EPIDEMIOLOGY OF STEMPHYLIUM BLIGHT ON LENTIL (*LENS CULINARIS*) IN SASKATCHEWAN.

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Abstract

Stemphylium blight is a defoliating fungal disease caused by *Stemphylium botryosum*. It has become more prevalent in Saskatchewan. Although not much is known about the biology of the fungus, increasing lentil (*Lens culinaris*) yield losses of up 62% have been reported in Bangladesh and India. The infection of lentil by *S. botryosum* was investigated under a range of temperatures (5 to 30°C), wetness periods (0 to 48 h) and wetness periods interrupted by dry periods of 6 to 24 h. The experiments involved testing the impact of environmental conditions on germination of conidia on glass slides and stemphylium blight infection on lentil (cv. CDC Milestone). Generalised linear models and non-parametric tests were used to determine the effects of these factors on conidial germination and disease development. Infection levels increased with increasing temperature and wetness duration. A latent period of 48 h was observed at 25°C and 30°C under continuous wetness. The duration of the latent period increased with decreasing temperatures and decreasing wetness duration. *S. botryosum* required warm temperatures (above 25°C) and a minimum wetness period of 8 h for optimal disease development. Low levels of infection were observed within the first 2 h of incubation at 10°C and increased with longer wetting periods up to 48 h and temperatures up to 30°C. The pathogen could maintain infectivity during interrupted wetness periods despite its requirement for prolonged wetness periods. Infection levels were not significantly affected by interrupting dry periods of 6 to 24 h although long dry periods (24 h) combined with higher temperatures (30°C) resulted in a decrease in stemphylium blight severity. Germination studies on glass slides supported these findings. Response surface models were developed that provided a good fit for the response of conidial germination to temperature and wetness duration. The coefficients of determination for the regression of observed against predicted effects ranged from 0.88 to 0.97. The general additive model could also be used to predict stemphylium blight severity responses to temperature and wetness duration (scaled deviance = 1.04). However, that model tended to overestimate infection levels especially at lower temperatures. The coefficients of determination for the observed against predicted effects at 5 to 30ºC ranged from 0.77 to 0.92 for the general additive model.

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1.0 Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest crops in the world, as it has been grown for over 8000 years (Oplinger et al., 1990). This food legume crop has been grown mainly as an inexpensive source of high quality protein in human diets, especially in West Asia (McVicar et al., 2005). Lentil is best adapted to the cooler temperate zones of the world, with more recent introductions to Australia, Canada, New Zealand and the USA (Bayaa and Erskine, 1998). The crop was introduced in 1969 to Canada and since then, Canada has experienced a rapid increase in lentil production. Canada is now the second largest lentil exporting country in the world (Saskatchewan Interactive, 2002). In 2003, Canada exported lentils worth C$ 200M, and about 98% of Canadian lentil production is in Saskatchewan (Statistics Canada, 2004). Exports are expected to exceed 600,000 tonnes in the 2005-2006 growing season as compared to 520,000 tonnes in the 2004-2005 season, signaling the growing importance of lentil to Saskatchewan (Statistics Canada and Industry Consultations, 2005). The increasing importance of lentil is expected to continue due to increasing world demand and innovation in lentil research such as the development of improved varieties and better agronomic practices (Skrypetz, 2000).

Diseases are a major constraint to lentil production all over the world (Bayaa, and Erskine, 1998). Stemphylium blight, caused by *Stemphylium botryosum* (Wallr.) is a relatively new disease in Saskatchewan. The disease has been observed with greater frequency in lentil fields in Saskatchewan over the past years (Morrall et al., 2004). Stemphylium blight has been reported on lentil in Bangladesh, Egypt, Syria and the USA (Bayaa, and Erskine, 1998). In Bangaladesh, it is a major disease causing large-scale defoliation (Erskine and Sarker, 1997). Preliminary studies in Bangladesh and India estimated yield losses of 62% and total crop failure have been reported in some

cases where the disease defoliated the crop in the early pod setting stage (Bakr, 1991; Erskine and Sarker, 1997). In Saskatchewan, no crop losses due to stemphylium blight have been estimated. There are concerns, however, that the disease may become more important once current primary diseases like anthracnose and ascochyta blight are controlled through resistance in lentil varieties.

Colhoun (1973) in his review, emphasized the important role played by the environment in the development of diseases. Understanding the effect of environmental factors on pathogen development and plant infection assists in the development of effective control measures. The diverse host range of *S. botryosum* that includes leguminous and non-leguminous crops in different parts of the world indicate its adaptability to different environments (Du Toit and Derie, 2001). However, little is known about the effect of the semi-arid conditions prevalent in Saskatchewan on stemphylium blight on lentil. Preliminary disease screening trials and the development of disease management strategies have been hampered by a lack of understanding what influence environmental factors have on the infection of lentil by *S. botryosum*.

Most of the research on infection by *Stemphylium* spp. of different hosts has confirmed that temperature and moisture are the most important environmental factors. In S.E. Asia and India, temperatures of 18 to 22°C and a relative humidity of over 85% have been reported to favour the development of the disease (Erskine and Sarker, 1997; Sinha and Singh, 1993). Most of the research published has been on *Stemphylium vesicarum* infecting spinach, asparagus, pear, garlic, onion and leek (Basallote-Ureba et al., 1999; Cowling and Gilchrist, 1981b; Johnson and Lunden, 1986; Montesinos and Vilardelli, 1992; Montesinos et al., 1995; Suheri and Price, 2001; Sutherland et al., 1990). Based on this research, infection levels increased with increasing leaf wetness to 24 h. The pathogen required at least 8 h of wetness at low temperatures (10°C) for successful infection (Bradley et al., 2003; Suheri and Price, 2000a,b). The infection of garlic and onion by *S. vesicarium* required wetness periods longer than 24 h for symptom development (Basallote-Ureba et al., 1999). However, Bashi and Rotem (1974) indicated that *S. botryosum* on tomato has the ability to maintain infectivity even with

dry periods of 24 h although this was associated with lower disease severity. The leaf wetness requirements and response to interrupted leaf wetness are expected to vary from region to region as the pathogen adapts to prevailing environmental conditions (Jhorar et al., 1998). This information is of importance for the development of disease management strategies. Information on moisture requirements for infection has been used to develop forecasting models for other host-pathogen systems. For example, the FAST forecasting system has been developed for *S. vesicarum* on pear resulting in reductions in spray applications (Montesinos and Vilardell, 1992). Lack of this information may limit the development of efficient and effective control methods.

Stemphylium blight on lentil has not been extensively studied especially under semi-arid conditions. The objectives of this project were; i) to determine the effects of temperature on conidial germination on *S. botryosum* of lentil, ii) to determine the effect of leaf wetness duration and temperature on disease development iii) to evaluate the effect of interrupted wetness periods and temperature on conidial germination and disease development.

2.0 Literature Review

2.1 Lentil production in Saskatchewan

Lentil is a pulse grain-legume crop that is adapted to cool growing conditions (Oplinger et al., 1990). The crop originated in the Near East, but spread to Egypt, Southern Europe and Asia where it has been cultivated since ancient times (Sonnante and Pignone, 2001). Production has spread to subtropical environments such as South Asia, as well as temperate climates like Canada. Lentils are an important component of the diet in Afro-Asian countries, where it serves as a cheap source of protein and carbohydrate (Iqbal et al., 2005; Muehlbauer and Tullu, 1997). Raw lentil seeds contain approximately 20.6g protein, 2.15g lipid, 2.80g ash, 6.83g crude fibre and 56.4g carbohydrates per 100g (Costa et al., 2006). Lentil is mainly consumed in soups, split lentil (dhal), stews, casseroles and salad dishes (Hnatowich, 2000; Oplinger et al., 1990). Lentil and other legumes will help reduce the increasing protein gap in the world population (Costa et al., 2006).

Lentils were first grown in Saskatchewan in 1969 at Richlea. Production has been increasing progressively, with Saskatchewan contributing 98% of Canada's lentil production (Hnatowich, 2000). Canada is currently the leading exporter and second largest producing nation after India, which is the largest consumer in the world (McVicar et al., 2005). Lentil remains a profitable crop with a farm gate value in Saskatchewan of more than $ 250 million (McVicar et al., 2005). A sufficiently high premium for lentil compared to most traditional Canadian prairie crops has contributed to a steady increase in production, aided by the decline in spring wheat production and summer fallow. Other factors that have contributed to importance of lentil are the reduction in the requirement to purchase fertilizers and its contribution residual soil N-fertility. Lentil and other pulse crops are expected to continue to be important in the

crop rotations in Saskatchewan (Carlyle, 2004; Campbell et al., 2002).

Lentil production in Saskatchewan is best suited to the brown and dark brown soil zones. It can also be grown in the black soil zones, if there is not excessive moisture (Slinkard and Holm, 1990). Diseases and delays in maturity associated with excess moisture hinder production of high quality lentil. The shorter growing season experienced in the black and grey soil zones of Saskatchewan are not suitable for the late maturing varieties (Hnatowich, 2000). However, research has been focused on expanding lentil production to the black and grey soil zones in Saskatchewan.

One of the major challenges of lentil production is the control of diseases. Diseases of lentil in Saskatchewan are ascochyta blight (*Ascochyta lentis* Vassil.), anthracnose (*Colletotrichum truncatum* Schwein.), botrytis grey mould (*Botrytis cinerea* Pers. Ex. Fr.), stemphylium blight (*Stemphylium botryosum* Wallr), and sclerotinia stem rot (*Sclerotinia sclerotiorum* [Lib.] de Bary). Ascochyta blight and anthrancose are presently the primary diseases and are being controlled by fungicide applications, crop rotation and varietal resistance. The production of the crop in more humid areas increases the risk of infestation by ascochyta blight and anthracnose (Campbell et al., 2002). The incidence of stemphylium blight has been on the increase recently (Morrall, pers. comm).

The introduction of ascochyta blight and anthracnose resistant varieties in Saskatchewan is likely to change the economic importance of diseases currently found in lentil fields. Varieties that are resistant to both races of anthracnose are not yet available, although varieties with moderate resistance to ascochyta blight are available. The interaction of different diseases in lentil fields is not well understood. Effective control of major diseases like ascochyta blight and anthracnose through varietal resistance may result in stemphylium blight becoming a more prominent disease. Ascochyta blight and anthracnose have been well studied under semi-arid conditions. These studies have shown that the pathogens require different climatic conditions for optimum disease development. Ascochyta blight is prevalent when conditions are wet and cool, particularly in August, as that result in severe pod and seed infection. Pedersen and

Morrall (1994) reported that temperatures of 10 to 15°C and leaf wetness durations of 24 or 48 hours were optimal for ascochyta blight development. In contrast, *C. truncatum* causing anthracnose requires warmer temperatures (20-24°C) for optimum disease development with a 24-hour wetness period (Chongo and Bernie, 2000). Significant yield losses due to anthracnose are mainly associated with high rainfall and warmer temperatures. As a result of these differences, the distribution and spread of these diseases varies depending on the environmental conditions. It is currently not known what environmental conditions are conducive to infection of lentil by *S. botryosum* and how the fungus interacts with *A. lentis* and *C. truncatum*.

The incidence, distribution and economic importance of stemphylium blight in Saskatchewan are poorly understood. The disease is not currently considered to be of importance in the province. However, Morrall et al. (2004) have reported that the disease is quite common in Saskatchewan and the incidence seems to be on the increase. Seed infection with *S. botryosum* has been observed yearly since 2000, as reported in the Canadian Plant Disease Survey (Dykstra et al., 2000). The disease seems to be well spread across lentil growing regions of Saskatchewan based on the origin of the isolates currently in the collection in the Department of Plant Sciences, University of Saskatchewan. The disease has widespread distribution in other crops. In a survey conducted on lupin (*Lupinus* spp.) in the United Kingdom in 2003, stemphylium blight was reported to be widespread (Landrock-White, 2003). Stemphylium leaf spot of alfalfa is one of the prominent diseases in Manitoba (Huebner, 2000). Several reports have been published on the effect of the disease on other crops but this has not been the case in the semi-arid areas (Cowling and Gilchrist, 1982b; Elmer et al., 1996; Llorente et al., 2002; Suheri and Price, 2000a; Prados-Ligero et al., 2003).

2.2 Macroscopic and microscopic features of *Stemphylium botryosum*

Stemphylium botryosum is the anamorph of *Pleospora herbarum,* an ascosporate teleomorph (Kiffer and Morelet, 2000). *Stemphylium* is a ubiquitous, dematiaceous filamentous fungus that belongs to the kingdom Fungi, phylum Ascomycota, class Ascomycetes, order Pleosporales, family Pleosporacea. The fungus is commonly referred to using the anamorph. Species of *Stemphylium* that have been documented as

plant pathogens include *S. botryosum, S. vesicarium, S. radicinum* and *S. solani. Stemphylium botryosum* is the causal organism for stemphylium blight on lentil. The biology of the fungus on lentil is not known. A number of diseases caused by *Stemphylium* spp. have been well documented examples include stemphylium leaf spot and purple spot of asparagus (*Asparagus officinalis* L.), where *S. botryosum* and *S. vesicarium* are the causal organisms, respectively; stemphylium leaf blight of onion (*Allium cepa* L.), caused by *S. vesicarium*; black rot of carrot (*Daucus carota* L. var. *sativa* DC), caused by *S. radicinum* and gray leaf spot of tomato (*Lycopersicon esculentum* Mill.), caused by *S. solani* (Falloon et al. 1987; reviewed by Shishkoff and Lorbeer 1989).

The macroscopic and microscopic features of *S. botryosum* on lentil have not been studied in detail. However, several studies on the fungus have been conducted in medical science, where the same fungus is considered an allergen (Larone, 2002). The features discussed in this section, are mainly derived from the medical science and from studies on other host plants. *Stemphylium* spp. can be compared to *Alternaria*, *Pithomyces* and *Ulocladium* in terms of microscopic and macroscopic features as they are closely related. Nevertheless, *Stemphylium* is differentiated from *Pithomyces* and *Ulocladium* by producing percurrent conidiophores (Sutton et al., 1998). The septate hyphae, conidiophores and conidia can be seen by microscopy. Larone (2002) and Sutton et al. (1998) reported that the dematiaceous conidiophores of *Stemphylium* are simple or occasionally branched, with a dark swollen terminus bearing conidia. The hyphae are light to dark brown. The conidiophores' noded appearance is due to successive periods of spore production and regrowth. Conidiophores bear a number of vesicular swellings or nodes that are readily produced by aging. Conidia (12-20 x 15-30µm) are described as solitary, rough or smooth walled and light brown to black in colour.

Stemphylium botryosum colonies grow rapidly on a variety of media. They mature in 5 days at 25°C on potato dextrose agar (Hashemi et al., 2005). On most media, the colonies are velvety to cottony in texture with a gray, brown or brownish-black colour

and black pigmentation on the colony reverse (Larone, 2002). The production of conidia in abundance under laboratory conditions is difficult, even when it is grown on PDA and / or V8 juice agar under alternate cycle of 12h light and 12h darkness (Chowdhury et al., 1996; Mehta, 1998). The use of mycelial suspensions in disease screening has been found to be as efficient and reliable as spore suspensions (Hashemi et al., 2005).

2.3 Symptoms of stemphylium blight

Disease symptoms have been well characterized in South Asia where *S. botryosum* has caused great devastation to the lentil crop. In Saskatchewan, it is suspected that stemphylium blight has not been correctly identified in the field, as the lesions closely resemble those of ascochyta blight (Morrall et al. 2004). Bakr (1991) reported that the symptoms of the disease in Bangladesh include the appearance of small pin-headed light brown to tan colored spots on the leaflets which later enlarge, covering the leaf surface within 2 to 3 days. The symptoms differ from other foliar lesions by being larger and spreading across or along the entire leaflet. A blighted dull yellow appearance is observed with infected foliage and branches. Defoliation occurs rapidly, leaving the branches with terminal leaves. The stems and branches also bend down, dry up and gradually turn ashy white, but the pods remain green. White mycelia growth can also be observed on the infected stems. In Saskatchewan, stemphylium blight symptoms have been similar to those described from Bangladesh. The symptoms seem to vary with the host. Stemphylium leaf spot symptoms on alfalfa (*Medicago sativa L.*) include small, oval, dark brown spots appearing at all stages of crop development. These spots later enlarge and often become zoned (University of Illinois Extension, 1988).

2.4 Host species for *S. botryosum*

Stemphylium spp. are pathogenic to a wide range of crops in varying geographic regions under diverse environmental conditions. Some of the host crops include cotton (Intergrata et al., 1998), tomato (Diener, 1955), lucerne (Irwin et al., 1991), garlic (Boiteux et al., 1994), mango (Johnson et al., 1990), pear (Montesinos et al., 1995), oilseed rape (reviewed in Singh et al., 1999) and asparagus (Menzies et al., 1992). Specifically, *S. botryosum* is pathogenic to lentil (Bakr and Ahmed, 1992), spinach

(Koike et al., 2001), onion (Aveling and Snyman, 1993), tomato (Bashi and Rotem, 1975), alfalfa (Cowling and Gilchrist, 1981b) and clover (Graham, 1957). The *S. botryosum* isolates prevalent in Saskatchewan were able to successfully infect most legumes including common bean (*Phaseolus vulgaris*), faba bean (*Vicia faba* L.) and lupin (*Lupinus* spp) under controlled conditions (P. Hashemi, pers comm.). The ability of pathogens to infect a wide range of crops provides a greater ability to survive harsh environmental conditions. The adaptability of the fungus to Saskatchewan's long winters and short summers is of importance, as this in combination with wide host range will increase the availability of inoculum in the next growing season.

2.5 Epidemiology of *S. botryosum* on lentil and other host species

2.5.1 Survival of S. botryosum

The increasing incidence of stemphylium blight in Saskatchewan suggests that the pathogen has mechanisms of surviving the long cold winters, and of reproducing during the short, hot summers experienced in Saskatchewan. Based on the studies on other crops on which *S. botryosum* is pathogenic, the fungus overwinters on infected plant debris, on seed and in the soil.

Researchers around the world have emphasized the importance of plant debris as the primary source of inoculum. Pseudothecia of *Pleospora herbarum* overwintering on garlic debris are an important source of inoculum in leaf spot epidemics (Basallote-Ureba et al., 1999). On alfalfa, *S. botryosum* has been reported to overwinter on seed and as mycelium on dead stems and leaves (University of Illinois Extension, 1988). The ascospores from pseudothecia on overwintering asparagus debris on the soil surface were the only source of inoculum for infection of spears (Elmer et al., 1996). Although the importance of plant debris as means of survival have been widely documented in many crops, very few studies have determined the conditions required for pseudothecia development. Prados-Ligero et al. (1998) reported that the pseudothecia of *Pleospora allii* on garlic leaf debris developed best at low temperature (5 to10°C) and high relative humidity (< 96%). High temperatures (15 to 20°C) and relative humidity less than 96% were associated with early degeneration of pseudothecia. Burial of the infected plant

debris is a recommended control measure targeting the reduction of overwintering inoculum of *S. botryosum*. The viability of ascospores and pseudothecia decreased rapidly on buried spinach debris over time as compared to surface debris, supporting the recommendation (Du Toit and Derie, 2004). On spinach, the burial of the plant debris did not fully prevent pseudothecial maturation although it impeded the release of ascospores (Prados-Ligero et al., 1998). In contrast, burial of asparagus residue fully prevented pesudothecial maturation and subsequent ascospore release (Johnson, 1990). Residue burial remains a vital component of integrated control of the disease.

Researchers have generally agreed that infected plant debris is normally the main source of primary inoculum and the pathogen can survive for long periods. Srivastava et al. (1996) reported that *S. vesicarum* remained viable for 4 months on diseased plant debris, for 3 months at soil depths of 2.5, 5.0 and 7.5 cm and for 2 months at soil depths of 10 and 15 cm. The burial of the infected plant debris reduced the disease severity in the coming season. However, *S. botryosum* should be able to survive for more than 8 months in Saskatchewan for successful infection in the following spring.

Infected seed is an important means of transmission of the disease from region to region and also serve as a source of initial inoculum early in the season (Agarwal and Sinclair, 1996). There is a need to determine the importance of lentil seed as a source of infection. *Stemphylium botryosum* was reported to cause in internal infection on spinach seed. Based on the results of a component seed assay, the pathogen was detected on 54% of the pericarps and 29% of the embryos. The internal infection rendered seed treatments ineffective (Du Toit and Derie, 2004). However, using a *Streptomyces*-based biofungicide was found to be effective against seed-borne *Stemphylium* (Marja-Leena, 2003).

2.5.2 Spread of S. botryosum

The conidia and ascospores produced on crop debris are responsible for the spread of stemphylium blight. As with other pathogens, the air-borne ascospores are discharged into the air from the protruding pseudothecia. The role of ascospore movement on the

distribution of the disease in Saskatchewan is not known. The prevalence of stemphylium blight seems to be randomly distributed in Saskatchewan (Morrall per. comm.). This random distribution can be attributed to seed transmission, although this has not been confirmed. Environmental factors have been found to affect the spread and release of ascospores of *Stemphylium* spp. Studies on *S. vesicarum* on onion revealed that rainfall was directly related to the spread of ascospores and conidia. Relative humidity played a role in the absence of rainfall (Prados-Ligero et al., 2003).

2.5.3 Environmental factors influencing the development of Stemphylium blight

The initiation and development of plant disease is affected by environmental factors through their influence on host susceptibility, pathogen infectivity and the host-pathogen interaction (Agrios, 2005; Campbell and Madden, 1990). A number of surveys and predictions on conducive environmental factors have been done in South Asia where stemphylium blight causes great devastation. The effects of environmental conditions have been well investigated in studies of *S. vesicarum* which causes leaf spot in garlic, onion and asparagus. These studies concluded that temperature, leaf wetness and relative humidity (RH) are the most important environmental factors affecting the development of the disease (Basallote-Ureba et al., 1999). The surveys and field experiments carried out in India and Bangladesh on *S. botryosum*, have confirmed the importance of temperature and RH for successful development of stemphylium blight in lentil (Bakr, 1991; Sinha and Singh, 1993). The effect of the environment has also been well described for *Alternaria* spp., a species closely related to *Stemphylium*. Somasundara and Anilkumar (1987) reported that the spores of *Alternaria helianthi* germinated at temperatures ranging from 10°C to 30°C, with the optimum germination at 25°C. Spores germinated under conditions of high moisture or high RH (<96%) whereas there was no germination at RH of 88.5%. Species of *Stemphylium* seem to occur over a wide range of environmental conditions (Basallote-Ureba et al., 1999; Suheri and Price, 2000b).

2.5.3.1 Temperature

Saskatchewan summers are generally hot and dry, with maximum temperatures ranging from 10°C to 30°C (Environment Canada, 2005). Variation in temperatures across the province accommodates most plant diseases. Moisture levels tend to be the limiting factor for epidemics to develop in Saskatchewan. Nonetheless, temperature remains an important variable as it has an effect on almost all biological components of a plant pathosystem (Campbell and Madden, 1990). It affects the extent of conidial germination and the time required for germination and germ tube elongation (Agrios, 2005). The rapid development of the disease will occur when the temperature is optimal.

The temperature preference for *S. botryosum* has been predicted from meteorological data in Bangaladesh and India. Sinha and Singh (1993) reported that an average mean temperature of 18±2°C and RH of 85 to 90% in the morning were favorable for the appearance, development and spread of stemphylium blight of lentil in India, whereas RH of >50% in the afternoon was essential. Being based solely on meteorological data analysis, these predictions may have neglected other variables. In Bangladesh, 97% humidity, cloudy weather and temperatures of 20 to 22°C favour the development of the disease (Bakr, 1991; Erskine and Sarker, 1997). The temperature requirements for optimum disease development seem to vary among populations of *S. botryosum* and from region to region. In the United States of America, *S. botryosum* causing stemphylium leaf spot of alfalfa is divided into two biotypes differing in their optimum temperatures and symptoms. Cool temperatures (18 to 20°C) favour the C-T biotype that is mainly a problem in spring and fall in California. Warm temperatures (23 to 27°C), favour the W-T biotype, which is mainly prevalent in the eastern U.S. (Cowling et al., 1981b). In New Zealand, stemphylium infection levels on asparagus were significantly higher at l4°C than at 20 or 26°C (Menzies et al., 1991). In surveys conducted in Spain, leaf spot outbreaks caused by *S. vesicarum* were favoured by high RH followed by dry, warm weather (Basallote-Ureba, 1999). Similar results have been reported from *Alternaria linicola*, where the minimum, optimum and maximum temperatures were 3, 25 and 35°C respectively (Vloutoglou et al., 1996). Although *A. helianthi* has the same optimum temperature of 25°C; the spores did not germinate at 5 and 35°C (Somasundara

and Anilkumar, 1987). It is not clear how temperature affects the *S. botryosum*-lentil interaction in Saskatchewan.

2.5.3.2 Moisture

The importance of moisture for the initiation and development of plant disease has been well documented for *Stemphylium* spp. (Basallote-Ureba et al., 1999; Johnson and Lunden, 1986; Montesinos and Vilardell, 1992; Montesinos et al., 1995; Suheri and Price, 2001). Moisture in the plant environment can include rain or water from irrigation, relative humidity in the air and dew. Most plant pathogens require moisture for spore germination and penetration of the host by the germ tube (Agrios, 2005). Splashing rain and running water promote the spread of most disease. As with most fungal diseases, the availability of moisture is important for successful infection by and development of *S. botryosum* on lentil. The moisture requirements can be specific, just as those required for the appearance of stemphylium leaf spot in alfalfa (Emery and English, 1994).

Saskatchewan is considered to have a semi-arid climate, characterized by short and warm summers interrupted by short rain periods, mostly in the form of thunderstorms. The province is generally considered dry with average total precipitation of between 300mm to 430mm (Environment Canada, 2005). In semi-arid environments, successful infection by fungal pathogens requires either rapid germination and penetration, or the ability of the germinating spores to survive dry periods (Bashi and Rotem, 1974). *Stemphylium* spp. were reported to require at least 8 h of leaf wetness for infection. Reviewing the effect of leaf wetness periods on *Stemphylium* spp., Bradley et al. (2003) reported on an association of longer wetness periods with increased sporulation, infection and disease severity. *Stemphylium botryosum* on lentil is known to require RH of more than 90% and prolonged periods of leaf wetness (Bakr, 1991). The infection of onion leaves by *S. vesicarum* occurred after 16 hours of leaf wetness at 15°C and 8 h of leaf wetness at 10 to 25°C. Infection increased with increasing leaf wetness duration to 24 h (Suheri and Price, 2000a). Similarly, Jakhar et al. (1996) reported that *S. vesicarium* required at least 16 h in a saturated atmosphere for initiation of disease development on

onion. When moisture was available, 76% of *S. vesicarum*'s conidia germinated after 32 h. The availability of long periods of moisture is important for infection of most *Stemphylium* spp.

Interrupted wetness periods which are typical of most semi-arid climate have an effect on the survival and success of infection by *S. botryosum*. Desiccation has been found to reduce the ability of conidia to germinate in *Stemphylium* spp. infecting clover spp. (*Trifolium* and *Medicago*, Fabaceae). Desiccation of *Stemphylium* conidia for 3h or 14h reduced maximum germination to 20% and 11% respectively, compared to 92% germination for continuously wet conidia (Bradley et al., 2003). In contrast, germinated spores of *S. botryosum* have been reported to survive several hours at 40% humidity although survival decreased with increasing germ tube length (Anonymous, 2004). Also, Bashi and Rotem (1974) reported that *S. botryosum* f. sp. *lycopersici* succeeds in the semi-arid climates because its germinating spores survive dry periods, maintaining infectivity during interrupted wetness periods. However, a dry period of 3h during a 12h wet period was adequate to prevent subsequent infections of pear (*Pyrus communis* L.) by *S. vesicarum* (Llorente and Montensinos, 2002). The effect of the interrupted leaf wetness on *Stemphylium* spp. is related to whether there is high or low RH during the dry interrupting periods. The prevalence of high RH during the dry interrupting periods is thought to enable the conidia of *S. vesicarum* to continue germ tube development (Llorente and Montensinos, 2002). However, the ability of the conidia to survive dry periods seems to be dependant on the species.

2.5.3.3 Light

Fungi exhibit different responses to light, depending on the light intensity, quality, and duration of exposure as well as temperature (Shahidul Alam et al., 2001). Light affects various processes in disease development including pathogen sporulation, germination and disease severity. However, light is not as important as temperature or moisture (Agrois, 2005). Studies in India with the aid of meteorological data concluded that an average of 7.7 or less sunshine hours favored the development of stemphylium blight on lentil. Eight or more sunshine hours per day were unfavorable for disease development (Sinha and Singh, 1993). However, the influence of sunshine on disease development is

confounded with low relative humidity. *Stemphylium* is assumed to be a diurnal sporulator; it requires an alternating light and dark cycle for spore development. In total darkness, it produces only a few spores and sterile conidiophores are formed under constant light (Warner, 2005). Earlier studies on *S. botryosum* f.sp. *lycopersici* of tomatoes indicated that sporulation was optimal in continuous darkness, with the highest spore yield occurring when a 12-h light period was followed by a 12-h dark period (Bashi and Rotem, 1975). Continuous light inhibits the sporulation of *Stemphylium solani* and conidiophores were formed but no conidia developed (Minussi et al., 1977). The release of *S. botryosum* spores borne singly or in chains on simple sporophores is favored by low RH in the presence of light (Leach, 1971). The timing and duration of light exposure influenced the germination of *Alternaria linicola* in the presence of wet leaf surfaces (Vloutoglou et al., 1996). The conidia of *A. solani* germinated in darkness at 25°C and RH above 96% (Stevenson and Pennypacker, 1988). The effect of light is obviously variable depending on the pathogen, stimulating spore germinating in some fungi and inhibiting in others.

2.5.3.4 Fungal culture age

Few studies have been carried out to determine the effect of culture age on germination and infection of *Stemphylium* spp., although the production of highly infective spores is important for inoculation and screening studies under controlled conditions. The age of a fungal culture is an important parameter for spore germination and infection in some fungi (RongRong et al., 2004). *In vitro*, cultures of *S. botryosum* from lentil sporulated better on a V-8 / PDA medium than on PDA (Hashemi et al., 2005). These findings were also reported from *S. solani*, where a V-8 medium performed better than MPA for conidia production under alternating light and dark conditions (Mussi and Kurozawa, 1996). It is thought that *S. botryosum* may react similarly to closely related fungal species. Based on studies of spore germination of *A. helianthi*, Somasundara and Anilkumar (1987) found that 16 day old culture had the highest spore germination (70.03%) whereas spores from 32 day old cultures had the lowest germination (11.99%). Germination of spores decreased significantly with an increasing age of the cultures. An increase in the culture age also resulted in a significant decrease in the germination and

virulence of *Verticillium lecanii* infecting *Trialeurodes vaporariorum* (RongRong et al., 2004).

2.6 Strategies for control of stemphylium blight

2.6.1 Chemical control

The use of fungicides has been effective in reducing the economic losses due to *Stemphylium* spp in a range of crops. Several fungicides (chlorothalonil, mancozeb, tebuconazole, procymidone and iprodione) have been found to provide effective control of diseases caused by *Stemphylium* spp. in various host species (Basallote-Ureba et al., 1998; Menzies et al., 1992; Meyer et al., 2000). The foliar fungicide Bravo 500 (chlorothalonil) which is known to control stemphylium blight is registered for the control of ascochyta blight and anthracnose of lentil in Canada (Hnatowich, 2000). The yield of asparagus spears was greatly increased with the application of chlorothalonil or mancozeb against stemphylium leaf spot (Menzies et al., 1992). Under severe disease pressure, fungicide application at 7-day regular intervals increased the stand as compared to applications every 10 or 14 days (Meyer et al., 2000). In addition to chlorothalonil, the application of tebuconazole or procymidone (alone or alternated with chlorothalonil) provided effective control for *S. vesicarum* on garlic (Basallote-Ureba et al., 1998). Mancozeb, iprodione and chlorothalonil are recommended for the control of stemphylium leaf blight and stalk rot (*S. vesicarium* and *S. botryosum*) in onion and lentil (Bakr and Ahmed, 1992; Davis et al., 2005). In South Africa, seed treatments with fungicides which included anilazine, benomyl, a carbendazim/flusilazole mixture, procymidone, tebuconazole and thiram did not eradicate *S. vesicarum* from onion seeds. The best way of controlling seed infections was soaking the seed at temperatures of 50°C for 20 minutes although this also reduced germination and emergence on onion seeds (Aveling et al., 1993). The method of application of foliar fungicides has also been found to affect their efficacy in control. Stemphylium control in onion was improved with the use of air-assisted placement spraying compared with cloud spraying (Gristein et al., 1988). Earlier, Kritzman (1983) also indicated air-assisted application was more effective than standard boom application, especially when applied in two sprayings in opposite directions. Timing and the choice of fungicide to use are also important in

achieving reasonable disease control. In Saskatchewan, the fungicides used to control ascochyta blight and anthracnose may also reduce stemphylium blight although this has not been confirmed (Bailey et al., 2003).

Forecasting models have been used to schedule fungicide sprays, with the main objective of reducing the number of sprays. A forecasting model (BSPcast) developed for prediction of brown spot (*S. vesicarium*) of pear significantly reduced the disease severity as effectively as fixed sprays. BSPcast was able to reduce the number of sprays by 20-70% for fungicides with a 15-day protection period (kresoxim-methyl or procymidone) and 20-50% when using a fungicide (thiram) with a 7-day protection period (Llorente et al., 2000). The FAST model was initially developed to time the fungicide sprays for *Alternaria solani* control on tomato (Madden et al., 1978). The use of the FAST model to predict stemphylium leaf spot development in pear reduced the sprays by 20% compared to the 7-day fixed spray schedules (Montesinos and Vilardell, 1992). Tom-Cast, which is derived from the FAST model was also effective in reducing fungicide sprays in controlling stemphylium leaf spot in asparagus. This program resulted in a 60% reduction in the number of fungicide applications when compared with the standard 7-day fixed interval (Meyer et al., 2000). The use of these models to schedule fungicide applications has been proved to provide effective and lower cost control of stemphylium leaf spot in different hosts (Chapman, 1998; Madden et al., 1978; Meyer et al., 2000). However, not all chemical control has been effective in reducing the disease under weather conditions which favour the fungus (Basallote-Ureba et al., 1998). In Saskatchewan, the economic feasibility of chemical control for stemphylium blight of lentil is still under consideration (Morrall et al., 2004).

2.6.2 Cultural controls

Cultural methods represent an effective and environmentally friendly way of disease control. Little research has been conducted to determine the effect of cultural methods on the incidence, severity and economic impact of stemphylium blight on lentil. Cultural control methods which have been employed to combat *Stemphylium* spp. in other hosts include crop rotation, residue incorporation, choosing the best planting and harvesting

dates; and the use of resistant varieties (Bayaa, and Erskine, 1998; Schwartz and Gent, 2005; Shanmugasundaram, 2001).

Long crop rotations with unrelated crops have been recommended for fields that are infected by *Stemphylium* spp. (Shanmugasundaram, 2001). However, crop rotation might not be effective because *S. botryosum* is a common saprophyte (Bailey et al., 2003). Low plant density and good field drainage has also been successful in reducing stemphylium blight severity in onion and garlic, as these factors reduce leaf wetness duration (Schwartz and Gent, 2005). High nitrogen application has been associated with increased disease intensity (Shanmugasundaram, 2001). Sowing date can be used to control the disease depending on the environmental conditions although this has not been determined for Stemphylium blight on lentil (Bayaa, and Erskine, 1998; Jakhar et al., 1994). The timing of harvesting is also important and earlier harvest prevents leaf loss and further spread of the disease (Willis and Stuteville, 1982).

Good sanitation such as incorporation of crop residue is an important disease management tool as *S. botryosum* overwinters in crop debris. Severity of stemphylium leaf spot of asparagus was reduced with the burial of debris from the previous season. The process also eradicated the volunteer seedlings that can serve as a source of inoculum for the next season (Johnson, 1990). The timing of the removal of crop residue is critical. The reduction of *S. vesicarium* inoculum through early removal of asparagus ferns is associated with low yields. However, late removal of the ferns did not affect yield in the following season (Kelly and Bai, 1999).

Resistant varieties provide a more effective and more consistent method of control. However, preliminary screening of lentil varieties at the Crop Development Centre has indicated that Crimson and Eston have moderate resistance to Stemphylium blight whilst CDC Glamis and CDC Milestone were susceptible (Beare, 2002). Sources of resistance to stemphylium blight in lentil have been identified in Bangladesh and resistant varieties Barimasur 3 and 4 are available to the farmers (Sarker et al., 1999a, b). Stemphylium blight resistant lines in Bangladesh mainly comprise local land races, exotic genotypes

and ICARDA lines (Sarker et al., 1991). Factors associated with resistance to stemphlium blight were studied in lentil. The resistant varieties were found to have a thicker cuticle, thicker epidermal cell layer, thicker cortical layers, fewer stomata and a large number of epidermal hairs compared to the susceptible lines (Chowdhury et al., 1997). Characteristics of the resistance genes in lentil are not known. However, lentil cultivars in Bulgaria (Naslada and Stella) are said to possess complex resistance to stemphylium blight (Mihov and Stoyanova, 1998). In lettuce, a dominant gene (*Sm1*) and recessive gene (*sm2*) control the resistance to *S. botryosum* (Netzer et al., 1985). In cotton, two genes were reported to be involved in the inheritance of resistance to leaf blight of cotton (*Gossypium hirsutum*) caused by *S. solani* (Mehta and Arias, 2001). The use of resistant varieties may provide long-term management of the disease, but there is need to understand the genetics of the stemphylium blight resistance in lentil.

3.0 Influence of temperature and wetness duration on the development of stemphylium blight of lentil

3.1 Introduction

Lentil has become an increasingly important crop in Saskatchewan mainly due to market expansion in Africa, Middle East, Asia and Central America (Saskatchewan Agridivision Corporation, 2002). Successful production of lentil is dependant on climatic conditions, with excessively cool and wet environments being associated with low yield and poor adaptation responses (Vandenberg, 1998). The optimal conditions for lentil production coincide with those conducive to disease outbreaks. Diseases are a major constraint to the production of lentil in Saskatchewan. Anthrancose, ascochyta blight and botrytis stem and pod rot of lentil have been associated with yield losses of up to 60% depending on the disease and prevailing conditions. Infection from *Stemphylium botryosum*, which causes stemphylium blight of lentil, has been observed more frequently over the years (Morrall et al., 2004). Crop devastation due to stemphylium blight has been reported in Bangladesh and S.E Asia, where this disease is a major constraint to lentil production (Bakr, 1991; Sinha and Singh, 1993).

The ability *S. botryosum* to infect lentil will be dependant on its adaptability to the environmental conditions prevalent in Saskatchewan. This fungus affects a wide host range under different environments, suggesting its ability to adapt to varying climatic conditions. As with most host-pathogen interactions, temperature and moisture are primary environmental factors affecting *S. botryosum* conidial germination and disease development (Campbell and Madden, 1990; Basallote-Ureba et al, 1999; Bashi and Rotem, 1974). The presence of free water on the leaf surface at optimal temperature promoted germination and disease development of *Stemphylium* spp. on clover (Bradley

al., 2003). In alfalfa, warm and cool biotypes of *S. botryosum* have been reported and these are separated geographically (Cowling et al., 1981b). The prevalence of warm temperatures (<25°C) and wetness duration longer than 24 h favours the appearance, development and spread of the stemphylium blight in S.E. Asia (Erskine and Sarker, 1997; Sinha and Singh, 1993). However, few studies have investigated the effects of temperature and moisture on conidial germination of *Stemphylium* spp. Most of the studies have been on *Alternaria* spp., which is closely related to *Stemphylium*. According to these studies, conidial germination occurs over a wide range of temperatures (3 to 35°C) and starts within 1 to 3 h of incubation at optimal temperatures (Jakhar et al., 1996; Somasundara and Anilkumar, 1987; Vloutoglou et al., 1996). *Stemphylium* spp. in general, require the presence of free water to germinate unlike *Alternaria* spp. that have the ability to germinate at 100% humidity (Bashi and Rotem, 1974; Montesinos et al., 1995). *Stemphylium* spp. are able to penetrate the host directly through the epidermis or indirectly through the stomata after the formation of the appressoria (Aveling and Snyman, 1993; Suheri and Price, 2000a).

Specific effects of environmental factors on *S. botryosum* of lentil under a semi-arid climate are not known. An understanding on the behaviour of *S. botryosum* on lentil in a semi-arid climate will assist in the development of effective management techniques. For example, the knowledge of the effect of temperature and wetness duration on germination and disease development is of paramount importance in determining the timing of fungicide applications (Grove, 2002). The objectives of this research were to determine the effect of temperature and leaf wetness duration on conidial germination, leaf penetration and development of stemphylium blight.

3.2 Materials and methods

3.2.1 Effect of temperature and incubation time on conidial germination

3.2.1.1 Isolates and inoculum production

Single conidium isolates of *S. botryosum* were collected from naturally infected lentil plants obtained at different locations in Saskatchewan. These isolates were initially screened for their ability to sporulate after 7 days of incubation in a growth chamber at

27°C. The two specified isolates of *S. botryosum* (SB 9 and SB 19) with the greatest spore production under the culture conditions were selected for further study. Stock cultures were stored in sterile deionised water at 7°C ± 2 and all the cultures used in these experiments were derived from a single stock.

Small pieces of mycelium from the selected *S. botryosum* stock culture were placed on 9 mm disposable Petri plates containing a V8 juice/potato dextrose medium, (150ml V8 juice, 10g Potato Dextrose Agar (Difco, Becton Dickinson and Company), 3g calcium carbonate and 850ml of distilled water). To achieve optimum sporulation, the cultures were incubated at 27°C under cool fluorescent light (44 µmol m^{-2} s^{-1}) using a photoperiod of 16 h light/day. The conidia were collected from the Petri dishes by flooding the plates with deionized water and then gently dislodging the conidia with a soft brush. A drop of the surfactant and spreading agent, Tween 20 was added to every 1000 ml of deionized water. All conidial suspensions were processed simultaneously to minimise bias. The conidial concentration was determined by counting on a Neubauer hemacytometer and adjusted to the desired concentration by diluting with deionized water.

3.2.1.2 Germination study

All germination tests were carried out on well slides under laboratory conditions. Conidial suspensions ($5x10^4$ conidia mL^{-1}) of the two isolates (SB 9 and SB 19) were prepared from 7-day old cultures. 100µl aliquot of conidial suspension was evenly spread over each well slide and the slides were placed in labeled Petri dishes, placed in plastic trays. Each slide was used only once for each treatment. High humidity (> 90%) was maintained in the plastic trays by positioning a moistened filter paper in each Petri dishes moistened paper towels were also positioned on the bottom of the tray. The Petri dishes were incubated at temperatures of 5, 10, 15, 20, 25 and 30°C in incubators under continuous cool fluorescent light (32 µmol m^{-2} s^{-1}). Light and age of the fungal cultures were shown not to have an effect on conidial germination of *S. botryosum* during preliminary studies (Appendix 1).

With the aid of a light microscope (Nikon Microphot FXA, Japan) at ×100 magnification, conidial germination was determined by evaluating 100 conidia within several fields of view selected randomly from left to right across each slide. Conidia were considered to have germinated when the germ tube was at least half the width of the spore (Degenhardt et al., 1982). Conidia were counted at two-hour intervals for 20 hours. The number of germinated conidia was expressed as a percentage of the total number of conidia counted. A split plot experimental design with four replications was used, with temperature as the main plot and incubation time as the subplot. The experiments were repeated twice.

3.2.1.3 Data analysis

The residuals of percentage germination were initially tested for normality and homogeneity of variance using UNIVARIATE and LEVENE TEST procedures. Arcsine square root transformation was used for all the data to satisfy the assumptions of ANOVA. The analyses of variance were performed on the transformed data using the MIXED procedure. The design of the experiment was associated with unavoidable subsampling which is sometimes referred to as pseudo-replication (Schabenberger and Pierre, 2002). Testing the effect of temperature and incubation time on conidial germination of all replications in the same growth chamber does not represent true replicates (Hulbert, 1984). To tackle the problem, the mixed model for the two-way factorial included a random statement that defined the experimental units repeat (temperature*incubated time) as clusters and columns of the Z_i matrix as having an intercept only (Schabenberger and Pierre, 2002). Conidial germination was related to temperature by means of linear regression using REG procedure. The relationship was defined for each temperature and the slope data were extracted. The observed data for germination after 20 h and the slopes were subjected to analysis of variance specifying subsampling when using the MIXED procedure. The two isolates did not respond differently to temperature in terms of observed maximum germination. Combined analyses for the two isolates and experimental runs were conducted. Linear contrasts were used to compare the germination response of the two isolates at different temperatures. The estimation of the variance components was by REML (Restricted or

Residual maximum likelihood) that employs least squares to estimate the treatment effects (Liao and Lipsitz, 2002; Littell et al., 1996).

A response surface for the percentage conidia germination as a function of temperature and wetness duration was computed. The response surface regression for each isolate was analysed using the two-step GLM procedure (Bowley, 1999). The least squares regression equation that included factor combinations was constructed based on the analysis. Linear, quadratic and cross factor terms were tested for their significance in the model. The estimated conidial germination from the least square regressions was compared with the actual observed conidia germination for the two isolates. The best least squares regression equation predicting conidial germination of *S. botryosum* as influenced by temperature and wetness duration was selected. The selection was based on the coefficient of determination, intercept and the scatter plots. All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary NC).

3.2.2 Effect of leaf wetness duration and temperature on infection and disease development

3.2.2.1 Planting and maintenance of lentils

The lentil cultivar CDC Milestone was grown in a growth chamber (model GR 48, Conviron, Winnipeg, MB, Canada) at 25°C with a photoperiod of 16 h light/day provided by fluorescent lamps with light intensities of between 315 and 325 $\mu molm^{-2}s^{-1}$. Preliminary research on disease resistance of lentil cultivars to stemphylium blight had confirmed the susceptibility of CDC Milestone to stemphylium blight (Beare, 2002; Vandenberg et al., 2001). The seeds were sown in 10 cm diameter plastic pots filled with a commercial potting mix (Terralite Redi-Earth®) and the seedlings were thinned to 4 plants per pot. The plants were fertilised with complete fertilizer solution (20:20:20 NPK + micronutrients) at two weeks after planting at a rate of 3 g per litre with 22 mL of the fertilizer solution were added to each pot. Whole plants were used in all the experiments and pots were randomly assigned to treatments.

3.2.2.2 Isolate selection and plant inoculation

A single isolate (SB 19) was used in this experiment and the conidial suspensions were prepared as described in section 3.2.1.1. The isolate SB 19 was selected based on its greater ability to sporulate relative to SB 9. Twenty one day old plants were placed in polythene plastic sheets and then inoculated until runoff (approx. 5 ml per plant), with a the conidial suspension ($2x10^5$ conidia mL^{-1}) using a CO_2 air brush (model RUH8210, Oxygen regulator, Uniweld, U.S.A) set at a pressure of 138 kPa. The inoculated plants were placed in mist chambers at various incubation temperatures. Deionised water solution was sprayed on non-inoculated checks.

3.2.2.3 Disease assessment

The disease severity assessments were conducted based on the Horsfall-Barratt (HB) scale, which is based on the percentage of affected leaves and stems (Horsfall and Barratt, 1945). The plants were individually assessed in the experiment and the class values (1 to 11) were converted to percentage of infected plant tissue (0, 1.17, 2.34, 4.68, 9.37, 18.75, 37.5, 62.5, 81.25, 90.63, 95.31, 97.66 and 98.82). The average level of percent infected tissue for the four plants was used in data analysis.

3.2.2.4 Experimental design and description of incubation treatments

The investigation was conducted under controlled conditions in a growth chamber (model PGV 56, Conviron, Winnipeg, MB. Canada). CDC Milestone plants were inoculated at 3 weeks with SB 19 conidial suspensions as described under section 3.2.2.2. The plants were placed in a plastic mist chamber built within the growth chamber. The chambers were operated at 10, 15, 20, 25 and 30°C. The plants were exposed to leaf wetness periods of 0h, 2h, 4h, 6h, 8h, 10h, 12h, 24h and 48h in the mist chamber. Leaf wetness was administered in the form of tiny water droplets from a humidifier (model 7075M, Herrmidifier, Sanford, NC). Relative humidity within the growth chamber and the mist chamber was maintained at 85% ± 3% and 95% ± 3%, respectively. Temperature and relative humidity were measured using a HOBO data logger. Leaf wetness duration was timed manually for each treatment at the termination of the leaf wetness period. The plants were allowed to dry in the same growth chamber under cool fluorescent light with light intensities of 315 μmol m^{-2} s^{-1}.

Disease severity was recorded at 3, 7 and 14 days using the HB scale as described before. The area under the disease progress curve (AUDPC) was calculated for ratings at 3, 7 and 14 days after inoculation using equation 1. The experiment was arranged as a split-plot analysis with temperature as the main plot and leaf wetness duration as the subplot. The experiment was conducted twice.

$$AUDPC = \Sigma_i^{n-1}(y_i + y_{i+1}/2)(t_{i+1} - t_i) \qquad \textit{(Eq. 1)}$$

where y_i is the percent severity observed for the i^{th} treatment, t_i is the date of the observation and observations were made on n dates (Shanner and Finney, 1977).

3.2.2.5 Data analysis

Non-parametric tests were used to analyse the data because of its nonconformance to equal variance, although the residuals approached normality (Garrett et al., 2004). The experimental design also involved subsampling but the data was analysed as a split plot because non-parametric statistics were used in the analysis. The MIXED procedure was conducted on the ranked means from the split plot design, using temperature as the main plot and incubation period as the sub-plot. Temperature levels were compared by means of contrasts. The LD_CI macro by Brumer was used to estimate the relative effects as well as their standard errors and confidence intervals. The significance of the temperature x wetness duration was determined by the F1_LD_F1 macro (Shah and Madden, 2004). The Generalised Additive Model (GAM) procedure was used to determine the form of the relationship between disease severity at 14 days from inoculation relative to temperature and leaf wetness duration. This procedure estimates generalized additive models and allows nonparametric relationships to be determined simultaneously due to the additive assumption (SAS Inst., 1999; Xiang, 2001; Hastie and Tibshirani, 1990). A less restrictive model was developed using the B-spline smoother with 3 degrees of freedom. Normal distribution with identity link was specified for the model. Computed estimated linear terms for temperature and wetness duration were added to their respective partial predictions to determine the entire prediction effect. The overall shape of the relationship between either temperature and wetness duration with disease severity was explored by plotting partial predictions. A

non-parametric GAM procedure was used to determine the form between the relationship of disease severity and each independent factor. Linear and quadratic terms for two independent factors were found to be significant. The corresponding parametric model was developed using GENMOD procedure, specifying the linear and quadratic terms for temperature and wetness duration determined with the GAM procedure (SAS Inst., 1993). After the specification of the terms there were no differences between the two models in the significance of the parameters. The parameter estimates from the GENMOD model were used for simplicity purposes in this study. Tests for combined linear and quadratic effects for each variable were conducted using contrasts. Goodness of fit was evaluated for the models by determining whether the scaled deviance divided by the degrees of freedom was less than one. The plot of the predicted residuals against observed severity residuals were also used to assess the fit of the model.

3.2.3 Infection studies of S. botryosum on lentil leaves

Microscopy studies on whole lentil plants were conducted to understand the infection process by *S. botryosum*. The plants were incubated at 25°C with RH of 85% ± 3% and 95% ± 3% in the growth chamber and mist chamber respectively. Leaf wetness was maintained in the same manner as described under section 3.2.2.4. Leaves were collected from separate plants after 2 h, 4 h, 6 h, 8 h, 10 h and 12 h of continuous leaf wetness. More than 20 leaves were randomly sampled from each of the three pots assigned for each wetness duration (2 to 12 h). Each pot had four plants. The leaves from each pot were stored separately in glass tubes containing a destaining solution of 3:1 glacial acetic acid – ethanol (Merck KGaA, Darmstadt, Germany and Brampton, Ontario, Canada respectively). The number of conidia that had germinated and the number of those that had successfully invaded the plant either through stomata or direct penetration were counted using ×100 magnification from eight randomly selected 8 leaves. The experimental design was a completely randomised design with the pots as 3 replications. The experiment was repeated twice.

The MIXED procedure was used to test for the significance of wetness duration on percentage germination and number of successful penetrations of the lentil leaves. Germination percentage was arsine square transformed to conform to the assumptions of

normality and equal variance. There were no differences between the two experimental runs and a combined analysis was conducted. The Tukey's Studentized Range (HSD) Test was used to compare the treatment means.

3.3 Results

3.3.1 Effect of temperature and incubation time on conidial germination

In the presence of free water, conidia of *S. botryosum* germinated over a wide range of temperatures (5 to 30°C). The conidia were polyspermic and produced up to six germ tubes depending on the temperature and the incubation time. Similar germination trends were observed over time for the two isolates. Generally, the percentage of conidia that had germinated increased with temperature and incubation time. After 20 h of incubation, the germination for the two isolates was over 30% at 5°C and above 80% for 25 or 30°C. At least 10% of the conidia germinated after 2h at the lowest temperature (5°C) and the number of germinated conidia increased to over 40% at 30°C after the same incubation period.

The rate of germination (slope) and maximum germination (after 20 h) significantly increased with temperature ($P = 0.0001$), with the highest germination being observed at 30°C for the rate of germination and 25°C for percent germination after 20h (Appendix 2 and 3). Comparison of percentage germination after 20h of incubation showed significant differences between temperatures except for 10ºC and 15ºC, and between 25ºC and 30°C (Table 3.1). The impact of the rate of germination increased as temperature increased above 15ºC. Generally, the slowest and fastest response was at 5°C and 30°C, respectively (Figure 3.1). Germination percentage after 20 h at 10ºC and 15ºC were not different but the rate of germination at 15ºC was higher than 10ºC suggesting a change in the nature of the germination response with temperature. There were differences in germination percentage after 20 h between 15ºC and 20ºC, but the rate of germination was similar in that temperature range. The rate of germination was different between 15ºC and 25ºC, and then the same at 15ºC and 30ºC. Although the percentage germination differed at 20h between 15ºC and 25ºC and 15ºC and 30ºC, the lack of any difference between 25ºC and 30ºC suggest that the optimum is at 25ºC or close by, rather than 30ºC.

Table 3.1 Contrasts of percentage conidial germination at different temperatures (5-30ºC) after 20 h and rate of germination (slope) of *Stemphylium botryosum* incubated on glass slides.

			Germination after 20 h		Rate of germination	
Source	Num DF	Den DF	F-test	Pr > F	F-test	Pr > F
5°C vs 10°C	1	12	13.82	0.003	0.05	0.827
5°C vs 15°C	1	12	26.31	<.0001	1.08	0.312
10°C vs 15°C	1	12	1.99	0.183	1.60	0.017
10°C vs 20°C	1	12	18.25	0.001	7.23	0.020
10°C vs 25°C	1	12	51.89	<.0001	39.59	<.0001
10°C vs 30°C	1	12	62.35	<.0001	8.88	0.012
15°C vs 20°C	1	12	8.18	0.014	2.03	0.180
15°C vs 25°C	1	12	33.54	<.0001	25.38	<.0001
15°C vs 30°C	1	12	42.01	<.0001	2.94	0.112
20°C vs 25°C	1	12	13.13	0.004	0.08	0.776
25°C vs 30°C	1	12	0.48	0.502	10.97	0.006

The response of conidial germination (*pcg*) to temperature (*t*) and incubation time (*h*) was described by least squares regression equation for SB 9 (Equation 2) and SB 19 (Equation 3). The parameter estimates and associated errors of the response surface analysis for the two isolates are presented in appendices 4 to 7. The linear and quadratic effects were significant for temperature and incubation time. Both parameters and their interaction terms are important in describing conidial germination of *S. botryosum* on lentil. The dependence of germination on temperature and incubation period was best described by the response surface of SB 19 (Equation 3), based on the r^2 and randomness of predicted germination response.

$$pcg = 7.855 + 0.289(t) + 0.013(t)^2 + 2.167(h) + -0.039(h)^2 + 0.061(t)(h) \qquad r^2 = 0.91$$

(Eq. 2)

$$pcg = -5.301 + 2.438(t) + -0.051(t)^2 + 2.182(h) + -0.076(h)^2 + 0.091(t)(h) \qquad r^2 = 0.94$$

(Eq. 3)

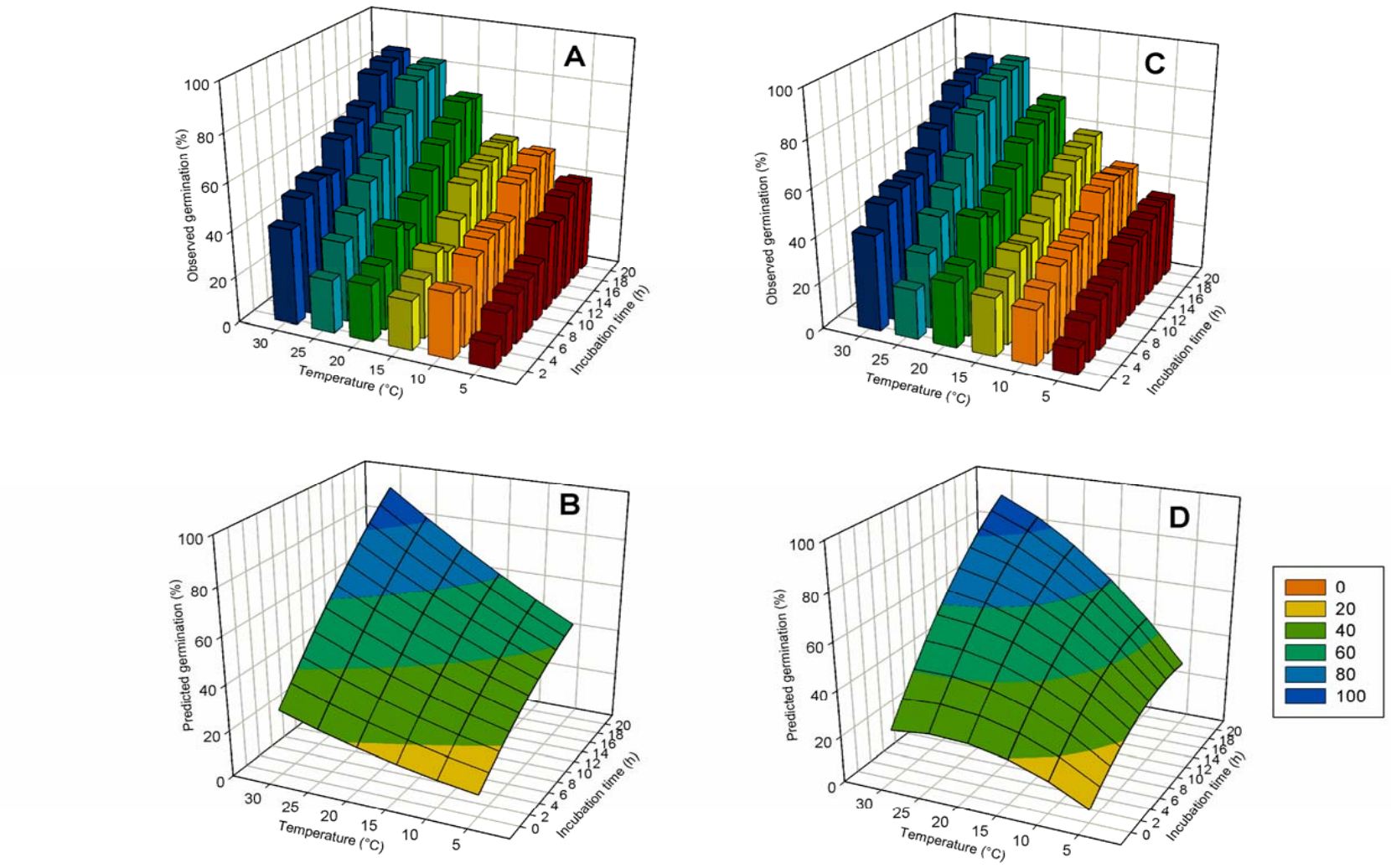


Fig 3.1 Effects of temperature (t) and incubation time (h) on conidial germination of *Stemphylium botryosum* isolates (SB 9 and SB 19). Graphs A and B denotes the observed conidial germination percentage and that predicted by $pcg = 7.855 + 0.289(t) + 0.013(t)^2 + 2.167(h) + -0.039(h)^2 + 0.061(t)(h)$ for SB 9 respectively. Graphs C and D denotes the conidial germination percentage and that predicted by $pcg = -5.301 + 2.438(t) + -0.051(t)^2 + 2.182(h) + -0.076(h)^2 + 0.091(t)(h)$ for SB 19 respectively.

Expected percentage germination of conidia on the response surface analyses described in Equations 2 and 3 were highly correlated with the observed percentage conidial germination (Appendices 8 and 9). Coefficient of determination for the two isolates ranged from 0.88 to 0.97 for the predicted conidial germination versus observed conidial germination. Also, root mean square errors of 9.28% and 8.36% for SB 9 and SB 19 respectively, supported the goodness of fit of the models. Conidial germination of 100% was predicted after 24 h of wetness duration at 25 and 30°C.

3.3.2 Effect of leaf wetness duration and temperature on disease development

Stemphylium blight was not observed on uninoculated plants but the disease was prevalent on all inoculated plants. There was a significant interaction between temperature and leaf wetness duration (P <0.001). Overally, there was a significant increase in disease severity with increasing duration of leaf wetness at temperatures of 10, 15, 20, 25 and 30°C (Appendix 10 and Fig 3.2). The maximum disease severity was observed at 25 and 30°C with leaf wetness periods of 48 h. A rapid increase in disease severity with an increase in leaf wetness duration was found at 25ºC. There was no significant difference in final disease severity at 25 and 30°C after 48 h of leaf wetness. The estimated relative marginal effects ranged from 0.03 to 0.95, with most of the higher values being observed at longer leaf wetness duration and higher temperatures (Appendix 11). Similar conclusions were drawn from the analysis of AUDPC. Comparison of means for different temperature levels and wetness durations revealed that there were significant differences among the different levels of the two parameters. The effect of leaf wetness duration on disease severity was significant at all levels except for 8 h and 10 h.

A regression model was developed that predicts the form of dependence of disease severity (ds) on temperature (t) and leaf wetness duration (w). Linear terms for temperature and leaf wetness duration were highly significant in the analysis (P <0.001). The quadratic terms were only significant in describing the effect of leaf wetness duration. The analysis of the parameter estimates is shown in Appendix 12. The linear and quadratic terms were incorporated in the GENMOD model and the results were consistent with those of the GAM procedure. The contrast tests between the linear and

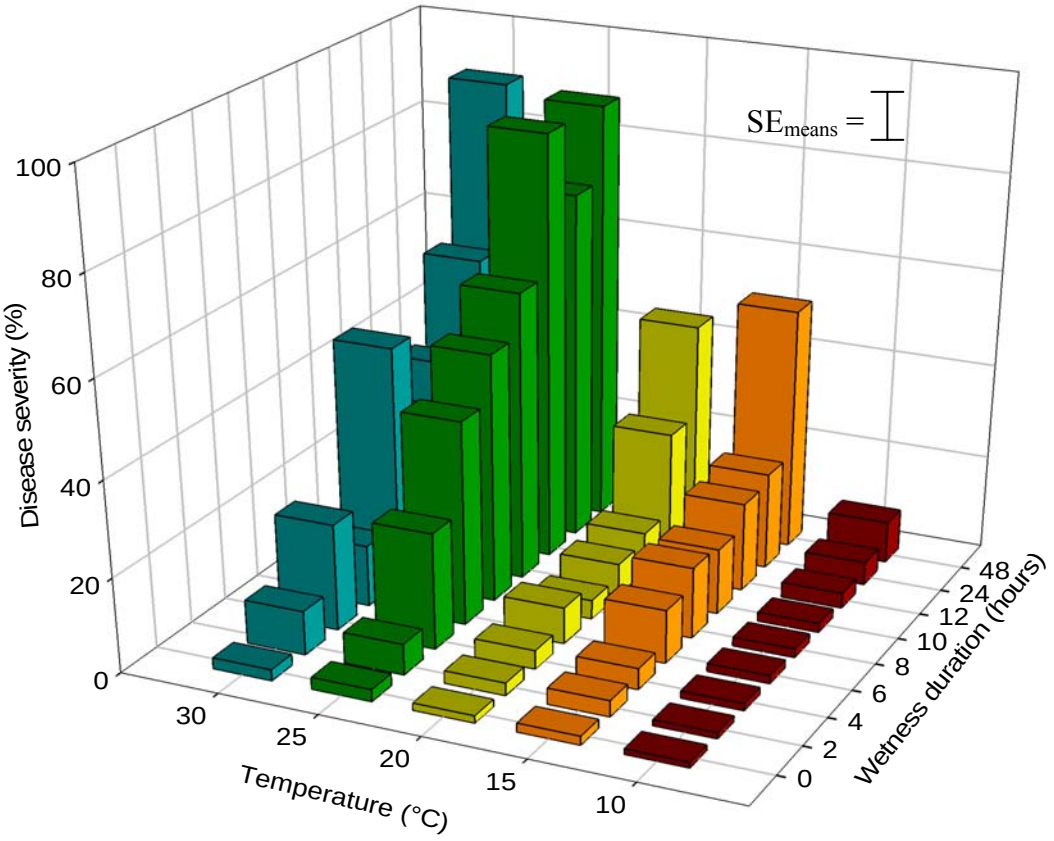


Fig 3.2 Average stemphylium blight severity on lentil inoculated with a suspension of *Stemphylium botryosum* conidia and incubated over a range of temperatures and leaf wetness duration. SE_{means} is the standard error of the interaction between temperature and wetness duration.

quadratic terms of either temperature or leaf wetness duration were highly significant (Appendix 13).

$$ds = -47.101 + 3.277\,(t) + 2.156\,(w) + -0.033\,(w)^2 \qquad \textit{(Eq. 4)}$$

The model (Equation 4) tends to overestimate disease severity, compared to the observed values. The scaled deviance divided by the freedom is almost equal to 1 and the R^2 of predicted against observed disease severity ranged from 0.77 to 0.92

(Appendix 15). The model accurately predicted the response at 10°C where the R^2 was 0.92. The model is useful in predicting the development of stemphylium in response to temperature and wetness periods although the overestimation of severity should be considered. A response surface was generated from Equation 4 (Fig 3.3).

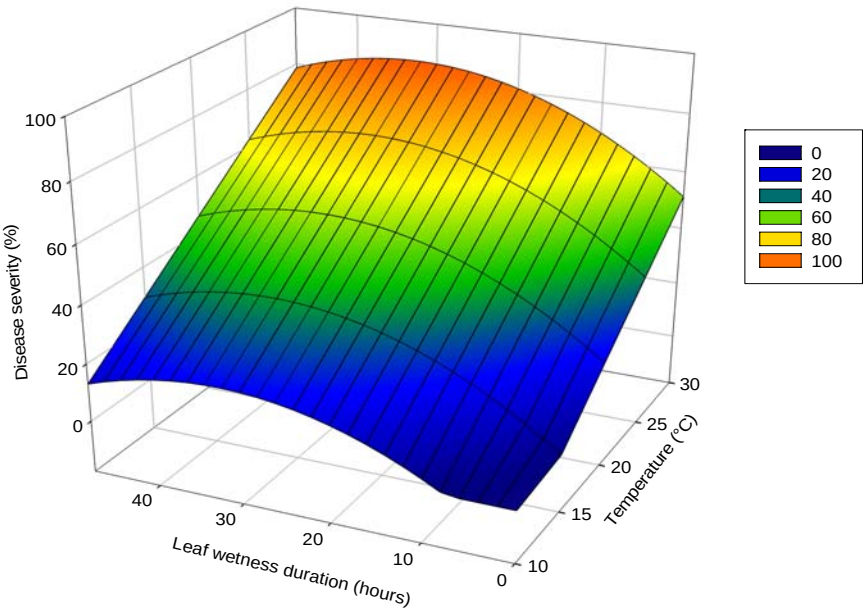
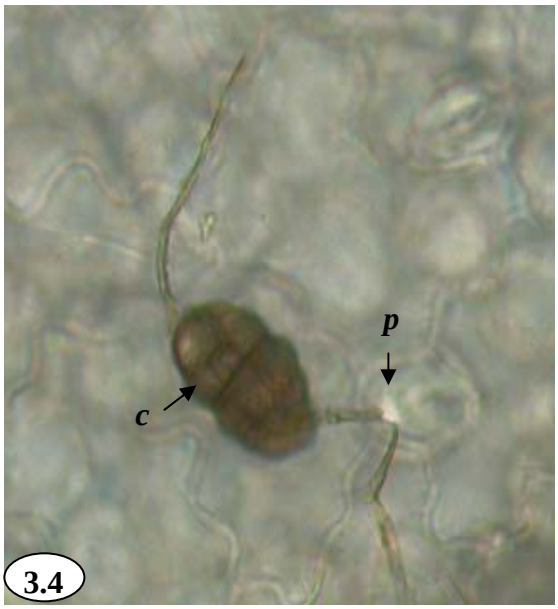
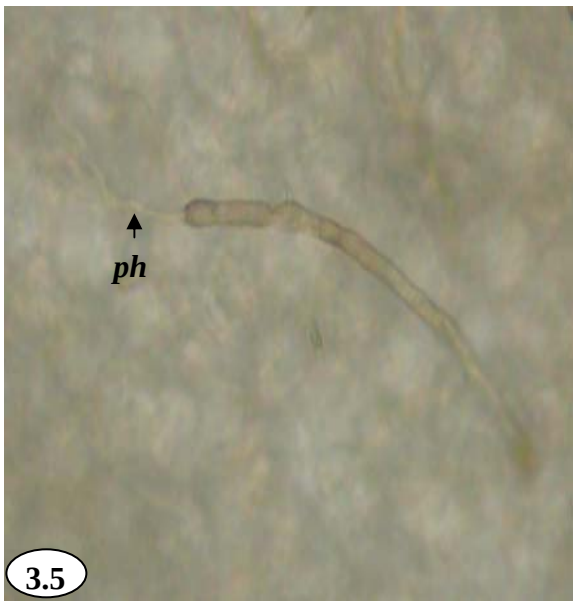


Fig 3.3 Response surface predicting disease severity of stemphylium blight over a range of leaf wetness durations (2 h - 48 h) and temperature (10°C to 30°C). The surface response was generated based on the least square regression model $ds = -47.101 + 3.277\ (t) + 2.156\ (w) + -0.033\ (w)^2$.

3.3.3 Infection studies of S. botryosum on lentil leaves

Germination occurred within the first 2 h of inoculation and conidia were polyspermic with germ tubes developing randomly in different directions (Fig 3.6). Most penetrations of the leaf surface were through the stomata (Fig 3.4). Appressorium formation was not observed in this experiment. Germ tubes passed close to or over the stoma, suggesting that there was no special orientation of germ tubes towards the stomata. Networks of primary hyphae developed over the inoculated region after 12 h (Fig 3.5).





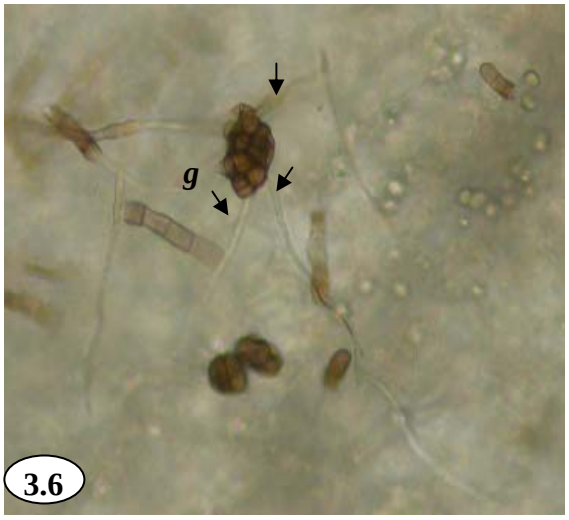


Fig 3.4 – 3.6 Infection of lentil leaves of CDC Milestone by *S. botryosum* conidia after 12 h wetness period at 25°C.
Fig 3.4 Germination of conidia (*c*) and penetration into epidermal cell layer through the stomata *(p*).
Fig. 3.5 Growth of the primary hyphae (*ph*) within the cell.
Fig 3.6 Polyspermic conidia with germtubes (*g*) developing randomly in different directions.

Germination percentage and the number of successful penetrations increased linearly with wetness duration at 25°C (P <0.001). The number of penetrations did not vary between 2 h and 4h, but higher numbers of penetrations were observed after 8 h of wetness (Fig 3.7). Most of the penetrations at 2 h and 4 h were through the stomata and they were mostly related to the position of the conidia with respect to the stomata. The highest numbers of successful penetrations were observed at 12 h and were expected to increase with time. The vegetative mycelium also developed hyphae that penetrated

through the stomata. Leaf wetness duration had an effect on the ability of mycelium to infect the lentil leaves.

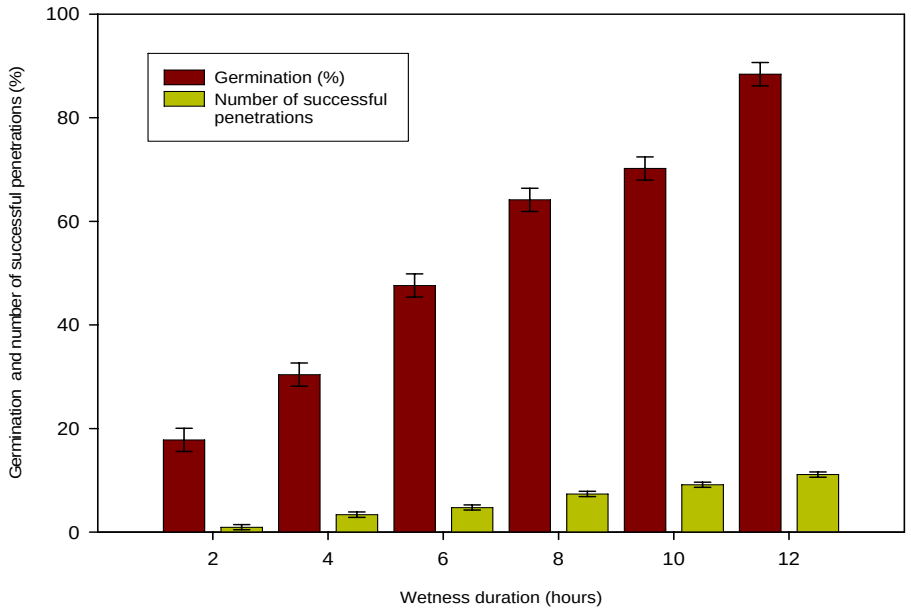


Fig 3.7 Percentage germination of conidia and the number of successful penetrations by *Stemphylium botryosum* on leaves incubated at 25°C over a period of 12 h of lentil plants of CDC Milestone.

3.4 Discussion

Germination experiments showed that conidia germinated under a varied range of temperatures (5 to 30ºC) and incubation times (2 to 20 h). Approximately 10% of the conidia germinated on glass slides after a wetness period of 2 h at 5ºC, although more than 20 h of incubation time was required for 50% conidial germination. Conidia of *A. helianthi* also germinated over a wide temperature range (3 to 35ºC), with the optimum temperature at 25°C. Results from the infection studies also showed that the penetration rate on lentil leaves increased with increasing wetness duration. Penetration of the lentil leaves by *S. botryosum* was mostly through the stomata, which was also reported on *S. vesicarum* on onion leaves (Aveling and Snyman, 1993). At temperatures between 20 and 30°C, 50% of *S. vesicarum* initiated germ tube formation within an hour of incubation in free water and most of the conidia germinated within 3 h of wetness

(Montensinos and Vilardell, 1992). In the present study, conidial germination responses with respect to temperature and incubation time between the two isolates (SB 9 and SB 19) were not significantly different. However, differences between isolates were observed in France where the optimum temperatures for conidial germination of different isolates of *S. vesicarum* ranged from 20 to 25ºC. Maximum disease severity occurred from 25 to 30ºC, again depending on the isolate.

Stemphylium spp. were reported to require prolonged periods of moisture (Basallote-Ureba et al., 1999; Bradley et al., 2003; Cowling and Gilchrist, 1982a; Llorente and Montesinos, 2002). Temperature requirements for *Stemphylium* spp. conidial germination varied depending on the species and varied from region and region, with a range from 20ºC to over 30ºC (Bakr, 1991; Cowling and Gilchrist, 1981b; Sinha and Singh, 1993; Menzies et al., 1991). Early research on *S. botryosum* on alfalfa indicated that the conidia required 3 h of incubation in free water at temperature of 20 to 23 ºC (Pierre and Millar, 1965). The importance of these factors in the epidemiology of *S. botryosum* on lentil and other host plants was emphasized in other studies (Bashi and Rotem, 1974; Cowling and Gilchrist, 1982; Degenhardt et al., 1982; Emery and English, 1994; Suheri and Price, 2000, 2001; Montesinos et al., 1995). *Alternaria* spp. have also been reported to be affected by temperature and incubation time in a manner similar to *Stemphylium* spp. (Montesinos and Vilardell, 1992).

In this study, stemphylium blight severity on lentil plants increased with increasing temperatures (5 to 30ºC) and durations of leaf wetness (2 h to 48 h). The rate of symptom development increased with increasing leaf wetness and symptoms were observed after 48 h of leaf wetness at temperatures of above 25ºC. This agrees with research on *S. botryosum* on alfalfa in California where severe stemphylium leaf spot was observed with incubation periods of 2 or more days under conditions of extended periods of RH greater than 90% (Falloon et al., 1987; Emery and English, 1994). Basallote-Ureba et al. (1999) also observed that leaf wetness periods longer than 24 h were required for symptom development of isolates of *S. vesicarum* from garlic, onion and asparagus at 22ºC. The present study shows that low disease levels (2 to 7%) were

observed after 2 h of leaf wetness at 10 to 20ºC. This demonstrates the ability of the pathogen to infect quickly even at low temperatures. The minimum leaf wetness period was dependent on the prevailing temperature, and 6 h of leaf wetness were adequate for severe disease development at 25 and 30ºC, but more than 24 h of wetness was required for severe stemphylium development at 10 to 20°C. Although the optimum temperature for stemphylium blight could not be clearly determined, *S. botryosum* requires warm temperatures (above 25ºC) for optimal disease development. The leaf wetness periods conform to other research. Suheri and Price (2000a) concluded that leaf wetness durations equal to or greater than 8 h produced an asymptotic infection response of onion leaves by *S. vesicarum* at 10 to 25ºC. In India, a minimum of 16 h of leaf wetness was required for infection of onion by *S. vesicarum* over the same temperature range (10 to 25°C). Optimal temperature requirements for stemphylium blight development seem to be dependant on the region and on the *Stemphylium* species. In Bangladesh, *S. botryosum* requires approximately 20ºC for appearance and development of stemphylium blight on lentil (Bakr, 1991). In New Zealand, severe stemphylium blight of asparagus was observed at lower temperatures of around 14ºC (Menzies et al., 1992). The adaptation of *S. botryosum* to the environment is clearly demonstrated by cool (C-T) and warm (W-T) biotypes of western and eastern regions of the United States of America. The optimal temperature requirements of 18 to 22ºC for the C-T biotype are similar to those prevalent in India and Bangladesh. Results of the present study suggest an optimum temperature range of 23 to 27ºC which is similar to the W-T biotype. Severe stemphylium blight development was observed at 30ºC with shorter wetness periods, which suggests rapid disease development at high temperatures.

Predictive models available for *Stemphylium* spp. such as BSPcast, FAST and Tomcast recognize the importance of temperature and leaf wetness duration on disease severity (Llorente et al., 2000; Montesinos and Vilardell, 1992; Meyer et al., 2000). Equation 3 and 4 generated in this study could be used to predict conidial germination and disease severity respectively at wetness duration of 0 to 48 h and temperatures of 5 to 30°C. The variation described by the components of the models was explained with R^2 of 0.94 and scaled deviance of 1.01 for Equation 3 and 4 respectively. The terms of the

predictive models were similar for germination response and disease severity, although the germination model predicted the observed response more precisely. However, the models for stemphylium blight development overestimated the observed severity and any interpretation of the predicted values should consider this overestimation. Descriptive and predictive models for *Stemphylium* and *Alternaria* spp. have shown a similar pattern although the optimum temperatures vary in each pathogen-host interactions (Canihos et al., 1999; Llorente et al., 2000; Montesinos et al., 1995).

These experiments were conducted under continuous wetness in controlled environments and may not reflect the complex situations encountered in the field. There is a need to test the effect of temperature above 30°C to confirm the optimum temperature for conidial germination and disease development. Similarly, prolonged period of wetness are not common in Saskatchewan, particularly in the semi-arid Brown and Dark Brown soil zones. The development of stemphylium blight on lentil in Saskatchewan will depend on the ability to infect when periods of leaf wetness conducive to disease development are interrupted by periods of leaf dryness. There is a need to investigate the effect of the interrupted leaf wetness on stemphylium blight development.

4.0 Influence of interrupted wetness and temperature on infection of lentil by *Stemphylium botryosum*.

4.1 Introduction

Lentil production in Saskatchewan is mostly confined to the semi-arid Brown and Dark Brown soil zones. Prolonged dry periods interspersed with short cycles of wetness periods during thunderstorms are frequent in these zones (Green and Bailey, 2000; Slinkard and Holm, 1990). Interrupted wetness periods have an important role in disease risk, influencing the infection process of most plant pathogens (Leonard and Fry, 1986). The conidia of *Stemphylium* spp. can germinate after 2 h of wetness at 5ºC, although higher temperatures and longer wetness periods resulted in significantly higher germination (Llorente et al., 2002, Montesinos et al., 1995; Bashi and Rotem, 1975). However, *S. botryosum* was reported to require minimal wetness periods of 8 h and warm temperature (20 - 30°C) for successful infection and disease development (Suheri and Price, 2000a). With warm temperatures and RH greater than 90%, *S. botryosum* had incubation periods of two or more days (Emery and English, 1994). Stemphylium blight has been reported to be successful in South Asia where these climatic conditions are common. The success of *S. botryosum* in the semi-arid climate of Saskatchewan is likely to be dependent on its ability to maintain infectivity under alternating wet and dry periods.

Several studies on the effect of the interrupted wetness periods have revealed different responses in different species. The mortality of *Venturia nashicola* conidia on pear increased with an increase in the length of the dry period, an increase in temperature and a decrease in humidity during the dry period (Li et al., 2005). Pustule development and

scab (*Venturia pirina*) severity were significantly decreased with an increase in the duration of the dry period (Villalta et al., 2000). The effect of interrupted leaf wetness was found to be dependant on the duration of the initial wet period in ascochyta blight of chickpea (*Cicer arietinum* L.) and pea (Armstrong-Cho et al., 2004; Roger et al., 1999). In a study on the development of tan spot in wheat, Sah (1994) reported that 18 h of wetness after various interrupting dry periods (1- 72 h) had higher infection levels compared to only 6 h wetness period after the dry period. The conidia of *S. botryosum* were able to withstand a 22 h dry period after initial wet periods of 2 to 6 h (Bashi and Rotem, 1975). Similar observations were reported on *Cercospora kikuchii* of soybean (Shuh, 1993). The ungerminated pycnidiospores of *Mycosphaerella pinodes* on pea could maintain infectivity after 2 to 21 days RH less than 70% (Roger et al., 1999). The severity of stemphylium leaf spot (*S. vesicarum*) on pear increased with increasing interrupted wetness period and high RH of more than 85% (Llorente and Montesinos, 2002). It is not clear whether the *S. botryosum* prevalent in Saskatchewan is tolerant or vulnerable to interrupted wetness periods. Such information would aid in the development of effective management tools. The objectives of this study were: i) to determine the effect of dry periods on conidial germination of *S. botryosum* ii) to assess the effect of varying wet and dry periods on conidial germination iii) to investigate the effects of interrupted leaf wetness periods and temperatures on stemphylium blight development on lentil.

4.2 Materials and methods

4.2.1 Effect of dry periods on conidial germination of S. botryosum

Conidial germination was tested using suspensions prepared from 7-day old fungal cultures of isolate SB 19. The germination assays were conducted on glass slides using the same procedure for testing germination as described under 3.2.5. The treatments included two controls involving continuous wetness of 8 h and 24 h. All other treatments were exposed to an initial wetness period of 2 h interrupted by dry periods of 12 h, 24 h, 48 h, 72 h and 96 h followed by a subsequent rewetting period of 6 h. In this study, dry period was defined as the unavailability of free water. High RH (≥ 90%) was maintained

in the Petri dishes during the wet and dry periods by placing moistened filter paper at the bottom of the dishes and by placing them in trays with moistened paper at the bottom. The slides were removed from the Petri dishes for drying under the fluorescent light (32 μmol m^{-2} s^{-1}). A gentle air breeze created by an air blower was used to shorten this drying process to 10 minutes. After the drying process, the slides were placed back into the Petri dishes. The well slides were rewetted by reintroducing 5 ml of distilled water onto the slide at the end of each dry period. Conidial suspensions on well slides were incubated at 25°C in an incubator. Percentage germination was recorded after 6 h of rewetting for all treatments with intermittent dry periods, using a light microscope (Nikon Microphot FXA, Japan) with ×100 magnification. Treatments were arranged in a completely randomised design with five replications for each treatment. This experiment was done twice.

UNIVARIATE procedure and LEVENE test were used to test for normality and homogenous variance respectively. The data were log transformed to conform to normality and homogenous variance. The effect of the interrupting dry periods on germination was determined by the MIXED procedure of SAS (SAS Inst., 1999). A random statement for interrupting dry periods nested in experimental repeats was used in the analysis of variance to account for subsampling as discussed in the previous chamber experiments (Schabenberger and Pierre, 2002). Contrasts were used to determine the significance of the difference between the dry periods and the control. A combined analysis was conducted for the repeated experiments, as there was no significant interaction between the experimental repeats and the treatments.

4.2.2 Effect of varying wet-dry-wet periods on conidial germination

In this experiment, the effect of the length of the initial wetness period before the dry period on conidial germination was investigated. The same general procedures as described under 4.2.1 were followed. Continuous wetness treatments of 12 h, 18 h and 24 h were used as controls. The treatments included varying wet-dry-wet durations as follows: 2h-6h-16h, 2h-12h-10h, 4h-6h-14h, 4h-12h-8h, 6h-6h-12h, 6h-12h-6h and 12h-6h-6h. The slides were dried under fluorescent light (32 μmol m^{-2} s^{-1}) and by air blower

as described under 4.2.1. The slides were rewetted by introducing 5 ml of distilled water at the end of each drying phase. Percentage germination was determined after the rewetting phase for all the treatments. A completely randomised design with four replications was used as the experimental design for the two experimental runs.

The effect of interrupted wetness periods on conidial germination was determined by the analysis of variance using the MIXED procedure (SAS Inst., 1999). In a combined analysis of the experimental repeats, a random statement for interrupted wetness periods nested in experimental repeats was specified (Schabenberger and Pierre, 2002). Treatments were compared using contrasts.

4.2.3 Effect of interrupted leaf wetness and temperature on disease development

Whole plants were exposed to either continuous or interrupted leaf wetness after inoculation with conidial suspensions of SB 19 based on the results of the conidial germination experiments. The plants were grown in the growth chamber under conditions described under section 3.2.1. The treatments consisted of varying wet-dry-wet durations as follows: 4h-6h-44h, 4h-12h-44h, 4h-24h-44h, 8h-6h-40h, 8h-12h-40h, 8h-24h-40h, 12h-6h-36h, 12h-12h-36h, 12h-24h--36h, 24h-6h-24h, 44h-12h-24h, 24h-24h-24h. All treatments were thus exposed to 48 h of total wetness. Inoculated plants exposed to 48 h of continuous wetness served as the control. The effect of interrupted leaf wetness on disease development was tested at 20, 25 and 30°C.

The plants were placed in a mist chamber within a growth chamber (model PGV 56, Conviron, Winnipeg, MB, Canada) for the initial wetness periods immediately after inoculation. Two humidifiers (model 7075M, Herrmidifier, Sanford, NC) were used to administer leaf wetness in the form of tiny deionised water droplets. The RH of 95% and 80% ± 3% was maintained in the plastic mist tent chamber and within the chamber, respectively. After the first initial wetness period, the plants were allowed to dry on the bench at each respective temperature under fluorescent light (315 µmol m^{-2} s^{-1}). The dry period took almost 15 minutes after the specified duration of exposure to wetness periods. A dry period in this experiment was defined as the absence of liquid moisture

on the lentil leaf surfaces. The plants were placed back into the mist chamber for the remainder of the second wet period. Temperature and humidity were measured using a sensor (HOBO H8 Pro, Onset Computer Corp., Pocasset, Mass.) and leaf wetness was determined visually. Disease severity was assessed after 14 days using the HB scale as described under 3.2.4. A split plot experimental design was used, with temperature as the main plot and wetness duration as the subplot. The experiment was repeated twice.

4.2.3.1 Data analysis

The effect of interrupted wetness on disease development was determined by analysis of variance using the MIXED procedure (SAS Institute Inc., Cary, NC). PROC UNIVARIATE and LEVENE TEST of SAS were used to test for the conformity of the data to normality and homogenous variance (SAS Inst., 1999). Arcsine transformation was used to stabilize variances and the analysis of variance was performed on the transformed data. Combined analysis was conducted for all the experiments. The experimental design involved subsampling and a corresponding random statement in the statistical model was used as described under section 3.2.1.5. In this experiment, uninoculated controls were not diseased and were not included in the analysis of variance. For ease of reading, the figures present untransformed data.

4.3 Results

4.3.1 Effect of increasing dry periods on conidial germination

In this experiment, an initial wetness period of 2 h was interrupted by dry periods of 12 to 96 h. These treatments were compared with continuous wetness duration of 8 h and 24h. The germination percentage after 24 h of continuous wetness reached 94% but it was only 46% when the conidia were exposed to just 8 h of continuous wetness. The conidial germination generally decreased with increasing durations of the interrupting dry periods (Fig. 4.1). For example, conidial germination of 27% was observed with 24 h interrupting dry period compared to 8% after 96 h interrupting dry period.

The exposure of the conidia to a 12 h interrupting dry period did not significantly reduce the germination rate compared to 8 h of continuous wetness. However, longer dry

periods resulted in a significant decline in germination rate. Under these longer dry intervals the germination rate was lower than the germination rate after 8 h of continuous wetness (Appendix 16). Germination rates for 48 h, 72 h and 96 h dry periods were not significantly different from each other. However, the germination rate after the 24 h interrupting dry period was significantly higher compared than 72 and 96 h, but it was similar to that after 48 h of interrupting dry period.

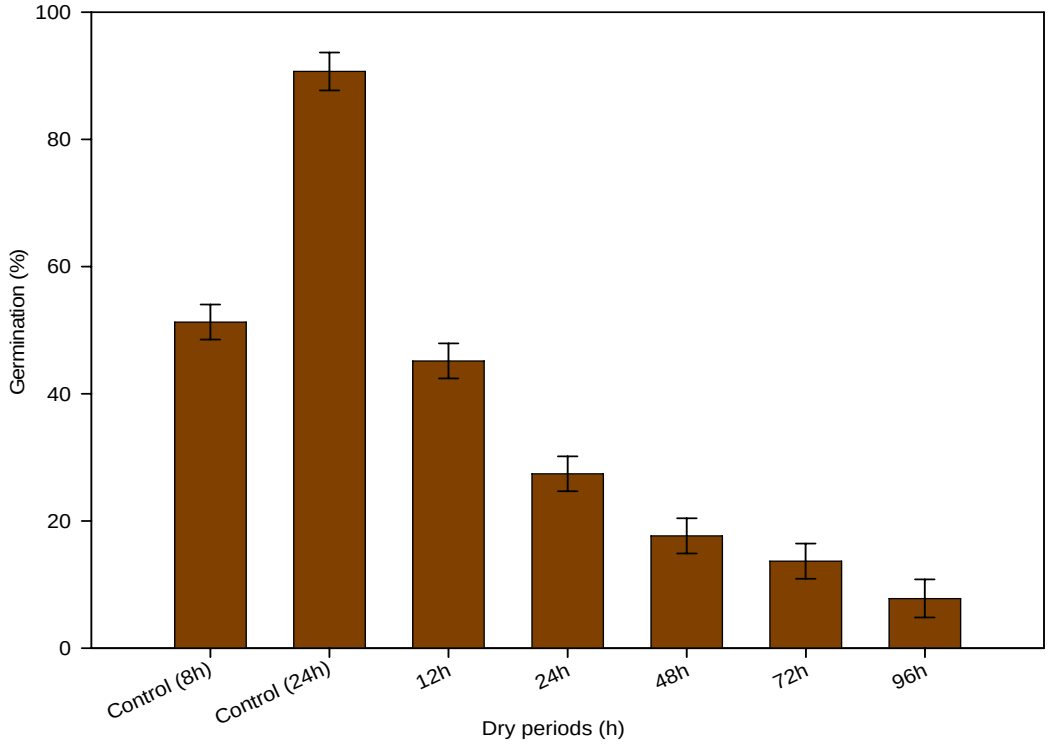


Fig 4.1 Percentage germination of conidia of *Stemphylium botryosum* isolate SB 19 on glass slides incubated at 25°C for an initial 2 h wetness period, followed by dry periods of 12, 24, 48, 72 and 96 h followed by another 6 h of wetness. Y- bars are standard errors of the means.

4.2.2 Effect of varying wet-dry-wet periods on conidial germination.

The effect of the duration of initial wetness period interrupted with dry period of 6 h or 12 h, with total wetness of 12 h and 18 h was investigated. The sensitivity of conidial germination to interrupted dry periods depended on the length of the initial wetness period. Germination rates on glass slides were generally greater after 18 h of total

wetness as compared to 12 h (Fig 4.2). Treatments with 12 h of total wetness interrupted by a dry period had significantly lower conidial germination rates compared to 12 h of continuous wetness. For example, the germination percentage of these treatments (4 h-12h-8h and 6h-12h-6h) were 71% and 70% respectively, compared to 85% for 12 h of continuous wetness. An increase in the duration of initial wetness period reduced the sensitivity of conidial germination to subsequent interrupting dry periods except for 6(6)12 and 6(12)6. Initial wetness duration and total wetness duration had a significant effect on percentage germination than did the duration of the dry period.

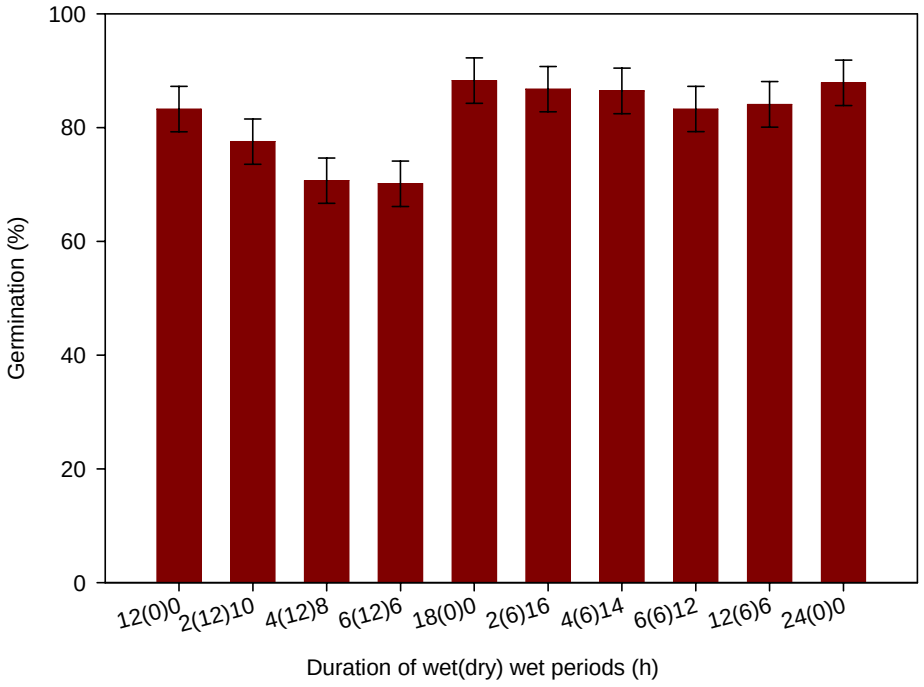


Fig 4.2 Percentage germination of conidia of *Stemphylium botryosum* isolate SB 19 incubated at varying wet-dry-wet conditions at 25°C on glass slides. Y-bars are standard errors of the means.

4.3.3 Effect of interrupted leaf wetness and temperature on disease development

The development of stemphylium blight on lentil leaves was affected by temperature and interrupted leaf wetness (Appendix 17). The interaction between temperature and varying periods of interrupted wetness period was not significant in the analysis of variance. Generally, higher disease levels were observed when plants were exposed to

48 h of continuous wetness compared to all other treatments. Disease severity increased with increasing initial wetness periods, and plants exposed to continuous 48 h of wetness developed the highest disease severity of 78% (Appendix 18). Although interrupting dry periods resulted in a general decrease in disease severity, there were no significant difference among the dry periods of 6 h, 12 h and 24 h (P = 0.4224). An increase in temperature from 20 to 30°C resulted in a significant increase in disease severity (Appendix 18). Stemphylium blight severity at 30°C was significantly higher compared to 20 and 25°C (P = <.0001). For example, disease severity levels of 15% were observed when an initial wetness period of 4h was interrupted by a dry period of 24 h at 30°C compared to 6% for the same treatment at 25°C. There were generally no significant differences between 48 h of continuous wetness period and most of the treatments with the initial wetness of 12 h, 18 h or 24 h at 25°C and 30°C. However, this trend was not observed with the initial wetness of 18 h and 24 h at 20°C. The differences in the stemphylium blight severity were mainly due to increasing initial wetness period and temperature rather than the interrupting dry period. However, interrupting dry periods had a significant effect when the initial wetness period was less than 8 h. This effect increased with increasing in temperature and duration of dry period.

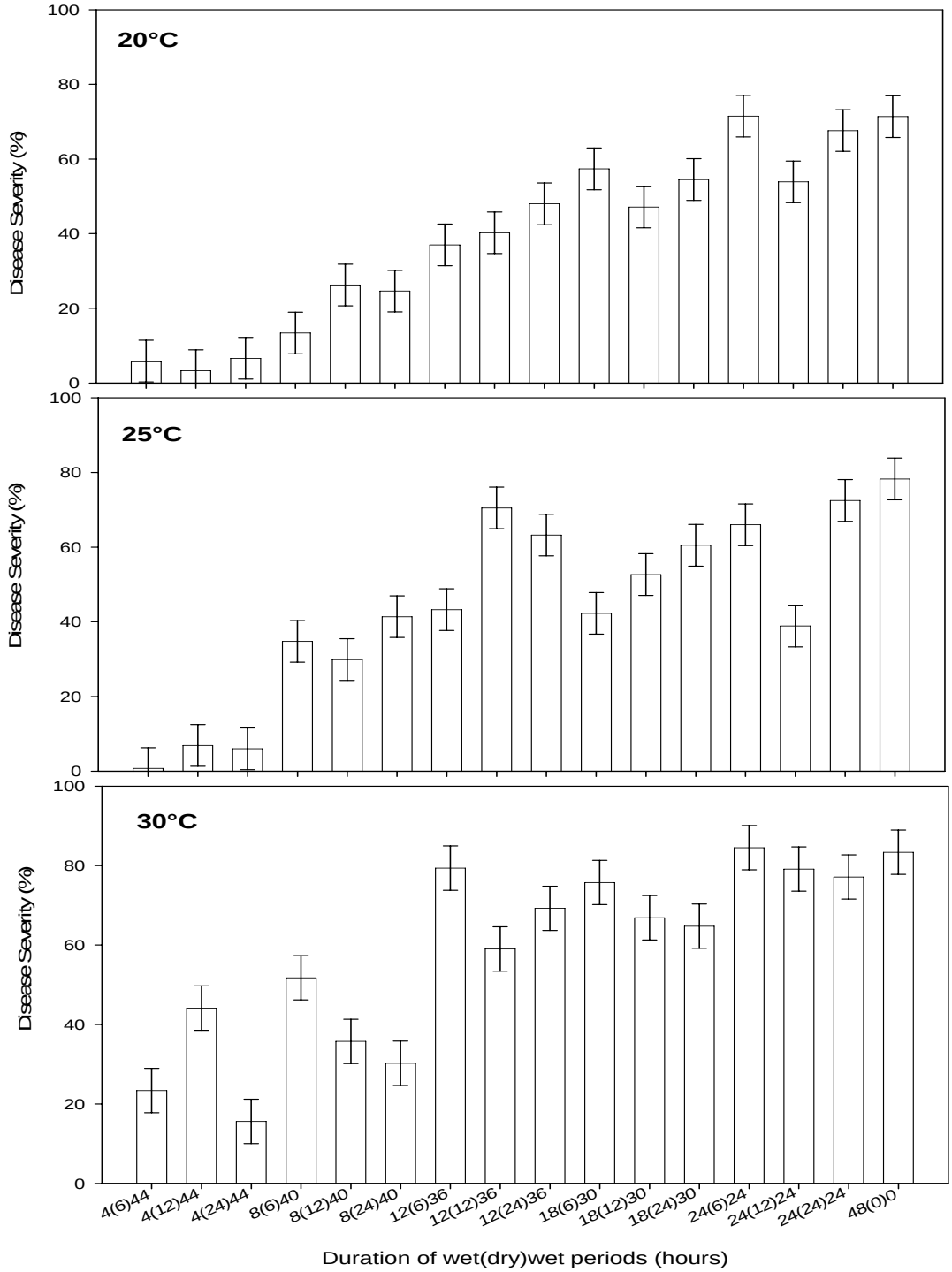


Fig 4.3 Percent severity of stemphylium blight on lentil as affected by varying wet-dry-wet periods and temperatures of 20, 25 and 30°C. Y-bars are standard errors of the means.

4.4 Discussion

Previous studies have indicated that *Stemphylium* spp. requires prolonged periods of leaf wetness for successful infection (Bradley et al., 2003). The present study under controlled conditions revealed that infection and disease development by *S. botryosum* may be reduced by moisture deficits during infection. Stemphylium blight severity was greater with 48 h of continuous wetness compared to treatments with an interrupting dry period of 6 to 24 h, even though the RH greater than 80%. However, conidia of *S. botryosum* were more tolerant to interrupting dry periods of 6 to 24 h if they had been exposed to initial wetness periods longer than 8 h prior to the dry event. The duration of the initial wetness periods and temperature were the most important factors in the development of stemphylium blight. Disease severity increased with the increase in the initial wetness periods irrespective of the length of any subsequent interrupting dry period. Previous studies have reported the survival of spores of other pathogens over prolonged dry periods in Saskatchewan. In studies of the spore survival of *Ascochyta rabei* of chickpea, Armstrong-Cho et al. (2004) concluded that the spores could maintain their infectiveness with dry periods longer than 24 h. The spores of *A. rabei* were resistant to interrupted wetness if the initial leaf wetness period was longer than 8 h. The 8 h initial wetness period was sufficient to allow germ tube elongation, appressoria formation and infection. Contrary to our results, the conidia of *Alternaria cirsinoxia* on Canada thistle (*Cirsium arvense*) in Saskatchewan were not only resistant to wet and dry periods, but the tolerance to interrupting dry periods increased with the decreasing duration of the initial wetness period (Green and Bailey, 2000).

Studies of other *Stemphylium* spp. have demonstrated similar results to the findings in the present work. In the study of the adaptation of *S. botryosum* f.sp. *lycopersici* to semi-arid conditions, Bashi and Rotem (1975) found that conidial germination was reduced by interrupting dry periods longer than 12 h after an initial wetness period of 2 h. However, conidia were able to resume germination in longer total wetness periods of 12 h and 18 h. The findings on the response of conidial germination to wet-dry-wet periods in the present study support the observations of an increase in disease severity with increases in initial wetness periods irrespective of the length of subsequent dry periods. Llorente

and Montesinos (2002) came to the same conclusion, finding that conidia of *S. vesicarum* resumed germ tube development after wetness periods greater or equal to 3 h with RH of 90%. Contrary to our study, disease severity of *A. porri* on onion leaves decreased when the interrupting dry periods were 8 to 12 h (Suheri and Price, 2000a). Unlike *S. botryosum, M. pinodes* on pea require longer initial wetness period (72 to 96 h) for severe disease development during subsequent dry periods (Roger et al., 1999).

Pathogens seem to vary in their ways of combating the effect of the interrupted wet periods. Success in infection under these conditions has been attributed to either the ability to rapidly germinate and penetrate the host during the initial wet period, or the ability of germinating spores to survive dry periods and resume growth when rewetted (Rotem and Bashi, 1975). In this study, conidia of *S. botryosum* exposed to a period of dryness germinated on glass slides after rewetting. Generally disease severity increased with an increase in the initial wetness period, irrespective of dry periods ranging from 6 to 24 h. This suggests that *S. botryosum* conidia can continue the infection process if wetness periods are interrupted by dry periods at high RH (≥ 80%). This phenomenon was reported on *M. pinodes* where moisture was essential for spore germination but appressorial formation, penetration and disease development could progress during a dry period (Roger et al., 1999). The particularly severe disease observed at 30°C with interrupted wetness periods in the present work could be attributed to either increased germination and penetration rates at the higher temperatures, or possibly through ultrastructure changes in the form of woronin bodies which plugs the fungal cells as observed with *M. pinodes* in pea (Roger et al., 1999). These factors have also been reported to complement each other as mechanisms for survival during interrupting dry periods (Becker and Burr, 1994). However, further histopathological studies are required to fully understand the mechanism of spore germination and tissue penetration by germinating conidia of *S. botryosum* after interrupting dry periods.

Based the results of this study, the duration of initial wetness periods and temperature are important factors influencing the ability of *S. botryosum* to cause an epidemic. Conidia are resistant to interrupting dry periods if the initial wetness period is longer

than 8 h. Low infection levels observed in the present study, after 4 h and 8 h of initial wetness periods may explain the low severity levels prevalent in Saskatchewan in most years. Prolonged periods of wetness are not common in the province and *S. botryosum* requires longer wetness periods for successful infection. The pathogen has the potential of being extremely destructive under conditions of high temperature (> 25°C) and prolonged wetness periods (> 8 h). However, this study was conducted under controlled conditions that may not reflect conditions prevalent under field conditions.

5.0 General Discussion

The adaptation of *S. botryosum* to the semi-arid Brown and Dark Brown soil zones of Saskatchewan is essential for it to be a major disease of lentil. The availability of moisture and prevalence of optimal temperature is important for the development of plant diseases. Morrall (1997) in his discussion of the evolution of lentil diseases in western Canada indicated that the major diseases of lentil (ascochyta blight and anthracnose) were first reported in the moister soil zones. The epidemiological studies on these diseases emphasized the importance of these factors. Ascochyta blight requires prolonged periods of wetness (24 or 48 h) for severe disease development. Temperature of 20°C reduced the latent period of ascochyta blight compared to 10°C. Infection levels were lower at 25°C (Pedersen and Morrall, 1994). Similarly, wetness periods of 24 h with prevailing temperatures of 20 - 24°C were optimal for the development of anthracnose on lentil (Chongo and Bernier, 2000). The present study shows that *S. botryosum* requires prolonged wetness periods (24 to 48 h) and temperature of 20 to 30°C for optimal infection of lentil. The disease levels were reduced by interrupting dry periods although longer initial wetness periods (> 8 h) reduced the effect of subsequent dry periods on disease development.

Free water is critical for germination and infection by *Stemphylium* spp. (Bradley et al., 2003). In the present study, *S. botryosum* conidia germinated rapidly in the presence of free water and the production of multiple germ tubes enhanced their potential for multiple infection. Highest germination rates and stemphylium blight severity were observed at 25 to 30°C and relative humidity of 85%. However, the ability of this pathogen to germinate up to 25% of conidia after 12 h of incubation at temperatures as low as 5°C increases its infection potential. This is an important factor considering that

most of the rain events in Saskatchewan are associated with lower temperatures. The optimum temperature of 25 to 30°C was in agreement with the optima seen for *S. botryosum* on lentil in South Asia (Bakr, 1991). Even higher germination rates (90 and 95%) were reported for *S. vesicarum* after 2 h of incubation at 25°C (Rossi et al., 2005). A similar trend has been reported in *A. cirsinoxia* of Canada thistle in Saskatchewan (Green and Bailey, 2000). However, these conditions of prolonged moisture levels with high temperatures are not common in Saskatchewan except under irrigation. It is also possible that they selection criteria of the isolates inadvertently selected for the warm biotype. The results can be different with use of a cool biotype, which requires lower temperatures for optimumal germination and disease development. The existence of such biotypes in Saskatchewan is another area which needs exploration in future research.

Rain events in Saskatchewan often occur as thunderstorms that accompanied by prolonged dry periods. These conditions are generally associated with low temperature (10- 15°C) and high relative humidity (> 90%). For example, in August 2004, lentil fields with high levels stemphylium blight infection were identified in southern Saskatchewan. Disease levels were considerable higher compared to the same period in 2005. The high disease levels in 2004 could be attributed to precipitation events, generally under cloudy conditions and prolonged hours (> 20 h) of high humidity (>80%) at average temperatures of 10 to 13°C.

The response of conidial germination and disease development to incubation temperature and leaf wetness duration was accurately predicted by least square regression equations in this study. Based on these models, this pathogen should have relatively low germination rates and low disease levels due to the low temperatures and short leaf wetness durations prevalent in Saskatchewan. Considering the tolerance of the pathogen to interrupting dry periods, repeated wetness periods (i.e night time dew) might result in the development of the disease. The survival mechanisms of the pathogen under these interrupting dry periods are poorly understood. However, a number of suggestions have been put forward to explain the maintenance of infectivity of conidia

under wet-dry-wet cycles. Suheri and Price (2000a) suggested that formation of lesions after an interrupting dry period was due to multiple germ tubes from multicelled conidia produced at different time periods with exposure to wetness. This school of thought may explain the observations in the present study, where the germ tubes had different lengths suggesting that they started to develop at different times.

The findings of the current work provide an explanation for the low levels of stemphylium typically observed in Saskatchewan lentil fields. With the adaptation of *S. botryosum* to interrupted wetness periods, epidemic proportions of the disease might be observed with longer wetness periods and warmer temperatures. Although high temperatures (25 to 30°C) are unlikely to be present during the thunderstorms, cloudy conditions with temperatures of 20°C after a thunderstorm or rainshowers in some seasons will promote disease outbreaks. Cloudy and foggy weather with temperatures of 20 to 22ºC have been reported to promote stemphylium blight outbreaks in India (Sinha and Singh, 1993). Relative humidity will be of importance in the development of the disease. The closed canopy of the crop might result in higher RH at the bottom of plants. Therefore, it is possible that a healthier thus denser canopy in anthracnose and ascochyta blight resistant varieties or in situations where sprays used to control these diseases may promote an increase in stemphylium blight on lentil. Following a cool, wet summers; inoculum level will likely increase from season to season. It is, however important to investigate the effect of these factors under field conditions. Under cool cloudy conditions, leaf wetness periods are prolonged compared to warm sunny conditions. It is also not known how *S. botryosum* interacts with anthracnose and ascochyta blight in the field. The general school of thought is that the introduction of anthracnose and ascochyta blight resistant varieties might eliminate competition, enabling *S. botryosum* to become a major lentil pathogen. To consolidate the present findings under controlled conditions, field studies will need to be conducted to determine how all these factors will interact and what effect they have on the stemphylium blight.

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7.0 Appendices

Appendix 1 Percent mean conidial germination of *Stemphylium botryosum* isolates (SB 9 and SB 19) at 25°C on glass slides as affected by age of fungal cultures and different light regimes.

Age of culture	*Isolate SB 9*	*Isolate SB 19*	*Light regimes*	*Isolate SB 19*
7 days	59.57c†	64.88bc†	24h light	61.55a†
14 days	63.23ab	67.57ab	24h darkness	61.79a
21 days	64.68a	68.76a	12h light and 12h dark	61.62a
28 days	61.04bc	62.77c	12h dark and 12h light	60.74a

†Means with the same letter in a column are not significantly different at $\alpha_{0.05}$.

Appendix 2 Analysis of variance for final germination after 20 h on glass slides as affected by temperature (5 to 30°C) and *Stemphylium botryosum* isolates (SB 9 and SB 19).

Effect	*Num DF*	*Den DF*	*F value*	*Pr > F*
Temperature (*T*)	5	12	39.60	<.0001
Isolate (*I*)	1	12	0.44	0.520
*T * I*	5	12	1.48	0.267

Appendix 3: Analysis of variance for the rate of conidial germination (slope) on glass slides as affected by temperature (5 to 30°C) and *Stemphylium botryosum* isolates (SB 9 and SB 19).

Effect	*Num DF*	*Den DF*	*F value*	*Pr > F*
Temperature (*T*)	5	12	10.88	<.0001
Isolate (*I*)	1	12	0.64	0.438
*T * I*	5	12	1.02	0.446

Appendix 4 Response surface variance analysis of conidial germination of *Stemphylium botryosum* SB 9 isolate as affected by temperature (t) and incubation time (h).

Source	*DF*	*Mean square*	*F value*	*Pr > F*
Intercept	1	106746.56	12391.60	<.0001
Block	7	1098.62	12.75	<.0001
Linear regressions	(2)			
T	5	13158.76	152.75	<.0001
H	9	10038.02	116.53	<.0001
Quadratic regressions	(2)			
t*t	1	304.26	4.01	0.046
h*h	1	630.87	8.31	0.004
Cross products	(1)			
t*h	1	4250.75	55.70	<.0001
Experimental error	200	86.14		

R^2: 91.30% CV: 19.68%

Appendix 5 Parameters of response surface variance analysis of conidial germination of *Stemphylium botryosum* isolate SB 9 affected by temperature (t) and incubation time (h).

Source	*DF*	*SS*	*Mean Square*	*F value*	*Pr > F*
Intercept	1	1067465.66	1067465.66	14054.8	<.0001
t	1	68711.56	68711.56	904.69	<.0001
*t*t*	1	304.26	304.26	4.01	0.046
h	1	88096.44	88096.44	88096.44	<.0001
*h*h*	1	630.87	630.87	630.87	0.004
*t*h*	1	4230.75	4230.75	55.70	<.0001

R^2: 81.81% CV: 18.48%

Appendix 6 Response surface variance analysis of conidial germination of *Stemphylium botryosum* isolate (SB 19) as affected by temperature (*t*) and wetness duration (*h*).

Source	*DF*	*Mean square*	*F value*	*Pr > F*
Intercept	1	1131025.69	16175.50	<.0001
Block	7	3717.26	53.16	<.0001
Linear regressions	(2)			
t	5	16345.10	233.76	<.0001
h	9	8479.72	121.27	<.0001
Quadratic regressions	(2)			
*t*t*	1	4761.73	64.80	<.0001
*h*h*	1	2366.10	32.60	<.0001
Cross products	(1)			
*t*h*	1	10186.93	138.63	<.0001
Experimental error	200	69.92		

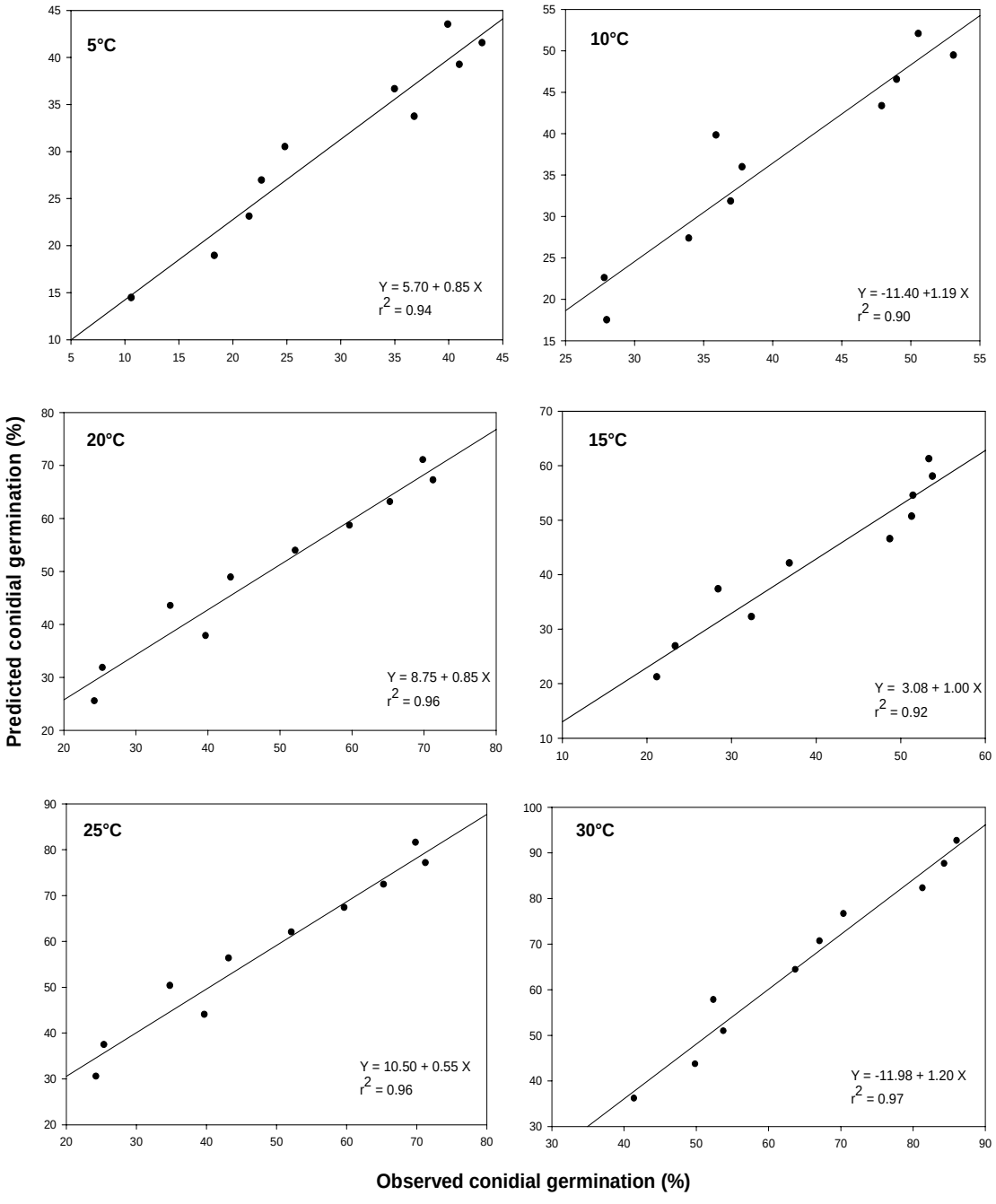
R^2: 93.82% CV: 17.22%

Appendix 7 Parameters of response surface variance analysis of conidial germination of *Stemphylium botryosum* isolate (SB 19) as affected by temperature (*t*) and wetness duration (*h*).

Source	*DF*	*SS*	*Mean Square*	*F value*	*Pr > F*
Intercept	1	1131025.69	1131025.69	15392.0	<.0001
t	1	101598.70	101598.70	1382.65	<.0001
*t*t*	1	4761.73	4761.73	64.80	<.0001
w	1	72902.33	72902.33	992.12	<.0001
*w*w*	1	2366.10	2366.10	32.20	<.0001
*t*w*	1	10186.93	10186.93	138.63	<.0001

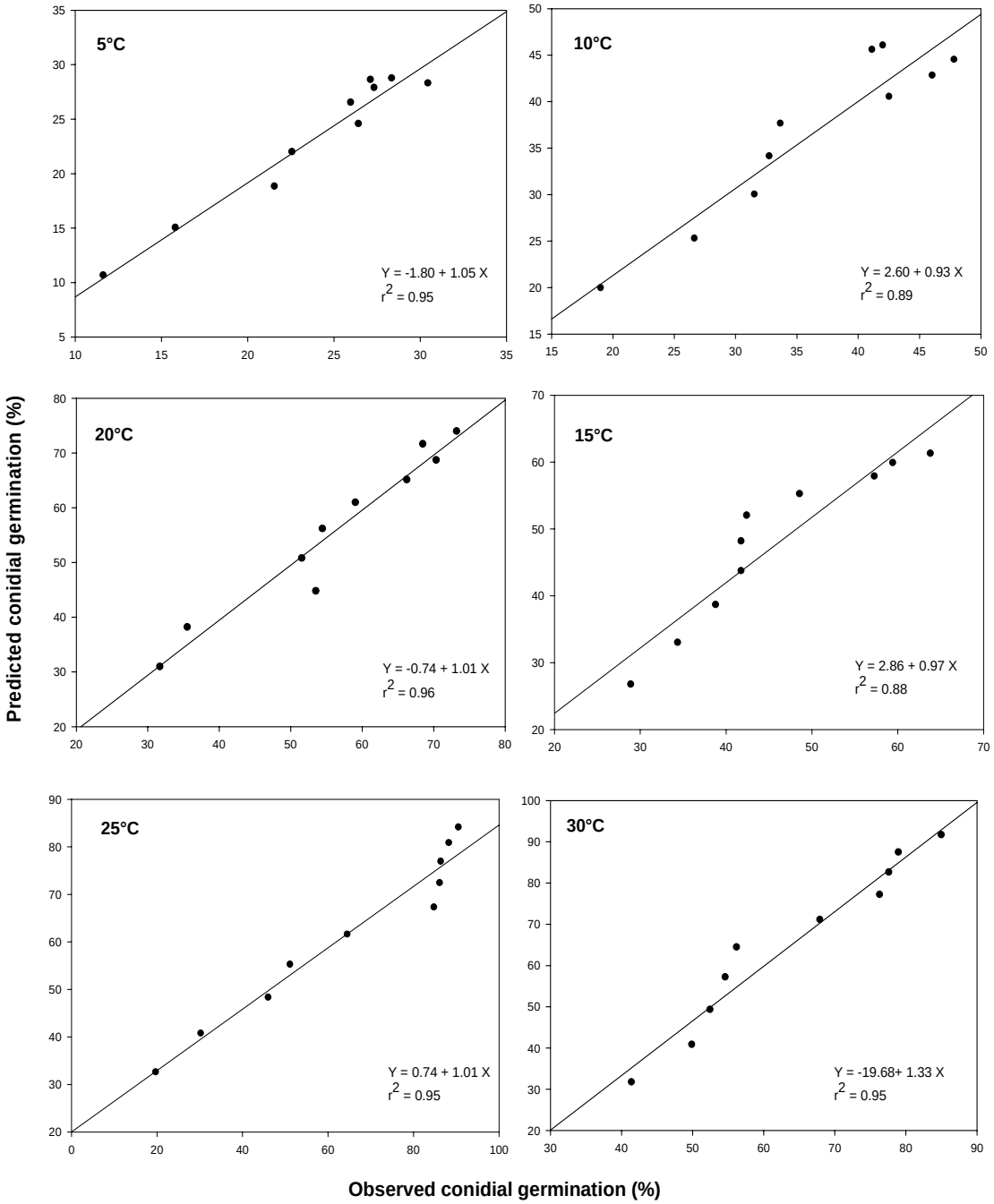
R^2: 84.63% CV:17.66%

Appendix 8 Predicted conidial germination versus the observed conidial germination for temperature levels of 5°C, 10°C, 15°C, 20°C, 25°C and 30°C for *Stemphylium botryosum* isolate SB 9. The germination response was predicted using the equation: $pcg = 7.855 + 0.289(t) + 0.013(t)^2 + 2.167(h) + -0.039(h)^2 + 0.061(t)(h)$



Appendix 9 Predicted conidial germination versus the observed conidial germination for temperature levels of 5°C, 10°C, 15°C, 20°C, 25°C and 30°C for *Stemphylium botryosum* isolate SB19. The germination response was predicted using the equation:

$pcg = -5.301 + 2.438(t) + -0.051(t)^2 + 2.182(h) + -0.076(h)^2 + 0.091(t)(h)$



Appendix 10 Test statistics for the effects of temperature and leaf wetness duration on stemphylium blight severity at 14 days from inoculation and as measured as Area Under the Disease Progress Curve (AUDPC) based on disease rating after 3,7 and 14 days after inoculation.

	ANOVA-type statistic (ATS)					
	Disease severity after 14 days			*AUDPC*		
Effect	*DF*	ATS	*P* value	*DF*	ATS	*P* value
Temperature (*t*)	3.10	378.21	<.0001	5.38	279.70	<.0001
Wetness duration (*w*)	5.62	237.75	<.0001	3.41	596.40	<.0001
t × *w*	8.56	8.56	<.0001	13.50	8.21	<.0001

Appendix 11 Mean rank and estimated relative effect (alpha = 0.05) for stemphylium blight disease severity in relation to temperature and leaf wetness duration.

	10°C		
Wetness duration	*Mean rank*	*Relative marginal Effect (RE)*	*RE standard error*
0	12.00	0.032	0.0001[a]
2	21.38	0.058	0.0001
4	35.56	0.097	0.0004
6	47.38	0.130	0.0005
8	52.94	0.146	0.0008
10	57.38	0.158	0.0006
12	97.44	0.269	0.0005
24	130.63	0.361	0.0002
48	170.75	0.473	0.0012
	15°C		
Wetness duration	*Mean rank*	*Relative marginal Effect (RE)*	*RE standard error*
0	50.56	0.139	0.0010
2	94.13	0.260	0.0017
4	119.88	0.332	0.0010
6	194.13	0.538	0.0004
8	201.69	0.559	0.0017
10	200.88	0.557	0.0013
12	224.69	0.623	0.0009
24	233.94	0.648	0.0509
48	295.38	0.819	0.0004

[a]Standard errors (*se*) are based on output of the LD_CI macro and calculated from: √(var/*N*). *N* is the total number of subjects (40 in this case), *not* the total number of observations.

Appendix 11 continuation. Mean rank and estimated relative effect (alpha = 0.05) for stemphylium blight disease severity in relation to temperature and leaf wetness duration.

	20°C		
Wetness duration	*Mean rank*	*Relative marginal Effect (RE)*	*RE standard error*
0	41.94	0.115	0.0003[a]
2	80.44	0.222	0.0010
4	111.25	0.307	0.0009
6	194.13	0.455	0.0006
8	201.69	0.309	0.0010
10	150.00	0.415	0.0022
12	172.38	0.477	0.0005
24	242.63	0.672	0.0012
48	285.25	0.791	0.0005
	25°C		
Wetness duration	*Mean rank*	*Relative marginal Effect (RE)*	*RE standard error*
0	80.31	0.222	0.0005
2	149.00	0.412	0.0010
4	242.31	0.672	0.0010
6	282.38	0.783	0.0004
8	297.38	0.825	0.0007
10	310.13	0.860	0.0004
12	344.31	0.955	< 0.0001
24	324.88	0.901	0.0006
48	344.25	0.955	0.0001

[a]Standard errors (*se*) are based on output of the LD_CI macro and calculated from: √(var/*N*). *N* is the total number of subjects (40 in this case), *not* the total number of observations.

Appendix 11 continuation. Mean rank and estimated relative effect (alpha = 0.05) for stemphylium blight disease severity in relation to temperature and leaf wetness duration.

	30°C		
Wetness duration	*Mean rank*	*Relative marginal Effect (RE)*	*RE standard error*
0	67.69	0.187	0.0012 [a]
2	180.75	0.501	0.0017
4	234.81	0.651	0.0009
6	197.63	0.548	0.0017
8	292.00	0.810	0.0012
10	258.50	0.712	0.0010
12	265.06	0.735	0.0022
24	302.88	0.840	0.0008
48	347.38	0.964	0.0001

[a]Standard errors (*se*) are based on output of the LD_CI macro and calculated from: √(var/*N*). *N* is the total number of subjects (40 in this case), *not* the total number of observations.

Appendix 12 Analysis of the parameter estimates for stemphylium blight severity after 14 days from inoculation as affected by temperature (*t*) and wetness duration (*w*).

Parameter	*DF*	*Estimate*	*Standard Error*	*Wald 95% Confidence Limits*		*Chi - Square*	*Pr > ChiSq*
Intercept	1	-47.101	8.585	-63.921	-30.280	30.12	<.0001
t	1	3.277	0.919	1.476	5.079	12.72	<.0001
*t*t*	1	-0.033	0.023	-0.077	0.012	2.08	0.149
w	1	2.156	0.254	1.659	2.653	72.32	<.0001
*w*w*	1	-0.022	0.005	-0.032	-0.012	19.50	<.0001
Scale	1	17.990	0.672	16.720	19.358		

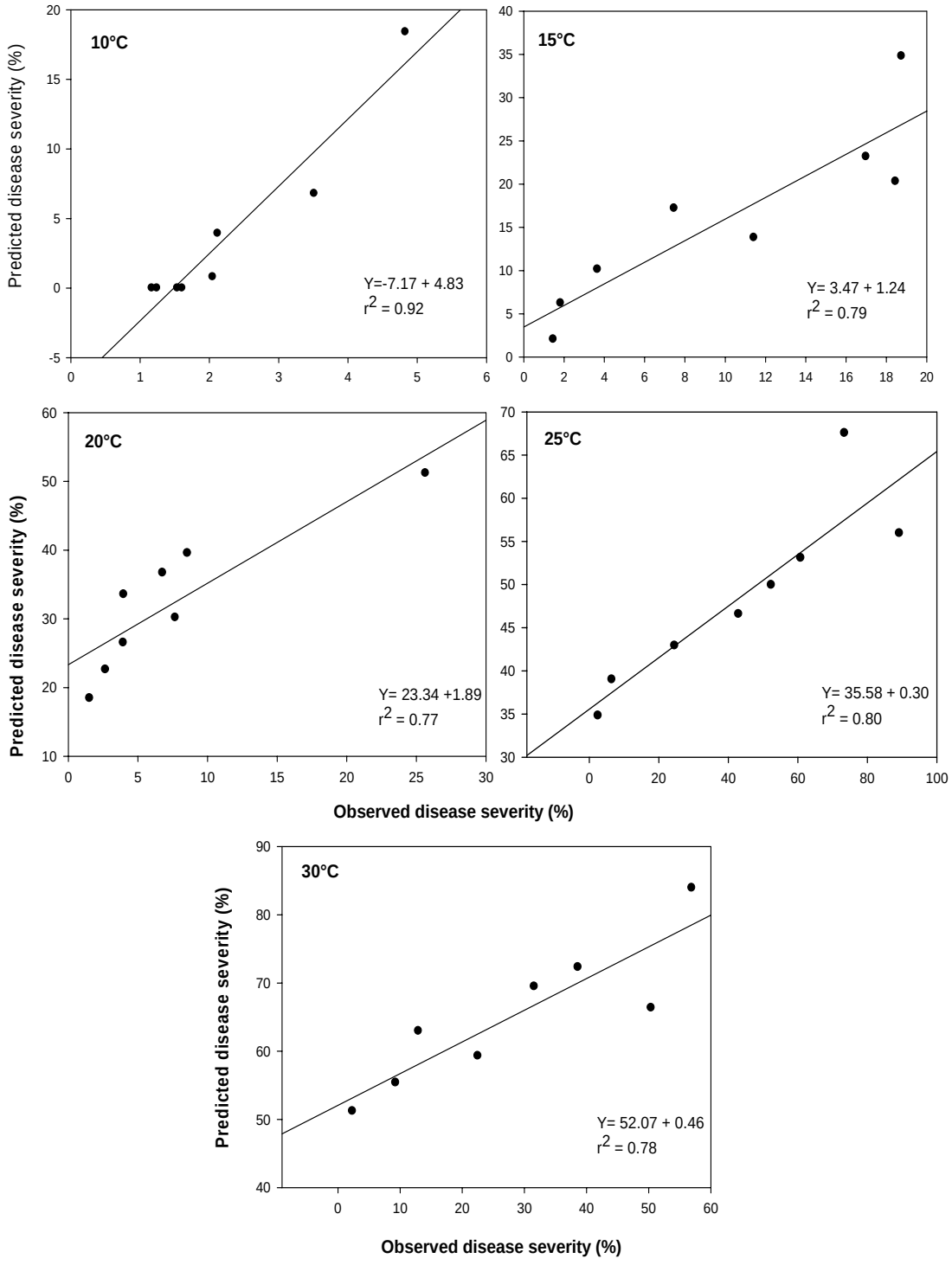
Appendix 13 Contrasts of linear and cubic effects of temperature and wetness periods estimates as they affect stemphylium blight severity.

Contrast	*DF*	*Chi-square*	*Pr > Chisq*	*Type*
*t vs t*t*t*	1	12.86	0.0003	LR
*w vs w*w*w*	1	66.96	<.0001	LR

Appendix 14 Contrasts of *Stemphylium botryosum* conidial germination at 25ºC after 8h of continuous wetness compared to interrupting dry periods of 12 to 96 h after an initial 2 h wetness period subsequently followed by 6 h wetness period after the interrupting dry periods.

Source	*Num DF*	*Den DF*	*F value*	*Pr > F*
8hwt vs 24hwt	1	7	7.09	0.032
8hwt vs 12hdry	1	7	0.20	0.670
8hwt vs 24hdry	1	7	7.23	0.031
8hwt vs 48hdry	1	7	21.12	0.003
8hwt vs 72hdry	1	7	31.55	0.001
8hwt vs 96hdry	1	7	65.25	<.0001

Appendix 15 Predicted stemphylium blight severity against the observed stemphylium blight severity at 10, 15, 20, 25 and 30°C.



Appendix 16 Contrasts of *Stemphylium botryosum* conidial germination at 25ºC after 12 h and 24 h of continuous wetness against varying wet-dry-wet periods.

Source	*Num DF*	*Den DF*	*F Value*	*Pr > F*
12h continuous vs 2w12d10w	1	10	2.72	0.130
12h continuous vs 4w12d8w	1	10	0.19	0.673
12h continuous vs 6w12d6w	1	10	0.11	0.742
18h continuous vs 12w6d6w	1	10	0.49	0.501
18h continuous vs 2w6d16w	1	10	0.07	0.800
18h continuous vs 4w6d14w	1	10	0.10	0.755
18h continuous vs 6w6d12w	1	10	0.79	0.396
12h continuous vs interrupted with 18 total wetness	1	10	7.94	0.018
18h continuous vs interrupted with 12 total wetness	1	10	14.19	0.004

Appendix 17 Type 3 tests of fixed effects for stemphylium blight severity as affected by temperature and varying wet-dry-wet periods on whole lentil plants under controlled conditions.

Effect	*Num DF*	*Den DF*	*F value*	*Pr > F*
Temperature (T)	2	48	30.42	<.0001
wet-dry-wet periods (IW)	15	48	26.98	<.0001
T x IW	30	48	1.64	0.062

Appendix 18 Contrasts of varying wet-dry-wet combinations and temperatures of 20, 25 and 30°C for stemphylium blight development on lentil.

Source	*Num DF*	*Den DF*	*F Value*	*Pr > F*
48h continuous wetness vs interrupted wetness	1	48	56.06	<.0001
48h continuous wetness vs 24h initial wetness	1	48	4.53	0.038
48h continuous wetness vs 18h initial wetness	1	48	17.30	<.0001
48h continuous wetness vs 12h initial wetness	1	48	19.61	<.0001
48h continuous wetness vs 8h initial wetness	1	48	89.57	<.0001
48h continuous wetness vs 4h initial wetness	1	48	176.96	<.0001
48h continuous wetness vs 6h dry	1	48	47.82	<.0001
48h continuous wetness vs 12h dry	1	48	55.85	<.0001
48h continuous wetness vs 24h dry	1	48	46.10	<.0001
4h and 8h initial vs 12h, 18h and 24h initial wetness	1	48	292.83	<.0001
4h, 8h and 12h initial vs 18h and 24h initial	1	48	168.24	<.0001
12h and 18 h initial vs 24 initial	1	48	12.48	0.001
18h initial vs 24h initial	1	48	8.23	0.006
6h and 12h dry vs 24h dry	1	48	0.65	0.422
20°C vs 25°C	1	48	4.22	0.0453
20°C vs 30°C	1	48	56.91	<.0001
25°C vs 30°C	1	48	30.12	<.0001
25°C and 20°C vs 30°C	1	48	56.61	<.0001