Effects of Environmental Factors on Teliospore Germination, Basidiospore Formation, and Infection of *Xanthium occidentale* by *Puccinia xanthii*

Louise Morin, John F. Brown, and Bruce A. Auld

Graduate student and associate professor, University of New England, Armidale, N.S.W. 2351, Australia, and principal research scientist, Agricultural Research and Veterinary Centre, Orange, N.S.W. 2800, Australia.

Portion of Ph.D. dissertation by the first author, the recipient of a Commonwealth Scholarship and Fellowship Plan award during the period in which these studies were made. The results reported in this paper arose from preliminary studies undertaken by M. A. Cholil.

We acknowledge H. Talbot and K. Radburn for their technical assistance and H. Nichols and A. Gilmour for statistical advice. This research was partially funded by a research grant from Sandoz Agro Ltd. (Switzerland) and the Australian Wool Corporation.

Accepted for publication 15 June 1992.

ABSTRACT

Morin, L., Brown, J. F., and Auld, B. A. 1992. Effects of environmental factors on teliospore germination, basidiospore production, and infection of *Xanthium occidentale* by *Puccinia xanthii*. Phytopathology 82:1443-1447.

The effects of temperature, dew, high relative humidity, and light regimes on teliospore germination, basidiospore production and germination, and infection of Noogoora burr (*Xanthium occidentale*) by *Puccinia xanthii* were examined. The optimum temperature for the germination of teliospores was between 20 and 30 C, and the optimum temperature for the production and germination of basidiospores was 20 C. The total number of basidiospores produced increased as the period under conditions of high relative humidity was increased. The highest levels of infection were observed at temperatures of 20 and 25 C. No infection occurred on plants not subjected to dew. A dew period of 2–3 h was sufficient to achieve a high level of infection. A period of darkness stimulated teliospore germination under high relative humidity. Light regimes preceded by a dark period had an inhibitory effect on the total number of basidiospores produced. Various light regimes had no effect on the germination of basidiospores and infection of plants.

Additional keywords: biological weed control, cocklebur.

The Noogoora burr (cocklebur) complex in Australia consists of four closely related *Xanthium* spp. (14): *X. occidentale* Bertol. (Noogoora burr), *X. italicum* Moretti (Hunter burr), *X. orientale* L. (California burr), and *X. cavanillesii* Schouw (South American burr). This complex contains important weeds of the temperate regions of the world (14,15,31). The microcyclic rust, *Puccinia xanthii* Schwein., has been suggested as a potential biological control agent for *Xanthium* spp. in Australia (13). This obligate parasite is widespread in North America and also occurs in Mexico, the West Indies, Japan, Europe (27), and India (16). This rust was first recorded in Australia in 1975, possibly as a result of accidental introduction (1).

P. xanthii causes severe symptoms in the form of telia. The production of telia causes distinct swellings on the abaxial surface of leaves and inside stems and petioles (13). Julien et al (17) demonstrated that *P. xanthii* infection reduced plant growth rate and productivity. They reported that the degree of infection and the rate of spread depended on climatic conditions. The effects of environment on the biology of *P. xanthii* are not well documented. The teliospores do not exhibit a dormant period and germinate while still attached to telia when exposed to high relative humidities (1,20,23). Hasan (13) briefly reported that teliospores germinated and infected the host at temperatures ranging between 10 and 30 C, with the optimum being 23 C. He also stated that initial infection occurred after 12 h of leaf wetness. In another study, teliospores germinated over a range of temperatures from 5 to 40 C (9), but the optimum temperature was 20 C (M. A. Cholil and J. F. Brown, *personal communication*).

A better description of the effect of environment on the early stages of *Xanthium* rust development should assist in understanding the progress and the seasonal variation of the disease. The objectives of this research were to examine the effects of temperature, light regimes, and dew period or high relative humidity on the germination of teliospores, production of basidiospores, germination of basidiospores in vitro and infection of Noogoora burr by *P. xanthii*.

MATERIALS AND METHODS

Plant production. Mature Noogoora burr fruits (*X. occidentale*), collected from plants harvested at Oxley Station, Warren, New South Wales, were soaked in 5% sodium hypochlorite for 30 min and rinsed in running tap water for 1 h. This surface sterilization procedure ensured that burrs were not infested with inoculum of any pathogens. Fruits were cut at the distal end to expose the tips of the seeds and were sown in a steam-sterilized soil-sand-peat mixture (1:3:1) in 10-cm-diameter plastic pots. Plants were grown in a temperature-controlled glasshouse at 25 ± 1 C fitted with an automatic watering system. Plants at the four- to five-leaf stage were used in all experiments unless stated otherwise.

Maintenance of the rust. Healthy plants were inoculated weekly with *P. xanthii* (isolate collected on 7 March 1990 near Darlington Point, New South Wales) to ensure a continuous supply of mature telia thoughout the experiments. To inoculate healthy plants, one heavily rust-infected plant was placed among four uninfected plants in a dark dew chamber (model I-60DL, Percival, Boone, Iowa) at 24 ± 1 C for 24 h. Plants were then transferred to the glasshouse for a week until telia started to erupt. Plants were transferred to a controlled-environment chamber (model E7H, Conviron, Winnipeg, Manitoba, Canada) set at 25 ± 1 C and 65% RH, with a 12-h photoperiod (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) to prevent germination of maturing teliospores in situ. Mature telia (3 wk after inoculation) were used in all experiments.

Teliospore germination and basidiospore production. Mature telia (8–10 mm in diameter) were cut into disks with a cork borer either 5 mm (teliospore germination experiments) or 3 mm (basidiospore production experiments) in diameter. Each disk was cut into eight equal sections and placed on the surface of 2% water agar in 9-cm-diameter petri dishes kept at room temperature (21 ± 1 C). Plates were placed in dark incubators set at 4, 10, 16,

20, 24, 28, 32, and 38 ± 1 C. Observations of teliospore germination and basidiospore production were made at 3, 6, 12, 18, 24, 36, and 48 h after telium sections were placed on the water agar. Plates containing the telium sections were also placed in a growth chamber (Sherer, model CEL-7H, Sydney, Australia) set at 20 ± 1 C and 55% RH, with a 24-h photoperiod (210 $\mu E \cdot m^{-2} \cdot s^{-1}$) with white (VHO, Coolwhite, Sylvania) and incandescent (25W, Osram, GEC, New Zealand) lights. The following light regimes were used: 6 h dark–18 h light; 12 h dark–12 h light; 24 h dark; 6 h light–18 h dark; 12 h light–12 h dark; and 24 h light. The dark period was provided by wrapping the plates in aluminium foil. Observations of teliospore germination and basidiospore production were made at 3, 6, 12, and 24 h after telium sections were placed on the agar surface. A completely randomized design with four replicates (sections from four different telia) was used in these factorial experiments. All experiments were performed twice.

Teliospores were killed and stained with lactophenol-cotton blue (29) after incubation. Each telium section was then transferred to a glass slide and placed in a drop of clear lactophenol. Teliospores were carefully separated from each telium section with fine needles under a dissecting microscope. Teliospores were considered to have germinated when the length of the germ tube was greater than the width of the teliospore cells. Several randomly selected fields of view were examined with a compound microscope (400×) until a total of 200 teliospores per replicate had been assessed.

For basidiospore production, one section of a telium was placed onto the center of a petri dish containing water agar. A glass slide to which a small block (approximately 3 × 1.5 × 0.3 cm) of 2% water agar had been placed was attached to the center of the inner side of a petri dish lid and held in position with adhesive tape. The plates were then inverted and incubated under the temperature or light conditions listed previously. This method is a modification of the one described by Bega (5). After incubation, a drop of lactophenol-cotton blue and a coverslip were placed on the agar block, and the number of basidiospores that had landed on the agar was determined. A new glass slide with water agar was placed in the petri dish lid immediately after the first one was removed. The total number of basidiospores produced by each telium section was estimated with a projection microscope (125×, Visopan, Reichert, Austria).

Basidiospore germination. Plates containing the telium section (2.45 mm^2) were placed in a dark incubator at 24 C for 6 h. A glass slide supporting a small block of water agar was placed on the center of the inner side of the petri dish lid, and the plate was inverted to allow the discharge of basidiospores onto the agar block for 30 min. The inoculated agar and the glass slides were then placed in empty, sterile petri dishes and placed in dark incubators set at 4, 10, 16, 20, 24, 28, 32, and 38 ± 1 C. Inoculated agar blocks were also placed in a growth cabinet (Sherer; conditions as previously stated) for 18-h light or darkness treatments. After incubation a drop of lactophenol-cotton blue was placed on the agar block to stop germination. The procedure used to assess basidiospore germination was similar to that described for teliospore germination. Ten representative germ tubes were measured in each of the four replicates. A completely randomized design was used, and the experiment was performed twice.

Controlled inoculation of plants. Fifteen to twenty disks, 9 mm in diameter, were cut from mature telia and placed onto the surface of 2% water agar contained in petri dishes. The plates were then placed in a dark incubator at 24 ± 1 C for 12 h to stimulate the production of basidiospores. After incubation, plates were gently flooded with distilled water to make a suspension of basidiospores. The basidiospore density was determined with the aid of a haemocytometer and adjusted to 1×10^5 basidiospores per milliliter. A 0.5-ml droplet of this suspension was placed on the middle of the upper surface of the third and fourth oldest leaves of *X. occidentale* and gently spread with a glass rod over an approximate area of 10 cm^2.

Inoculated plants were placed in a dark dew chamber set at 10, 20, 25, or 30 ± 1 C for 0, 2, 4, 6, 10, 12, or 18 h. Plants were then transferred to a walk-in controlled environment chamber (Conviron, model PGV 36) set at 25 ± 1 C and 55% RH, with a 12-h photoperiod (425 $\mu E \cdot m^{-2} \cdot s^{-1}$). The four dew chamber temperatures were randomly allocated to four consecutive days in each of 4 wk. A new population of plants was prepared for each week so that they were at the same leaf stage when inoculated. A new suspension of inoculum was prepared each day. A split-plot model in a randomized complete block design consisting of five replicates was used, with week as blocks, temperature as whole plots, and dew period as subplots. The total number of lesions that developed on both inoculated leaves were evaluated at 1 wk after inoculation.

To investigate the effect of light on infection, controlled inoculated plants were placed in plastic trays (33 × 48 cm) filled to a 5-cm depth with water. Trays were then covered with plastic bags (to provide dew and high relative humidity) and placed in a lighted growth chamber (Sherer; conditions as previously stated). The following light regimes were simulated by covering the trays with transparent or opaque plastic bags: 6 h dark–18 h light; 12 h dark–12 h light; 24 h dark; 6 h light–18 h dark; 12 h light–12 h dark; and 24 h light. A completely randomized design with six replicates was used. The experiment was performed three times. Post–dew period conditions and assessment of infection were similar to the previous experiment.

Natural inoculation of plants. Disks, 5 mm in diameter, were cut from mature telia and placed on the surface of 2% water agar in petri dishes (four disks per plate). Plates were then placed in a dark incubator at 24 ± 1 C for 6 h. After incubation, plates without a lid were inverted over the opening of small inoculation chambers for 1 h. Inoculation chambers were made by inverting 15-cm-diameter plastic pots, with a 6-cm-diameter area cut from their base, on other intact pots of similar size containing a 10-cm-diameter pot into which a healthy plant at the two-leaf stage was growing. The inside of the inoculation chambers and the surface of the plants were sprayed with a fine mist of water, except for the control (no dew) treatment, before inoculation. After the inoculation period, the chambers were dismantled and plants were placed in a dark dew chamber set at 23 ± 1 C for 2, 4, 6, 10, 14, 18, and 24 h. Plants were then transferred to the walk-in controlled environment chamber set at 20 ± 1 C and 55% RH, with a 12-h photoperiod (315 $\mu E \cdot m^{-2} \cdot s^{-1}$). The number of lesions that developed on the two first true leaves was evaluated at 5 days after inoculation. Counts were made for each leaf on two 1-cm^2 areas located over the midvein of the leaf, 1 and 2.5 cm from the petiole. A completely randomized design was used, and the experiment was performed twice.

Analysis of data. Data were subjected to a logarithmic transformation prior to an analysis of variance when a relationship was observed between the variances and the means of each treatment (28). Curvilinear functions were fitted to the data, where appropriate, by generating polynomial regression equations using the transformed data. A locally weighted, three-dimensional surface was fitted where appropriate, using the computer program SYSTAT (Evanston, IL). In this method, every patch of the surface is generated by four weighted multiple regressions of all the points. Results for the trials of each experiment were pooled when homogeneity of variances was detected with Barlett's test (28) or when no significant difference between trials was observed, or both. Differences between treatment means were assessed using Tukey's HSD test ($\alpha = 0.05$).

RESULTS

Teliospore germination and basidiospore production. Teliospore germination occurred over the complete range of temperatures tested (4–38 C). However, there were significant differences ($P < 0.001$) among the temperature treatments. The optimum temperature for teliospore germination was between 20 and 30 C after 24 h of incubation (Fig. 1). Simultaneous germination of both teliospore cells was sometimes observed. Low (<10 C) and high (38 C) temperatures slowed the germination process. At 28 C abnormal germination occurred, in which teliospores

produced very long germ tubes and metabasidia with long sterigmata bearing no basidiospores (data not shown). Teliospores germinated quickly (< 3 h) upon exposure to high relative humidity (approximately 100%) and suitable temperatures. Extended exposure to high relative humidities did not significantly affect germination rate (data not shown). Teliospores immersed in water germinated abnormally by producing a very long germ tube and no metabasidia (data not shown). The range of temperatures that enabled basidiospores to be produced by the germinating teliospores was relatively narrow. No basidiospores were produced at 4, 32, and 38 C. The production of basidiospores was significantly ($P < 0.01$) affected by temperature and was greatest at 20 C (Fig. 2). The total number of basidiospores produced increased significantly ($P < 0.01$) as the period under high relative humidity was extended (Fig. 2).

Continuous darkness and a period of 12 h of darkness at the beginning of the incubation period significantly stimulated teliospore germination in one trial (Table 1). Light regimes beginning with a dark period had a significant ($P < 0.001$) inhibitory effect on the total number of basidiospores produced after 24 h in one trial (Table 1). Orthogonal contrasts comparing light regimes beginning with a dark period versus regimes beginning with a light period were significant ($P < 0.01$) in these experiments on teliospore germination and basidiospore production. A similar trend was observed in the second trial of each experiment, but the difference between the light regime treatments was not statistically significant (Table 1).

Basidiospore germination. Basidiospores germinated directly by producing a thin germ tube at 3–6 h after landing on the agar. Germination was significantly ($P < 0.001$) affected by temperature (Fig. 1). The optimum temperature was between 16 and 28 C. Germ tube length was reduced at temperatures above and below 20 and 25 C (Fig. 1). Basidiospores germinating on the telial surface often produced secondary basidiospores (data not shown). Germination of basidiospores was similar under continuous light or darkness and reached a maximum of 91%.

Effects of environment on infection. The optimum dew period temperatures for infection were 20 and 25 C (Fig. 3). There was no and very low infection when controlled inoculated plants were subjected to 10 and 30 C, respectively. No significant difference ($P = 0.4$) in infection was detected between 20 and 25 C. The controlled inoculation method used generated variable results,

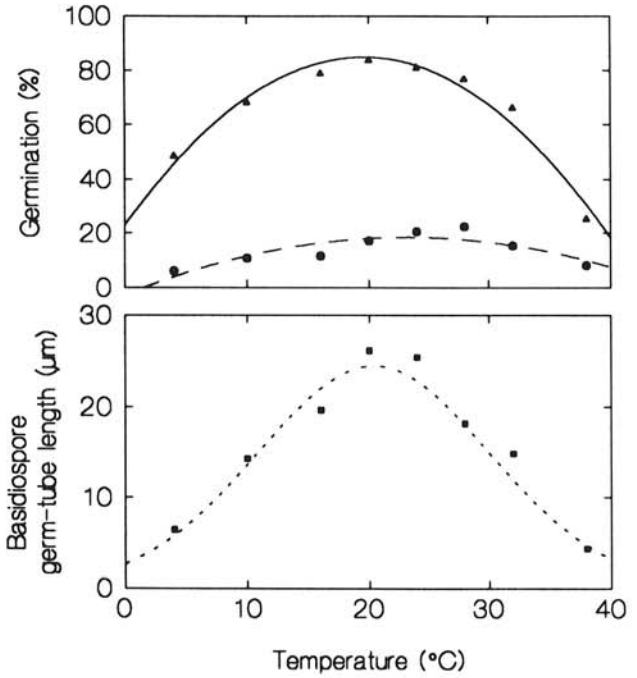

Fig. 1. Effect of temperature on germination of teliospores (– – –) and basidiospores (——) of *Puccinia xanthii* after 24 and 18 h, respectively, of exposure to high relative humidity. Results are from pooled trials. Data points represent means of four replicates. Regression equation for teliospore germination (– –) : $Y = -2.661 + 1.814x - 0.039x^2$; for basidiospore germination (——): $Y = 22.917 + 6.322x - 0.161x^2$; for length of basidiospore germ tubes (– – – –): $\log_e Y = 0.967 + 0.218x - 0.005x^2$, where Y = percentage of germination or length of germ tube and x = temperature.

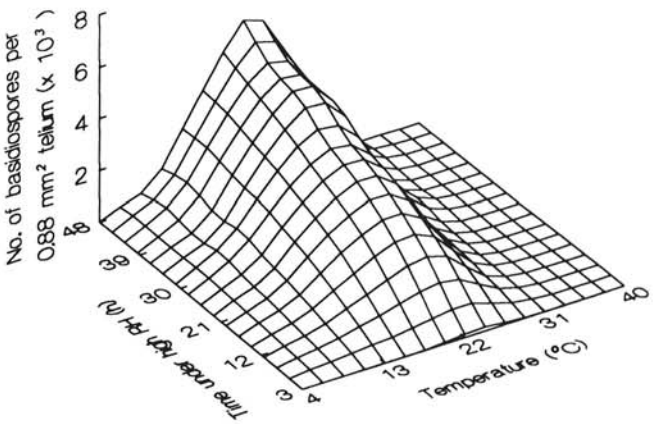

Fig. 2. Effect of temperature and duration of the period under high relative humidity on the production of basidiospores by telia of *Puccinia xanthii*. Results are from pooled trials. A locally weighted three-dimensional surface was fitted on the means of the cumulative data over time.

TABLE 1. Effect of light regimes on teliospore germination, basidiospore production and germination, and infection of *Xanthium occidentale* by *Puccinia xanthii*.

Light regimes[v] (h)	Teliospore germination[w,x,y] (%) Exposure to high relative humidity (h)				Basidiospores per 0.88-mm² telium[w,y] (× 10³) Trial		Infection[w,y] (lesions per 10-cm² leaf area) Trial		
	3	6	12	24	1	2[z]	1	2[z]	3
24D	33.50	35.25	40.25	43.00 a	1.20 a	0.23	2.00 ab	7.75	36.33 a
12D/12L	34.50	48.75	30.25	44.50 a	1.12 a	0.80	5.17 ab	15.00	14.08 ab
6D/18L	18.50	32.75	33.00	30.50 ab	3.55 a	0.98	5.92 a	16.00	17.50 ab
6L/18D	10.00	13.00	27.00	33.50 b	4.06 a	1.77	1.42 ab	25.75	9.83 ab
12L/12D	13.00	17.25	22.25	37.75 b	15.71 b	1.68	0.67 ab	13.08	8.08 b
24L	20.75	18.00	26.50	31.50 ab	11.11 b	6.77	0.17 b	22.33	33.58 a

[v] L = light period, D = dark period.
[w] Experiments were not combined, because variances were not homogeneous or a significant difference between trials was observed, or both.
[x] Results from one trial are presented. Similar trends, however, were observed for both trials. Since there was no significant ($P = 0.513$) interaction between light regimes and exposure to high relative humidity, comparison among light regimes was performed on the mean values averaged over all levels of exposure to high relative humidity.
[y] Light regime treatments associated with the same letter have means that are not significantly different ($P > 0.05$), according to Tukey's HSD test.
[z] No significant difference detected.

as indicated by the highly significant ($P < 0.001$) difference between weeks and the significant interactions between temperature or dew period and weeks for the 20 and 25 C treatments. Consequently, it was difficult in this experiment to describe with certainty the effect of dew period on infection, because of the large standard errors associated with the treatment means (Fig. 3). The natural inoculation method of plants was then designed to allow plants to be inoculated by the natural discharge of basidiospores, which was more representative of the field situation. No infection occurred on plants that were not misted with water during the inoculation period and not subjected to a dew period. There were no significant differences between all the other dew period treatments (2–24 h). For these treatments, the total mean number of lesions that developed per cm^2 leaf area had a 95% confidence interval of 16–21 and 33–45 for the first and second trials, respectively.

No consistent trend in lesion numbers was observed among the different light regimes to which the controlled inoculated plants were subjected (Table 1). In the experiments using the controlled inoculation method, a consistently higher (2–8 times) number of lesions developed on the fourth leaf compared with the third leaf when conditions were favorable for infection (data not shown).

DISCUSSION

P. xanthii is an autoecious, microcyclic rust without pycnia, aecia, and uredinia (27). The disease cycle is maintained by a continuous succession of telial generations in which basidiospores are the infective propagules. Individual teliospores are not easily harvested, because they are strongly attached to the telium by their pedicels (13). A large proportion of teliospores was consistently observed germinating in the telium. In contrast, teliospores in a suspension (made from maceration of telia embedded in leaf tissue) and spread over the surface of water agar showed low percentage of germination. Similar observations have been reported with *P. graminis* Pers.:Pers. (30) and *P. malvacearum* Bertero ex Mont. in Gay, the latter being a microcyclic rust (10). This phenomenon may have resulted from a stimulatory effect of the telia on germination of individual teliospores or from the impairment of germination caused by the physical damage inflicted to the teliospores during the maceration process. The method of germinating teliospores in situ that was used in this study is considered to reflect the natural situation, in which teliospores are not readily airborne and remain attached to the telia during the entire life cycle of the rust (1).

Heavy infection of plants with *P. xanthii* has been achieved by attaching diseased leaves to healthy plants (17) or by resting rusted leaf fragments onto leaves. Alcorn (2) reported that the latter method led to the development of many more pustules on plants than those that were sprayed with suspensions of teliospores and basidiospores in water. These findings, together with those obtained during preliminary experiments were taken into account when developing the inoculation techniques used in this study. The controlled inoculation of leaves with basidiospore suspensions allowed a direct quantitative evaluation of the effects of environmental factors on infection. The high variation observed between weeks when the experiment was performed using this method, however, made the latter method adequate only to detect major differences between treatments. Results obtained with the natural inoculation method were more representative of what happens in the field, since the method mimics the natural discharge of basidiospores on the leaves.

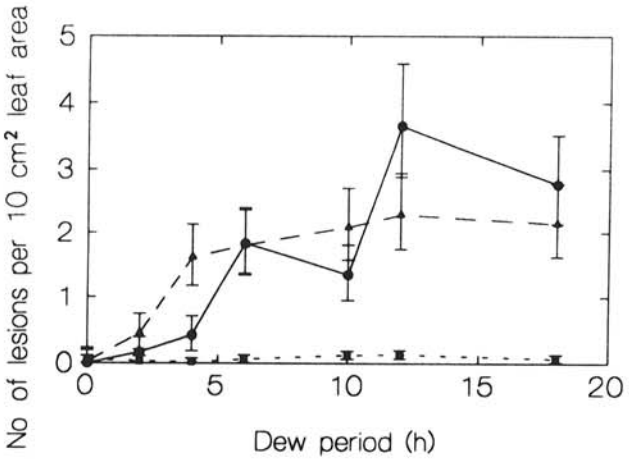

Fig. 3. Effect of dew period duration and temperature on infection of *Xanthium occidentale* by *Puccinia xanthii*. The blocks (weeks) are pooled for graphing purposes. Data points represent means of five replicates. Points for each temperature are connected with various lines: 20 C (——), 25 C (– –), and 30 C (– – – –). Bars indicate overall standard errors of each mean. No lesions developed when plants were incubated at 10 C.

Mature teliospores of *P. xanthii*, like *P. malvacearum* (3) and *P. heterospora* Berk. & M. A. Curtis (19), germinated without a rest period when conditions were favorable. Teliospore germination in each telium was not synchronized and was spread over several hours or days. This nonsynchronicity may be related to the order in which the teliospores were formed and reached maturity (22) and the conditions at the time of formation (21). Abnormal germination occurred when teliospores were immersed in water. The same phenomenon has also been observed with teliospores of *P. graminis, Phragmidium rubi* Wint., *Uromyces fabae* (Grev.) Fuckel (6), and *P. malvacearum* (3) and suggests that oxygen is required for normal germination of teliospores and subsequent production of basidiospores (22).

The range of temperatures under which teliospores of *P. xanthii* germinated was similar to the findings of Cholil (9) and much wider than that stated by Hasan (13) and those previously reported with teliospores of other Uredinales (10,22). Teliospore germination with abnormal metabasidia and practically no basidiospores was recorded at 28 C. A similar effect was observed with *Puccinia helianthi* Schwein. (22). However, as pointed out by Doran (10), teliospores may germinate over a wide range of temperatures, but normal germination leading to the production of basidiospores and subsequent host infection may occur only at a much narrower range of temperatures.

Teliospores of *P. xanthii* began to produce basidiospores after 3 h under conditions of high relative humidities and optimum temperatures. Similar observations have been reported for *P. malvacearum* (10). There was still a high production of basidiospores after 48 h of incubation, confirming the nonsynchronous pattern of germination of teliospores. The optimum temperature for production of basidiospores was found to be 20 C, which differs slightly from that reported by Hasan (13) and from that observed for *P. malvacearum*, which germinated best at 14 C (10). The optimum temperature for basidiospore production by *P. xanthii* corresponds to the temperature that stimulated the highest number of basidiospores to germinate on water agar and to the optimum temperature for infection of the host.

Indirect germination of basidiospores to produce secondary basidiospores often occurred on the surface of telia a few hours after spore formation but was never observed on the surface of water agar. Bega (5) considered this phenomenon to be a means of vegetative perpetuation, which may be significant in the dispersal and longevity of basidiospores.

Anikster (4) did not consider light, in general, as an influence on teliospore germination. Gold and Mendgen (11), however, referred to several reports in which teliospore germination of various rust species differed in the species' response to light regimes. Maneval (23) found that continuous darkness was less favorable than alternate light and darkness for the production of basidiospores of *P. xanthii*. The results reported in this paper agree with Maneval's findings but further indicate that maximum production of basidiospores occurred when telia were first exposed to light after being placed under conditions of high relative humidity. Anikster (4) also found that darkness depressed germination of teliospores of *P. graminis* f.sp. *avenae* Eriks. & E. Henn.

A short dew period (2–3 h) after inoculation was sufficient for the prepenetration development and penetration of Noogoora burr by *P. xanthii* when plants were inoculated by the natural discharge of basidiospores. On the other hand, a consistent low

number of lesions developed on controlled inoculated plants subjected to short dew periods (2 and 4 h). This observation indicates that basidiospores inoculated with the controlled inoculation method were possibly stressed following inoculation and consequently slower to germinate and penetrate plant tissue. The optimum dew period temperatures for infection were 20 and 25 C. Hasan (13) reported similar observations for the optimum temperature for infection but found that initial infection occurred only after a 12-h dew period. However, it is difficult to compare his results with those reported here, since his experimental methodology was not specified. In this study, *P. xanthii* did not appear to have an absolute requirement for dew to infect plants, since we have often observed new infections occurring in the glasshouse where rust-infected plants were kept and the relative humidity was high.

The artificial light regimes tested during the first 24 h after inoculation did not appear to affect the ability of basidiospores of *P. xanthii* to cause infection of Noogoora burr. Other authors have reported that the presence of light during the early stages of infection may stimulate (12,18), inhibit (7,12,21), or cause no difference in infection of plants by rust fungi (12,18). Hart and Forbes (12) related the different responses of rust fungi to darkness during the infection process to their ability to penetrate closed stomata. This statement does not apply to basidiospore-derived infections like those of *P. xanthii*, since the basidiospore germ tubes penetrate directly through the cuticle and epidermis (26).

Infection of controlled inoculated Noogoora burr plants by *P. xanthii* was always more severe on younger leaves. The cuticle thickness may have been responsible for this difference, since *P. xanthii* directly penetrates the host's cuticle. It has been demonstrated that an increase in the thickness of the cuticle from young to old leaves of *Berberis vulgaris* L. corresponded with increased resistance to infection by *P. graminis* resulting from basidiospore inoculation (24). However, Mendgen (25) commented that this study did not consider possible physiological differences in leaves of different ages.

It is essential to relate the experimental results describing the effects of environmental factors on the biology of *P. xanthii* to the possible behavior of epidemics on Noogoora burr under field conditions. In natural environments, basidiospores must be produced and released under conditions of high relative humidity and during dew formation or rain. It is possible that basidiospores may show a periodicity of release, as has been shown to occur in *P. malvacearum* (8), since more basidiospores were produced when telia were exposed to light during initiation of teliospore germination. High relative humidity environments or the presence of available water on healthy plant tissue combined with warm temperatures (20–25 C) should ensure infection. Providing that optimum conditions are present, this succession of events should happen anytime during a day, since the early phases of the infection process do not seem to be affected by light. Consequently, epidemics of *P. xanthii* on the Noogoora burr complex depend on environmental conditions that vary seasonally and geographically within the range of the host in Australia. Severe epidemics of *P. xanthii* have been observed in the northeastern regions of Australia, particularly in Queensland, where the summer dominant rainfall may provide adequate water and warm temperatures for the production of basidiospores and infection of plants by the fungus. The dependence of *P. xanthii* on favorable environmental conditions to cause epidemics on the *Xanthium* weeds is likely to limit its scope as a classical biocontrol agent. Field studies on the spread of the fungus are needed to add to our knowledge of the epidemiology of this rust.

LITERATURE CITED

1. Alcorn, J. L. 1975. Rust on *Xanthium pungens* in Australia. Australas. Plant Pathol. Soc. Newsl. 4:14-15.
2. Alcorn, J. L. 1976. Host range of *Puccinia xanthii*. Trans. Br. Mycol. Soc. 66:365-367.
3. Allen, R. F. 1933. A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum*. Phytopathology 23:572-586.
4. Anikster, Y. 1986. Teliospore germination in some rust fungi. Phytopathology 76:1026-1030.
5. Bega, R. V. 1960. The effect of environment on germination of sporidia in *Cronartium ribicola*. Phytopathology 50:61-69.
6. Blackman, V. H. 1903. On the conditions of teleutospore germination and of sporidia formation in the Uredineae. New Phytol. 2:10-14.
7. Burrage, S. W. 1970. Environmental factors influencing the infection of wheat by *Puccinia graminis*. Ann. Appl. Biol. 66:429-440.
8. Carter, M. V., and Banyer, R. J. 1964. Periodicity of basidiospore release in *Puccinia malvacearum*. Aust. J. Biol. Sci. 17:801-802.
9. Cholil, M. A. 1984. Studies on *Puccinia xanthii*, a rust fungus on Noogoora burr (*Xanthium strumarium* L.). B.Sc. Honors thesis, Department of Botany, University of New England, Armidale, NSW, Australia.
10. Doran, W. L. 1919. The minimum, optimum, and maximum temperatures of spore germination in some Uredinales. Phytopathology 9:391-402.
11. Gold, R. E., and Mendgen, K. 1983. Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus*. II. Light and host volatiles. Phytopathol. Z. 108:281-293.
12. Hart, H., and Forbes, I. L. 1935. The effect of light on the initiation of rust infection. Phytopathology 25:715-725.
13. Hasan, S. 1974. *Xanthium* rust as a possible biological control agent of Bathurst and Noogoora burrs in Australia. Pages 137-140 in: Proc. Int. Symp. Biol. Control Weeds, 3rd. A. J. Wapshere, ed. CAB, Farnham Royal, Slough, England.
14. Hocking, P. J., and Liddle, M. J. 1986. The biology of Australian weeds. 15. *Xanthium occidentale* Bertol. complex and *Xanthium spinosum* L. J. Aust. Inst. Agric. Sci. 52:191-221.
15. Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu, Hawaii. 609 pp.
16. Jadhav, A. N., and Somani, R. B. 1978. *Puccinia xanthii* Schw.—A first report from India. Curr. Sci. 23:905.
17. Julien, M. H., Broadbent, J. E., and, Matthews, N. C. 1979. Effects of *Puccinia xanthii* on *Xanthium strumarium* [Compositae]. Entomophaga 24:29-34.
18. Kochman, J. K., and Brown, J. F. 1976. Host and environmental effects on the penetration of oats by *Puccinia graminis avenae* and *Puccinia coronata avenae*. Ann. Appl. Biol. 82:251-258.
19. Kotwal, S. N. 1970. The mode of germination of teliospores of *Puccinia heterospora*. Curr. Sci. 12:285-286.
20. Kuhnholtz-Lordat, M. 1942. *Puccinia xanthii* Schw. Bull. Trimest. Soc. Mycol. Fr. 58:192-198.
21. Lambert, E. B. 1929. The relation of weather to the development of stem rust in the Mississippi Valley. Phytopathology 19:1-71.
22. Maneval, W. E. 1922. Germination of teliospores of rusts at Columbia, Missouri. Phytopathology 12:471-488.
23. Maneval, W. E. 1927. Further germination tests with teliospores of rusts. Phytopathology 17:491-498.
24. Melander, L. W., and Craigie, J. H. 1927. Nature of resistance of *Berberis* spp. to *Puccinia graminis*. Phytopathology 17:95-114.
25. Mendgen, K. 1984. Development and physiology of teliospores. Pages 375-398 in: The Cereal Rusts. Vol. 1. W. R. Bushnell and A. P. Roelfs, eds. Academic Press, Orlando. 546 pp.
26. Morin, L., Brown, J., and Auld, B.A. Teliospore germination, basidiospore formation and the infection process of *Puccinia xanthii* on *Xanthium occidentale*. Mycol. Res. 96:661-669.
27. Parmelee, J. A. 1969. The autoecious species of *Puccinia* on Heliantheae ['Ambrosiaceae'] in North America. Can. J. Bot. 47:1391-1402.
28. Steel, R. G. D., and Torrie, H. T. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York. 633 pp.
29. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess, Minneapolis. 239 pp.
30. Waterhouse, W. L. 1921. Studies in the physiology of parasitism. VII. Infection of *Berberis vulgaris* by sporidia of *Puccinia graminis*. Ann. Bot. 35:551-564.
31. Weaver, S. E., and Lechowicz, M. J. 1983. The biology of Canadian Weeds. 56. *Xanthium strumarium* L. Can. J. Plant Sci. 63:211-225.