The Effect of Temperature on the Germination of Teliospores of *Puccinia punctiformis*

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ABSTRACT

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Germination of teliospores was analyzed as a time-dependent process. Nonlinear regression was used to fit germination progress curves for 5, 10, 15, and 20 C. Germination was characterized by the maximum fraction of teliospores germinated, the mid germination time (one-half of the maximum germinated), and the rate of germination at the mid germination time. Temperature had a major effect on the mid germination time and, to a smaller extent, on the rate of germination. The mid germination time was shortest at 15 C, longer at 10 and 20 C, and longest at 5 C. The rate of germination was highest at 10 and 15 C and lower at 5 and 20 C. No effect of temperature was detected on the maximum fraction of teliospores germinated. The consequences of the germination dynamics for infection of *Cirsium arvense* root buds by *Puccinia punctiformis* are discussed.

Additional keywords: biological weed control.

Puccinia punctiformis (F. Strauss) Röhl. infects *Cirsium arvense* (L.) Scop. systemically by means of teliospores (4,12). Teliospores are considered as a potential bioherbicide for *C. arvense* (4,11,12). The clonal plant *C. arvense*, with the common names Californian thistle and Canadian thistle, is a troublesome weed on arable land and grassland (2). Current research demonstrated that root buds of *C. arvense* act as ports of entry for the rust (J. Frantzen & W. Van der Zweerde, *unpublished*). Root buds of *C. arvense* develop in the range of 5–30 C (8). To evaluate the practical use of teliospores as a bioherbicide, knowledge of the influence of temperature on root bud infection is required. Quantitative data on germination are necessary to conduct infection experiments adequately.

Germination of *P. punctiformis* teliospores is stimulated by volatile extracts from *C. arvense* roots (5). I have demonstrated (*unpublished*) that instead of extracts of the roots, root pieces may be used to stimulate germination.

French and Lightfield (4) reported that only a low fraction of teliospores germinated at temperatures below 11 C or above 24 C after 7 days of incubation. They did not consider the dynamic component of germination, i.e., the change of the fraction of teliospores germinated with time. The present study was designed to determine the influence of temperature on the dynamics of germination.

MATERIALS AND METHODS

General. An isolate of *P. punctiformis* was collected from infected *C. arvense* shoots in a grassland in the vicinity of the town of Nijmegen (51°48′N, 6°02′E) and cultured on a *C. arvense* clone from the same site in a climate room (20 C, 60–70% RH, 35–45 W m^{-2} for 14 h day^{-1}). Root pieces were inoculated with spores, and the resulting systemically infected shoots were, following Waters (13), placed at 10–13 C and low light intensity (<5 W m^{-2}) to stimulate the production of teliospores. Spore samples were collected with a cyclone spore collector and stored for 2–4 mo in a refrigerator (about 5 C) at 45% RH. Teliospores were rehydrated at 80% RH for at least 24 h before being used in the experiments.

Teliospores were evenly deposited on slides by means of a settling tower. The slides had been covered previously with 1% water agar (prepared with distilled water). Each slide was placed in a separate petri dish with wetted blotting paper on the bottom.

Root pieces of *C. arvense* (total weight 0.6–0.7 g) were placed next to a slide to stimulate germination of teliospores by means of volatile components of the roots. The petri dishes were placed in the dark at various temperatures in incubators. Deviations from the chosen temperatures were within the range of ±1 C. After incubation, the fraction of teliospores germinated was determined by observing at least 150 teliospores per slide. Teliospores were considered to be germinated if a metabasidium had been formed by one or both of the telial cells.

Temperature selection. The range of temperature to be tested was selected in a preliminary experiment. Teliospores, collected from one systemically infected shoot on one date, were dispersed over 33 slides. The slides were randomly assigned to an incubation temperature of 5, 10, 15, 20, or 25 C. Teliospores were incubated at 5, 10, and 25 C for 7, 14, and 21 days and at 15 and 20 C for 7 days. For each temperature and incubation time, three slides (replicates) were used.

Germination dynamics. On the basis of results of the preliminary experiment, the effect of temperature on the dynamics of germination was determined for 5, 10, 15, and 20 C. Teliospores were dispersed over eight series of 15 slides each. For each series a subsample of a total spore sample, collected from several systemically infected shoots and on several dates, was used. Two series were randomly assigned to each temperature and served as replicates. Because the two replicates of a temperature were placed together in the same incubator, they were not considered as independent in the statistical analysis. Germination was determined after various times of incubation. Germination for 15 and 20 C was determined at daily intervals until 14 days after the beginning of incubation, for 10 C at 2-day intervals until 20 days after the beginning of incubation, and for 5 C at 2-day intervals until 30 days after the beginning of incubation. At each time of observation, germination of teliospores was determined on one slide for each temperature and replication. This experiment (experiment I) was repeated (experiments II and III). The total spore sample used in experiment I was also utilized in experiments II and III. In each experiment, temperature was randomly assigned to the incubators.

Data analysis. Data analysis of the three experiments was conducted in two steps. First, curves were fitted to the data by nonlinear regression using Genstat (9). Log-logistic curves (1) were fitted:

$$y = c^*g \tag{1}$$

and

$$g = 1/\{1 + \exp(-b^*\ln(t/\tau))\}, \tag{2}$$

where y = the fraction of teliospores germinated, c = the upper asymptote of y (the germinable fraction of teliospores), g = the function describing the germination of germinable teliospores, b = the shape parameter, t = the time in days, and τ = the mid germination time, at which one-half of the germinable spores had germinated. To restrict values of c to the range 0–1, the parameter γ was estimated in the curve fitting routine and transformed into c using the equation:

$$c = 1/(1 + \exp(-\gamma)). \tag{3}$$

The curves were fitted using a binomial distribution with correction for overdispersion (7). The binomial distribution accounted for the differences in variance of germination depending on incubation time. Overdispersion accounted for factors affecting the independence of teliospores with respect to germination, i.e., deviations from the binomial distribution.

Curves were fitted to data of each temperature and replicate within an experiment. The rate of germination (dg/dt) of germinable teliospores at the mid germination time (τ) was computed:

$$v = b/(4^*\tau). \tag{4}$$

For each temperature and experiment, the mean values of the parameters were computed from the values of the two replicates. These mean values were used in the subsequent statistical analysis, in which the three experiments were treated as blocks in a randomized complete block design with four temperature treatments.

The effect of temperature on the parameters was first tested by a distribution-free permutation F test (6) because, according to Ross (10), parameter estimates need not be exactly normally distributed. The results of the permutation test were compared with the results of an ANOVA. ANOVA was performed to test the temperature effect on linear and nonlinear trends (6), providing additional information about the effect of temperature on the germination dynamics.

RESULTS

Temperature selection. Hardly any of the teliospores incubated at 25 C had germinated after 21 days of incubation (Table 1). The fraction of teliospores germinated was about 0.65 or higher for teliospores incubated at 5, 10, 15, and 20 C.

Germination dynamics. The quality of fit of curves varied, as reflected by the standard errors of the estimates of the parameters (Table 2). Estimates of parameter b were less accurate than those

TABLE 1. The fraction of teliospores of *Puccinia punctiformis* germinated at various temperatures and incubation times in the preliminary experiment[a]

Temperature (C)	Incubation time (days)[b]		
	7	14	21
5	0.00 (0.00)	0.01 (0.01)	0.66 (0.13)
10	<0.01 (<0.01)	0.79 (0.02)	0.84 (0.03)
15	0.78 (0.01)	...	...
20	0.84 (0.03)	...	...
25	0.00 (0.00)	0.01 (<0.01)	0.04 (0.01)

[a] Means of three replicates.
[b] SE in parentheses.

TABLE 2. Estimated parameters of a log-logistic model[a] fitted to data on germination of *Puccinia punctiformis* teliospores, depending on temperature

Experiment	Temperature (C)	Replicate	Parameter[b]		
			c	τ	b
I	5	1	0.64 (0.04)	20.0 (0.6)	13.0 (2.8)
		2	0.60 (0.02)	19.4 (0.3)	14.7 (1.6)
	10	1	0.70 (0.02)	9.0 (0.2)	8.7 (1.2)
		2	0.62 (0.06)	8.6 (0.7)	9.3 (4.5)
	15	1	0.72 (0.11)	6.9 (0.9)	3.8 (1.2)
		2	0.53 (0.05)	6.4 (0.6)	5.2 (1.5)
	20	1	0.72 (0.16)	9.5 (1.5)	3.6 (0.8)
		2	0.81 (0.21)	8.7 (1.8)	3.4 (1.1)
II	5	1	0.59 (0.06)	17.2 (0.9)	7.5 (1.9)
		2	0.56 (0.05)	16.9 (0.7)	8.5 (1.9)
	10	1	0.56 (0.06)	8.2 (0.6)	8.2 (3.2)
		2	0.55 (0.04)	7.6 (0.3)	14.6 (5.3)
	15	1	0.59 (0.05)	5.5 (0.4)	8.7 (3.4)
		2	0.66 (0.02)	5.4 (0.1)	9.2 (1.1)
	20	1	0.69 (0.14)	6.8 (1.3)	3.3 (1.3)
		2	0.37 (0.06)	5.2 (0.9)	5.5 (3.0)
III	5	1	0.44 (0.05)	21.6 (0.7)	10.8 (1.7)
		2	0.55 (0.10)	22.7 (1.2)	9.4 (1.7)
	10	1	0.56 (0.10)	10.6 (1.1)	4.9 (1.5)
		2	0.55 (0.03)	8.3 (0.2)	21.8 (8.6)
	15	1	0.48 (0.05)	6.1 (0.4)	7.9 (2.9)
		2	0.44 (0.03)	5.4 (0.3)	12.0 (4.4)
	20	1	0.50 (0.06)	6.0 (0.6)	6.3 (2.3)
		2	0.37 (0.06)	5.7 (0.9)	5.8 (3.3)

[a] $y = c^*g$ and $g = 1/\{1 + \exp(-b^*\ln(t/\tau))\}$, in which y = the fraction of teliospores germinated, c = the germinable fraction of teliospores, g = the function describing the germination of germinable teliospores, b = the shape parameter, t = the time in days, and τ = the mid germination time, at which $y = 0.5^*c$.
[b] SE in parentheses.

of parameters c and τ. Fitted curves for 20 C in experiment I replicate 2 and in experiment II replicate 1 had relatively large errors for the estimates of all three of the parameters. Examples of curves with better and poorer fit, respectively, are presented in Figure 1.

The significance levels of the effect of temperature on germination determined by ANOVA differed only slightly from those of the permutation test (Table 3). Analysis of variance was, therefore, used to test the temperature effect on linear and nonlinear trends. No effect of temperature could be detected on parameter c. Parameter τ depended both linearly and nonlinearly on temperature; the value of τ differed significantly between lower and higher temperatures and suggested a trough around 15 C. Parameter b depended linearly on temperature; the value of b differed significantly between lower and higher temperatures. Parameter ν depended nonlinearly on temperature; the value of ν showed a hump at 10–15 C.

Substitution of the parameter estimates in equations 1 and 2 resulted in curves with a delay of germination at 5 and 10 C and relatively high rates of germination at 10 and 15 C (Fig. 2). At 20 C, the maximum level was reached late relative to the early onset of germination, reflecting the relative low ν.

DISCUSSION

The temperature optimum curve for germination of teliospores of *P. punctiformis* reported by French and Lightfield (4) was based on data obtained after 7 days of incubation. Germination after 7 days of incubation in the preliminary experiment (Table 1) corresponded well with the optimum curve of French and Lightfield (4). Incubation of teliospores during 14 and 21 days at 10 and 5 C, respectively, resulted in fractions of teliospores germinated that approximated those obtained at 15 and 20 C during 7 days. Based on the results, a time dependency of germination is assessed. At 25 C, the fraction of teliospores germinated was still low after 21 days of incubation. Incubation time seemed to have relatively little influence on germination at this temperature. Therefore, 25 C was not included in experiments I–III.

The results of experiments I–III confirmed that time is a major factor influencing germination of fungal spores, as has been shown by other studies, e.g., that by Eisensmith et al (3). The log-logistic model used to describe the germination dynamics was applied to all data. Some curves had a poor fit, especially at 20 C, and another model might have been applied in those cases. To compare data of the various temperatures and experiments, however, the same model was applied to all data.

The model was chosen because of the biological meaning of the parameters. The parameter c indicated the germinable fraction of teliospores. In the present study, the parameter had, independent of temperature, a mean value of about 0.6. This intermediate value, on a scale of 0–1, reflects mixing several spore samples into one large total sample. Variation in the conditions of the climate room or genetic variation within the *P. punctiformis* isolate probably caused differences in ripening or vitality of teliospores sampled from various shoots and on various dates.

TABLE 3. Effects of temperature on parameters of a log-logistic model[a] fitted to data on germination of *Puccinia punctiformis* teliospores in experiments I–III

Temperature (C)	Parameter[b]			
	c	τ	b	ν[c]
5	0.57	19.7	10.6	0.14
10	0.59	8.7	11.2	0.34
15	0.57	6.0	7.8	0.34
20	0.58	7.0	4.7	0.18
P (permutation)	0.98	0.009	0.064	0.051
P (ANOVA)	0.98	<0.001	0.058	0.035
Linear	0.95	<0.001	0.016	0.490
Nonlinear	0.91	0.001	0.400	0.019

[a] $y = c*g$ and $g = 1/\{1 + \exp(-b*\ln(t/\tau))\}$, in which y = the fraction of teliospores germinated, c = the germinable fraction of teliospores, g = the function describing the germination of germinable teliospores, b = the shape parameter, t = the time in days, and τ = the mid germination time, at which $y = 0.5*c$.

[b] Means of three estimates of the parameters, one estimate per experiment.

[c] Rate of germination (dg/dt) at the mid germination time: $\nu = b/(4*\tau)$.

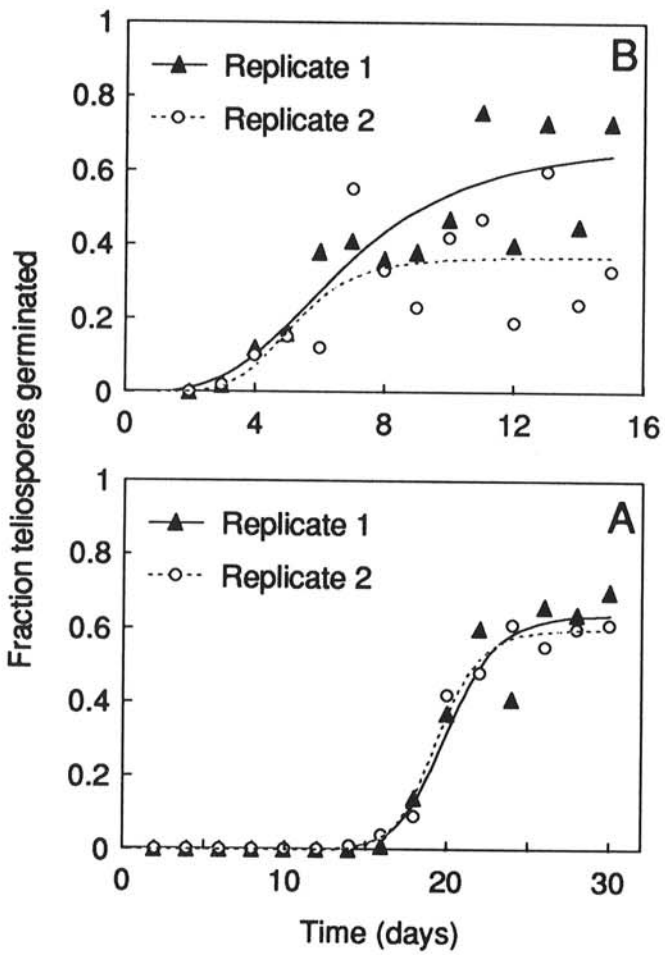

Fig. 1. Examples of fitting curves to data of germination of *Puccinia punctiformis* teliospores at **A**, 5 C in experiment I and **B**, 20 C in experiment II. Entries were observed fractions, and lines were fitted to the data with a log-logistic model (see Table 2 for parameter estimates).

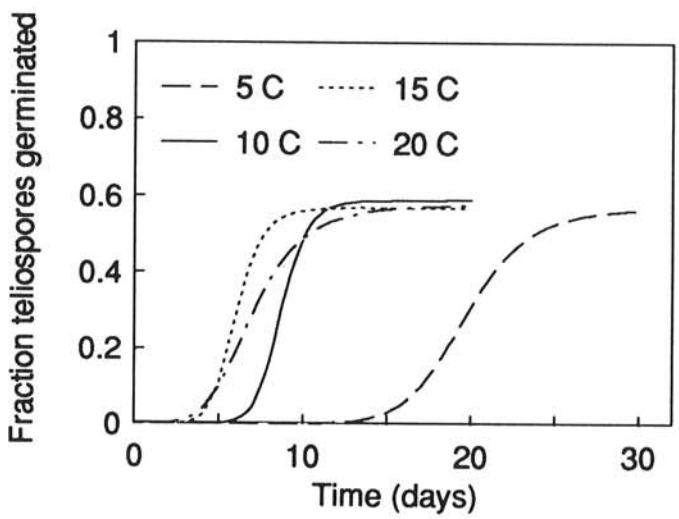

Fig. 2. Germination of teliospores of *Puccinia punctiformis* (see Table 3 for parameter estimates).

Heterogeneity of germination in the present study agrees with that reported for teliospore samples of *P. punctiformis* collected from the field (11).

The function g described the germination dynamics of the germinable teliospores. The parameter τ of this function indicated the time at which one-half of the germinable teliospores had germinated, the mid germination time. The major effect of temperature was on this parameter. Germination of teliospores was strongly delayed at 5 C. Experiments directed toward infection of *C. arvense* root buds by *P. punctiformis* have to take this temperature effect into account. Infection has to be expected at a much later time at low temperature than at high temperature.

The shape parameter b had biological meaning because it determined, together with τ, the rate of germination at the mid germination time. The rate of germination showed a nonlinear trend, with relatively high values at intermediate temperature (10 or 15 C) and relatively low values at the extremes (5 and 20 C). According to these results, an optimum for the rate of germination may result in a higher infection rate (more infections per unit time) around 10–15 C than at 5 or 20 C.

On the basis of the germination characteristics of teliospores, biological control of *C. arvense* by *P. punctiformis* should occur in the range of 5–20 C. Infection experiments are required to conclude which temperature will be best for biological control. The germination dynamics described here provide a basis to conduct the infection experiments.

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