Effects of Light, Temperature, and Leaf Wetness Duration on Daylily Rust

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ABSTRACT

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Experiments in controlled environments were completed to determine the influence of light intensity, temperature, and leaf wetness duration on daylily rust caused by *Puccinia hemerocallidis*. As light intensity increased, there was a significant decrease in urediniospore germination ($R^2 = 0.88$ and $Y = 96 - 0.6x$). Urediniospores germinated in vitro between 7 and 34°C with an optimal temperature of 22 to 24°C. To test the effect of temperature on infection efficiency, plants were inoculated with urediniospores, incubated under high relative humidity at 4, 10, 22, 30, or 36°C, and then transferred to a greenhouse at 23°C for 15 days. Plants incubated at 22°C had an average of 13 lesions cm leaf⁻¹. Incubation temperatures of 4, 10, 30, or 36°C resulted in less than 1.5 lesions cm leaf⁻¹. Plants were inoculated, incubated at 22°C for 24 h, and then incubated at different temperatures for 15 days to test the effect of temperature on disease development. There were no significant differences in disease development at 22 and 30°C; however, there were significantly fewer lesions at 10°C and no lesions developed at 36°C within 15 days. Five to six h of leaf wetness were required for lesion development and as the duration of leaf wetness increased, there was a significant increase in the number of lesions that developed. These studies indicate that for disease development of *P. hemerocallidis* on daylily, temperatures around 22°C and 5 h of leaf wetness are required during infection. However, once a daylily is infected, disease development is not as sensitive to environmental conditions.

A new disease on daylilies (*Hemerocallis* spp.), daylily rust, has become an increasing problem for daylily growers. Daylily rust, caused by *Puccinia hemerocallidis* Thüem, was first reported in the United States in August 2000 (17). First found in Georgia, *P. hemerocallidis* spread rapidly throughout the United States in 2001 and has been reported as far north as Minnesota and west as Oregon and California. At present, very little is known about the biology of *P. hemerocallidis* on daylily. *P. hemerocallidis* is a heteroecious rust that needs two different hosts to complete its life cycle. The uredinial/telial host is daylily in the Hemerocallidaceae family and the alternate (spermagonial/aecial) host is the herbaceous perennial, *Patrinia* spp. in the Valerianaceae family (6). However, the alternate host is not required for daylily infection. Similar to other heteroecious rusts (1), the uredinial or repeating spore stage on daylily can reinfect the same host plant leading to epidemics.

Knowledge of environmental conditions needed for growth and multiplication of pathogens is a key step to understanding the epidemiology and control of plant diseases. Determining how environmental factors such as temperature and moisture

affect spore germination and infection is important for the creation of favorable conditions for inoculation of plants in the greenhouse. Lesion development by *P. hemerocallidis* will occur on new daylily leaves when exposed to inoculum, 100% relative humidity, and leaf wetness for 24 h (3). However, the minimum length of leaf wetness required for infection and the effect of light and temperature on infection are not known. Leaf moisture, light, and temperature have been found to be particularly important in spore germination and subsequent infection of the host plant by many other rust pathogens (4,5,7-16). Environmental factors influence species of *Puccinia* differently. For example, the optimal temperature for in vitro germination of *P. graminis* urediniospores is 10°C (7), whereas the optimal temperature for *P. substriata* var. *indica* is 19 to 22°C (14). Photo-inhibition of urediniospore germination has been demonstrated at different levels of light intensity for several *Puccinia* spp. (5,12,14).

The objectives of this study were to provide information on (i) the effect of light and temperature on urediniospore germination; (ii) the effect of temperature on infection efficiency and disease development; and (iii) the minimum length of leaf wetness needed for lesion development to occur. Preliminary results from this study have been reported (2).

MATERIALS AND METHODS

Plant and pathogen maintenance. *P. hemerocallidis* was collected from an infected daylily plant cultivar Pardon Me

received by the University of Georgia disease diagnosis clinic in 2000 (17). This isolate was maintained on daylily plants of the same cultivar in a greenhouse with average night/day temperatures of 22 and 26°C, respectively. Cv. Pardon Me daylilies were maintained in Metro-Mix 400 (The Scotts Company, Marysville, OH) in 2-liter pots. Plants were irrigated as needed and fertilized with Osmocote Plus 15-9-12 controlled release fertilizer (The Scotts Co.). Insect and arthropod pests were managed using standard practices.

Effect of light and temperature on germination of *P. hemerocallidis* urediniospores in vitro. Urediniospores were collected by vacuum from 10- to 12-day-old pustules, suspended in 0.05% Tween 20 (J. T. Baker, Philipsburg, NJ) solution, and filtered through sterile cheesecloth. Spores were counted with a hemocytometer and adjusted to 1 to 2×10^5 spores ml⁻¹ in 0.05% Tween 20. A 50- μ l aliquot of spore suspension was placed on potato dextrose agar (approximately 20 ml in 9-cm petri dishes) amended with 100 μ g ml⁻¹ of chloramphenicol (C-PDA, Sigma, St. Louis, MO). Four replicate petri dishes were used for each light intensity. Photosynthetically active radiation (PAR) was measured with a LI-189 Quantum/Radiometer/Photometer using a LI-190SA Quantum sensor (LI-COR, Lincoln, NE). Treatments included 0 (no light), 21, 43, 64, 86, and 112 μ mol s⁻¹ m⁻². The no light treatment was achieved by wrapping each petri dish with aluminum foil. For each light treatment, continuous illumination was maintained throughout the incubation. Light was provided only by cool-white fluorescent light bulbs for each of the intensity levels. Lights were arranged 110 cm directly above the petri dishes, except at 112 μ mol s⁻¹ m⁻² where lights were placed 50 cm above the petri dishes. The numbers of germinated and nongerminated spores from each plate were counted after 18 h with the aid of a microscope ($\times 200$ magnification). Urediniospores with germ tube length greater than or equal to spore diameter were considered germinated. Enough fields of view were counted to include at least 250 total urediniospores from each plate. Data were converted to percent germination. The experiment was repeated.

Fifty-microliter aliquots of spore suspension (1 to 2×10^5 spores ml⁻¹) were placed on C-PDA as done previously and incubated at 4, 7, 10, 16, 18, 22, 26, 28, 30, 32, 34, or 36°C to study the effect of temperature on urediniospore germination.

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Each dish was wrapped with aluminum foil and incubated at each temperature treatment for 18 h. Plates were stored at 4°C to prevent further germination. The numbers of germinated and nongerminated spores from four plates for each treatment temperature were counted after 18 h as described above. Data were converted to percent germination. The experiment was repeated.

Effect of temperature and leaf wetness on infection efficiency and disease development. Urediniospores were collected from 10- to 12-day-old pustules and suspended as described above. Fifteen daylilies were watered to saturation, sprayed with a urediniospore suspension in 0.05% Tween 20 (2×10^5 spores ml^{-1}), sealed in clear plastic $20 \times 8 \times 38$ -cm bags to maintain high relative humidity, and incubated at either 4, 10, 22, 30, or 36°C with no light to study the effect of temperature on infection efficiency (16). After 24 h, the bags were removed, and plants were transferred to a research greenhouse under ambient light with an average daily temperature of 23°C. For the disease development study, 12 daylilies were watered to saturation, sprayed with urediniospore suspension (2×10^5 spores ml^{-1}), sealed in clear plastic bags, and incubated at 22°C with no light. After 24 h, the bags were removed, and plants were transferred to growth chambers set at 10, 22, 30, and 36°C with a 12-h light period ranging from 102 to 112 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Fifteen days after inoculation, rust lesions were counted on six leaves on each plant. The number of lesions cm^{-1} of leaf was calculated. Plants were chosen that had similar size and number of leaves to limit leaf-to-leaf variability within each experiment. Both experiments were repeated.

Eighteen daylilies were watered to saturation, sprayed with a urediniospores in 0.05% Tween 20 (2×10^5 spores ml^{-1}), sealed in clear plastic bags, and stored at 22°C with no light to study the effect of leaf wetness duration on infection efficiency. After 1 to 8, 11 to 13, 15, 17, or 24 h the bags were removed, and plants were transferred to a research greenhouse under ambient light and an average daily temperature of 23°C. Fifteen days after inoculation, rust lesions were enumerated on six leaves on each plant and the number of lesions cm^{-1} of leaf was calculated. Data were converted to a percentage of the lesions observed at 24 h (e.g., average lesions at 5 h per average lesions at 24 h $\times 100\%$). The experiment was completed three times.

Data analysis. For the in vitro urediniospore germination studies, the experiments were set up as a completely randomized design with four replications. A regression analysis, performed with the PROC REG procedure of SAS (SAS Institute Inc., Cary, NC), was used to determine the relationship of light intensity and urediniospore germination. A regression equation

was computed using PROC REG. Percent germination of urediniospores incubated at different temperatures was expressed as means $\pm$ standard errors.

Plant experiments on temperature and leaf wetness were arranged in randomized complete block designs with three replications. Data were subjected to analysis of variance (ANOVA) using general linear models procedure (PROC GLM) of SAS (SAS Institute Inc.). Trials and replications were considered random variables, whereas temperatures and hours of leaf wetness were considered fixed variables. Means were separated by Fisher's protected least significant differences (LSD) at $P \leq 0.05$. The number of lesions formed with different leaf wetness durations was analyzed using PROC REG.

RESULTS

In vitro germination—light. ANOVA indicated that there was no effect ($P = 0.96$) of trial or the interaction between trial and germination ($P = 0.27$). Therefore, data from the two trials were combined. Germination was observed at all PAR levels evaluated (Fig. 1). There was a signifi-

cant decrease in germination with increase in PAR. The percent germination ranged from 89% at 0 and 21 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ to 1.2% at 112 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Regression analysis showed a significant linear relationship between PAR and urediniospore germination ($P < 0.01$). The R^2 value was 0.88 and the model was $Y = 96 - 0.6x$.

In vitro germination—temperature. ANOVA indicated that there was no effect ($P = 0.12$) of trial or the interaction between trial and germination ($P = 0.88$). Therefore, data from the two trials were combined. Urediniospore germination occurred from 7 to 34°C but was not observed at 4 and 36°C (Fig. 2). The percent germination ranged from 87% at 24°C to less than 1% at 34°C. Over 60% of the urediniospores germinated when incubated at 16 to 30°C.

Effect of temperature on infection efficiency and disease development. For both experiments, there was a significant difference between trials ($P < 0.01$); however, there was not a significant trial by temperature interaction. Therefore, data from the two trials for both experiments were combined (Table 1). Differences

Fig. 1. Effect of photosynthetically active radiation (PAR) on in vitro germination of *Puccinia hemerocallidis* urediniospores. Bars indicate standard errors of the means. Data points represent the mean values after 18 h incubation over two experiments and four replications of at least 250 urediniospores each.

Fig. 2. Effect of temperature on in vitro germination of *Puccinia hemerocallidis* urediniospores. Bars indicate standard errors of the means. Data points represent the mean values after 18 h incubation over two experiments and four replications of at least 250 urediniospores each.

between the trials were due to increased disease levels in the second trial of both the infection efficiency and disease development experiment. For the infection efficiency experiment, 13.0 lesions cm leaf⁻¹ developed at 22°C, whereas less than 1.5 lesions cm leaf⁻¹ developed at 4, 10, 30, and 36°C (Table 1). For the disease development study, there was no significant difference ($P \leq 0.05$) between 22°C (15.9 lesions cm leaf⁻¹) and 30°C (13.3 lesions cm leaf⁻¹). There was significantly less disease observed at 10 and 36°C compared to the other temperatures (Table 1).

Leaf wetness. There was a significant difference in the number of lesions formed between the three trials ($P < 0.01$). However, there were no significant differences between trials ($P = 0.42$) and the interaction between trial and hour ($P = 0.18$) when data were converted to a percentage of total lesions observed after 24 h leaf wetness. Therefore, percentage data from the three trials were combined. The percentage of lesions after 0 to 2 h leaf wet-

ness was less than 1% of the 24 h incubation (Fig. 3). Five and 17 h of leaf wetness resulted in 11 and 80%, respectively, of the number of lesions observed after 24 h incubation. The average number of lesions from 24 h of leaf wetness was 6.9 lesions cm leaf⁻¹. Regression analysis showed a significant linear relationship between length of leaf wetness and number of lesions ($P < 0.01$). The R^2 value was 0.36 and the model was $Y = 3.9 + 6.0x$.

DISCUSSION

Determining how environmental factors such as temperature and moisture affect spore germination and infection is important for the creation of favorable conditions for inoculation of plants in the greenhouse. The in vitro germination of *P. hemerocallidis* urediniospores as well as lesion development on daylily was affected by the environmental factors tested. Our studies indicate that germination of urediniospores is maximized under light conditions less than 21 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ and at 24°C. Also, for

disease development of *P. hemerocallidis* on daylily, temperatures approximately 22°C and 5 h of leaf wetness are required during infection. However, once a daylily is infected, disease development is not as sensitive to environmental conditions.

Under natural conditions, light and temperature will not be constant, but continuous light and temperature allows us to observe how each of these factors affect urediniospore germination independently. Urediniospore germination was not reduced at low light levels (less than 21 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$); however, continuous light at greater than 43 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ reduced germination. As a comparison, the PAR for a full sunlight, cloudless day at noon on 18 June 2002 and 4 September 2002 in Griffin, GA was 510 and 440 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, respectively. Light inhibition of urediniospore germination has been previously reported on other *Puccinia* spp. (6,12,14). These studies have evaluated shorter incubation periods and suggest that the first 2 h of incubation may be the most important (6,14). Temperature also affected urediniospore germination as the range was 7 to 34°C and the optimal temperature was 22 to 24°C. These data are similar to temperature ranges and optimal temperatures of other *Puccinia* spp. (4,7,14). Tapsoba and Wilson (14) observed urediniospore germination to be greater than 90% between 19 and 22°C and less than 1% germination occurred at 35°C for *P. substriata* var. *indica*. A wide range of in vitro germination has been observed with *P. graminis* and *P. recondite* where germination exceeded 95% from 6 to 28°C after 17 h of incubation (7). High temperatures (>34°C) appear to inhibit germination of many *Puccinia* spp. (this study, 4,7,14).

Temperature affected infection efficiency and disease development differently than in vitro urediniospore germination. Infection efficiency on daylily leaves is more sensitive to temperature during inoculation than urediniospore germination. Several lesions developed when the temperature was at the optimal temperature of 22°C, whereas very few lesions developed when the temperature was at 10 and 30°C. This was not expected because over 40 and 60% of the *P. hemerocallidis* urediniospores germinated at 10 and 30°C, respectively. At 10°C, percent germination of *P. substriata* var. *indica* urediniospores increased over time (14). It is possible in our system that even though the urediniospores germinated, a longer leaf wetness period than 24 h is required at 10°C to infect the plant. The effect of temperature on infection has been reported for alfalfa rust, where infection efficiency was approximately 20 times greater at 17.5°C than at 28°C (16). In our disease development study, daylily plants were inoculated at 22°C and then placed at different temperatures for the remainder of the study. Temperature did not have as much effect on

Table 1. Effect of temperature on infection efficiency and disease development on daylily during and after infection by *Puccinia hemerocallidis* urediniospores

Temperature	Pustules cm ⁻¹ leaf length ^a	
	Infection efficiency ^b	Disease development ^c
4	0.1	...
10	1.5	8.0
22	13.0	15.9
30	0.2	13.3
36	0.2	0.0
LSD _(0.05)	1.9	3.8

^a Mean values calculated at 15 days after inoculation over two experiments, three plants per temperature, and six leaves from each plant.

^b For the infection efficiency experiment, plants were incubated in high relative humidity at the respective temperatures for 24 h after inoculation, and then placed in the greenhouse (average daily temperature of 23°C) for 15 days.

^c For the disease development experiment, plants were incubated at 22°C for 24 h after inoculation, and then placed in growth chambers set at each temperature treatment for 15 days.

Fig. 3. Percentage of *Puccinia hemerocallidis* lesions on daylily after different lengths of leaf wetness. Data are the ratio of lesions observed at each wetness treatment to the number of lesions observed on plants incubated for 24 h of leaf wetness. Mean values calculated at 15 days after inoculation over two experiments, three plants per temperature, and six leaves from each plant.

disease development of daylily rust. Unlike the infection efficiency study, there were no differences in lesion development at 22 and 30°C. However, there were significantly less disease development at 10°C and no disease development at 36°C. Plants incubated at 10°C had smaller lesions that developed late in the study (*personal observation*). These data agree with other reports that suggest postinoculation temperatures strongly influence disease development by different rust species (4,8).

Our final study determined the minimum length of leaf wetness required for infection of daylily by *P. hemerocallidis*. Less than one lesion cm leaf⁻¹ was observed at the 0- to 3-h wetness periods even in the presence of inoculum and optimal temperatures, indicating that wetness is required for disease development. A minimum of 5 to 6 h of continuous leaf wetness was required for significant numbers of daylily rust lesions to develop. Infection efficiency increased as duration of leaf wetness increased. These results are similar to other rust diseases caused by *Puccinia* spp. (9,13,16).

From our data, we suggest that infection of daylilies by *P. hemerocallidis* occurs during cooler temperatures early or late in the day or at night when leaf wetness is present, and potentially inhibitory PAR is not present. These studies provide useful information about how the light, temperature, and leaf wetness affect *P. hemerocallidis*. Additional research looking at the

interactions between these factors is needed to better understand when outbreaks of daylily rust may occur.

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