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Effect of environmental conditions on germination and survival of teliospores and basidiospores of the pear rust fungus (*Gymnosporangium asiaticum*)

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Abstract

Pear rust, caused by *Gymnosporangium asiaticum*, is an important disease of pear (*Pyrus spp.*) in China in areas where cypress (*Juniperus spp.* and *Sabina spp.*), the alternate host, is present. Controlled environment experiments were conducted to study the effects of temperature, relative humidity (RH) and duration of free water on germination of teliospores and basidiospores of *G. asiaticum*. Teliospores, sampled from *Juniperus chinensis*, germinated at temperatures ranging from 5 to 28 °C, with an optimum between 16 and 20 °C. For teliospores to germinate, telial horns required free water: soaking in water for 30 sec triggered teliospore germination. After initial wetting, RH had little effect on germination of teliospores except at extreme temperatures (5 and 30 °C) where germination needed near-saturation moisture. The minimum time for telial horns to produce basidiospores was about 3 h at optimum temperatures. Teliospores in all telial horns germinated at 12–24 °C, but at 8 and 28 °C only those from a third of telial horns germinated. Basidiospores germinated at 5–30 °C with the optimum around 15 °C; they needed free water or saturated moisture to germinate. Germination in free water was about eight times greater than at 100% RH. Logistic models described well the germination dynamics of basidiospores. Basidiospore survival declined exponentially with storage time, and linearly with increasing temperature and decreasing RH. Basidiospores survived for at least six days in dry conditions with RH as low as 45%.

Introduction

Pear rust, caused by *Gymnosporangium asiaticum*, is an important disease of pear (*Pyrus spp.*) in China in areas where cypress trees (*Juniperus spp.* and *Sabina spp.*), the alternate host, are present (Wang and Hu, 1997; Xu and Zhu, 1997; Li and Chen, 2000). *Gymnosporangium asiaticum* overwinters as mycelium in cypress trees and produces telial horns next spring. Telial horns begin to maturate and teliospores acquire the ability to germinate just before pear bloom in spring (Xu and Zhu, 1997; Ma, 1998). Infected *Juniperus* twigs may release basidiospores over many years (Aldwinckle, 1990). Teliospores germinated in free water from rain and produce basidiospores, which infect young tissues of pear, such as young leaves, fruits and twigs. The fungus forms spermagonia, receptive hyphae and spermatia on pear trees; after fertilization, it forms dikaryotic mycelia and produces aecia and aeciospores. The aeciospores then infect cypress trees, completing the disease cycle. A minimum distance between the two hosts (pear and cypress trees) of 5000 m may be required to reduce

disease transmission sufficiently for practical disease management (Wang and Hu, 1997; Zhang and Huang, 1997). Two races of *G. asiaticum* have been described based on their behaviour on cultivars of *P. pyrifolia* (Sakuma, 1992).

Until recently, pear rust was mainly controlled by cutting down the cypress trees near pear orchards in China (Zhang and Huang, 1997). Recently, many cypress trees have been planted in cities and along highways to improve the general environment, which has led to serious outbreaks of pear rust (Xu and Zhu, 1997; Li and Chen, 2000). Currently, the disease is mainly controlled by routine application of systemic fungicides, starting from the time when new lesions are first observed. Generally, pear rust symptoms become visible about 7 days after infection. Recent studies suggest that current approved systemic fungicides, such as triadimefon, have a high control efficacy only when applied within 7 days after infection (Xu and Zhu, 1997; Ma, 1998). Hence, accurate predictions of potential infection periods are needed to time fungicide applications for optimum control efficacy.

Teliospores in mature telial horns of *G. asiaticum* normally germinate when free water is available, and no telial horns remain on the cypress trees after several successive rains (Wang and Hu, 1997; Xu and Zhu, 1997; Ma, 1998). Optimum temperature for teliospore germination is between 15 and 20 °C (Lee, 1990). Lack of quantitative epidemiological knowledge, however, has hampered the development of models forecasting potential infections of pear rust for improving current control efficacy. Progress and severity of pear rust are the results of several underlying fungal development processes from germination of telial horns, production and subsequent dispersal of basidiospores, and infection of host tissues by basidiospores. Environmental conditions can greatly influence these pathogens or related rust pathogens. For example, the optimum temperature for germination of teliospores of *Gymnosporangium juniperi-virginianae*, causal agent of cedar apple rust, is between 14 and 24 °C and germination is not possible when the temperature is below 6 °C (Pearson et al., 1977). Basidiospores of *G. juniperi-virginianae* are formed within 4 h at 12–24 °C, but not at 26–30 °C, despite the fact that teliospores can germinate at these temperatures (Pearson et al., 1977). Discharge of *G. juniperi-virginianae* basidiospores normally begins within a few hours of the start of rainfall (Pearson et al., 1980). Infection of apple leaves by basidiospores is critically dependent on the length of wetness period and the temperature during such a period (Aldwinckle et al., 1980).

This study aimed to obtain basic epidemiological data on pear rust, specifically on the effects of temperature, relative humidity (RH) and duration of free water on germination of teliospores and basidiospores, and on the effects of temperature, RH and the length of storage time on the viability of basidiospores.

Material and methods

Source of telial horns and production of basidiospores

In 2003 and 2004, mature telial horns were collected from cypress trees (*J. chinesis*) in Xianhe Park, Laiyang, Shandong Province in a mixed planting of pear (*P. bretschneideri* cv. Tse) and cypress. The telial horns were placed immediately into plastic bags and kept in a refrigerator at 5 °C for up to 40 days, i.e. all experiments in each year were completed within 40 days after the sampling of telial horns.

In order to obtain basidiospores, telial horns were soaked in distilled water for 30 min, then placed into an open Petri dish and incubated at room temperature (normally between 15 and 25 °C) for 5 h. Abundant basidiospores were produced by teliospores from the germinated telial horns. Distilled water was added to the Petri dish and final concentration of the basidiospore suspension was adjusted to *c.* 1×10^5 basidiospores ml^{-1} using a haemocytometer.

Effects of wetness duration on germination of teliospores

Germination of teliospores was examined at seven lengths of wetness duration (0, 0.5, 5, 10, 20, 30 and 60 min) at each of three temperatures (5, 15 and 30 °C). Three telial horns were randomly chosen for each treatment. The telial horns were placed into three jars, 21 in each jar; each closed jar was placed in one of the three incubators, set to 5, 15 or 30 °C. An additional single jar containing

100 ml distilled water was placed into each incubator at the same time. After 1 h preconditioning, three telial horns were taken out from each jar and placed into a Petri dish, which contained 20 ml water agar (WA) to maintain 100% RH. The Petri dishes were immediately sealed with parafilm and placed upside down in the incubator set to 15 °C. This was the zero wetness duration treatment. At the same time, all remaining telial horns in each jar were placed into the corresponding jar containing distilled water to soak. At the end of each of the six designated wetness durations, three telial horns were randomly taken out from each jar, dried with filter paper and placed onto a Petri dish as described above. After 24 h incubation at 15 °C, each telial horn was washed in 1 ml distilled water and the water was examined for the presence of basidiospores with a microscope. Teliospores from a telial horn were considered germinated when basidiospores were found. The experiment was repeated twice.

Effects of temperature and RH on germination of teliospores

Germination of teliospores was examined at six temperatures (5, 10, 15, 20, 25 and 30 °C) at each of the four RH levels (100, 85, 75 and 45%). RH of 100, 85, 75 and 45% was obtained by using 30 ml distilled water or saturated solution of K_2CO_3, NaCl and KCl in four 200 ml sealed jars. All 72 telial horns were first soaked in distilled water for 30 min and then dried with filter paper. Three telial horns selected at random were then hung in each jar of each treatment using adhesive tape and the jars were then sealed. The jars were immediately placed into incubators, each set to one of the experimental temperatures. The telial horns were taken out and examined as described above after 24 h incubation. The experiment was repeated twice. For each replicate experiment, each incubator was randomly assigned to one of the experimental temperatures.

Temporal dynamics of basidiospore production

The temporal dynamics of basidiospore production by telial horns was studied at 8, 12, 16, 20, 24 and 28 °C. At each temperature, the production of basidiospores was examined eight times. For each combination of temperature and incubation time, there were three telial horns chosen randomly. Each of the six jars, which contained 100 ml distilled water, was placed in an incubator set to one of the experimental temperatures. After 30 min, three telial horns were placed in each jar to soak for 30 min. The jars were then removed and the distilled water was discharged; the jars were returned to the same incubator. From 2 h until 9 h after wetting, three telial horns were examined hourly for the presence of basidiospores. The experiment was repeated twice. For each replicate experiment, each incubator was randomly assigned to one of the experimental temperatures.

Effect of temperature and RH on basidiospore germination

Germination of basidiospores was investigated at six temperatures (5, 10, 15, 20, 25 and 30 °C) at each of the five RH levels (100, 99, 97 and 95%, free water). RH levels of 100, 99, 97 and 95% were obtained by amending WA with 0, 0.3, 0.9 and 1.5 M NaCl, respectively, inside sealed Petri dishes (Lang, 1967; Harris et al., 1970; Alderman and Beute, 1986; Xu et al., 2001). Two glass slides were placed on the lid of each Petri dish containing approximately 20 ml of WA amended with the appropriate amount of NaCl to achieve the desired RH. Two separate 10 µl droplets of basidiospore suspension were placed on each slide (26 × 76 mm) using a micropipette and air-dried at room temperature (normally between 15 and 25 °C). On average, it took about 20 min from making the basidiospore suspension to the time when the suspension droplets had dried. The plate was then sealed with parafilm and placed upside down in an incubator set to an experimental temperature. For the free water treatment, a water agar plate without NaCl (i.e. same as for the 100% RH treatment) was sealed immediately after the suspension was placed on the slides without being air-dried.

Germination was recorded after 24 h. A drop of cotton blue in lactophenol (lactic acid:phenol: glycerin:distilled water = 1:1:2:1 (v/v/v/v)) was placed on each suspension droplet to prevent further germination and preserve basidiospores. Percentage germination was estimated by examining 100 basidiospores in each suspension droplet under a microscope; a basidiospore was considered to have germinated when its germination tube was longer than the basidiospore width. There was

one plate (i.e. 400 basidiospores examined for germination) for each combination and the experiment was repeated four times. Incubators were randomly assigned to one of the experimental temperatures in each replication.

Effects of temperature on the temporal dynamics of basidiospore germination

The temporal dynamics of basidiospore germination were studied at 5, 10, 15, 20, 25 and 30 °C. Two glass slides (26 × 76 mm) were placed on the lid of a Petri dish, containing 20 ml water agar to maintain 100% RH. Two separate 10 µl droplets of basidiospore suspension were placed on each slide using a micropipette. The plate was then sealed with parafilm (i.e. suspension droplets were not dried) and placed upside down in an incubator set to an experimental temperature. There were 13 plates at each temperature; a single plate was assigned randomly to one of the following 13 assessment times: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 24 h after incubation. At each assessment time, a single Petri dish was taken out and a drop of cotton blue in lacto-phenol was placed on each suspension droplet to halt germination and to preserve basidiospores. Percentage germination was estimated by examining 100 basidiospores in each suspension droplet under a microscope. The experiment was repeated twice and incubators were randomly assigned in each replication.

Effects of temperature and RH on basidiospore survival

Viability of basidiospores was examined 24, 72 and 144 h after incubation in each combination of six temperatures (5, 10, 15, 20, 25 and 30 °C) and three RH levels of (100, 75 and 45%). RH of 100, 75 and 45% were obtained by adding 30 ml distilled water or saturated solution of NaCl and KCl in sealed jars of 200 ml capacity, respectively. Eighteen telial horns chosen randomly were soaked in water for 30 min; each telial horn was then placed on a piece of wet filter paper (2 × 2 cm) inside a Petri dish and incubated at room temperature for 5 h to produce basidiospores. Each filter paper with germinated telial horns was removed from the dish, air-dried and hung using adhesive tape in a jar at an experimental RH. Three jars (i.e., 100, 75 and 45% RH) were then placed into each of the six incubators set to one of the six temperatures. At each assessment, one jar was taken out for each combination of temperature and RH; basidiospores were retrieved with wet brushes and deposited onto two sites on each of two glass slides. The basidiospores were wetted with a drop of distilled water on each deposition site and then incubated in sealed Petri dishes under 100% RH at 20 °C for 24 h. Percentage germination was then estimated by examining 100 basidiospores at each deposition site as described before. After each assessment, the telial horns were immediately returned to the same jar incubator.

Data analysis

Logistic regression analysis (Cox and Snell, 1989) was used to measure effects of treatment factors (temperature, RH etc.) on the germination (p) of telial horns or basidiospores. In this form of analysis, the number of telial horns or basidiospores germinated per treatment is assumed to be binomially distributed and the logit transformation of the proportion, *i.e.*, $\ln(p/1-p)$, is expressed as a multiple linear regression on the treatment factors.

The effect of treatment factors (incubation time and temperature) on the dynamics of telial horn or basidiospore germination was described by a logistic model:

$$p = \frac{K}{1 + \exp(-\beta(w - M))} \quad (1)$$

where w is the duration of incubation (h), and K, M and β are parameters to be estimated from observed data. For telial horn germination, p, K, M and β are the number of telial horns germinated, the maximum number of telial horns germinated, the length of elapsed time (h) until $p = K/2$, and the rate of germination (h^{-1}), respectively. For basidiospore germination, p, K, M and β are the % germination, the maximum % germination, the length of elapsed time (h) until $p = K/2$, and the rate of germination ($\%h^{-1}$), respectively. Logistic models were fitted to each temperature treatment over all replicate experiments. All analyses were carried out using Genstat™ version 6.1 (Payne, 2002).

Results

Effects of wetness duration on germination of teliospores

Germination of teliospores from mature telial horns required free water (Table 1). None of the 27 telial horns in the 'zero-hour' wetness treatments produced basidiospores. Soaking in distilled water for as little as 0.5 min led to the production of basidiospores in 16 of the 27 telial horns (Table 1). Out of the 27 telial horns that were soaked in water for 5 or 10 min, 24 telial horns produced basidiospores. All of the telial horns soaked for longer than 10 min produced basidiospores. Logistic regression analysis showed that only the main effect of soaking duration had significantly ($P < 0.01$) affected the germination of teliospores, accounting for 68% of the total deviances. Germination was significantly ($P < 0.01$) less for the zero soaking treatment than for the soaking of 0.5 min, which, in turn, was significantly ($P < 0.01$) less than the other treatments.

Effects of temperature and RH on germination of teliospores

Teliospores from all the telial horns incubated at 15 and 20 °C germinated and none of the 36 telial horns incubated at 30 °C produced basidiospores (Table 2). At 5 °C, only the telial horns incubated at 100% RH produced basidiospores. Except for five telial horns incubated at 45% RH and one at 75% RH, all telial horns incubated at 10–25 °C produced basidiospores (Table 2). Logistic regression analysis showed that both the main effects of temperature and RH were significant ($P < 0.01$), accounting for 77.6% and 22.3% of the total deviances, respectively. Germination at 100% RH was significantly greater ($P < 0.01$) than at the other three RH levels; germination at 30 °C was significantly less ($P < 0.01$) than that at 5 °C, which, in turn, was significantly less ($P < 0.01$) than at 10–25 °C.

Temporal dynamics of basidiospore production

The minimum time required for teliospore germination was 5, 4, 3, 3, 3 and 5 h at 8, 12, 16, 20, 24 and 28 °C, respectively (Table 3). All telial horns produced basidiospores at 12–24 °C. At each temperature, the temporal pattern of % germination was described well by a logistic equation; overall, the six logistic equations accounted for 99% of the total variation. Parameter K estimates did not differ significantly from 12–24 °C, and was close to the maximum (= 9), but was significantly greater ($P < 0.01$) than that at 8 and 28 °C (both close to 3). The rate estimates initially increased with increased temperature then decreased with increasing temperature; the maximum rate was between 16 and 20 °C. The relationship of parameter M estimates with temperature was opposite to that of the rate with temperature (Table 3).

Effect of temperature and RH on basidiospore germination

Overall, germination increased with increasing RH (Table 4). Germination was greatest under free water and reached 90% at 15 °C. For temperatures above and below 15 °C, % germination decreased. Logistic regression analysis showed that only the main effect of RH on germination of *G. asiaticum* basidiospores was significant ($P < 0.01$), accounting for 68% of the total

Table 1. Number of *Gymnosporangium asiaticum* telial horns (from nine) that produced basidiospores after soaking in water for various durations at three temperatures when assessed 24 h after incubation at 15 °C

Water temperature (°C)	Soaked duration in water (min)						
	0	0.5	5	10	20	30	60
5	0	5	9	9	9	9	9
15	0	5	7	6	9	9	9
30	0	6	8	9	9	9	9

Table 2. Number of *Gymnosporangium asiaticum* telial horns (from nine) that produced basidiospores after incubation at six temperatures and five levels of RH when assessed 24 h after incubation

Relative humidity (%)	Temperature (°C)					
	5	10	15	20	25	30
100	9	9	9	9	9	0
85	0	9	9	9	9	0
75	0	8	9	9	9	0
45	0	6	9	9	7	0

Table 3. Number of *Gymnosporangium asiaticum* telial horns (from nine) that produced basidiospores after incubation at six temperatures for various durations, together with parameter estimates of logistic models describing such temporal dynamics of germination of teliospores at each of the six temperatures. The parameter K is the maximum possible number of telial horns that germinated, M is the elapsed time until $p = K/2$, and β is the rate (h^{-1})

Temperature (°C)	Incubation duration (h)								Parameter estimates		
	2	3	4	5	6	7	8	9	K	β	M
8	–[a]	0	0	1	2	3	3	3	3.06 ± 0.113	1.79 ± 0.302	5.53 ± 0.114
12	0	0	3	5	8	9	9	–	9[b]	1.49 ± 0.217	4.71 ± 0.111
16	0	4	8	9	9	9	9	–	9[b]	2.71 ± 0.300	3.11 ± 0.038
20	0	4	8	9	9	9	9	–	9[b]	2.71 ± 0.300	3.11 ± 0.038
24	0	1	5	6	9	9	9	–	9[b]	1.44 ± 0.301	4.12 ± 0.163
28	–	0	0	1	2	2	3	3	2.99 ± 0.366	1.23 ± 0.443	5.73 ± 0.366

[a]Germination of telial horns were not tested at the treatment.
[b]Parameter was not estimated but set to the maximum possible value (= 9).

deviances. Average germination was 4.6%, 4.7%, 4.9%, 8.0% and 57.4% at 95%, 97%, 99%, 100% RH and free water, respectively. Germination under free water was significantly ($P < 0.01$) greater than at the other four levels of RH. These four levels did not differ significantly from each other. Despite significant interactions for two treatments (5 and 30 °C under wet), the overall effects of temperature and its interactions with RH were not significant ($P > 0.05$).

Effects of temperature on the temporal dynamics of basidiospore germination

Basidiospore germination was first observed 2 h after incubation at all temperatures. Thereafter, germination increased rapidly, particularly at 10, 15 and 20 °C. The average % germination 24 h after incubation was 54.7%, 79.1%, 88.0%, 79.7%, 46.7% and 12.1% at 5, 10, 15, 20, 25 and 30 °C, respectively.

Table 4. Average percentage of *in vitro* germination of basidiospores of *Gymnosporangium asiaticum* (based on 2400 basidiospores per treatment) at combinations of temperature and relative humidity, assessed 24 h after incubation

Relative humidity (%)	Temperature (°C)						
	5	10	15	20	25	30	Mean
Wet	33.8	78.9	90.0	79.1	48.3	14.4	57.4
100	5.7	9.9	12.5	11.1	4.1	4.4	8.0
99	4.9	5.3	6.1	5.1	3.9	3.9	4.9
97	5.0	5.6	5.3	4.1	4.5	3.8	4.7
95	3.5	5.2	4.0	4.9	5.2	4.6	4.6
Mean	10.6	21.0	23.6	20.9	13.2	6.2	15.8

Logistic models satisfactorily described the temporal dynamics of basidiospore germination at 5, 10, 15, 20 and 25 °C. Less variance was accounted for by the fitted logistic model at 5 °C (53.1%) than at other temperatures. An overall logistic model failed to fit germination data at 30 °C, which arose from the observed variability between the three replicate experiments. In one of the replicates, germination was close to zero at 30 °C; a logistic model described well the germination dynamics observed in the other two replicates, explaining 74.4% of the total variation. The parameter estimates of the logistic models are given in Table 5; these fitted models, together with observed values, are shown in Figure 1. The estimated maximum possible % germination (K) increased with increasing temperature, was maximum at 15 °C, and then decreased with increasing temperature. A similar relationship with temperature was also observed for the parameter estimates of M, except that the maximum value was obtained at 10 °C (Table 5). The relationship of the estimated β values with temperature was similar to that of K with temperature (Table 5).

Effects of temperature and RH on basidiospore survival

Overall % germination of basidiospores decreased with increasing incubation time (Table 6). Six days after incubation, the average % germination of basidiospores was 23.2%, compared to an average of 57.9% one day after incubation. The average germination decreased nearly linearly with increasing incubation temperature, from 50.1% at

Table 5. Parameter estimates of logistic models describing the temporal dynamics of *in vitro* proportion of germination (p) of *Gymnosporangium asiaticum* basidiospores at each of the six temperatures. The parameter K is the maximum possible percentage of germination, M is the elapsed time until $p = K/2$, and β is the rate (h^{-1}). These models are also shown in Figure 1

Temperature (°C)	K	β	M	% variance accounted for
5	0.513 ± 0.059	0.334 ± 0.153	4.709 ± 1.317	53.1
10	0.802 ± 0.039	0.387 ± 0.054	5.376 ± 0.392	89.2
15	0.849 ± 0.041	0.595 ± 0.119	3.798 ± 0.335	82.4
20	0.762 ± 0.039	0.531 ± 0.104	3.848 ± 0.361	82.6
25	0.458 ± 0.025	0.399 ± 0.091	3.120 ± 0.465	76.5
30[a]	–	–	–	–

[a]a logistic model described well the germination dynamics observed in two of the three replicate experiments, explaining 74.4% of the total variation but model details are not given here because germination was close to zero in the other replicate.

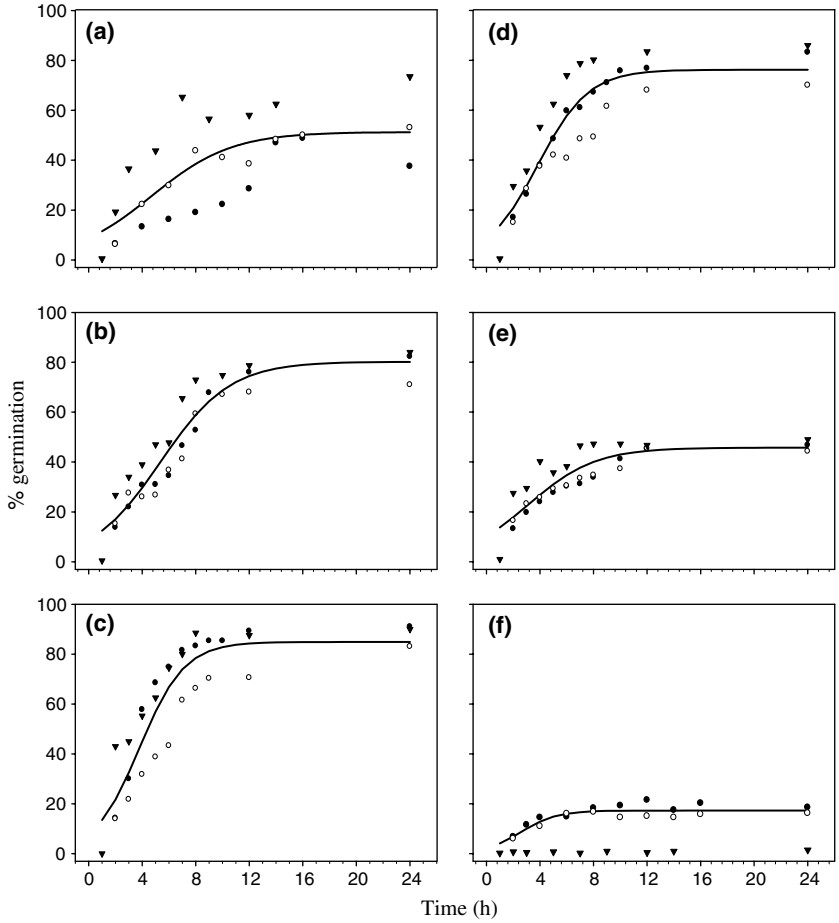

Figure 1. Germination dynamics of *Gymnosporangium asiaticum* in free water at (a) 5; (b) 10; (c) 15; (d) 20; (e) 25 and (f) 30 °C. Symbols represent the observed % germination of three replications. Solid line is the fitted logistic model with parameter values given in Table 5; at 30 °C, the logistic model described well the germination dynamic observed in two of the three replicate experiments

5°C to 23.6% at 30°C. Overall, increasing RH led to increased germination; average germination was 32.8%, 38.5% and 41.4% at RH values of 45%, 75% and 100%, respectively.

Logistic regression analysis showed that all main effects of the treatment factors (incubation duration, temperature and RH) and their pair-wise interactions on the survival of *G. asiaticum*

Table 6. Average percentage germination of basidiospores of *Gymnosporangium asiaticum* (based on 400 basidiospores) after incubation at various combinations of temperature and RH for 1, 3 or 6 days. After incubation, basidiospores were provided with free water at 20 °C for 24 h before germination was assessed

Dry duration (days)	Relative humidity (%)	Temperature (°C)						
		5	10	15	20	25	30	Mean
1	45	63.5	64.5	49.5	44.8	39.8	32.3	49.1
	75	79.8	66.3	59.5	56.5	52.0	47.8	60.3
	100	83.0	70.0	64.8	58.3	57.3	52.0	64.2
3	45	37.8	33.8	34.3	28.5	23.5	9.8	28.0
	75	45.8	37.8	36.5	29.8	24.8	17.0	32.0
	100	50.0	43.8	39.5	32.5	26.3	18.0	35.0
6	45	28.8	28.3	25.3	21.5	16.5	7.3	21.3
	75	30.3	30.8	26.0	21.8	18.0	12.5	23.2
	100	31.8	29.5	28.3	24.8	20.5	15.5	25.1
Mean		50.1	45.0	40.4	35.4	31.0	23.6	37.6

basidiospores were significant at the 1% level except the interaction between temperature and RH, which was significant at the 5% level. However, these interactions jointly accounted for little variation in the observed data, explaining $< 3.2\%$ of the total deviances. The main effects of incubation duration, temperature and RH accounted for 62.3%, 24.9% and 4.3% of the total deviance, respectively. The following non-linear model fitted well the observed germination data, accounting for 91.2% of the total variation in the data:

$$p = 28.07 - 1.026\text{Temp} + 0.159\text{RH} + 69.12 \times 0.522^{D} \quad (2)$$

where D was the duration of incubation (days). The standard errors of the corresponding parameter estimates were 1.72, 0.043, 0.017, 4.19 and 0.030, respectively. Thus the germination of treated spores decreased linearly with increasing temperature and with decreasing RH, and exponentially with increasing incubation duration.

Discussion

This is the first study aiming to understand key aspects of pear rust epidemiology. Results from these controlled environment experiments demonstrated that germination of teliospores and basidiospores of the pear rust fungi is greatly affected by temperature, free water and RH. Telial horns were stored for up to 40 days at 5 °C before being used in experiments. All experiments were repeated twice; telial horns varied considerably in the length of storage time between replicate experiments. However, the overall effect of environmental conditions on germination and mortality of teliospores and basidiospores appeared to be consistent over replicate experiments. Thus we conclude that the length of time of storing telial horns at 5 °C was unlikely to affect the main results reported in this paper.

Telial horns consist of pectin and teliospores; the pectin needs to be thoroughly wetted before teliospores can germinate to produce basidiospores. A soaking period as short as 30 sec is long enough to enable teliospores to germinate; 10–20 min of soaking in water enabled germination of teliospores in all telial horns. Teliospores can germinate and produce basidiospores at temperatures ranging from 5 to 28 °C with an optimum between 16 and 20 °C, similar to the results obtained by Lee (1990). The effect of RH on teliospore germination was small except at extremely low or high temperatures where germination occurred at 100% RH. The minimum time for telial horns to produce basidiospores was about 3 h at 16–20 °C. At extremely low or high temperatures, such as 8 and 28 °C, about 75% of telial horns failed to germinate and produce basidiospores, as indicated by the logistic models fitted to the data. This indicates that germination of teliospores was greatly inhibited at these extreme temperatures. The behaviour of teliospore germination in relation to temperature was very similar to that of apple cedar rust (*G. juniperi-virginianae*) (Pearson et al., 1977). Field studies on cedar apple rust showed that the concentration of basidiospores tended to be greater when average temperature was in the range 13–20 °C and that the length of this delay of basidiospore discharge from the start of rainfall appeared to be inversely related to temperature, suggesting that basidiospore production is inhibited at low temperature (Pearson et al., 1980). These observations can also be explained on the basis of the relationship of teliospore germination with temperature as quantified in this study. Therefore, we infer that the discharge and concentration of basidiospores of pear rust would follow the same pattern as observed for cedar apple rust (Pearson et al., 1980).

Basidiospores of *G. asiaticum* can germinate over a temperature range of 5–30 °C with an optimum around 15 °C. As for many other pathogens, e.g., pear scab (Li et al., 2003) and apple brown rot (Xu et al., 2001), the key requirement for basidiospores to germinate is the availability of free water. Germination even under 100% RH was only about 13% of that under free water. The low but consistent germination of 4–5% observed at 95%, 97% and 99% RH might be due to the way the basidiospore inoculum was made. It took about 20 min for an inoculum droplet to dry. During that interval most basidiospores may have already initiated germination processes and some of these spores may have already been in an advanced stage of their germination particularly because our results indicated that some teliospores will have already germinated to produce basidiospores with 3 h of soaking the telial horns. Hence, RH as high as 95% may be sufficient to enable these 'advanced' germinating spores to complete the germination process. However, further experiments are needed to determine whether these successfully germinated spores can continue to develop and infect pear tissues under these dry but near-saturated moisture conditions.

Logistic models indicated that most basidiospores (about 80%) failed to germinate at 30 °C and to a lesser extent at 5 and 25 °C. These results may suggest that basidiospores are experiencing mortality even in free water under extreme low or high temperatures. The greatest variability between replicate experiments was found at 5 and 30 °C. Less variance was accounted for by the fitted logistic model at 5 °C (53.1%) than at other temperatures except 30 °C. This lack of fit was not because the germination dynamics did not follow a logistic-model shape/pattern; rather it arose from the fact that there was considerable variation between the logistic models fitted to each individual replicate data set. The % variance explained increased to 89.4% when logistic models were fitted to individual replicate data sets. At 30 °C, no germination was observed in one replicate whereas about 18% germination was obtained in the other replicates. However, 30 °C is not likely to occur in spring when infection takes place; hence the results obtained at such a temperature are of academic interest only. The rate of germination as well as the maximum possible germination was greatest when temperature was around 15 °C.

The rate of basidiospore mortality during the dry period differed significantly among the six temperatures (5–30 °C) and three RH values (45%, 75% and 100%). Basidiospore mortality linearly increased with increasing temperature and decreasing RH. An increase of 1 °C led to an additional mortality of 1.0% and a decrease of 10% in RH led to an additional 1.6% mortality. Mortality increased exponentially with dry duration. Overall, basidiospores appeared to be tolerant of dry periods. For example, about 50% and 20% of the basidiospores remained viable even after 24 and 144 h respectively at an RH of 45%. Under field conditions in spring, given the fact that RH during the night is generally very high, at least 20% of discharged basidiospores may remain viable for a week. Under natural conditions, sunlight (especially UV) may also play an important role in affecting the survival of basidiospores, in addition to the effects of temperature and RH.

In China, spring temperature in April-May is usually between 10 and 25 °C when infection by *G. asiaticum* normally takes place. Thus, temperature is not likely to be a limiting factor for initial infection of pear tissues by rust basidiospores. The key limiting factor for telial horns to germinate and the initiation of infection by basidiospores is rainfall, followed by a sufficient duration of free water on pear tissue surfaces. Present results suggest that within 6 h from the start of rainfall about 10–20% of basidiospores will have germinated at temperatures between 10 and 24 °C: 3 h are needed for teliospores to produce basidiospores. Further research is needed to obtain the precise duration of free water required from germination to a successful penetration of host tissues in relation to temperature. Extrapolating from experience with cedar apple rust (Aldwinckle et al., 1980), we may reasonably assume that it may take another few hours for germinated basidiospores to penetrate the host tissues at these temperatures; hence considerable infections may have already taken place within 8–9 h from the start of rainfall. In addition to the information on precise infection requirements, further research is also needed to investigate whether dew is sufficient to trigger telial horns to produce basidiospores.

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