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Effects of temperature and humidity on the survival of urediniospores of gladiolus rust (*Uromyces transversalis*)

Gary L. Peterson · Dana K. Berner

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Abstract *Uromyces transversalis* is an autoecious microcyclic rust mainly infecting *Gladiolus* spp. The pathogen is considered of plant quarantine importance in Europe and the USA. In 2006, the pathogen was found for the first time in the USA in several commercial nurseries in Florida and California. The US Department of Agriculture (USDA) initiated an eradication programme that recommended the immediate removal and destruction of infected plants followed by a host-free period, use of a fungicide treatment schedule, and equipment decontamination. In support of this plan, a study was conducted to determine how long urediniospores of *U. transversalis* would continue to germinate at temperatures of 2.8, 15.0, 18.8 and 25.0°C under controlled relative humidities (RH) of 11, 23, 43, 75, 93 and 100%. Choice of temperature and humidity parameters were mostly based on historical multi-year climate data from areas where the disease was detected in California and Florida. Analysis of variance (ANOVA) indicated no significant effect of RH on urediniospore germination but a highly significant effect of temperature. No germinating urediniospores were detected after 79 days for any treatment, but the 15°C treatment was more likely to be the result of germination independent of any low or high temperature-induced spore quiescence. Thus, lack of germination after 79 days was probably a good indicator of the lack of viable spores after this time for the 15°C treatment.

Keywords Host-free · Rust eradication · Gladiolus

G. L. Peterson (✉) · D. K. Berner
USDA, ARS,
Foreign Disease-Weed Science Research Unit,
1301 Ditto Ave, Fort Detrick,
Detrick, MD, USA 21702
e-mail: gary.peterson@ars.usda.gov

Gladiolus rust caused by the fungal plant pathogen *Uromyces transversalis* is indigenous to southern and eastern Africa. Over the last 100 years, it has spread into southern Europe, Australia, New Zealand, and South and Central America (Beilharz et al. 2001; OEPP/EPPO 1982). This autoecious microcyclic rust is reported to infect members of the Iridaceae family, including species of *Crocosmia*, *Freesia*, *Gladiolus*, *Melasphaerula, Tritonia* and *Watsonia* (Schubert et al. 2006; Beilharz et al. 2001).

Uromyces transversalis is of serious concern to commercial greenhouse and nursery enterprises growing hybrid gladiolus cultivars for cut flower production and is considered to be of plant quarantine importance in Europe and the USA (Hernández 2004). Although infected plants have been intercepted in shipments and passenger baggage from Mexico and Brazil (Anonymous 2007) since 2004, the disease had not been reported to occur in the USA. In 2006, the pathogen was observed for the first time in several commercial cut flower operations in Florida (Schubert et al. 2006), and later that year on several commercial

production farms in southern California (Blomquist et al. 2007). Outbreaks were reported again from isolated sites in both states in 2007 and 2008. (Kosta, K.L., *personal communication*).

In response, the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) implemented a national management plan for exclusion and eradication. The eradication approach focuses on the removal and destruction of infected plant material, establishment of a host-free period, implementation of scheduled fungicide applications, and decontamination of equipment (Rizvi et al. 2007). In support of these eradication steps, a study was conducted to determine how long individual urediniospores of *U. transversalis* will survive under a number of temperature and relative humidity (RH) conditions.

Four rusted gladiolus plants were collected by the County Plant Pathologist of San Diego County Department of Agriculture, Weights & Measures, and shipped bare-root to the USDA, Agricultural Research Service (ARS) biosafety level-3 plant disease containment facility at Fort Detrick, Maryland under Federal and State permits. These plants, with sporulating uredinia, were replanted in 7.5 l clay pots and placed in a greenhouse (24.0°C±3°C). After 7 days, urediniospores were harvested from the infected plants with a microcyclone spore collector (Ternet et al. 1951). Urediniospores were suspended in 25 ml water containing one drop of Tween-20 (polyoxyethylene sorbitan monolaurate, Sigma, St. Louise, MO) per 100 ml, and the concentration adjusted to 5.0×10^4 urediniospores ml^{-1}. Twelve 6 week-old gladiolus plants (cvs: Tampico and Jester) were inoculated by spraying until run-off with a atomiser at 0.0732 kg cm^{-2} (DeVilbiss Healthcare, Somerset, PA), immediately transferred to a dew chamber for 18 h at 17°C, and then returned to the greenhouse for disease development. After 21–25 days, profusely sporulating uredinia were observed on all plants. Urediniospores were harvested with a microcyclone spore collector and aliquoted equally among 28 plastic Petri dishes (10×35 mm) using a camel hair brush to dust them across the bottom of the dishes. An additional aliquot was suspended in 0.5 ml of water and 300 µl pipetted onto each of three Petri dishes containing 2% water agar (WA). The dishes were incubated for 16 h at 17.0°C to establish the baseline germination potential.

Average daily temperature and RH data for San Diego County, California from 2001 to 2009 (CIMIS 2008) and Clewiston, Florida 2005 to 2007 (USGS 2008) were used to determine the range of temperature and RH conditions occurring during December through until May. In San Diego County temperatures ranged from 1.7 to 22.7°C and RH from 20 to 95%, while Florida temperatures ranged from 13.3 to 26.3°C and RH from 44.1 to 93.5%. These parameters and the availability of growth chambers were used in choosing the temperature and RH treatments applied in the study.

To achieve six different RHs at the four selected temperatures, 250 ml of five different saturated salt solutions (Rockland 1960) and distilled water (Table 1) contained in 13.5×13.5×8.0 cm airtight plastic boxes (Lock & Lock, Heritage Mint, Ltd, Scottsdale, AZ) were used. The Petri dishes containing the urediniospores alone were placed on a wire rack suspended over the saturated salt solutions. One box of each RH treatment was placed in each of four growth chambers programmed for temperatures of 2.8, 15.0, 18.8 and 25.0°C with no light. One dish of urediniospores was placed directly inside each growth chamber to monitor the effects of ambient chamber RH on spore germination. Small self-contained electronic temperature/RH recorders (Watchdog 450 data logger; Spectrum Technologies, Plainfield, IL, USA) were placed in each of the RH boxes, except the 11% RH (below sensitivity range of the recorder), to confirm RH level and stability. Ambient relative RH between the four growth chambers did not differ significantly and ranged from 24.0 to 82.4% with a mode of 30.0% and a mean of 31.0%.

Table 1 Saturated salt solutions and the RHs achieved in airtight boxes at specific temperatures

	% RH at °C			
Saturated Salt Solution	2.8°C	15.0°C	18.8°C	25.0°C
Lithium chloride	16	13	12	11
Potassium acetate	25	24	23	23
Potassium carbonate	47	45	44	43
Sodium chloride	76	75	75	75
Potassium nitrate	96	95	94	93
Distilled water	100	100	100	100

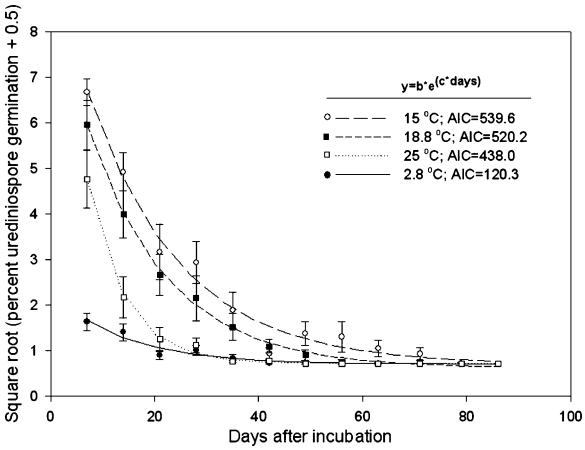

Fig. 1 Results of fitting the pooled urediniospores germination data for each temperature to the non-linear exponential decay model. Akaike's information criterion (AIC) establish goodness-of-fit for the model

At seven-day intervals, an aliquot of urediniospores was removed via camel hair brush and suspended in 0.5 ml of water in a 1.5-ml microfuge tube, vortexed for 15 s and 300 µl transferred to a 10× 35 mm Petri dish containing 2% WA. Dishes were incubated at 17°C for 16–18 h. Three replicate counts of >100 urediniospores each were made using a compound microscope to determine the percent germination for each treatment. Sampling continued until no urediniospore germination was detected in any treatments for two consecutive samplings. The experiment was repeated twice. Between the two studies, results of the analysis of variance (ANOVA) showed no significant difference ($P \leq 0.05$) in urediniospore germination at the time of harvest; data were therefore combined to show an average of 49.1% germination before treatment.

To normalise the data, urediniospore germination was transformed by square root of the percent germination plus 0.5 (Steel and Torrie 1980), and the averages of the three samples per experiment were used in the statistical analyses. A repeated measures ANOVA was conducted on the complete dataset with a mixed model and auto-regressive variance-covariance error structure based on the experiment× temperature×RH interaction. This was done to determine the significance of the sources of variation in the model. Subsequently, the data on RHs were pooled, and the temperature effects on germination over time were modeled as individual exponential decay models for each temperature and analysed as generalised non-linear models with the NLMIXED procedure of SAS (SAS Institute Inc. 2004, Cary, NC) to obtain parameter estimates and goodness-of-fit for each temperature. The model used was $y=b\times e^{(c\times days)}$ where y=transformed percent germination, days= days after incubation, e=the natural log, and b and c were parameters estimated from the analyses.

The parameter estimates for each temperature were then used as starting values in a larger generalised non-linear model in which all temperatures were included. This model allowed statistical comparisons of the differences in the estimated parameters for each temperature. The NLMIXED procedure uses maximum likelihood estimation. Areas under germination curves (AUGCs) were calculated using percent germination for each temperature over time. These areas were then subjected

Table 2 Parameter estimates, standard errors, and AUGCs for germination of *Uromyces transversalis* urediniospores at 2.8, 15, 18.8, and 25°C. All parameter estimates were significantly ($P \leq 0.001$) different than zero. Urediniospore germination was transformed by square root (% germination+0.5) and fit to the model: $y=b*e^{(c*days)}$, where y=transformed germination percentage, days=days after start of incubation, e=the natural log, and b and c=parameters estimated from the model

	2.8°C		15.0°C		18.8°C		25.0°C	
Parameter	Est[1]	SE[2]	Est	SE	Est	SE	Est	SE
b	1.502	0.229	8.453	0.406	7.691	0.451	6.525	0.782
c	−0.012	0.004	−0.039	0.002	−0.044	0.003	−0.061	0.009
AUGC[3]	61 A[4]		2389 B		1949 C		1246 D	

[1] Estimate

[2] Standard error of estimate

[3] Area under the germination curve

[4] AUGC values followed by different letters are significantly different at $P \leq 0.05$

to ANOVA to generate least squares means and comparisons among temperatures.

Results of the repeated measures ANOVA showed no significant ($P \leq 0.05$) effect of RH or the interaction of RH and temperature on urediniospore germination. The effect of temperature was highly significant ($P \leq 0.0001$). Results of fitting the pooled data for each temperature to the non-linear exponential decay model are shown in Fig. 1, and parameter estimates for these models are shown in Table 2. Comparisons of the parameter estimates showed that the rate of decline (parameter c) in urediniospore germination was significantly greater at 25.0°C than at 15.0°C and 2.8°C but was the same as 18.8°C. The rates of decline and germination on the first day (day 7) of the test (parameter b) for 18.8°C and 15.0°C were statistically the same, but both were statistically greater than these estimates for 2.8°C. Germination on the first day of the test was significantly greater for 15.0°C than for 25.0°C, and AUGCs were the highest for 15.0°C followed by 18.8, 25.0, and 2.8°C (Table 2).

Results observed in these controlled growth chamber studies suggest that once the infected gladioli are removed, RH would not be a significant factor in determining the length of time an infected production facility or field should remain host-free. In contrast, temperature was shown to have a significant effect on the longevity of urediniospore germination, a fact that could be utilised to shorten the required host-free time period for greenhouse production (Shlevin et al. 2004), or in the case of field production, permit decisions to be made based on the prevailing environmental conditions.

Although the most rapid initial rate of decline in urediniospore germination occurred at 25°C, the second most rapid rate of decline was at 15.0°C. The highest amount of germination on the first day of the test was also at 15.0°C and AUGC was significantly greater for 15.0°C than for any other temperature. Melching et al. (1991) reported cold-induced dormancy in urediniospores of *Puccinia graminis* f. sp. *tritici* and other rust fungi that could be overcome by heat-shocking at 40°C for 5 min. Prior to this study it was determined that this heat shock treatment to urediniospores of *U. transversalis* incubated for 7 and 14 days at 2.8°C did not increase the germination rate beyond that of non-heat shocked urediniospores (Peterson, G.L., *personal communication*). A literature review found no reference to heat-induced dormancy in urediniospores of other *Uromyces* species. This would suggest that in the absence of temperature-induced dormancy, the greatest number of viable urediniospores would be present for the longest period of time at 15.0°C. Conversely, the 15.0°C treatment resulted in the most germination and was more likely to result in germination independent of any low or high temperature-induced spore quiescence. Thus, lack of germination after 79 days was probably a good indicator of the lack of viable spores after this time for the 15.0°C treatment. No germinating urediniospores were detected after 79 days for any treatment. Further tests would need to be conducted at each temperature to determine the cause(s) of decline in germination from the time of urediniospore harvest (49.1%) to the first sampling at 7 days.

This study does not address the longevity or role of the *U. transversalis* teliospore stage. It has been speculated that this stage plays a role in the dispersal and survival of the pathogen (Smith et al. 1997) and clearly warrants further investigation. Since the formation of teliospores occurs in the leaf tissue within and surrounding the uredinia much later in the season, eradication of infected plants at symptom onset would reduce the risk of teliospore formation and potential soil contamination.

In 2008, APHIS reduced the recommended host-free period for nursery production facilities from 120 to 90 days (Rizvi et al. 2007, 2008). The results of this study confirm that this time period would be adequate for preventing infection of new nursery stock from residual onsite urediniospores of *U. transversalis.*

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