44
Genesis	NO35	1	2	6	10	19	22
Genesis	Mock	1	0	0	0	0	0
LaSalle	OO55	1	8	4	7	13	15
LaSalle	OO54	1	3	4	7	13	15
LaSalle	OA46	1	8	9	16	31	35
LaSalle	OO27	1	10	10	18	34	39
LaSalle	NO35	1	4	5	8	15	18
LaSalle	Mock	1	0	0	0	0	0
Pontiac	OO55	1	8	3	5	10	11
Pontiac	OO54	1	2	4	7	14	16

Pontiac	OA46	1	16	2	4	7	8
Pontiac	OO27	1	8	14	24	44	51
Pontiac	NO35	1	5	2	4	7	8
Pontiac	Mock	1	0	0	0	0	0
Stanley	OO55	2	5	17	30	55	63
Stanley	OO54	2	1	10	18	33	51
Stanley	OA46	2	3	9	15	29	44
Stanley	OO27	2	10	14	25	46	72
Stanley	NO35	2	1	5	9	17	26
Stanley	Mock	2	0	0	0	0	0
Prince	OO55	2	8	9	16	29	45
Prince	OO54	2	3	7	12	23	36
Prince	OA46	2	8	5	8	15	24
Prince	OO27	2	3	14	24	45	70
Prince	NO35	2	4	4	7	14	22
Prince	Mock	2	0	0	0	0	0
Highlander	OO55	2	5	17	30	55	85
Highlander	OO54	2	3	13	23	42	66
Highlander	OA46	2	10	2	3	6	9
Highlander	OO27	2	10	31	48	90	100
Highlander	NO35	2	1	5	8	14	22
Highlander	Mock	2	0	0	0	0	0
Hendrix	OO55	2	4	11	18	33	51
Hendrix	OO54	2	2	15	23	42	66
Hendrix	OA46	2	9	11	17	32	49
Hendrix	OO27	2	10	7	11	18	28
Hendrix	NO35	2	5	7	10	17	27
Hendrix	Mock	2	0	0	0	0	0
Hamlet	OO55	2	2	16	26	43	67
Hamlet	OO54	2	1	10	16	27	42
Hamlet	OA46	2	8	9	14	24	37
Hamlet	OO27	2	7	20	31	53	82
Hamlet	NO35	2	1	6	10	17	26
Hamlet	Mock	2	0	0	0	0	0
Trailblazer	OO55	2	2	14	22	36	56
Trailblazer	OO54	2	4	8	12	20	30
Trailblazer	OA46	2	10	11	17	29	45

Trailblazer	OO27	2	8	26	39	65	68
Trailblazer	NO35	2	1	6	9	16	25
Trailblazer	Mock	2	0	0	0	0	0
Madras	OO55	2	2	8	12	20	31
Madras	OO54	2	7	9	13	22	35
Madras	OA46	2	8	4	6	10	16
Madras	OO27	2	11	17	25	43	69
Madras	NO35	2	1	3	4	7	11
Madras	Mock	2	0	0	0	0	0
Patterson	OO55	2	10	7	11	19	30
Patterson	OO54	2	3	10	15	25	40
Patterson	OA46	2	6	4	6	11	17
Patterson	OO27	2	16	20	30	51	81
Patterson	NO35	2	0	4	7	11	18
Patterson	Mock	2	0	0	0	0	0
Milestone	OO55	2	3	8	11	21	34
Milestone	OO54	2	6	7	10	19	31
Milestone	OA46	2	9	3	4	8	13
Milestone	OO27	2	10	19	29	55	87
Milestone	NO35	2	3	4	7	13	20
Milestone	Mock	2	0	0	0	0	0
Genesis	OO55	2	6	6	9	18	29
Genesis	OO54	2	2	10	15	28	46
Genesis	OA46	2	18	2	3	6	10
Genesis	OO27	2	13	13	19	37	59
Genesis	NO35	2	6	4	6	12	20
Genesis	Mock	2	0	0	0	0	0
LaSalle	OO55	2	1	5	8	15	23
LaSalle	OO54	2	5	10	15	28	46
LaSalle	OA46	2	12	4	6	11	18
LaSalle	OO27	2	10	16	24	46	74
LaSalle	NO35	2	2	7	10	20	32
LaSalle	Mock	2	0	0	0	0	0
Pontiac	OO55	2	2	3	4	8	12
Pontiac	OO54	2	2	6	9	18	29
Pontiac	OA46	2	5	4	6	12	20
Pontiac	OO27	2	6	10	16	30	47

Pontiac	NO35	2	1	10	15	29	46
Pontiac	Mock	2	0	0	0	0	0
Stanley	OO55	3	13	5	7	14	22
Stanley	OO54	3	6	3	4	7	12
Stanley	OA46	3	5	3	5	10	16
Stanley	OO27	3	15	10	15	28	45
Stanley	NO35	3	9	5	7	14	23
Stanley	Mock	3	0	0	0	0	0
Prince	OO55	3	9	7	10	18	29
Prince	OO54	3	10	14	20	39	62
Prince	OA46	3	16	5	7	13	21
Prince	OO27	3	16	14	21	40	64
Prince	NO35	3	3	3	4	8	13
Prince	Mock	3	0	0	0	0	0
Highlander	OO55	3	10	10	15	29	46
Highlander	OO54	3	8	8	12	23	45
Highlander	OA46	3	5	10	14	27	55
Highlander	OO27	3	18	22	32	60	62
Highlander	NO35	3	3	6	9	18	32
Highlander	Mock	3	0	0	0	0	0
Hendrix	OO55	3	9	3	5	9	16
Hendrix	OO54	3	3	5	7	17	31
Hendrix	OA46	3	6	3	5	11	20
Hendrix	OO27	3	9	11	17	39	50
Hendrix	NO35	3	3	2	4	8	11
Hendrix	Mock	3	0	0	0	0	0
Hamlet	OO55	3	12	13	20	46	60
Hamlet	OO54	3	9	12	18	42	55
Hamlet	OA46	3	17	7	10	22	29
Hamlet	OO27	3	10	22	32	74	96
Hamlet	NO35	3	8	8	11	26	33
Hamlet	Mock	3	0	0	0	0	0
Trailblazer	OO55	3	11	10	14	34	44
Trailblazer	OO54	3	12	9	12	29	38
Trailblazer	OA46	3	15	6	9	22	28
Trailblazer	OO27	3	7	20	30	69	90
Trailblazer	NO35	3	6	11	17	39	51

Trailblazer	Mock	3	0	0	0	0	0
Madras	OO55	3	12	8	12	29	37
Madras	OO54	3	12	6	8	19	25
Madras	OA46	3	5	6	9	20	28
Madras	OO27	3	6	13	19	45	62
Madras	NO35	3	10	5	7	17	23
Madras	Mock	3	0	0	0	0	0
Patterson	OO55	3	11	9	12	29	40
Patterson	OO54	3	12	4	7	16	21
Patterson	OA46	3	5	5	9	20	27
Patterson	OO27	3	17	23	43	58	60
Patterson	NO35	3	6	8	14	33	42
Patterson	Mock	3	0	0	0	0	0
Milestone	OO55	3	18	6	11	25	31
Milestone	OO54	3	8	3	6	15	19
Milestone	OA46	3	12	5	10	23	29
Milestone	OO27	3	13	11	20	47	61
Milestone	NO35	3	10	3	5	13	16
Milestone	Mock	3	0	0	0	0	0
Genesis	OO55	3	5	6	12	28	36
Genesis	OO54	3	3	9	16	37	47
Genesis	OA46	3	10	7	12	29	37
Genesis	OO27	3	7	16	30	71	90
Genesis	NO35	3	10	7	13	31	40
Genesis	Mock	3	0	0	0	0	0
LaSalle	OO55	3	4	10	19	28	36
LaSalle	OO54	3	10	9	17	25	32
LaSalle	OA46	3	16	10	19	29	37
LaSalle	OO27	3	15	13	24	36	46
LaSalle	NO35	3	12	6	11	16	21
LaSalle	Mock	3	0	0	0	0	0
Pontiac	OO55	3	10	2	4	6	8
Pontiac	OO54	3	6	5	10	15	19
Pontiac	OA46	3	16	5	10	15	19
Pontiac	OO27	3	13	10	18	26	34
Pontiac	NO35	3	7	4	7	10	13
Pontiac	Mock	3	0	0	0	0	0

Stanley	OO55	4	4	13	24	36	44
Stanley	OO54	4	2	10	18	27	33
Stanley	OA46	4	3	7	12	18	22
Stanley	OO27	4	4	18	34	50	60
Stanley	NO35	4	1	11	25	37	44
Stanley	Mock	4	0	0	0	0	0
Prince	OO55	4	3	21	46	69	82
Prince	OO54	4	4	18	39	58	70
Prince	OA46	4	4	5	11	16	19
Prince	OO27	4	3	15	32	47	57
Prince	NO35	4	4	10	21	32	38
Prince	Mock	4	0	0	0	0	0
Highlander	OO55	4	3	23	50	74	88
Highlander	OO54	4	3	28	60	66	72
Highlander	OA46	4	11	13	28	42	51
Highlander	OO27	4	7	28	60	63	76
Highlander	NO35	4	4	15	32	47	57
Highlander	Mock	4	0	0	0	0	0
Hendrix	OO55	4	2	8	18	26	32
Hendrix	OO54	4	7	11	25	37	44
Hendrix	OA46	4	7	7	14	21	25
Hendrix	OO27	4	7	25	53	79	95
Hendrix	NO35	4	2	9	19	29	35
Hendrix	Mock	4	0	0	0	0	0
Hamlet	OO55	4	4	21	46	69	82
Hamlet	OO54	4	2	18	39	58	70
Hamlet	OA46	4	4	10	21	32	38
Hamlet	OO27	4	6	23	50	74	88
Hamlet	NO35	4	2	16	35	53	63
Hamlet	Mock	4	0	0	0	0	0
Trailblazer	OO55	4	3	8	18	26	32
Trailblazer	OO54	4	1	11	25	37	44
Trailblazer	OA46	4	6	7	14	21	25
Trailblazer	OO27	4	8	15	32	47	57
Trailblazer	NO35	4	2	2	4	5	6
Trailblazer	Mock	4	0	0	0	0	0
Madras	OO55	4	8	13	28	42	51

Madras	OO54	4	6	5	11	16	19
Madras	OA46	4	5	11	25	45	53
Madras	OO27	4	9	25	53	96	100
Madras	NO35	4	3	7	14	25	31
Madras	Mock	4	0	0	0	0	0
Patterson	OO55	4	3	15	32	57	69
Patterson	OO54	4	2	7	14	25	31
Patterson	OA46	4	11	7	14	25	31
Patterson	OO27	4	9	18	39	70	84
Patterson	NO35	4	3	3	7	13	15
Patterson	Mock	4	0	0	0	0	0
Milestone	OO55	4	1	11	25	45	53
Milestone	OO54	4	4	3	7	13	15
Milestone	OA46	4	6	3	7	13	15
Milestone	OO27	4	9	16	35	64	76
Milestone	NO35	4	2	3	7	13	15
Milestone	Mock	4	0	0	0	0	0
Genesis	OO55	4	7	13	28	51	61
Genesis	OO54	4	2	15	32	57	69
Genesis	OA46	4	9	6	12	22	27
Genesis	OO27	4	5	11	23	41	50
Genesis	NO35	4	6	5	11	20	24
Genesis	Mock	4	0	0	0	0	0
LaSalle	OO55	4	6	6	13	24	28
LaSalle	OO54	4	1	4	8	15	18
LaSalle	OA46	4	3	6	13	24	28
LaSalle	OO27	4	10	26	56	60	72
LaSalle	NO35	4	1	5	11	20	24
LaSalle	Mock	4	0	0	0	0	0
Pontiac	OO55	4	2	9	18	33	40
Pontiac	OO54	4	1	3	7	13	16
Pontiac	OA46	4	2	3	7	13	16
Pontiac	OO27	4	8	9	19	34	41
Pontiac	NO35	4	7	3	6	11	14
Pontiac	Mock	4	0	0	0	0	0

Table A2. 5 2015 field trials: ANOVA of incidence on onion cultivars

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0.83	1.96	0.42	0.3357
Residual	33.01	7.89	4.18	<.0001
Fixed effects	Num df	Den df	F	Pr > F
Cultivar	11	35	4.41	.0004

Table A2. 6 Raw data field trials- 2015 data: incidence - onion cultivars

		Assessment date				
Cultivar	Block	29June	06July	13July	20July	14August
Stanley	1	33	41	57	82	92
Prince	1	31	43	54	91	95
Highlander	1	24	33	45	62	74
Hendrix	1	33	41	57	61	83
Hamlet	1	48	60	70	100	100
Trailblazer	1	24	47	65	98	100
Madras	1	39	47	57	61	82
Patterson	1	31	43	52	71	92
Milestone	1	58	62	70	87	100
Genesis	1	51	56	65	79	93
LaSalle	1	26	39	53	76	93
Braddock	1	.	.	.	.	.
Pontiac	1	14	26	44	57	70
Stanley	2	39	45	54	64	82
Prince	2	40	49	59	76	89
Highlander	2	29	45	51	64	81
Hendrix	2	45	55	63	78	88
Hamlet	2	54	61	67	73	92
Trailblazer	2	28	46	67	82	86
Madras	2	53	53	63	86	100
Patterson	2	49	56	65	70	83
Milestone	2	61	64	70	94	100
Genesis	2	31	41	54	74	80
LaSalle	2	37	44	53	86	100
Pontiac	2	24	37	49	63	70
Stanley	3	27	42	54	73	87
Prince	3	29	39	49	66	79
Highlander	3	41	55	61	86	96
Hendrix	3	35	45	53	72	86
Hamlet	3	41	54	65	79	83
Trailblazer	3	23	45	57	81	96
Madras	3	46	53	59	74	93
Patterson	3	41	50	55	69	83

Milestone	3	54	61	68	87	91
Genesis	3	46	49	60	81	100
LaSalle	3	39	45	55	78	94
Pontiac	3	27	41	52	66	84
Stanley	4	27	43	56	79	95
Prince	4	32	46	58	75	78
Highlander	4	39	54	63	78	98
Hendrix	4	39	51	57	73	93
Hamlet	4	43	52	61	85	88
Trailblazer	4	29	54	63	80	97
Madras	4	48	52	57	79	93
Patterson	4	47	54	62	80	100
Milestone	4	56	64	70	89	100
Genesis	4	47	57	64	84	100
LaSalle	4	35	51	59	86	100
Pontiac	4	30	48	58	68	81

Table A2. 7 2016 field trials: ANOVA of incidence in onion cultivars

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	35.63	8.77	4.06	<.0001
Fixed effects	Num df	Den df	F	Pr > F
Cultivar	10	32	2.93	.0010

Table A2. 8 Raw data field trials- 2016 data: incidence - onion cultivars

		Assessment date				
Cultivar	Block	11July	19July	25July	03August	11August
Stanley	1	17	18	26	29	37
Prince	1	13	17	20	20	29
Highlander	1	12	16	20	20	21
Hendrix	1	2	9	14	22	23
Hamlet	1	17	17	23	31	39
Trailblazer	1	9	17	25	31	35
Braddock	1	13	21	27	30	30
Patterson	1	15	17	20	20	28
Milestone	1	18	25	31	37	41
Genesis	1	9	13	19	28	28
Madras	1	.	.	.	.	.
Pontiac	1	.	.	.	.	.
LaSalle	1	12	17	17	23	26
Stanley	2	12	16	23	29	31
Prince	2	16	19	24	25	25
Highlander	2	15	21	23	29	35
Hendrix	2	5	7	9	10	10
Hamlet	2	0	3	3	8	12
Trailblazer	2	14	22	29	30	34
Braddock	2	16	21	21	26	29
Patterson	2	12	16	19	24	26
Milestone	2	21	28	36	39	44
Genesis	2	5	14	24	29	32
Madras	2	.	.	.	.	.
Pontiac	2	.	.	.	.	.
LaSalle	2	13	13	14	16	16
Stanley	3	8	14	20	22	26
Prince	3	11	13	23	27	34
Highlander	3	8	14	17	22	29
Hendrix	3	3	6	11	16	24
Hamlet	3	14	19	25	29	32
Trailblazer	3	16	20	27	29	33
Braddock	3	12	14	23	23	25

Patterson	3	4	9	13	13	15
Milestone	3	16	23	28	33	35
Genesis	3	8	13	19	20	20
Madras	3	.	.	.	.	.
Pontiac	3	.	.	.	.	.
LaSalle	3	11	14	17	28	31
Stanley	4	15	20	27	27	27
Prince	4	8	11	21	24	30
Highlander	4	11	19	26	26	27
Hendrix	4	4	8	12	17	23
Hamlet	4	18	22	27	31	36
Trailblazer	4	9	16	20	30	34
Braddock	4	9	14	19	27	27
Patterson	4	13	20	23	27	27
Milestone	4	14	25	28	32	37
Genesis	4	18	23	23	29	31
Madras	4	.	.	.	.	.
Pontiac	4	.	.	.	.	.
LaSalle	4	11	15	18	19	25

Table A2. 9 Raw data field studies- 2015: lesions per leaf - onion cultivars

		Assessment date	
Cultivar	Block	29June	06July
Stanley	1	4	7
Prince	1	4	7
Highlander	1	4	5
Hendrix	1	4	7
Hamlet	1	5	6
Trailblazer	1	3	7
Madras	1	3	4
Patterson	1	4	6
Milestone	1	7	9
Genesis	1	4	5
LaSalle	1	7	9
Braddock	1	.	.
Pontiac	1	3	5
Stanley	2	5	6
Prince	2	4	5
Highlander	2	4	6
Hendrix	2	4	6
Hamlet	2	4	8
Trailblazer	2	4	8
Madras	2	4	6
Patterson	2	5	5
Milestone	2	7	9
Genesis	2	4	6
LaSalle	2	6	7
Braddock	2	.	.
Pontiac	2	3	5
Stanley	3	6	7
Prince	3	6	8
Highlander	3	5	7
Hendrix	3	4	6
Hamlet	3	4	6
Trailblazer	3	3	3
Madras	3	5	7

Patterson	3	4	6
Milestone	3	7	8
Genesis	3	5	6
LaSalle	3	4	7
Braddock	3	.	.
Pontiac	3	3	5
Stanley	4	4	7
Prince	4	4	7
Highlander	4	4	7
Hendrix	4	4	7
Hamlet	4	5	8
Trailblazer	4	3	8
Madras	4	5	7
Patterson	4	5	7
Milestone	4	8	10
Genesis	4	4	7
LaSalle	4	4	9
Pontiac	4	3	5

Table A2. 10 Raw data field studies- 2016: lesions per leaf - onion cultivars

		Assessment dates		
Cultivar	Block	07July	11July	19July
Stanley	1	0	1	3
Prince	1	0	4	4
Highlander	1	0	1	3
Hendrix	1	0	2	3
Hamlet	1	2	4	5
Trailblazer	1	0	5	8
Braddock	1	0	2	7
Patterson	1	1	3	4
Milestone	1	4	4	8
Genesis	1	0	1	5
Madras	1	.	.	.
Pontiac	1	.	.	.
LaSalle	1	2	2	3

Stanley	2	1	2	5
Prince	2	0	3	3
Highlander	2	4	4	5
Hendrix	2	0	1	5
Hamlet	2	0	0	3
Trailblazer	2	7	7	7
Braddock	2	2	5	5
Patterson	2	2	2	3
Milestone	2	5	5	5
Genesis	2	0	1	5
Madras	2	.	.	.
Pontiac	2	.	.	.
LaSalle	2	4	5	5
Stanley	3	0	3	6
Prince	3	0	2	4
Highlander	3	2	2	3
Hendrix	3	0	2	3
Hamlet	3	4	5	6
Trailblazer	3	3	5	8
Braddock	3	4	7	9
Patterson	3	0	5	6
Milestone	3	1	5	8
Genesis	3	1	3	6
Madras	3	.	.	.
Pontiac	3	.	.	.
LaSalle	3	0	1	7
Stanley	4	4	4	6
Prince	4	1	5	5
Highlander	4	1	2	5
Hendrix	4	0	1	5
Hamlet	4	2	5	6
Trailblazer	4	0	6	6
Braddock	4	1	4	7
Patterson	4	4	4	5
Milestone	4	4	7	7
Genesis	4	1	5	6
Madras	4	.	.	.

Pontiac	4	.	.	.
LaSalle	4	2	5	5

Table A2. 11 Raw data field studies- 2015: leaf dieback on onion cultivars

		Assessment dates			
Cultivar	Block	20July	27July	04August	14August
Stanley	1	10	18	37	56
Prince	1	19	32	38	50
Highlander	1	30	50	60	78
Hendrix	1	12	33	39	46
Hamlet	1	29	35	41	50
Trailblazer	1	7	33	44	62
Madras	1	15	56	64	72
Patterson	1	24	35	43	49
Milestone	1	14	31	42	42
Genesis	1	11	32	49	58
LaSalle	1	27	38	42	70
Braddock	1	.	.	.	.
Pontiac	1	9	20	31	55
Stanley	2	22	41	41	61
Prince	2	13	26	43	55
Highlander	2	32	56	65	87
Hendrix	2	13	25	38	48
Hamlet	2	24	37	45	67
Trailblazer	2	7	21	37	69
Madras	2	13	27	27	44
Patterson	2	23	33	43	56
Milestone	2	13	28	50	53
Genesis	2	12	23	45	55
LaSalle	2	20	31	43	68
Braddock	2	.	.	.	.
Pontiac	2	12	23	36	51
Stanley	3	7	27	40	55
Prince	3	10	36	45	55
Highlander	3	39	56	64	79
Hendrix	3	12	24	42	52
Hamlet	3	33	45	50	61
Trailblazer	3	16	28	43	53
Madras	3	21	32	47	49

Patterson	3	18	33	44	61
Milestone	3	11	25	40	61
Genesis	3	13	24	34	47
LaSalle	3	22	35	44	70
Braddock	3	.	.	.	.
Pontiac	3	12	24	36	50
Stanley	4	11	25	33	60
Prince	4	17	30	44	64
Highlander	4	31	47	60	86
Hendrix	4	7	24	27	68
Hamlet	4	31	42	47	52
Trailblazer	4	13	32	44	52
Madras	4	18	37	45	53
Patterson	4	10	29	38	61
Milestone	4	15	31	39	57
Genesis	4	18	29	39	59
LaSalle	4	11	26	34	53
Pontiac	4	13	26	37	58

Table A2. 12 Raw data field studies- 2016: leaf dieback on onion cultivars

		Assessment dates			
Cultivar	Block	19July	25July	03August	11August
Stanley	1	3	8	13	21
Prince	1	0	3	5	13
Highlander	1	0	19	26	41
Hendrix	1	0	4	8	10
Hamlet	1	7	17	23	33
Trailblazer	1	2	9	20	26
Braddock	1	0	0	14	19
Patterson	1	10	13	17	22
Milestone	1	0	7	16	17
Genesis	1	12	15	25	25
Madras	1		.	.	.
Pontiac	1		.	.	.
LaSalle	1	9	14	14	23
Stanley	2	5	14	17	27
Prince	2	8	13	15	24
Highlander	2	9	19	23	29
Hendrix	2	0	4	8	21
Hamlet	2	0	0	12	19
Trailblazer	2	7	15	18	26
Braddock	2	4	14	20	33
Patterson	2	9	11	18	22
Milestone	2	8	13	18	25
Genesis	2	0	0	3	15
Madras	1		.	.	.
Pontiac	1		.	.	.
LaSalle	2	5	12	19	31
Stanley	3	0	0	6	15
Prince	3	0	3	10	33
Highlander	3	2	12	20	24
Hendrix	3	4	7	19	24
Hamlet	3	13	22	30	28
Trailblazer	3	4	11	25	33
Braddock	3	7	16	18	25

Patterson	3	0	5	13	20
Milestone	3	5	9	9	17
Genesis	3	7	16	22	27
Madras	1		.	.	.
Pontiac	1		.	.	.
LaSalle	3	0	4	13	18
Stanley	4	10	17	17	26
Prince	4	0	5	8	14
Highlander	4	6	10	23	34
Hendrix	4	0	0	5	11
Hamlet	4	8	19	23	25
Trailblazer	4	3	9	23	29
Braddock	4	0	8	19	22
Patterson	4	4	10	10	17
Milestone	4	8	13	17	20
Genesis	4	2	8	23	27
Madras	1		.	.	.
Pontiac	1		.	.	.
LaSalle	4	15	24	29	34

Table A2. 13 Raw data field studies- 2015 data: yield - onion cultivars

Cultivar	Block	Mkt yield (tha^{-1})	% Mkb	%Jumbo	%Medium	%small
Stanley	1	65	96.55	2.30	94.25	3.45
Prince	1	43.3	91.76	1.18	90.59	8.24
Highlander	1	74	92.52	3.74	88.79	7.48
Hendrix	1	50.1	70.00	3.00	67.00	30.00
Hamlet	1	59.15	94.25	8.05	86.21	5.75
Trailblazer	1	62.05	95.10	7.84	87.25	4.90
Madras	1	51.75	95.24	2.86	92.38	4.76
Patterson	1	62.5	90.48	7.14	83.33	9.52
Milestone	1	58.6	92.16	6.86	85.29	7.84
Genesis	1	56.3	93.59	6.41	87.18	6.41
LaSalle	1	39	75.38	6.15	69.23	24.62
Pontiac	1	54	98.72	10.26	88.46	1.28
Stanley	2	49.7	86.96	11.59	75.36	13.04
Prince	2	65.3	96.47	16.47	80.00	3.53
Highlander	2	56.75	94.68	3.19	91.49	5.32
Hendrix	2	45.6	52.50	2.50	50.00	47.50
Hamlet	2	48.5	85.87	2.17	83.70	14.13
Trailblazer	2	47	84.62	7.69	76.92	15.38
Madras	2	56.55	91.36	6.17	85.19	8.64
Patterson	2	70.75	96.04	7.92	88.12	3.96
Milestone	2	53.15	96.61	18.64	77.97	3.39
Genesis	2	46.5	77.33	2.67	74.67	22.67
LaSalle	2	64.25	93.86	7.89	85.96	6.14
Pontiac	2	64.1	91.59	13.08	78.50	8.41
Stanley	3	43.4	94.00	22.00	72.00	6.00
Prince	3	74.05	95.24	3.57	91.67	4.76
Highlander	3	58	91.78	5.48	86.30	8.22
Hendrix	3	57.05	92.11	4.39	87.72	7.89
Hamlet	3	52.5	90.22	2.17	88.04	9.78
Trailblazer	3	47.9	92.86	3.57	89.29	7.14
Madras	3	45.6	86.42	7.41	79.01	13.58
Patterson	3	63.05	97.30	4.50	92.79	2.70
Milestone	3	56.65	97.30	12.16	85.14	2.70
Genesis	3	33.4	77.14	2.86	74.29	22.86
LaSalle	3	63.95	93.64	2.73	90.91	6.36
Pontiac	3	53.2	94.05	3.57	90.48	5.95
Stanley	4	61.75	94.57	6.52	88.04	5.43
Prince	4	57.85	92.31	4.40	87.91	7.69
Highlander	4	49.55	96.74	1.09	95.65	3.26
Hendrix	4	54.5	83.13	8.43	74.70	16.87
Hamlet	4	67.45	95.37	2.78	92.59	4.63

Trailblazer	4	43.9	85.07	1.49	83.58	14.93
Madras	4	59.7	93.20	3.88	89.32	6.80
Patterson	4	57.35	87.18	17.95	69.23	12.82
Milestone	4	45.5	97.30	2.70	94.59	2.70
Genesis	4	35.95	92.68	14.63	78.05	7.32
LaSalle	4	30.5	78.43	7.84	70.59	21.57
Pontiac	4	37.85	87.30	3.17	84.13	12.70

Table A2. 14 Raw data field studies- 2016 data: yield - onion cultivars

Cultivar	Block	Mkt yield (tha^{-1})	% Mkb	%Jumbo	%Medium	%small
Braddock	1	27.45	72.34	10.64	61.70	27.66
Braddock	2	26.75	71.15	0.00	71.15	28.85
Genesis	1	49.50	90.48	12.70	77.78	9.52
Genesis	2	32.85	73.85	4.62	69.23	26.15
Hamlet	1	40.00	71.62	6.76	64.86	28.38
Hamlet	2	32.20	84.29	0.00	84.29	15.71
Hamlet	3	28.65	78.26	0.00	78.26	21.74
Hendrix	1	34.50	92.54	2.99	89.55	7.46
Hendrix	2	36.90	88.24	25.49	62.75	11.76
Hendrix	3	41.30	83.02	22.64	60.38	16.98
Highlander	1	32.60	81.58	0.00	81.58	18.42
Highlander	2	38.60	77.03	4.05	72.97	22.97
LaSalle	1	28.20	83.33	0.00	83.33	16.67
LaSalle	2	26.90	80.85	8.51	72.34	19.15
LaSalle	3	49.50	90.48	12.70	77.78	9.52
Milestone	1	47.70	81.25	7.50	73.75	18.75
Milestone	2	43.90	85.71	17.46	68.25	14.29
Patterson	1	34.15	69.86	2.74	67.12	30.14
Patterson	2	26.15	76.81	0.00	76.81	23.19
Patterson	3	23.20	64.86	0.00	64.86	35.14
Prince	1	31.75	71.43	3.17	68.25	28.57
Prince	2	32.00	77.63	0.00	77.63	22.37
Prince	3	29.70	81.67	1.67	80.00	18.33
Prince	4	28.20	80.70	8.77	71.93	19.30
Stanley	1	22.90	76.00	0.00	76.00	24.00
Stanley	2	35.60	75.00	6.25	68.75	25.00
Stanley	3	27.30	78.43	9.80	68.63	21.57
Trailblazer	1	31.75	88.31	0.00	88.31	11.69
Trailblazer	2	36.70	81.16	2.90	78.26	18.84
Trailblazer	3	23.00	68.12	0.00	68.12	31.88
Trailblazer	4	35.55	80.88	4.41	76.47	19.12

N.B: Missing data due to poor emergence

APPENDIX 3: SUPPLEMENTARY TABLES FOR CHAPTER FOUR

Table A3. 1 Analysis of variance – Overall incidence (AUDPC) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	34.88	13.18	2.65	0.0041
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	1.05	.
treatment	5	14	12.04	0.0001

Table A3. 2 Raw data- spray-timing comparison - 2015 incidence

		Assessment date				
Treatment		02July	09July	16July	31July	AUDPC
BOTCAST	1	56	63	85	89	84
TOMCAST 15	1	36	46	73	75	67
STEMCAST	1	59	67	87	89	86
CP1	1	27	39	64	69	58
LDR	1	63	68	89	89	88
UNSPRAYED	1	66	70	92	94	92
BOTCAST	2	48	54	76	80	74
TOMCAST 15	2	32	38	63	77	61
STEMCAST	2	52	62	83	83	80
CP1	2	34	46	72	84	68
LDR	2	72	73	95	94	95
UNSPRAYED	2	58	68	93	100	92
BOTCAST	3	42	50	74	83	72
TOMCAST 15	3	38	48	76	81	71
STEMCAST	3	43	45	72	78	69
CP1	3	36	36	64	70	60
LDR	3	54	62	83	87	82
UNSPRAYED	3	59	69	91	97	91
BOTCAST	4	55	63	87	87	84
TOMCAST 15	4	41	52	80	83	74
STEMCAST	4	53	63	86	92	84
CP1	4	34	43	66	73	63
LDR	4	47	56	82	88	79
UNSPRAYED	4	59	69	94	100	93

Table A3. 3 Analysis of variance – Overall incidence (AUDPC) - spray-timing comparison, 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	34.88	13.18	2.65	0.0041
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	1.05	.
treatment	5	14	12.04	0.0001

Table A3. 4 Raw data- spray-timing comparison - 2016 incidence

		Assessment dates			
Treatment	Block	11July	19July	25July	09August
BOTCAST	1	0	0	17	30
TOMCAST 15	1	0	0	6	19
TOMCAST 1525	1	0	5	15	26
TOMCAST 15R	1	4	4	8	13
CP2	1	3	9	20	22
UNSRAYED	1	7	9	32	39
BOTCAST	2	0	0	11	19
TOMCAST 15	2	0	2	15	16
TOMCAST 1525	2	0	2	11	21
TOMCAST 15R	2	0	8	16	27
CP2	2	3	5	14	26
UNSRAYED	2	0	12	19	24
BOTCAST	3	0	0	10	16
TOMCAST 15	3	0	0	0	8
TOMCAST 1525	3	0	0	8	12
TOMCAST 15R	3	4	7	18	25
CP2	3	0	4	7	9
UNSRAYED	3	0	13	17	39
BOTCAST	4	0	4	8	13
TOMCAST 15	4	0	0	6	15
TOMCAST 1525	4	0	0	9	14
TOMCAST 15R	4	6	8	17	23
CP2	4	0	0	3	8
UNSRAYED	4	4	17	31	35

Table A3. 5 Analysis of variance – Lesions per leaf - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.56	0.21	2.65	0.0041
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	2.07	.
treatment	5	14	14.14	<.0001

Table A3. 6 Analysis of variance –overall severity (AUDPC) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	23.60	8.92	2.65	0.0041
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	5.08	.
treatment	5	14	11.31	0.0002

Table A3. 7 Raw data- spray-timing comparison – 2015 lesions per leaf and severity.

			Severity assessment dates				
Treatment	Block	Lesions	23July	31July	06August	13August	AUDPC
BOTCAST	1	8	6	15	21	27	17
TOMCAST							
15	1	4	10	17	23	30	20
STEMCAST	1	7	7	16	24	33	20
CP1	1	4	7	18	25	38	21
LDR	1	9	18	23	31	43	29
UNSPRAYED	1	7	22	30	40	61	39
BOTCAST	2	5	14	21	34	57	31
TOMCAST							
15	2	4	9	20	34	50	28
STEMCAST	2	7	23	42	52	64	46
CP1	2	4	14	21	34	57	31
LDR	2	6	11	21	32	45	27
UNSPRAYED	2	7	23	42	52	64	46
BOTCAST	3	6	8	25	34	42	28
TOMCAST							
15	3	4	11	20	31	47	27
STEMCAST	3	6	13	19	32	45	27
CP1	3	4	12	21	21	45	24
LDR	3	7	11	18	30	48	26
UNSPRAYED	3	7	34	45	52	62	51
BOTCAST	4	7	11	28	31	45	29
TOMCAST							
15	4	4	10	18	28	42	24
STEMCAST	4	7	16	28	34	44	31
CP1	4	4	15	27	35	43	31
LDR	4	7	16	29	35	43	32

UNSPRAYED	4	6	31	44	51	72	51

Table A3. 8 Analysis of variance – Lesions per leaf - spray-timing comparison, 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	2.94	1.08	2.74	0.0031
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	0.55	.
treatment	5	15	3.59	0.0247

Table A3. 9 Analysis of variance – overall severity (AUDPC) - spray-timing comparison, 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	40.21	14.69	2.74	0.0031
Fixed effects	Num df	Den df	F	Pr > F
Block	3	0	0.81	.
Treatment	5	15	1.96	0.1442

Table A3.10 Raw data- spray-timing comparison – 2016 lesions per leaf and severity

			Severity assessments dates				
Treatment	Block	Lesions	25July	02August	09August	16August	AUDPC
BOTCAST	1	0	0	7	28	40	19
TOMCAST 15	1	0	0	5	23	33	16
TOMCAST 1525	1	1	10	10	18	28	18
TOMCAST 15R	1	2	0	7	17	20	12
CP2	1	6	5	13	23	23	18
UNSRAYED	1	4	18	27	43	57	39
BOTCAST	2	0	7	8	17	23	15
TOMCAST 15	2	4	5	12	20	35	18
TOMCAST 1525	2	0	7	7	22	27	16
TOMCAST 15R	2	3	0	10	18	25	14
CP2	2	4	0	10	23	30	17
UNSRAYED	2	4	5	3	25	45	19
BOTCAST	3	0	15	18	18	30	22
TOMCAST 15	3	0	0	5	15	25	11
TOMCAST 1525	3	0	7	7	10	22	11
TOMCAST 15R	3	2	3	15	22	22	17
CP2	3	1	0	7	18	20	12
UNSRAYED	3	5	12	30	35	47	34
BOTCAST	4	1	0	3	12	18	8
TOMCAST 15	4	0	10	10	15	22	15
TOMCAST 1525	4	0	5	7	13	20	12
TOMCAST 15R	4	6	15	15	20	27	21
CP2	4	0	0	10	28	38	20
UNSRAYED	4	3	3	8	13	25	13

Table A3. 11 Analysis of variance – Marketable yield (tha^{-1}) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	69.02	25.2034	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.95	.
Treatment	5	15	0.38	0.8562

Table A3. 12 Analysis of variance – Yield (percentage jumbo) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	107.05	39.09	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.11	.
Treatment	5	15	0.71	0.6269

Table A3. 13 Analysis of variance – Yield (% medium) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	100.94	36.86	2.74	0.003
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.43	.
Treatment	5	15	0.89	0.51

Table A3. 14 Analysis of variance – Yield (% culls) - spray-timing comparison, 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	6.28	2.29	2.74	0.003
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.61	.
Treatment	5	15	1.83	0.17

Table A3. 15 Raw data – yield, spray-timing comparison, 2015

Treatment	Block	Yield (t ha^{-1})	Bulb size distribution (%) Jumbo	Medium	small
BOTCAST	1	46	28	66	6
TOMCAST 15	1	46	21	73	6
STEMCAST	1	49	24	69	6
CP1	1	54	30	64	7
LDR	1	42	26	70	4
UNSPRAYED	1	48	18	80	2
BOTCAST	2	48	30	62	8
TOMCAST 15	2	44	29	60	11
STEMCAST	2	49	22	73	5
CP1	2	56	41	52	7
LDR	2	31	23	69	8
UNSPRAYED	2	40	18	73	8
BOTCAST	3	42	11	81	8
TOMCAST 15	3	42	10	83	7
STEMCAST	3	53	44	52	4
CP1	3	44	32	57	11
LDR	3	35	19	71	10
UNSPRAYED	3	46	32	66	2
BOTCAST	4	50	40	53	7
TOMCAST 15	4	43	40	49	11
STEMCAST	4	34	23	74	2
CP1	4	24	25	67	8
LDR	4	51	22	69	9
UNSPRAYED	4	35	11	78	11

Table A3. 16 Analysis of variance – Marketable yield (t ha^{-1}) - spray-timing comparison, 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	19.6861	7.1884	2.74	0.003
Fixed effects	**Num df**	**Den df**	**F value**	**Pr > F**
Block	3	0	1.02	.

Treatment	5	15	1.03	0.44

Table A3. 17 Analysis of variance – Yield (percentage jumbo) - spray-timing comparison - 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	2.1528	0.7861	2.74	0.003
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.38	.
Treatment	5	15	0.95	0.48

Table A3. 18 Analysis of variance – Yield (percentage medium) - spray-timing comparison - 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	76.85	28.06	2.74	0.003
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.07	.
Treatment	5	15	1.04	0.43

Table A3. 19 Analysis of variance – Yield (percentage culls) - spray-timing comparison - 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	88.94	32.48	2.74	0.003
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	0.09	.
Treatment	5	15	0.75	0.60

Table A3. Raw data- yield, spray-timing comparison, 2016

Treatment	Block	Yield ($t\ ha^{-1}$)	Bulb size distribution (%)		
			Jumbo	Medium	Small
BOTCAST	1	70	0	65	35
TOMCAST 15	1	83	4	59	37
TOMCAST 1525	1	76	0	64	36
TOMCAST 15R	1	75	2	52	47
CP2	1	87	1	68	30
UNSRAYED	1	83	0	78	22
BOTCAST	2	81	1	63	36
TOMCAST 15	2	83	0	58	42
TOMCAST 1525	2	84	1	63	36
TOMCAST 15R	2	83	2	59	39
CP2	2	84	1	62	37
UNSRAYED	2	89	3	73	23
BOTCAST	3	90	0	76	24
TOMCAST 15	3	78	0	59	41
TOMCAST 1525	3	77	0	61	39
TOMCAST 15R	3	88	5	73	22
CP2	3	87	0	70	30
UNSRAYED	3	68	0	43	57
BOTCAST	4	87	0	72	28
TOMCAST 15	4	76	1	53	46
TOMCAST 1525	4	80	2	58	40
TOMCAST 15R	4	79	0	58	42
CP2	4	91	0	71	29
UNSRAYED	4	79	0	61	39

APPENDIX 4: SUPPLEMENTARY TABLES FOR CHAPTER FIVE

Table A4. 1 Analysis of variance – Cultivar disease incidence (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	20.43	5.03	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.60	.
cultivar	11	33	5.46	<.0001

Table A4. 2 Analysis of variance – Cultivar NDVI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	126.99	.
cultivar	11	33	5.60	<.0001

Table A4. 3 Analysis of variance – Cultivar GNDVI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	23.78	.
cultivar	11	33	8.00	<.0001

Table A4. 4 Analysis of variance – Cultivar CI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	18.33	.
cultivar	11	33	8.00	<.0001

Table A4. 5 Analysis of variance – Cultivar PSRI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	432.46	.
cultivar	11	33	8.00	<.0001

Table A4. 6 Raw data- Cultivar-Remote Sensing (13 July, 2015)

Cultivar	Block	IncJy13	NDVIjy13	GNDVIjy13	PSRIjy13	CIjy13
Stanley	1	57	0.0403	0.0964	0.2100	0.1642
Prince	1	54	0.0415	0.0971	0.2392	0.1687
Highlander	1	45	0.0998	0.0179	0.1790	0.0202
Hendrix	1	57	0.1026	0.0103	0.1889	0.0094
Hamlet	1	70	0.1052	0.0005	0.2213	0.0090
Trailblazer	1	65	0.0817	0.0466	0.2306	0.0772
Madras	1	57	0.0566	0.0830	0.2910	0.1451
Patterson	1	52	0.0697	0.0647	0.2484	0.1116
Milestone	1	70	0.0808	0.0396	0.1882	0.0631
Genesis	1	65	0.1032	0.0077	0.1673	0.0026
LaSalle	1	53	0.0820	0.0279	0.1888	0.0437
Pontiac	1	44	0.0920	0.0237	0.1817	0.0355
Stanley	2	54	0.0453	0.1014	0.43	0.1692
Prince	2	59	0.0465	0.1021	0.4884	0.1737
Highlander	2	51	0.0616	0.0229	0.368	0.0252
Hendrix	2	63	0.0747	0.0153	0.3878	0.0144
Hamlet	2	67	0.0858	0.0055	0.4526	0.014
Trailblazer	2	67	0.0867	0.0516	0.4712	0.0822
Madras	2	63	0.087	0.088	0.592	0.1501
Patterson	2	65	0.097	0.0697	0.5068	0.1166
Milestone	2	70	0.1048	0.0446	0.3864	0.0681
Genesis	2	54	0.1076	0.0127	0.3446	0.0076
LaSalle	2	53	0.1082	0.0329	0.3876	0.0487
Pontiac	2	49	0.1102	0.0287	0.3734	0.0405
Stanley	3	54	0.0906	0.4056	0.86	0.3384
Prince	3	49	0.093	0.4084	0.9768	0.3474
Highlander	3	61	0.1232	0.0916	0.736	0.0504
Hendrix	3	53	0.1494	0.0612	0.7756	0.0288
Hamlet	3	65	0.1716	0.022	0.9052	0.028
Trailblazer	3	57	0.1734	0.2064	0.9424	0.1644
Madras	3	59	0.174	0.352	1.184	0.3002
Patterson	3	55	0.194	0.2788	1.0136	0.2332
Milestone	3	68	0.2096	0.1784	0.7728	0.1362
Genesis	3	60	0.2152	0.0508	0.6892	0.0152
LaSalle	3	55	0.2164	0.1316	0.7752	0.0974
Pontiac	3	52	0.2204	0.1148	0.7468	0.081
Stanley	4	56	0.1812	0.2028	0.215	0.6768
Prince	4	58	0.186	0.2042	0.2442	0.6948
Highlander	4	63	0.2464	0.0458	0.184	0.1008
Hendrix	4	57	0.2988	0.0306	0.1939	0.0576
Hamlet	4	61	0.3432	0.011	0.2263	0.056
Trailblazer	4	63	0.3468	0.1032	0.2356	0.3288

Madras	4	57	0.348	0.176	0.296	0.6004
Patterson	4	62	0.388	0.1394	0.2534	0.4664
Milestone	4	70	0.4192	0.0892	0.1932	0.2724
Genesis	4	64	0.4304	0.0254	0.1723	0.0304
LaSalle	4	59	0.4328	0.0658	0.1938	0.1948
Pontiac	4	58	0.4408	0.0574	0.1867	0.162

Table A4. 7 Analysis of variance – Cultivar disease incidence (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	49.44	12.17	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.68	.
cultivar	11	33	2.74	0.0124

Table A4. 8 Analysis of variance – Cultivar NDVI (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	70.05	.
cultivar	11	33	8.00	<.0001

Table A4. 9 Analysis of variance – Cultivar GNDVI (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	19.50	.
Cultivar	11	33	8.00	<.0001

Table A4. 10 Analysis of variance – Cultivar CI (August 04) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.01	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	19.60	.
Cultivar	11	33	8.00	<.0001

Table A4. 11 Analysis of variance – Cultivar PSRI (August 04) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	4.06	<.0001
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	535.61	.
Cultivar	11	33	8.00	<.0001

Table A4. 12 Raw data- Cultivar-Remote Sensing (04 August, 2015)

Cultivar	Block	IncAug4	NDVIag4	GNDVIag4	PSRIag4	CIag4
Stanley	1	84	0.0535	0.0488	0.3034	0.0848
Prince	1	87	0.0349	0.0771	0.3033	0.1365
Highlander	1	66	0.1131	0.0147	0.2292	0.0407
Hendrix	1	76	0.0982	0.0027	0.2135	0.0042
Hamlet	1	92	0.0819	0.0308	0.1971	0.0503
Trailblazer	1	92	0.0752	0.0401	0.2583	0.0672
Madras	1	74	0.0038	0.1197	0.2952	0.2032
Patterson	1	84	0.0584	0.0604	0.2765	0.1026
Milestone	1	92	0.0913	0.0084	0.2454	0.0093
Genesis	1	85	0.1103	0.0176	0.2134	0.0423
LaSalle	1	86	0.0607	0.0339	0.2190	0.0591
Pontiac	1	62	0.1027	0.004	0.2262	0.0145
Stanley	2	74	0.0585	0.0538	0.6168	0.0898
Prince	2	81	0.0399	0.0821	0.6166	0.1415
Highlander	2	73	0.1181	0.0197	0.4684	0.0457
Hendrix	2	80	0.1032	0.0077	0.437	0.0092
Hamlet	2	84	0.0869	0.0358	0.4042	0.0553
Trailblazer	2	78	0.0802	0.0451	0.5266	0.0722
Madras	2	92	0.0088	0.1247	0.6004	0.2082
Patterson	2	75	0.0634	0.0654	0.563	0.1076
Milestone	2	92	0.0963	0.0134	0.5008	0.0143
Genesis	2	72	0.1153	0.0226	0.4368	0.0473
LaSalle	2	92	0.0657	0.0389	0.448	0.0641
Pontiac	2	62	0.1077	0.009	0.4624	0.0195
Stanley	3	79	0.117	0.1076	1.2336	0.1796
Prince	3	71	0.0798	0.1642	1.2332	0.283
Highlander	3	88	0.2362	0.0394	0.9368	0.0914
Hendrix	3	78	0.2064	0.0154	0.874	0.0184
Hamlet	3	75	0.1738	0.0716	0.8084	0.1106
Trailblazer	3	88	0.1604	0.0902	1.0532	0.1444
Madras	3	86	0.0176	0.2494	1.2008	0.4164
Patterson	3	76	0.1268	0.1308	1.126	0.2152
Milestone	3	83	0.1926	0.0268	1.0016	0.0286
Genesis	3	92	0.2306	0.0452	0.8736	0.0946
LaSalle	3	86	0.1314	0.0778	0.896	0.1282
Pontiac	3	76	0.2154	0.018	0.9248	0.039
Stanley	4	87	0.234	0.2152	0.3084	0.3592
Prince	4	71	0.1596	0.3284	0.3083	0.566
Highlander	4	90	0.4724	0.0788	0.2342	0.1828
Hendrix	4	85	0.4128	0.0308	0.2185	0.0368
Hamlet	4	81	0.3476	0.1432	0.2021	0.2212
Trailblazer	4	89	0.3208	0.1804	0.2633	0.2888

Madras	4	86	0.0352	0.4988	0.3002	0.8328
Patterson	4	92	0.2536	0.2616	0.2815	0.4304
Milestone	4	92	0.3852	0.0536	0.2504	0.0572
Genesis	4	92	0.4612	0.0904	0.2184	0.1892
LaSalle	4	92	0.2628	0.1556	0.224	0.2564
Pontiac	4	73	0.4308	0.036	0.2312	0.078

Table A4. 13 Analysis of variance – Spray-timings- disease incidence (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	30.51	11.14	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.26	.
Treatment	5	15	11.28	0.0001

Table A4. 14 Analysis of variance – Spray-timings NDVI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	8.72	.
Treatment	5	15	1.28	0.3252

Table A4. 15 Analysis of variance – Spray-timings GNDVI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	3.29	.
Treatment	5	15	4.30	0.0125

Table A4. 16 Analysis of variance – Spray-timings CI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	10.82	.
Treatment	5	15	13.40	<.0001

Table A4. 17 Analysis of variance – Spray-timings PSRI (July 13) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	42.12	.
Treatment	5	15	7.06	0.0014

Table A4. 18 Raw data - Spray-timing trails- Cultivar-Remote Sensing (13 July, 2015)

Treatment	Block	Incjy13	NDVIjy13	GNDVIjy13	PSRIjy13	CIjy13
BOTCAST	1	77	0.1025	0.0583	0.2094	0.1113
TOMCAST	1	65	0.0965	0.0965	0.2024	0.1066
STEMCAST	1	79	0.0812	0.1935	0.1964	0.0591
CP1	1	57	0.0693	0.2791	0.2136	0.0365
LDR	1	81	0.0690	0.2697	0.2476	0.0273
Unsprayed	1	84	0.0846	0.1693	0.256667	0.0828
BOTCAST	2	68	0.0887	0.1203	0.242533	0.1261
TOMCAST	2	55	0.0924	0.0991	0.2013	0.1227
STEMCAST	2	75	0.0731	0.1497	0.181433	0.0711
CP1	2	64	0.0613	0.1739	0.179267	0.0179
LDR	2	87	0.0511	0.1509	0.266833	0.0369
Unsprayed	2	85	0.0609	0.0935	0.3667	0.0821
BOTCAST	3	66	0.0740	0.0291	0.4288	0.1163
TOMCAST	3	68	0.0824	0.0483	0.414733	0.1116
STEMCAST	3	64	0.0865	0.0967	0.4028	0.0641
CP1	3	57	0.0902	0.1395	0.4372	0.0415
LDR	3	75	0.0963	0.1349	0.505267	0.0323
Unsprayed	3	83	0.1031	0.0847	0.523333	0.1425
BOTCAST	4	79	0.1069	0.0601	0.495067	0.2421
TOMCAST	4	72	0.1087	0.0495	0.4126	0.2454
STEMCAST	4	78	0.1030	0.0616	0.372867	0.1422
CP1	4	58	0.0979	0.0574	0.368533	0.0357
LDR	4	74	0.1023	0.2697	0.540333	0.0737
Unsprayed	4	86	0.1219	0.1693	0.736733	0.1642

Table A4. 19 Analysis of variance – Spray-timings incidence (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	21.73	7.94	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.16	.
Treatment	5	15	12.52	<.0001

Table A4. 20 Analysis of variance – Spray-timings NDVI (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.72	.
Treatment	5	15	6.98	0.0015

Table A4. 21 Analysis of variance – Spray-timings GNDVI (August 4) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.27	.
Treatment	5	15	0.40	0.8410

Table A4. 22 Analysis of variance – Spray-timings CI (August 04) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	16.52	.
Treatment	5	15	10.97	0.0001

Table A4. 23 Analysis of variance – Spray-timings PSRI (August 04) – remote sensing 2015

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	123.87	.
Treatment	5	15	3.96	0.0173

Table A4. 24 Raw data-Spray-timing trails- Remote Sensing (04 August, 2015)

Cultivar	Block	IncAug4	NDVIag4	GNDVIag4	PSRIag4	CIag4
BOTCAST	1	89	0.0672	0.0469	0.250	0.1050
TOMCAST	1	75	0.0821	0.0315	0.277	0.0514
STEMCAST	1	89	0.0977	0.0161	0.272	0.0369
CP1	1	69	0.0851	0.0245	0.245	0.0386
LDR	1	89	0.0536	0.0635	0.226	0.0545
Unsprayed	1	94	0.0458	0.0734	0.220	0.0819
BOTCAST	2	80	0.0512	0.0628	0.354	0.0923
TOMCAST	2	77	0.0867	0.0288	0.487	0.0655
STEMCAST	2	83	0.0874	0.0200	0.567	0.0367
CP1	2	84	0.0912	0.051867	0.507	0.0456
LDR	2	94	0.0740	0.0365	0.437	0.1119
Unsprayed	2	100	0.0670	0.021067	0.456	0.1293
BOTCAST	3	83	0.0722	0.029533	0.510	0.1100
TOMCAST	3	81	0.0871	0.068533	0.563	0.0564
STEMCAST	3	78	0.1027	0.0784	0.555	0.0419
CP1	3	70	0.0901	0.067833	0.500	0.0436
LDR	3	87	0.0586	0.0338	0.462	0.0877
Unsprayed	3	97	0.0508	0.024967	0.449	0.1607
BOTCAST	4	87	0.0562	0.0235	0.715	0.1847
TOMCAST	4	83	0.0917	0.051833	0.976	0.1309
STEMCAST	4	92	0.0924	0.0936	1.135	0.0735
CP1	4	73	0.0962	0.103733	1.015	0.0911
LDR	4	88	0.0968	0.073	0.873	0.2238
Unsprayed	4	100	0.0842	0.042133	0.912	0.2587

Table A4. 25 Analysis of variance – Spray-timings- disease incidence (July 25) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	31.57	11.53	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.40	.
Treatment	5	15	4.84	0.0078

Table A4. 26 Analysis of variance – Spray-timings NDVI (July 25) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	7.17	.
Treatment	5	15	3.60	0.0244

Table A4. 27 Analysis of variance – Spray-timings GNDVI (July 25) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.03	0.01	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	9.42	.
Treatment	5	15	0.79	0.5743

Table A4. 28 Analysis of variance – Spray-timings CI (July 25) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	3.70	.
Treatment	5	15	13.94	<.0001

Table A4. 29 Analysis of variance – Spray-timings PSRI (July 25) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	3.70	.
Treatment	5	15	13.94	<.0001

Table A4. 30 Raw data Spray-timing trails- Remote Sensing (25 July, 2016)

Treatment	Block	incjy25	NDVIjy25	GNDVIjy25	PSRIjy25	CIjy25
BOTCAST	1	17	0.1288	0.1461	0.0834	0.3710
TOM15	1	6	0.1352	0.0455	0.0922	0.3553
TOM1525	1	15	0.1410	0.0754	0.0908	0.1970
TOM15R	1	8	0.1504	0.1511	0.0817	0.1216
CP2	1	20	0.1611	0.2180	0.0753	0.0909
Unsprayed	1	32	0.1670	0.2107	0.0732	0.2760
BOTCAST	2	11	0.1698	0.1323	0.1180	0.4204
TOM15	2	15	0.1609	0.0940	0.1622	0.4090
TOM1525	2	11	0.1530	0.0774	0.1891	0.2370
TOM15R	2	16	0.1076	0.0963	0.1691	0.0596
CP2	2	14	0.1283	0.0897	0.1455	0.1229
Unsprayed	2	19	0.3217	0.2839	0.1520	0.1283
BOTCAST	3	10	0.2706	0.1782	0.1701	0.1817
TOM15	3	0	0.2311	0.0455	0.1878	0.1744
TOM1525	3	8	0.2301	0.1782	0.1849	0.1002
TOM15R	3	18	0.2819	0.1942	0.1667	0.0648
CP2	3	7	0.2956	0.3218	0.1540	0.0504
Unsprayed	3	17	0.3080	0.6449	0.1497	0.2227
BOTCAST	4	8	0.2437	0.9302	0.2382	0.3783
TOM15	4	6	0.2042	0.8991	0.3255	0.3834
TOM1525	4	9	0.1704	0.5644	0.3782	0.2222
TOM15R	4	17	0.0952	0.4009	0.3382	0.0558
CP2	4	3	0.1157	0.3302	0.2910	0.0776
Unsprayed	4	31	0.3418	0.4991	0.3040	0.1728

Table A4. 31 Analysis of variance – Spray-timings- disease incidence (August 02) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	38.41	14.02	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.40	.
Treatment	5	15	4.74	0.0085

Table A4. 32 Analysis of variance – Spray-timings NDVI (August 02) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	27.41	.
Treatment	5	15	9.62	0.0003

Table A4. 33 Analysis of variance – Spray-timings GNDVI (August 02) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.03	0.01	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	1.08	.
Treatment	5	15	0.21	0.9546

Table A4. 34 Analysis of variance – Spray-timings CI (August 02) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.00	0.00	2.74	0.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	16.54	.
Treatment	5	15	10.99	.0001

Table A4. 35 Analysis of variance – Spray-timings PSRI (August 02) – remote sensing 2016

Random effects	Estimate	Standard error	Z value	Pr > Z
Block	0	.	.	.
Residual	0.01	0.00	2.74	.0031
Fixed effects	Num df	Den df	F value	Pr > F
Block	3	0	2.82	.
Treatment	5	15	2.90	.0503

Table A4. 36 Raw data Spray-timing trails- Remote Sensing (2 August, 2016)

Treatment	**Block**	incjy25	NDVIag1	GNDVIag1	PSRIag1	CIag1
BOTCAST	1	19	0.2239	0.1562	0.6710	0.0350
TOM15	1	7	0.2736	0.1050	0.6048	0.0171
TOM1525	1	17	0.3258	0.0536	0.5976	0.0123
TOM15R	1	9	0.2837	0.0818	0.8894	0.0129
CP2	1	22	0.1788	0.2118	0.5730	0.0182
Unsprayed	1	35	0.1527	0.2447	0.6700	0.0273
BOTCAST	2	12	0.1706	0.2094	0.6480	0.0308
TOM15	2	17	0.2889	0.0960	0.6294	0.0218
TOM1525	2	12	0.2914	0.0666	0.6831	0.0122
TOM15R	2	18	0.3041	0.1729	0.7895	0.0152
CP2	2	15	0.2466	0.1217	0.8177	0.0373
Unsprayed	2	21	0.1047	0.0329	0.7735	0.0431
BOTCAST	3	11	0.1128	0.0461	0.6447	0.0367
TOM15	3	0	0.1360	0.1071	0.5826	0.0188
TOM1525	3	9	0.1605	0.1225	0.5758	0.0140
TOM15R	3	20	0.1408	0.1060	0.5688	0.0145
CP2	3	8	0.0916	0.0528	0.7755	0.0292
Unsprayed	3	19	0.0794	0.0390	0.6980	0.0536
BOTCAST	4	9	0.0878	0.0367	0.6746	0.0616
TOM15	4	7	0.1432	0.0810	0.6547	0.0436
TOM1525	4	10	0.1444	0.1463	0.7120	0.0245
TOM15R	4	19	0.1504	0.1621	0.8254	0.0304
CP2	4	3	0.1019	0.0768	0.8556	0.0746
Unsprayed	4	34	0.0887	0.0444	0.8084	0.0862