Eur J Plant Pathol (2010) 126:153–163
DOI 10.1007/s10658-009-9529-9

Influence of environmental factors on conidial germination and survival of *Sphaeropsis pyriputrescens*

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Received: 8 April 2009 / Accepted: 10 August 2009 / Published online: 2 September 2009
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Abstract *Sphaeropsis pyriputrescens* is the cause of Sphaeropsis rot in apples and pears. In this study, effects of temperature, wetness duration, relative humidity (RH), dryness, and interrupted wetness duration on conidial germination of the fungus were evaluated. Conidial germination and germ tube elongation occurred at temperatures from 0°C to 30°C. The optimum temperature for germination and germ tube elongation appeared to be 20°C, at which a minimum wetness period of 5 h was required. Conidia germinated at RH as low as 92% after 36 h at 20°C, but not at 88.5% RH. The effect of dry periods on germination depended on RH. Conidial germination at 85% RH was higher than that at 25% RH within a 4-h dry period, after which time no difference was observed. Less than 10% conidia germinated after a 10-day dry period at both 20°C and 28°C. Conidial germination decreased as the wetness duration prior to dryness increased. Conidia wetted for 6 h prior to dryness died within a 1-h dry period. After a 12-h dry period, no or few conidia germinated at 25% RH, whereas 3% to 10% of the conidia germinated at 85% RH and no further decrease was observed as the dry period increased. The results contribute to our understanding of conditions required for conidial germination of *S. pyriputrescens* and infection of fruit leading to Sphaeropsis rot.

Keywords Dryness · Interrupted wetness · Postharvest disease · Sphaeropsis rot

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Introduction

Sphaeropsis pyriputrescens is the causal agent of Sphaeropsis rot, a recently reported postharvest fruit rot disease of apples and pears (Xiao and Rogers 2004; Xiao et al. 2004). This disease was initially observed on d'Anjou pears, but later findings indicated that this disease causes more problems on apples (Kim and Xiao 2008). In one instance observed in 2003 in which neither preharvest nor postharvest fungicides were applied to the fruit, 24% of Red Delicious apple fruit in storage bins were rotted by *S. pyriputrescens* (Xiao et al. 2004). A survey for postharvest diseases of apples conducted in Washington State during 2003–2005 indicated that Sphaeropsis rot accounts for approximately 20% of the total fruit rots in stored apples (Kim and Xiao 2008). This disease has been observed on various cultivars of apple, including Red Delicious, Golden Delicious, Granny Smith, Gala and Fuji (Xiao et al. 2004; Kim and Xiao 2008).

Infection of apple fruit by *S. pyriputrescens* occurs in the orchard, but symptoms develop during storage (Xiao et al. 2004). In apple orchards, *S. pyriputrescens* has also been found to be responsible for a twig

dieback and canker of apple and crabapple trees (crabapple is commonly used as pollenisers in commercial apple orchards) (Xiao and Boal 2005). Pycnidia of *S. pyriputrescens* are commonly present on diseased twigs and branches of the trees (Xiao and Boal 2005). No teleomorph of the fungus has been observed in the orchard (Xiao and Rogers 2004). Pycnidia formed on the diseased twigs or branches of the infected trees are believed to be the inoculum responsible for infection of apple fruit in the orchard leading to Sphaeropsis rot during storage, and viable pycnidia and conidia are available throughout the apple-fruit growing season (unpublished data). Because availability of inoculum of *S. pyriputrescens* appears not to be a limiting factor for infection of apple fruit in the orchard, environmental conditions required for dispersal and germination of conidia seem to be important in initiating infection of apple fruit.

The effects of environmental factors on mycelial growth of this fungus have been reported (Kim et al. 2005). Mycelial growth of *S. pyriputrescens* occurs at temperatures ranging from −3°C to 25°C, with the optimum at 20°C. No mycelial growth occurs at 30°C, but the fungus survives at 30°C (Kim et al. 2005). However, environmental conditions required for conidial germination of the fungus have not been reported. Knowledge about environmental factors that influence conidial germination would help us to better understand the conditions required for fungal infection of fruit leading to Sphaeropsis rot and may also lead to the development of disease management strategies or tactics in the orchard.

The objectives of this study were to determine the influence of environmental factors including temperature, wetness duration, and relative humidity (RH) on conidial germination and the effects of dryness and interrupted wetness duration on survival and conidial germination of *S. pyriputrescens*.

Materials and methods

Isolates and pycnidial production

Two single-spored isolates of *S. pyriputrescens* were used in this study. Isolate CLX2142 was recovered from a twig with dieback symptoms sampled from a crabapple tree in a commercial apple orchard in Manson, WA, USA. Isolate CLX2145 was recovered from a decayed 'Red Delicious' apple fruit sampled from a commercial apple packinghouse in Yakima, WA, USA. Identification of these isolates was based on the descriptions of the fungus by Xiao and Rogers (2004). Conidia from 3 week-old cultures on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) were streaked on oatmeal agar (OMA; 60 g of finely ground iron- and zinc-fortified oatmeal [Gerber, Fremont, MI, USA], 15 g agar in 1 l of deionised water, autoclaved at 121°C for 90 min). All plates were sealed with Parafilm, four holes cut (5 mm) in the Parafilm along the rim of the plate and incubated at 20°C under a 12-h light/12-h dark cycle (10 Wm^{-2}) for 3 weeks (Kim et al. 2005).

Preparation of conidial suspensions

Conidia grown on OMA were obtained by scraping oozing pycnidia into 20 ml of sterile deionised water. The resulting conidial suspensions were filtered through four layers of cheesecloth. Preliminary studies indicated that conidial germination of *S. pyriputrescens* was poor in sterile water, and adding apple juice into the suspension (TreeTop, Selam, WA, USA) stimulated the germination of the fungus. In this study, unless otherwise specified, a 10% apple juice solution was used to make conidial suspensions. The final concentration of conidial suspension was adjusted to 2×10^5 conidia ml^{-1} with a hemacytometer.

Effects of temperature and wetness duration

A 10-µl drop of conidial suspension was placed on a cover glass using a micropipette. Three cover glasses were placed on a moist filter paper (70 mm diam, Whatman Ltd, England) in a 95-mm plastic Petri plate. The plates were sealed with Parafilm and incubated at 0°C to 35°C (5°C increment) in the dark. Conidial germination was evaluated after 4, 5, 6, 8, 10, 12, 16, 20, and 24 h of incubation at 10°C to 25°C. A preliminary observation indicated that conidia of the fungus germinated very slowly at temperatures <10°C or >25°C. Thus, in addition, the evaluation was extended to 36, 48, 72, 96, 120, and 144 h of incubation at 0°C, 5°C, 30°C, and 35°C. Percent conidial germination was determined by examining 100 conidia on each cover glass. A

conidium was considered germinated if the germ tube was at least one-half the length of the conidium. Germ tube length was measured after 4, 5, 6, 8, 10, 12, 16, 20, and 24 h of incubation by measuring 20 randomly selected germ tubes on each cover glass. Because it took >1 h to complete the evaluation of germination and measurement of germ tube length, some germination plates containing cover glasses were kept in a refrigerator at 4°C before examination to slow down the germination process and minimise the influence of delay of examination on conidial germination.

Effect of RH

The agar dish equilibration technique, described by Harris et al. (1970) and modified by Alderman and Beute (1986), was used to control the RH inside sealed agar plates. The RH in this sealed plate is related to the NaCl molality according to the value given by Lang (1967). Water agar (2%) amended with sodium chloride was prepared, and 35 ml of the medium was poured into the base of plastic Petri plates (95×15 mm), leaving a 6-mm air space. The levels of RH were 100%, 99%, 98%, 95%, 92% and 88.5%, obtained by amending the agar with 0, 0.3, 0.6, 1.5, 2.2, and 3.1M NaCl, respectively. The RH chambers were equilibrated at the respective temperatures for at least 12 h prior to use. Two free-water controls were included; one in which the conidial suspension was not air-dried and the other in which the conidial suspension was air-dried for 20 min at ambient conditions (24±0.45°C and 20.7±2.4% RH measured by a small data logger [Watchdog® 450, Spectrum Technologies, Inc., Plainfield, IL, USA]) and rewetted immediately after drying. A 2-µl drop of the conidial suspension was placed on a cover glass and air-dried for 20 min except the first free-water control. Three cover glasses were placed on the lid of the RH chamber. The cover glasses for the free-water control were placed on a moist filter paper in the Petri plate. The RH chambers were sealed with Parafilm, placed upside down in plastic crispers, and incubated at 0°C to 30°C (5°C increment) in the dark. Conidial germination was evaluated after 3, 4, and 5 days of incubation at 0°C; 12, 24, 36, and 48 h at 5°C, 10°C, 25°C, and 30°C; and 4, 6, 8, 10, 12, 16, 20, 24, 36, and 48 h of incubation at 15°C and 20°C. Germ tube length was measured after 3, 4, and 5 days of incubation at 0°C, and 12 and 24 h of incubation at 5°C to 30°C. Percent conidial germination and germ tube length were determined as described above.

Effects of dryness and interrupted wetness

The effects of dryness and interrupted wetness duration on conidial germination were evaluated. A 2-µl drop of conidial suspension was placed on each of the three cover glasses per treatment and air-dried for 20 min. After the drops evaporated, cover glasses were placed in either empty Petri plates, corresponding to 25% RH measured by the data logger, or RH chambers amended with 4 M NaCl, corresponding to 85% RH. The plates were incubated at 20°C and 28°C in the dark for 2, 4, 8, 12, 24, 48 h, 3, 5, and 10 days after drying. Conidia rewetted immediately after 20-min air-drying were used as a control. Following dry periods, conidia on cover glasses were rewetted with sterile water and allocated on a moist filter paper in the Petri plates. The plates were sealed with Parafilm and incubated at 20°C for another 24 h to obtain the maximum germination of conidia.

For the interrupted wetness study, conidia were subjected to various sequential wetness and dryness treatments. Three cover glasses each with a 2-µl drop of conidial suspension were placed on a moist filter paper in a Petri plate, sealed with Parafilm, and incubated at 20°C and 28°C in the dark for 1, 2, 4, and 6 h. After the wet periods, the cover glasses were removed from the plates and air-dried for 20 min; they were then placed in 25% RH and 85% RH chambers as described above and incubated at 20°C and 28°C in the dark for 0, 1, 2, 4, 8, 12, and 24 h. Following dry periods, conidia on cover glasses were rewetted with sterile deionised water and allocated on a moist filter paper in the Petri plates. The plates were sealed with Parafilm and incubated at 20°C for another 24 h to achieve maximum germination of conidia. Conidia air-dried for 20 min after 0, 1, 2, 4, and 6-h wet periods and rewetted immediately after drying were used as controls of each treatment. Percent germination was evaluated as described above. Because some conidia already germinated in the 6-h wet treatment prior to dry treatments, percent germination in 6-h wet treatments prior to dryness was subtracted by the percent germination in 6-h continuous-wet treatment to eliminate conidia that germinated prior to dry periods.

Data analysis

All experiments were performed twice. An *F*-test was used to determine if variance of the two runs of each experiment was homogeneous and if data could be pooled. The homogeneity of variance test indicated that the data from both runs of each experiment could be pooled, and thus all further analyses were conducted on pooled data. Effects of temperature and wetness duration on conidial germination were subjected to a logistic regression analysis, performed with SAS PROC LOGISTIC (version 9.2, SAS Institute, Cary, NC, USA). Analysis of variance was performed to determine the effects of RH on conidial germination using PROC GLM of SAS and the treatment means were separated by Fisher's protected least significant difference ($P=0.05$). Spore germination data in percentages were logit-transformed using $Y = \ln[(y + 0.0001)/(1 - y)$ prior to analysis.

Linear regression was performed with a log-transformation of conidial germination in response to dryness and interrupted wetness duration using SAS PROC REG. To determine whether the effects of RH on conidial germination and germ tube elongation correlated, Pearson's correlation coefficient was calculated using SAS PROC CORR.

Results

Effects of temperature and wetness duration

Logistic regression analysis indicated that temperature and wetness duration significantly ($P<0.05$) affected conidial germination of *S. pyriputrescens*, accounting for 90.2% and 94.7% of the total deviances for isolate CLX2142 and CLX2145, respectively. There was a significant interaction between temperature and wetness duration for both isolates ($P<0.0001$).

Conidia of both isolates germinated at temperatures ranging from 0°C to 30°C (Fig. 1). Conidial germination reached the maximum at 20°C for both isolates after 20 h of incubation except for isolate CLX2145; the germination at 15°C at 20 and 24 h of incubation appear to be higher than that at 20°C, but the difference was not statistically significant ($P>0.05$). Conidial germination increased as temperature increased up to 20°C and then decreased gradually from 20°C to 30°C (Fig. 1). At 0°C, conidia germinated extremely slowly starting after 48 h of incubation; the germination reached 69% and 64% after 6 days of incubation for isolates CLX2142 and CLX2145, respectively (data not shown). The maximum germination (>90%) of isolate CLX2142 occurred at 15°C and 20°C after 16 h of incubation. Isolate CLX2145 reached the maximum germination (86%) at 20°C after 16 h of incubation, whereas the germination at 15°C reached the maximum (>96%) after 20 h of incubation. There was no difference ($P>0.05$) in the percent germination after 3 days of incubation at 10°C, 15°C, and 20°C for isolate CLX 2142 and at 5°C, 10°C, 15°C, and 20°C for isolate CLX2145, with the maximum germination >90% (data not shown). No germination occurred at 35°C for both isolates.

Regardless of temperature, no germination was observed in <5 h of wetness duration (Fig. 1). Minimum wetness durations of 6 and 5 h were required for germination at 15°C and 20°C, respectively. Minimum wetness durations of 72, 24, and 12 h were required for germination at 0°C, 5°C, and 10°C, respectively; 5 and 8 h were required at 25°C and 30°C, respectively. The percent germination increased as wetness duration lengthened until germination reached its maximum.

Germ tubes elongated at temperatures from 0°C to 30°C, with the optimum growth at 20°C for both isolates (data not shown). The average lengths of germ tubes were 113.3 and 83.9 μm after 12 h of incubation at 20°C for isolates CLX2142 and CLX2145, respectively. At 0°C, germ tubes elongated very slowly, with averages of 43.0 and 39.5 μm after 5 days of incubation for isolates CLX2142 and CLX2145, respectively (data not shown). We also observed a change in conidial morphology at high temperatures. Conidia appeared swollen and germ tubes thickened at temperatures >25°C after 12 h of inoculation.

Effect of RH

RH significantly affected conidial germination at all temperatures tested (Figs. 2 and 3). Because conidial germination and germ tube elongation were highly correlated, data for the germ tube elongation were not presented. Pearson's correlation coefficients were $r=0.87$ ($P<0.0001$) and $r=0.88$ ($P<0.001$) for isolates

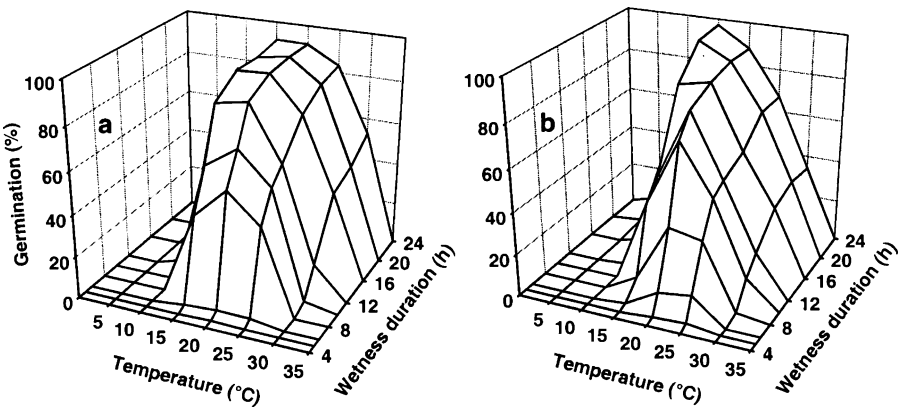

Fig. 1 Effects of temperature and wetness duration on conidial germination of *Sphaeropsis pyriputrescens*. Each data point represents the mean of two experimental runs each with three replicates. **a** isolate CLX2142, **b** isolate CLX2145

CLX2142 and CLX2145, respectively. Conidia germinated at RH ≥ 95% at 20°C, 25°C, and 30°C after 24 h of incubation, and no germination was observed at 88.5% RH. At 92% RH, conidia germinated after 36 h at 20°C and 25°C (data not shown). Conidial germination generally increased as RH increased from 95% to 100% at 10°C, 15°C, and 20°C after 24 h of incubation, except for isolate CLX2142 at 15°C and isolate CLX2145 at 20°C. At 30°C, germination at 98% RH was higher than that at 100% RH for isolate

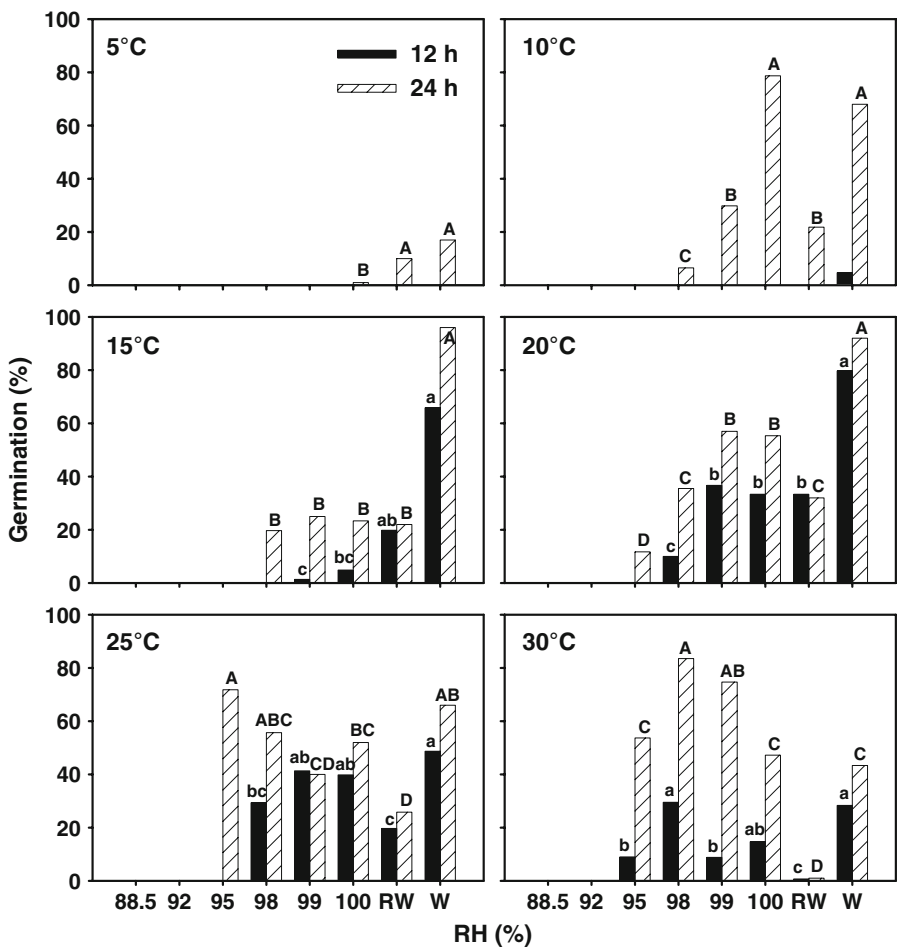

Fig. 2 Effect of relative humidity (RH) on conidial germination of *Sphaeropsis pyriputrescens*, isolate CLX2142, after 12 or 24 h incubation at different temperatures. Each column represents the mean of two experimental runs each with three replicates. **W**: wet treatment in free water without air-drying, **RW**: rewetted after drying for 20 min. Columns within the same temperature group followed by the same letters (lowercase letter = 12 h and capital letter = 24 h) are not significantly different according to Fisher's least significant difference (P=0.05)

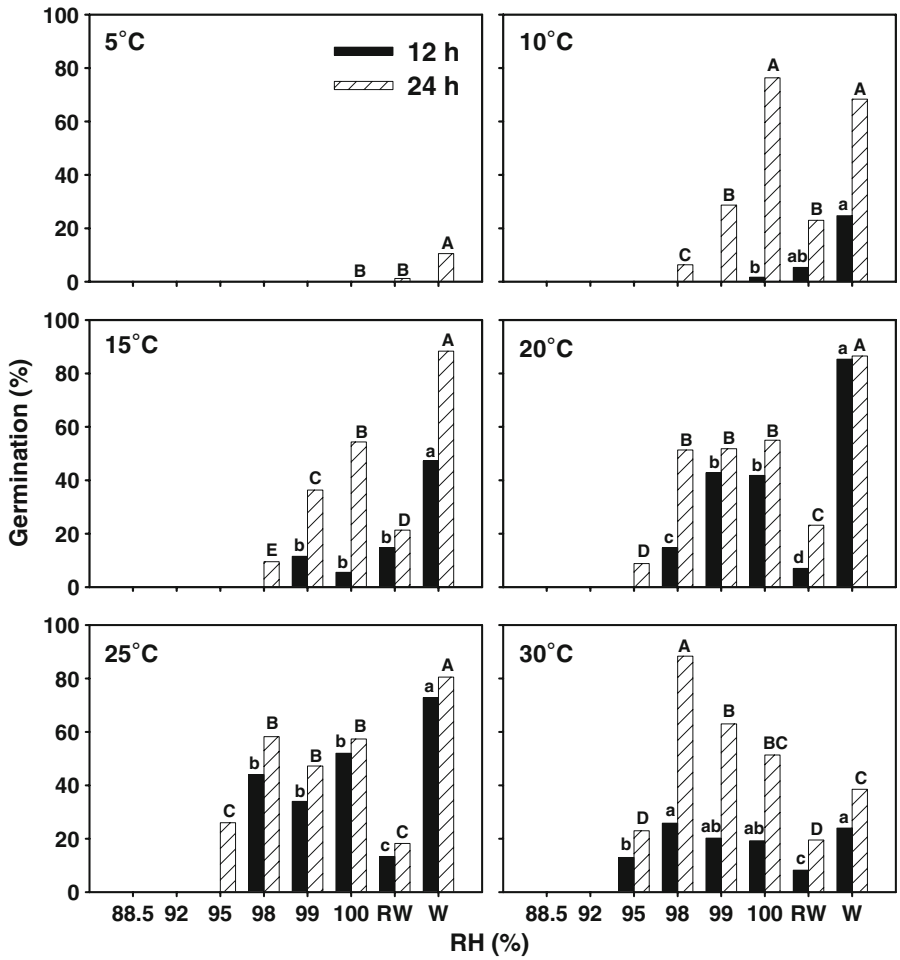

Fig. 3 Effect of relative humidity (RH) on conidial germination of *Sphaeropsis pyriputrescens*, isolate CLX2145, after 12 or 24 h incubation at different temperatures. Each column represents the mean of two experimental runs each with three replicates. **W**: wet treatment in free water without air-drying, **RW**: rewetted after drying for 20 min. Columns within the same temperature group followed by the same letters (lowercase letter = 12 h and capital letter = 24 h) are not significantly different according to Fisher's least significant difference (P=0.05)

CLX2142 and that at 99% and 100% RH for isolate CLX2145. At 25°C, germination of isolate CLX2142 decreased as RH increased from 95% to 100% after 24 h of incubation. At 0°C, germination occurred at 99% and 100% RH after 72 h of incubation; no germination was observed at RH <98% after 5 days for both isolates (data not shown).

Air-drying for 20 min at ambient conditions significantly reduced conidial germination at all temperatures tested (Figs. 2 and 3). The germination in continuous wet treatments was significantly higher than that of rewetted treatments except conidia of isolate CLX2142 at 5°C. In rewetted treatments, average reductions in germination relative to the continuous wet after 24 h were 68.3% and 71.8% for isolates CLX2142 and CLX2145, respectively. Conidial germination increased from 12 to 24 h in the rewetted treatments at all temperatures except for isolate CLX2142 at 20°C. After 24 h of incubation, the percent germination in the rewetted treatments was significantly lower than that of 100% RH treatments at all temperatures except at 5°C for both isolates and at 15°C for isolate CLX2142 (Figs. 2 and 3). This phenomenon was also observed in the germ tube lengths at 25°C and 30°C; however, the germ tube length at other temperatures was not comparable because it was out of the measurable range (data not shown).

Effects of dryness and interrupted wetness

The effect of dry period on conidial germination of *S. pyriputrescens* depended on RH (Fig. 4). Conidial germination was significantly higher at 85% RH than that at 25% RH within 4 h of dry periods for all treatments. Regardless of temperature, germination

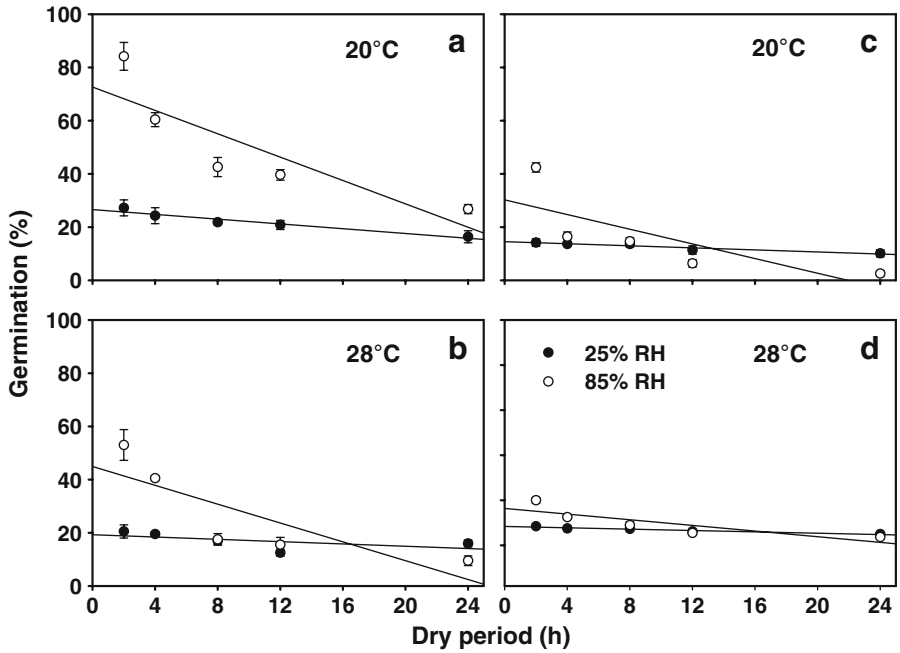

Fig. 4 Effect of dryness on conidial germination of *Sphaeropsis pyriputrescens*, isolates CLX2142 (**a** and **b**) and CLX2145 (**c** and **d**). Each data point represents the mean of two experimental runs each with three replicates. *Bars* = standard errors

decreased rapidly as dry periods increased at 85% RH (isolate CLX2142: y=95.1−22.6x, R^2=0.96 at 20°C, y=63.9−18.7x, R^2=0.93 at 28°C; isolate CLX2145: y=45.8−14.9x, R^2=0.85 at 20°C, y=23.0−6.6x, R^2=0.95 at 28°C). At 25% RH, conidial germination decreased slowly as dry periods lengthened (isolate CLX2142: y=30.3−4.2x, R^2=0.98 at 20°C, y=22.1−2.6x, R^2=0.61 at 28°C; isolate CLX2145: y=15.8−1.7x, R^2=0.82 at 20°C, y=9.6−1.4x, R^2=0.94 at 28°C). There were no significant differences in the slopes between 20°C and 28°C at the same RH for both isolates (P> 0.05). The percent germination after 10 days of incubation was <10%, but conidia were still viable at all treatments tested (data not shown).

The effect of interrupted wetness duration on conidial germination depended on wet periods prior to dryness (Figs. 5 and 6). Germination decreased as wet periods prior to dryness increased. Regardless of temperature and RH, conidia with 6-h wet treatment prior to dryness did not germinate after 1 h of dry period with the exception of isolate CLX2145 at 28°C, 85% RH. Conidial germination decreased as dry periods increased up to 12 h, and these relationships were well described by linear regression models (not shown). After 12 h of dry period, conidial germination remained constant and no or few conidia germinated at 25% RH. Germination was significantly higher at 20°C than at 28°C and higher at 85% RH than at 25% RH (P<0.0001). For both isolates, conidia with 1- to 4-h wet treatments prior to dryness, except for the 4-h wet treatment of isolate CLX2145, showed a higher germination rate after a 2-h dry period at 85% RH than those rewetted immediately after air-drying (Figs. 5 and 6).

Discussion

The optimum temperature for conidial germination and germ tube elongation was the same as that for mycelial growth of *S. pyriputrescens* (Kim et al. 2005). However, mycelium of the fungus is not able to grow at 30°C (Kim et al. 2005), while in the present study conidia germinated at 30°C, with maximum germination of 52–69% after 48–72 h of incubation, depending on isolate. The results suggest that conidia of the fungus have adapted to high temperatures better than mycelia.

Apples are commercially stored around 0°C (Meheriuk 1993). Because both mycelial growth and conidial germination of *S. pyriputrescens* can occur at apple storage temperatures, low temperatures commercially used for storage of apple fruit cannot prevent development of Sphaeropsis rot during

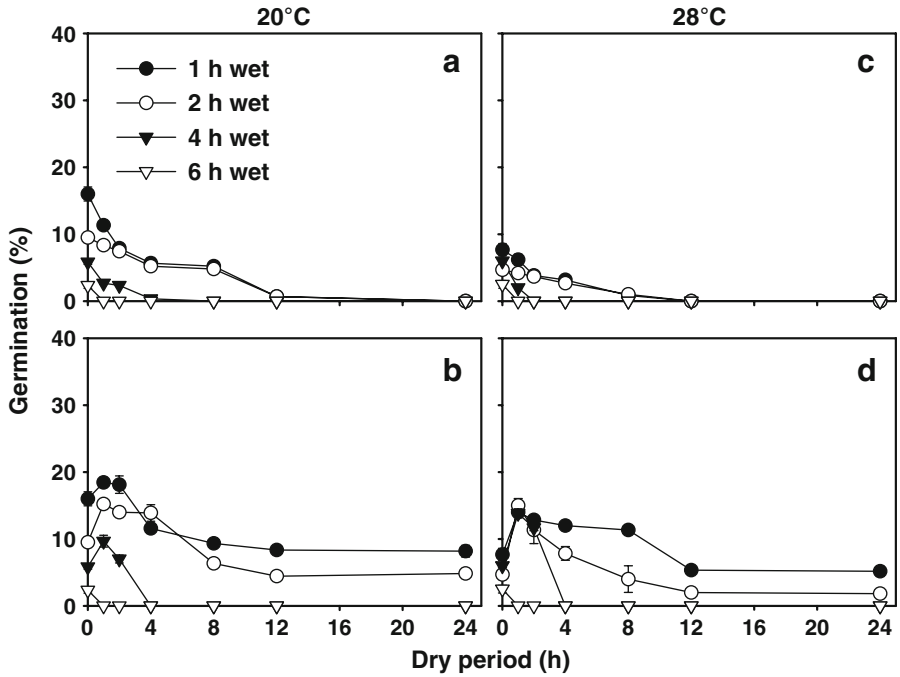

Fig. 5 Effect of interrupted wetness duration on conidial germination of *Sphaeropsis pyriputrescens*, isolate CLX2142 at 20°C (**a** 25% RH and **b** 85% RH) and 28°C (**c** 25% RH and **d** 85% RH). Conidia were wetted for 1–6 h prior to drying. Each data point represents the mean of two experimental runs each with three replicates. *Bars* = standard errors

storage. This is different from other decay-causing fungi on apples, such as *Botryosphaeria obtusa* (the cause of black rot of apple fruit) and *Botryosphaeria dothidea* (the cause of white rot of apple fruit) because these fungi are not able to grow at the apple-storage temperatures (Arauz and Sutton 1989; Sutton and Arauz 1991). Development of black rot and white rot can be prevented by storing apple fruit around 0°C, but decay may resume at temperatures >10°C (Snowdon 1990).

Wetness requirements for conidial germination of *S. pyriputrescens* were temperature-dependent, but a

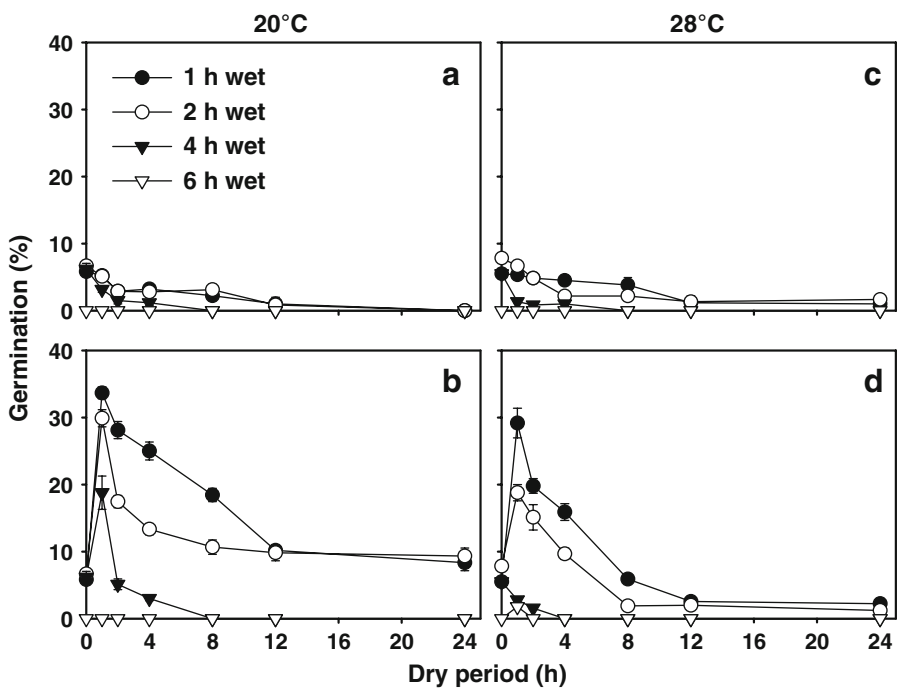

Fig. 6 Effect of interrupted wetness duration on conidial germination of *Sphaeropsis pyriputrescens*, isolate CLX2145 at 20°C (**a** 25% RH and **b** 85% RH) and 28°C (**c** 25% RH and **d** 85% RH). Conidia were wetted for 1–6 h prior to drying. Each data point represents the mean of two experimental runs each with three replicates. *Bars* = standard errors

minimum 5 h of continuous wetness was required for conidial germination. In a preliminary study on field inoculation of apple fruit, fruit incubated for 12 to 15 h of wetness duration after inoculation resulted in high percentages of fruit infection by the fungus, leading to Sphaeropsis rot in storage, regardless of temperatures at which the fruit were inoculated during the apple-growing season (unpublished data). However, the minimum wetness duration required for fruit infection by this fungus in the field remains to be determined.

In this study, we found that conidia of *S. pyriputrescens* were able to germinate at a RH as low as 92%, depending on temperature. In most cases, >50% of conidia of *S. pyriputrescens* incubated at RH ≥98% germinated when the temperature was higher than 20°C. In eastern Washington State, apples are grown in a semiarid climate. Precipitation is often limited during the apple growing season. The ability of conidia to germinate at high RH without continuous wetness suggests that orchard practices that lead to high RH in the orchards may create conducive conditions for infections of apple fruit by this fungus. In commercial apple orchards in central Washington State, over-tree evaporative cooling of fruit in summer is a common practice for protecting fruit from sunburn, a non-infectious disorder on the peel of apple fruit caused by high temperature and long exposure to solar radiation (Flore and Dennis 1990; Parchomchuk and Meheriuk 1996). Over-tree cooling may run for several hours, creating a long wetness period on the fruit and a high RH microenvironment in the orchard. Alternatives to over-tree evaporative cooling, such as spraying the fruit with clay or film-forming products for sunburn prevention (Schrader et al. 2006), could avoid creating conducive conditions for fungal infections.

In this study, conidial germination generally increased with increasing RH at temperatures <25°C. However, germination decreased as RH increased from 98 to 100% at temperatures >25°C. We observed water condensation on most treatments (cover glass slides) maintained at RH ≥ 99% and a temperature of ≥25°C. Thus, one potential contributing factor may be the formation of water condensation around the air-dried conidia. When condensation was present on the cover glass, a low level of oxygen was available for conidia to germinate, resulting in a low percentage of conidial germination. Spore germination and mycelial growth of the same fungus may have different sensitivities to reduced oxygen (Griffin 1994). Reduced conidial germination in free water than at high RH (≥98%) has also been observed in conidial germination of *Potebniamyces pyri* (Liu and Xiao 2005).

In this study, we chose 28°C as a high temperature in the evaluation of effects of dryness and interrupted wetness on survival and germination of conidia because the average high temperature from August to September is around 28°C in the Wenatchee area, WA, USA. Air-drying for 20 min at ambient conditions significantly reduced conidial germination of *S. pyriputrescens*. However, conidia air-dried and maintained at both the optimum temperature (20°C) and relatively high temperature (28°C) survived for at least >10 days, though after which time only <10% of the conidia survived and germinated when optimal conditions for germination were given. *Sphaeropsis pyriputrescens* has thick-walled, melanised conidia, which may help the fungus to survive under adverse conditions (Xiao and Rogers 2004). Our results may suggest that *S. pyriputrescens* has adapted well to the semiarid climatic conditions in central Washington where apples are commercially grown. This may also explain why the fungus is widespread in the major apple-production counties in the region (Kim and Xiao 2008).

Conidia wetted for 4 to 6 h prior to dryness were more sensitive to dryness than those wetted for <2 h prior to dryness. This may be due to the differences in germination status of conidia after being exposed to different durations of wetness prior to dryness. Because conidia of *S. pyriputrescens* required 5 to 6 h of wetness periods for germination at the optimum temperature, we considered that conidia wetted for 4 to 6 h were ready to germinate or had germinated. Germinating or germinated conidia are likely to be more sensitive to dryness than conidia wetted for <2 h because cell walls of the conidia disintegrate when germ tubes are ready to elongate and germ tubes or mycelia may be more sensitive to dryness than conidia.

In the dryness and interrupted wetness studies, RH during the dry periods affected the conidial germination of *S. pyriputrescens* after rewetting. Conidia maintained at 85% RH after drying showed a higher

percent germination than those at 25% RH, especially within a 12-h dry period. The effect of low RH on conidial survival and germination during a dry period has been reported in other fungal pathogens. Conidia of *Botrytis cinerea*, *Cercospora musae*, and *Monilinia fructicola* did not survive when conidia were maintained at a low RH during a dry period (Good and Zathureczky 1967). Similarly, significant reductions in infections at low RH during a dry period were observed in other fruit tree pathogens including *Stemphylium vesicarium* in pear (Llorente and Montesinos 2002) and *Coccomyces hiemalis* in sour cherry (Eisensmith et al. 1982). In contrast, Arauz and Sutton (1990) reported that the RH during a dry period did not affect the ability of germ tube elongation of *B. obtusa* after rewetting. However, we could not compare our results with that of Arauz and Sutton (1990) because our study evaluated conidial germination rather than germ tube elongation.

Because conidia (pycnidia) of *S. pyriputrescens* are believed to be the major inoculum responsible for fruit infection in apple orchards and infections of apple fruit by the fungus are believed to take place in the orchard (Xiao et al. 2004), microclimatic conditions in the orchard likely play an essential role in infections of apple fruit by the fungus. In this study we defined relationships between conidial germination of *S. pyriputrescens* and some key environmental factors that affect conidial germination and survival. However, effects of these factors on infections of apple fruit by this fungus under orchard conditions have not yet been determined. In orchard conditions, temperatures fluctuate and ultraviolet light may also affect survival and germination of conidia. Moreover, because conidia of *S. pyriputrescens* are water-dispersed, although apples are grown in a semiarid climate in central Washington State, over-tree evaporative cooling likely plays an important role in spore release and dispersal of *S. pyriputrescens* in summer when rainfall is limited and creates a microenvironment conducive to fungal infections of fruit in the orchards. The impact of over-tree cooling and over-tree irrigation on infections of apple fruit by *S. pyriputrescens* remains to be determined. Nonetheless, the results of this study will help us to understand orchard conditions required for infection of fruit by the fungus leading to Sphaeropsis rot in storage and thus may help us develop effective measures for disease control.

Acknowledgements This research was supported in part by the Washington Tree Fruit Research Commission. This manuscript is Plant Pathology New Series 0519, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences Agricultural Research Centre, Project No. WNP00367, Washington State University, Pullman, WA 99164-6430, USA.

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