Influence of Temperature and Wetness Periods on the Development of Stemphylium Blight on Lentil

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ABSTRACT

Mwakutuya, E., and Banniza, S. 2010. Influence of temperature and wetness periods on the development of Stemphylium blight on lentil. Plant Dis. 94:1219-1224.

Stemphylium blight of lentil (*Lens culinaris* subsp. *culinaris*) caused by *Stemphylium botryosum* has become more prevalent in the Canadian prairies. Germination of conidia, appressorium formation, and infection of lentil plants were evaluated under controlled conditions at temperatures from 5 to 30°C and increasing incubation periods under wet conditions to elucidate the epidemiology of this disease. On glass slides, conidial germination increased steadily with temperature up to 25 and 30°C, and reached more than 80% after 20 h at these temperatures, compared with around 30% at 5°C. The response of germination on glass slides to temperature was nonlinear, as evident in significant linear, quadratic single factor, and linear, quadratic, and cubic cross factor temperature effects in the model. On lentil leaves, 18% of conidia had germinated after 2 h of incubation at 25°C, and a few germ tubes penetrated into the tissue through stomata. Germination reached 89% after 12 h, and 12% of germ tubes had penetrated into the leaves. Stemphylium blight severity reached more than 80% at 25 and 30°C with leaf wetness periods of 48 h. A simple logistic model with linear temperature, leaf wetness period, and cross factor effects described disease development on lentil plants.

Lentil (*Lens culinaris* Medik. subsp. *culinaris*) is mainly grown in the cooler temperate zones of the old world, with more recent introductions into Australia, Canada, New Zealand, and the United States (6). Diseases are a major constraint to the production of lentil in the Canadian prairies. Anthracnose (*Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore), Ascochyta blight (*Ascochyta lentis* Vasilievsky), and Botrytis stem and pod rot (*Botrytis cinerea* Pers.:Fr.) of lentil have been associated with yield losses of up to 60% depending on the disease and prevailing conditions (26). Infection by *Stemphylium botryosum* Wallr., causal agent of Stemphylium blight of lentil, has been observed more frequently in recent years, most notably on lentil seed tested for various fungal infections in commercial seed testing laboratories (27). Initial symptoms on leaves consist of small, light beige lesions that enlarge and coalesce, thus blighting entire leaves and shoots. Pedicels and flowers can also be infected, the latter resulting in flower abortion. Symptoms are prominent in the upper canopy, but entire plants can be blighted under severe infestations. Significant leaf drop, loss in biomass

and seed yield, and a reduction in seed size can occur. Infected seeds are often stained and can have low germination rates. The pathogen spreads by airborne conidia that develop in successive generations on distinct conidiophores on the leaf surface.

S. botryosum can infect a wide range of plant species under different environmental conditions. Host plants other than lentil are spinach (*Spinacia oleracea* L.) (18), onion (*Allium cepa* L.) (1,17), tomato (*Lycopersicon esculentum* L.) (5), alfalfa (*Medicago sativa* L.) (8), and clover (*Trifolium repens* L.) (13). Whether host specialization occurs among isolates from the different host plants has not been explored in detail. Crop devastations in lentil due to Stemphylium blight have been reported from Bangladesh and India, where this disease is a major constraint to production (2,16,30). The economic impact of this disease in Canadian lentil production has not been assessed, and very little is known about the biology of the pathogen in this environment. In many years, Stemphylium blight appears toward the end of the lentil growing season in Canada, so that the main impact is to hasten desiccation. Early infection observed in some years may, however, result in yield reductions and loss of seed quality. Preliminary experiments under controlled conditions have shown that seed size can be significantly reduced and seed staining similar to that caused by *Ascochyta lentis* can be induced by *S. botryosum* (S. Banniza, unpublished data).

Temperature and moisture are primary environmental factors affecting conidial

germination of *S. botryosum* and further development of disease on other hosts (4,5,11). The presence of free water on the leaf surface at optimal temperatures promoted germination and disease development of *Stemphylium* spp. on clover (7). In alfalfa, geographically separated warm and cool biotypes of *S. botryosum* have been identified (9). The prevalence of moderate to warm temperatures below 25°C and wetness duration longer than 24 h favor the appearance, development, and spread of Stemphylium blight in northeastern India and in west-central Bangladesh (3,30). In southern Bangladesh, higher temperatures up to 35°C, maximum and minimum relative humidity (RH) of 78 to 100% and 48 to 80%, and 1.2 to 14.8 mm of precipitation through periodic rainfalls were considered favorable for Stemphylium blight severity (16). However, detailed studies investigating the effects of temperature and moisture on conidial germination of *Stemphylium* spp. are lacking. Data on the closely related genus *Alternaria* suggest that conidial germination occurs at temperatures ranging from 3 to 35°C, and is initiated within 1 to 3 h of incubation at optimal temperatures (31,33). Conidia of *Alternaria* spp. have the ability to germinate at 100% humidity, whereas those of *Stemphylium* spp. appear to require the presence of free water (5). In some host species, *Stemphylium* spp. are able to penetrate directly through the epidermis or indirectly through the stomata, whereas in others appressoria are formed (1,32). For example, *S. vesicarium* differentiates appressoria on onion leaves (1), but not on asparagus (12).

It is currently not understood what factors have triggered the higher occurrence of Stemphylium blight on lentil in the Canadian prairies in recent years. Specific effects of environmental factors on *S. botryosum* of lentil in the semiarid climate of the Canadian prairies are not known; hence this study was initiated with the objective to gain a better understanding of the epidemiology of this pathogen. As a first step, experiments were conducted to determine the effect of temperature and leaf wetness duration on conidial germination, leaf penetration, and the development of Stemphylium blight on a susceptible lentil cultivar. Data generated from these experiments were utilized to model germination and disease development under varying environmental conditions.

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Accepted for publication 11 June 2010.

MATERIALS AND METHODS

Inoculum production. Two monoco-nidial isolates of *S. botryosum* (SB9 and SB19) were selected from a collection of isolates obtained from naturally infected lentil plants collected at different locations in the province of Saskatchewan, Canada, where most Canadian lentil production is concentrated. Preliminary experiments on a larger set of isolates had shown that yields of conidia in general were low, irrespective of the growth medium used (*S. Banniza, unpublished data*). SB9 and SB19 were among the better sporulating isolates, and both were used for the germination experiments on glass slides. SB19 with slightly higher conidiation was selected for further plant inoculations, which required greater amounts of inoculum.

Stock cultures of monoco-nidial isolates were stored in sterile deionized water at $7 \pm 2^\circ\text{C}$, and all cultures used in these experiments were derived from a single stock. Isolates were grown on V8 juice/potato dextrose medium (150 ml of V8 juice [Campbell Soup Company, Camden, NJ, USA], 10 g of potato dextrose agar [Difco, Becton Dickinson and Co., Franklin Lake, NJ, USA], 3 g of calcium carbonate, and 850 ml of distilled water) in petri dishes. To achieve optimum sporulation, cultures were incubated for 7 to 10 days at 27°C under cool fluorescent light ($44 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a photoperiod of 16 h light per day. Conidia were collected from petri dishes by flooding the plates with deionized water and gently dislodging the conidia with a soft brush. A drop of the surfactant Tween 20 (Sigma, Saint Louis, MO, USA) was added to every 1,000 ml of deionized water. All conidial suspensions were processed simultaneously. The concentration of conidia was determined by counting on a Neubauer hemacytometer and was adjusted to the desired concentration by diluting with deionized water.

Plant material. The small green 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 per day provided by fluorescent lamps with light intensities of between 315 and $325 \mu\text{mol m}^{-2} \text{s}^{-1}$. Preliminary experiments on disease resistance of lentil cultivars to *Stemphylium* blight had confirmed that CDC Milestone was susceptible to the disease (*S. Banniza, unpublished data*). The seeds were sown in 10-cm-diameter plastic pots filled with a commercial potting mix (Terralite Redi-Earth, Scotts Sierra Horticultural Products Co., Marysville, OH, USA), and the seedlings were thinned to four plants per pot. The plants were fertilized with complete fertilizer solution (20:20:20 NPK + micro-nutrients) (Plant-Prod All Purpose 20-20-20, Plant Products Co. Ltd., Brampton, ON, Canada) 2 weeks after planting. Three-week-old whole lentil plants were inoculated in all the experiments as de-

scribed below, and pots were arbitrarily assigned to treatments.

Effect of temperature and incubation period on conidial germination. All germination tests were carried out on culture slides with single depressions of 18 mm diameter (Culture Micro Slide, Single Depression, VWR, Mississauga, ON, Canada) under laboratory conditions. Conidial suspensions of 5×10^4 conidia per ml of isolates SB9 and SB19 were prepared from 7-day-old cultures. A high concentration of conidia was used to simplify the counting of germinated spores. An aliquot of 100 μl of conidial suspension was evenly spread over each well slide depression before placing them on top of moistened filter paper in labeled petri dishes. Dishes were kept in plastic trays lined with moistened paper towels to maintain high humidity and were incubated at temperatures of 5, 10, 15, 20, 25, and 30°C in incubators (Sanyo Versatile Environmental Test Chamber Model MLR-350H, Sanyo Electric Co., Ltd., Gunma, Japan) under continuous cool fluorescent light ($32 \mu\text{mol m}^{-2} \text{s}^{-1}$). Preliminary studies had shown that light and the age of the fungal cultures had no effect on conidial germination of *S. botryosum* (data not presented). Germination of conidia was assessed through destructive sampling at 2-h intervals for 20 h by removing four replicate slides of each isolate and temperature treatment. With the aid of a light microscope (Nikon Microphot FXA, Japan) at $\times 100$ magnification, conidial germination was determined by evaluating 100 conidia within several fields of view selected arbitrarily 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 conidium (10). The number of germinated conidia was expressed as a percentage of the total number of conidia counted. The experiment was arranged as a split-plot with temperature as the main plot and leaf wetness duration as the subplot. The experiment was conducted twice.

All statistical data analyses of these and subsequent experiments were conducted in SAS (Version 9.1, SAS Institute, Cary, NC, USA).

Data of conidial germination on glass slides of both isolates were analyzed by repeated measures analysis using the mixed model procedure with the Kenward-Roger correction (19). Heterogeneity of variances and dependencies among repeated measures were modeled by specifying a generalized first-order autoregressive structure with heterogeneous variances which had the best fit among several covariance structures tested (19) (data not presented). Simple, logistic, and negative exponential models were assessed for their goodness-of-fit based on visual evaluation of residuals (22). Neither the logistic nor the negative exponential model improved goodness-of-fit, so further analyses were

conducted with untransformed data. Means were separated using Fisher's least significant differences.

Overall, germination of isolates responded similarly to temperature, so data were pooled and a response surface model for conidial germination as described by temperature and incubation time was developed. As before, a generalized first-order autoregressive covariance structure was specified. Initially, a model was specified with linear, quadratic, cubic, and cross factor terms. Nonsignificant components of the model were deleted in subsequent steps, and models were compared based on Akaike's information criterion (AIC), the corrected AIC (AICC), and the Bayesian information criterion (BIC), as well as by visual assessment of residuals. Fixed effect estimates were obtained with the SOLU-TION option in the model statement.

Infection studies of *S. botryosum* on lentil leaves. Microscopy studies on attached leaves of CDC Milestone were conducted to understand the infection process of *S. botryosum*. Eighteen pots with four plants each of CDC Milestone grown as described above were inoculated with isolate SB19 at 2×10^5 conidia per ml. In preliminary experiments, this concentration had been identified as optimal for disease development (data not presented). A CO_2 air brush (Model RUH8210, Oxygen regulator, Uniweld, USA) at a pressure of 138 kPa was used to apply the spore suspension. Deionized water was sprayed on noninoculated control plants. Plants were incubated at 25°C in a mist tent within the growth chamber. Relative humidity was kept above 95% by saturating the air with water droplets from a humidifier (Humidifier model 7075M, Sanford, NC, USA). Temperature and relative humidity were monitored using a HOBO data logger (HOBO[®]H8 Pro Series, Onset Computer Corporation, Bourne, MA, USA). Three arbitrarily selected pots were assigned to each of six treatments: 2, 4, 6, 8, 10, and 12 h of incubation with continuous leaf wetness. At the end of each incubation period, more than 20 leaves were arbitrarily sampled from each of the three replicate pots assigned to each incubation treatment, and were stored separately for each pot in glass tubes containing a destaining solution of 3:1 glacial acetic acid and ethanol (Merck KGaA, Brampton, ON, Canada). Eight arbitrarily selected leaves of the 20 collected from each pot were examined under $\times 100$ magnification of a Nikon Microphot-FXA microscope (Nikon Inc., New York, USA). The number of conidia that had germinated and the number of those that had invaded the leaves (through stomata or by direct penetration) were counted. Penetration by several germ tubes generated by one polyspermic conidium was counted as one. Data were expressed as percentage for statistical analysis. The experimental de-

sign was a completely randomized design with three replications and two repetitions.

Numbers of germinated conidia and successful invasion from the leaf infection study were analyzed by simple linear regression with the regression procedure of SAS. The same set of data of isolate SB19 was extracted from the experiment on germination on glass slides, and was analyzed with the same procedure for the purpose of comparison.

Effect of leaf wetness duration and temperature on disease development.

The experiment was conducted under controlled conditions in growth chambers (Model PGV 56, Conviron, Winnipeg, MB, Canada). Twenty-one-day-old plants of CDC Milestone were inoculated until runoff (approximately 5 ml per plant) with a conidial suspension of isolate SB19 (2×10^5 conidia per ml) as described before, and pots were wrapped with polythene plastic sheets to promote high humidity. Deionized water was sprayed on noninoculated control plants. Plants were placed in mist tents at various incubation temperatures as described before. The chambers were operated at 10, 15, 20, 25, and 30°C. The plants were exposed to leaf wetness periods of 0, 2, 4, 6, 8, 10, 12, 24, and 48 h. After removal from the mist tents, plants were allowed to dry and incubated until 14 days postinoculation in the same growth chamber under cool fluorescent light with light intensities of $315 \mu\text{mol m}^{-2} \text{s}^{-1}$ and at $85 \pm 3\%$ RH to promote disease development. The experiment was arranged as a split-plot with temperature as the main plot and leaf wetness duration as the subplot. The experiment was conducted twice.

Disease severity was recorded at 3, 7, and 14 days postinoculation using the Horsfall-Barratt scale (15). Individual plants were assessed using the grades and data were transformed into percent disease using the ELANCO conversion.

Stemphylium blight severity on plants of lentil (cv. CDC Milestone) as described by temperature and leaf wetness period was assessed by repeated measures analysis using the mixed model procedure with the Kenward-Roger correction (19). Heterogeneous variances and dependencies among repeated measures were modeled by specifying a first-order antedependence structure that showed the best fit among several models evaluated, and accommodated unequal spacing in repeated measures (19,29,34). Based on the shape of response curves of observed disease severity values, the fit of logistic, Gompertz, and Richards models were explored (22). The logistic model had the better fit (data not presented) and was used for detailed analyses. Means were separated using Fisher's least significant differences.

A response surface model for Stemphylium blight severity on plants of lentil cultivar CDC Milestone in response to temperature and leaf wetness was devel-

oped based on the logistic model and an unstructured covariance matrix which had the best fit. The mixed model procedure was used, and as before a model was initially specified with linear, quadratic, cubic, and cross factor terms. Nonsignificant components of the model were deleted in subsequent steps, and models were compared based on AIC, AICC, and BIC, as well as residuals. Fixed effects estimates were obtained with the SOLUTION option in the model statement.

RESULTS

Effect of temperature and incubation period on conidial germination. In the presence of free water, conidia of *S. botryosum* germinated over a wide range of temperatures (5 to 30°C) (Fig. 1). Generally, the percentage of germinated conidia increased with temperature and incubation period. After 20 h of incubation, the conidial germination for the two isolates was above 30% at 5°C and above 80% at 25 or 30°C. At least 10% of conidia germinated after 2 h at 5°C, whereas over 40% had germinated at 30°C after the same period (Fig. 1).

Significant effects on germination were observed for isolates ($P = 0.0201$), temperature ($P < 0.0001$), incubation period

($P < 0.0001$), and the isolate $\times$ temperature interaction ($P < 0.0001$). To explore differences between the isolates further, models were fitted for each temperature. Isolate SB9 had a somewhat higher germination rate at 5°C beyond 10 h, and at 10°C beyond 18 h of incubation, whereas isolate SB19 had higher conidial germination during the first 10 h of incubation at 15 and 20°C (Fig. 1). SB19 also had higher conidial germination between 12 and 18 h of incubation at 25°C compared to 30°C, but final germination after 20 h of incubation was similar.

Overall, germination trends over time for the two isolates were considered similar enough to pool data for further modeling. The relationship of temperature, incubation period, and conidial germination of *S. botryosum* was best described by the following model:

$$G = b_0 + b_1I + b_2I^2 + b_3T + b_4T^2 + b_5TI + b_6T^2I + b_7T^3I + E \quad (1)$$

where G is the proportion of germinated conidia, T is temperature, and I is incubation period. Although complex, the model showed a good fit overall, and the majority of predicted values were within 2% of the observed germination values (Fig. 2). The highest deviations were observed at 10°C,

Fig. 1. Conidial germination of *Stemphylium botryosum* isolates SB19 and SB9 on glass slides in response to temperature and incubation period. Asterisks indicate significant differences between the isolates at $P \leq 0.05$ for *, $P \leq 0.01$ for **, and $P < 0.0001$ for ***.

where predicted germination was up to 7% lower than observed.

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. Up to six germ tubes were observed developing from one conidium. Most penetrations of the leaf surface were through the stomata; however, germ tubes also passed close to or over stomata, suggesting that there was no specific orientation of germ tubes toward stomata. Appressorium for-

mation was not observed in this experiment. The development of hyphae inside the leaf tissue was detectable after 12 h. Percent germination and the number of successful penetrations increased linearly with wetness duration at 25°C ($P < 0.0001$) (Fig. 3). Germination of conidia had reached 88% after 12 h of incubation, and an average of 11 successful penetrations per leaflet were observed.

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. Temperature, leaf wetness period, and the interaction between these factors in the repeated measures analysis had significant effects on Stemphylium blight severity ($P < 0.0001$). In general, there was a significant increase in disease severity with increasing leaf wetness duration (Fig. 4). Disease severity was lower on plants incubated at 10°C than on plants incubated at any other temperature except for plants incubated under dry conditions after inoculation (0 h) at 15 and 20°C, and after 2 h of incubation at 20°C (Table 1). Disease progress was similar at 15 and 20°C, except for the periods between 8-, 10-, and 12-h leaf wetness periods. Disease severity was significantly higher on plants incubated at 25 compared with 30°C after leaf wetness periods of 6 h and 12 to 24 h (Table 1). Maximum disease severity of more than 80% was observed at 25 and 30°C with leaf wetness periods of 48 h.

A regression model was developed to predict the form of dependence of disease severity (L_{DS} = logit transformed disease severity) on temperature (T) and leaf wetness duration (W). Linear terms for temperature and leaf wetness duration and their interaction were highly significant in the analysis ($P < 0.0001$ for T and W , $P = 0.0003$ for $T \times W$). None of the quadratic and cubic terms examined were significant. The relationship of temperature, leaf wetness period, and Stemphylium blight severity was described by the following model:

$$L_{DS} = b_0 + b_1T + b_2W + b_3TW + E \quad (2)$$

Overall, the model predicted disease adequately. Stemphylium blight severity was under-predicted by 9 to 13% at 15°C with leaf wetness periods between 10 and 48 h, and significantly over-predicted at 20°C after 48 h of leaf wetness (Fig. 4). Lower predicted values were also observed at 25°C with wetness periods between 4 and 24 h, and at 30°C at 8 h leaf wetness. At 25 and 30°C, the model predicted most rapid increases in disease severity after leaf wetness periods of 10 and 35 h, before and beyond which the rate of disease increase declined. The model also suggests that this temperature range represents the temperature optimum, but further data beyond 30°C would be required to confirm this. At the other end of the temperature spectrum, very little disease development would be expected at temperatures below 10°C irrespective of prolonged wetness periods.

DISCUSSION

Stemphylium blight of lentil is a poorly understood disease on the Canadian prairies. Germination experiments under controlled conditions showed that conidia of *S. botryosum* from lentil germinated at temperatures ranging from a low of 5°C up to 30°C. These results were confirmed on

Fig. 2. Conidial germination (G) of *Stemphylium botryosum* on glass slides in response to temperature (T) and incubation period (I). Symbols represent observed germination at 5, 10, 15, 20, 25, and 30°C. Response surface mesh plot represents predicted germination based on the polynomial function $G = 0.071710 + 0.02330I - 0.00054I^2 + 0.00508T + 0.00006T^2 - 0.000747I + 0.00014T^2I - 3.23E^{-6}T^3I$.

Fig. 3. Germination of conidia of *Stemphylium botryosum* isolate SB19 on attached leaves of lentil cultivar CDC Milestone and on glass slides, and penetration of the fungus into leaves in a growth chamber at 25°C during a 12-h wetness period.

glass slides and on lentil leaves. A similar temperature range for conidial germination of 3 to 35°C was reported for the related species *A. helianthi* with an optimum at 25°C (31). Penetration of the lentil leaves by the pathogen was mostly through the stomata, which was also reported for alfalfa (28), and from *S. vesicarium* on onion leaves (1) and asparagus (12).

Earlier research on *S. botryosum* infecting alfalfa showed that 95% of conidia had germinated on alfalfa leaves incubated for 3 h at 20 to 23°C (28). This was significantly higher than for *S. botryosum* on lentil, where at 25°C, 20 and 30% of conidia had germinated after 2 and 4 h of incubation, respectively. Higher germination rates were also observed for *S. vesicarium* incubated at 25°C on water agar where 50% of conidia had germinated within an hour of incubation (25).

Despite high conidial germination rates after relatively short incubation periods in wet conditions, longer wetness periods appear to be required for *Stemphylium* spp. to cause significant disease on host plants, in particular at suboptimal temperatures. When *S. botryosum* f. sp. *lycopersici* was inoculated onto tomatoes, disease severity reached 2.5 on a scale of 0 to 5 (5 representing completely wilted leaves) after 48 h of incubation under wet conditions at 20°C (5). Similarly, alfalfa plants inoculated with *S. botryosum* and incubated at 18 to 20°C required 4 days of misting for disease severity on leaves to reach more than 40% (8). Inoculation of asparagus spears with *S. vesicarium* also required 48 h of incubation in high humidity at 25°C for first symptoms to appear (12), and leaf wetness periods longer than 24 h were required for symptom development on garlic after inoculation with *S. vesicarium* and incubation at 22°C (4). Observations in northeastern India indicated that 18 ± 2°C, 85 to 90% RH in the morning, and more than 50% RH in the afternoon (indicative for prolonged dew periods), as well as less than 7.7 h of sunshine per day were considered favorable for *Stemphylium* blight development in winter-grown lentil (30). Similarly, in west-central Bangladesh, *Stemphylium* blight development was most dramatic when maximum temperatures in the canopy ranged from 20 to 22°C, combined with high maximum humidity above 90% RH (3). In field experiments in south-central Bangladesh, first *Stemphylium* blight symptoms were observed in late January or early February when maximum temperatures were between 21 and 29°C, minimum temperatures between 13 and 15°C, maximum RH between 78 and 85%, and minimum RH between 48 and 63% (16). Disease severity increased steadily and was highest in March when maximum temperatures reached 35°C and minimum temperatures did not drop below 20°C, combined with RH ranging between 78 and 100%. In the

current study, 48 h of incubation under wet conditions at 20°C resulted in disease levels on lentil of 44%. However, at higher incubation temperatures, similar disease levels were observed after wetness periods of 6 to 8 h. An effect of temperature similar to this was also observed when studying disease severity on onion after inoculation with *A. porri* in response to wetness duration in relation to temperature (32).

The adaptation of *S. botryosum* to particular environments was clearly demonstrated by cool (C-T) and warm (W-T) biotypes found in populations on alfalfa in western and eastern regions of the United States (9). Optimal temperature requirements of 18 to 22°C were identified for the C-T biotype originating from California, whereas the W-T isolates from the eastern United States thrived at temperatures of 23 to 27°C. The most severe symptoms of

Stemphylium blight on lentil plants in the current study were observed at temperatures of 25 and 30°C, suggesting an optimum temperature for western Canadian lentil isolates of *S. botryosum* in this temperature range. This optimum temperature would be similar to that of field isolates in southern Bangladesh and that of the W-T biotype of alfalfa isolates. In contrast, west-central Bangladeshi lentil isolates of *S. botryosum* required 20 to 22°C for maximum infection of lentil (3), and the temperature optimum for *Stemphylium* blight on lentil in northern India was identified as 18 ± 2°C (30). Isolates in those regions could thus represent cool biotypes of the pathogen adapted to the environmental conditions of lentil grown throughout the winter in those locations. More isolates need to be tested to confirm whether lentil isolates of *S. botryosum* can

Fig. 4. Severity of *Stemphylium* blight after inoculation of lentil cultivar CDC Milestone with *Stemphylium botryosum* isolate SB19 and incubation for up to 48 h at temperatures of 10, 15, 20, 25, and 30°C. Symbols represent observed disease severity. Response surface mesh plot represents predicted germination based on the function $L_{DS} = -4.7547 + 0.003096T + 0.0708W + 0.004307TW$, where L_{DS} is logit transformed disease severity, T is temperature, and W is wetness duration.

Table 1. *Stemphylium* blight severity (back-transformed logit transformed least-squared estimates) on lentil cultivar CDC Milestone in response to incubation at different temperatures and leaf wetness periods

Wetness period (h)	Temperature (°C)				
	10 ^z	15	20	25	30
0	1.17 a	1.73 ab	1.52 ab	2.43 b	2.15 b
2	1.27 a	2.93 b	2.47 ab	5.99 c	8.36 c
4	1.47 a	4.12 b	3.69 b	22.88 c	20.67 c
6	1.66 a	11.07 b	7.31 b	42.73 c	11.61 b
8	1.76 a	12.85 c	3.72 b	52.26 d	37.56 d
10	1.87 a	12.36 c	6.23 b	63.10 e	29.81 d
12	3.11 a	17.49 c	8.20 b	90.77 e	35.21 d
24	4.82 a	19.61 b	23.39 b	79.02 e	57.44 d
48	8.00 a	51.34 b	44.43 b	91.13 c	92.76 c

^z Estimates followed by the same letter in a row are not significantly different based on LSD at 5%.

indeed be separated into two biotypes with distinct temperature optima. The present study demonstrated that low levels of disease (2 to 7% severity) can develop after 2 h of leaf wetness at 10 to 20°C, indicating that the pathogen can cause at least some infection at low temperatures.

Predictive models used for *Stemphylium* spp. such as BSPcast, FAST, and TOM-CAST recognize the importance of temperature and leaf wetness duration on disease severity and have been used successfully in the timely scheduling of fungicide applications (20,23,25). For example, FAST incorporates hours of leaf wetness, average temperature during the wetness period, mean air temperature, hours of relative humidity greater than 90%, and total precipitation into the model (21). Although this model was developed for *Alternaria solani* on tomato, it accurately predicted infection periods for *S. vesicarium* on pear and could reduce fungicide sprays by 28 to 38% when used to schedule fungicide applications. However, it was not accurate at forecasting disease levels (25). Even higher reductions in the number of fungicide applications of up to 60% were achieved when using TOM-CAST, a model derived from FAST, to control purple spot of asparagus (23). This forecasting model relies on wetness periods and temperature to assign disease severity values.

BSPcast was specifically developed to forecast infection by *S. vesicarium* on pear by relating disease severity to temperature and daily wetness duration (20). Although isolates of *S. vesicarium* causing brown spot on pear clearly have a temperature optimum around 20°C compared to an optimum of 25 to 30°C for *S. botryosum* on lentil as determined in this study, disease progress appears to be similar, characterized by a logarithmic increase in disease severity toward the optimum temperature. This was recognized in both studies by using a log₁₀ transformation in the model for brown spot on pear (24), and the logit transformation in the current model.

This study could be used for the prediction of conidial germination and disease severity at wetness duration of 0 to 48 h and temperatures of 5 to 30°C under field conditions. Further experiments beyond 30°C would be required to confirm that the temperature optimum for *S. botryosum* on lentil is indeed between 25 and 30°C, and to explore to what degree infection is inhibited beyond the maximum temperature. Prolonged continuous periods of wetness are infrequent during the growing season in the Canadian prairies, particularly in the semiarid region where the majority of lentil is grown. Significant rain events often occur in the form of thunderstorms characterized by very intense rain in short periods of time, followed by dry and warm or possibly hot periods. Conidia of the related *A. cirsinioxia*, evaluated as a poten-

tial bioherbicide of Canada thistle in the Canadian prairies, were shown to be well adapted to this environment and remained infectious, albeit at decreasing rates, even after five cycles of intermittent leaf wetness (14). It is likely that survival during repeated intermittent leaf wetness periods is also characteristic for conidia of *S. botryosum*, an area which therefore warrants further evaluation in the development of forecasting models.

ACKNOWLEDGMENTS

Funding for this project through the Agricultural Development Fund of the Saskatchewan Ministry of Agriculture and the Saskatchewan Pulse Growers is gratefully acknowledged.

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