

Environmental Influences on the Establishment of *Puccinia recondita* Infection Structures

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ABSTRACT

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Optimum temperature for completion of the infection process (as measured by the development of infection structures: appressoria, substomatal vesicles, and infection hyphae with at least one haustorium) by urediniospores of *Puccinia recondita* was 16 C. At 16 C, 39% of the urediniospores germinated within approximately 1 hr after inoculation, 35% of the germinated spores formed appressoria in approximately 3 hr, 50% of the appressoria formed substomatal vesicles within approximately 8 hr, and 50% of the substomatal vesicles formed infection hyphae with at least one haustorium in approximately 12 hr. Infection structures developed much slower at temperatures above or below 16 C. The minimum length of dew period for trace infection (one uredinium per leaf) was 3 hr at 16 C and 4 hr at temperatures above or below 16 C. Penetration and infection (development of substomatal vesicles and infection hyphae with haustoria) increased with increasing dew period. Light intensity provided by 40W cool-white VHO fluorescent lights reduced spore germination during the first 3 hr of incubation on both water agar and leaf surfaces but failed to inhibit germination completely. As a result, rate of development of infection structures was slower in the light than in the dark.

Additional key words: modeling, wheat, wheat leaf rust

Establishment of infections after inoculation of wheat (*Triticum aestivum* L.) plants with urediniospores of *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* depends on the environmental conditions during the infection process. Defining the influence of exogenous factors on the germination of urediniospores and subsequent development of infection structures is essential to our understanding of wheat leaf rust epiphytotics. Temperature, duration of surface wetness, and light all may have a pronounced influence on the development of infection structures.

Clifford and Harris (2) found that the amount and rate of germination and appressorium formation were highest over a temperature range of 15–20 C,

with an optimum at 15 C. Penetration was delayed and reduced at 10 C and negligible below 10 C and above 20 C. Wiese and Ravenscroft (8) reported that temperatures above 30 C were especially detrimental to both germinability and infectivity of *P. recondita*. Delay or inhibition of germination of *P. recondita* urediniospores by light has been reported by several workers (1,3). However, Stock (7) detected no depression of urediniospore germination by light. Data of Wiese and Ravenscroft (8) indicated that light did not reduce germination of *P. recondita* urediniospores in the temperature range of 15–20 C. Infectivity of urediniospores was reduced at temperatures above 15 C in their studies.

The objectives of the present study were to determine the influence of temperature, light, and duration of free moisture on urediniospore germination, formation of appressoria and substomatal vesicles, and subsequent development of uredinia in a compatible *P. recondita*/*T. aestivum* combination.

MATERIALS AND METHODS

Seedling wheat plants (12–15 per pot) for use in four replications of studies of effects of temperature, dew period, and light on development of infection structures were grown and inoculated in the following manner: Wheat cultivar Trison was planted in 5.5-cm-diameter pots and grown for 2 wk in the greenhouse at 21 ± 3 C to the two-leaf stage. On the evening before inoculation,

all plants were placed in dew chambers to accrue abundant dew on the leaf surfaces by the time of inoculation. Fresh *P. recondita* urediniospores (ATCC PR76), grown on Trison at 20 ± 1 C in an environmental chamber, were collected 3–4 hr before inoculation and kept in an open vial in the laboratory at 21 ± 2 C until used. Seedlings with fully developed primary leaves were inoculated by dusting with a 1:10 urediniospore:talc mixture that resulted in $67 \pm 6/\text{cm}^2$ urediniospores being deposited on a primary leaf in a 12-in.-diameter settling tower.

Effects of temperature on development of infection structures. Sixty pots of the wet seedlings were inoculated for each replication and placed in dew chambers set at 12, 14, 16, 18, or 20 C. Starting 1 hr after inoculation and at 1-hr intervals for the first 8 hr and 2-hr intervals for the next 8 hr, a pot was removed from each dew chamber.

Starting 3 hr after inoculation, one randomly selected inoculated primary leaf was excised from the plants of each pot removed from the dew chambers and placed in fixative, stained in lactophenol-cotton blue, and cleared in chloral hydrate after the method of Shipton and Brown (6). Starting at the leaf base and moving toward the tip, at least 100 urediniospores were counted to provide percentage germination, percentage of germinated spores that produced appressoria, percentage of those with appressoria that produced substomatal vesicles, and percentage of substomatal vesicles that produced infection hyphae with haustorium mother cells. Remaining leaves in the pots removed from the dew chambers for each time interval were air-dried with an electric fan to prevent further germination and appressorium development.

Three pots were placed in a growth chamber in the dark at the same temperature as the dew chamber in which they were incubated. Plants were kept there for the remainder of the 16-hr period, when all plants were removed to growth chambers at 24 C with 14-hr photoperiods. The number of uredinia observed 10–14 days after inoculation was counted and recorded.

Effects of light and length of dew period on development of infection structures. Six pots for each of four

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replications were inoculated as described above, then three pots were placed in a dark compartment and three in a lighted compartment of the same growth chamber at 16 C. Light intensity was

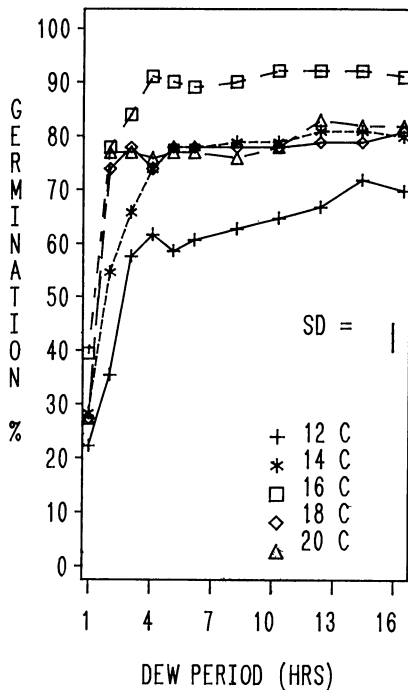


Fig. 1. Percentage germination of *Puccinia recondita* urediniospores (ATCC PR76) at 12, 14, 16, 18, and 20 C under various periods of dew. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

provided by three 40W cool-white VHO fluorescent tube lamps situated approximately 25 cm from plants on the dew chamber shelves. Water agar on 25 x 75 mm microscope slides was inoculated at the same time as plants and placed in both the dark and the light compartments of the dew chamber as controls for germination.

At 2-hr intervals over a period of 12 hr, one water-agar slide and eight leaves were sampled. Four of the leaves were used to prepare leaf surface replicas for comparing spore germination on leaves and water agar. Replicas were made by spreading a thin layer of Duco cement over the leaf surface, allowing the cement to dry for approximately 10 min, then peeling the cement from the leaf and mounting it in lactophenol-cotton blue. Entrapped spores, germ tubes, and appressoria, along with impressions of the epidermal cells of the leaf, were removed with the cement. The other four leaves were fixed, stained in lactophenol-cotton blue, and cleared in chloral hydrate (6) for microscopic examination to determine comparative numbers of infection structures.

Starting near the leaf base and moving toward the tip, urediniospores and fungal structures were counted to provide percentage germination, percentage of germinated spores that formed appressoria, and percentage of those with appressoria that formed substomatal vesicles. Data were analyzed for each

temperature vs. stage of fungal development.

RESULTS

Effects of temperature on development of infection structures. Urediniospore germination reached 39% within 1 hr after inoculation and 80-90% within 3 hr at 16 C (Fig. 1). Germination was slower at temperatures either higher or lower than 16 C. Germination at each temperature remained relatively constant with 4-16 hr of free moisture. A higher percentage formation of appressoria, substomatal vesicles, and infection hyphae occurred at 16 C (Figs. 2, 3, and 4). At 16 C, 35% of germinated spores formed appressoria within 3 hr and 80% within 7 hr (Fig. 2), whereas at 14, 18, and 20 C the time required for 50% of germinating spores to form appressoria was 5-6 hr and at 12 C, 14 hr (Fig. 2).

Substomatal vesicles were formed by 50% of the germinated spores with appressoria within 8 hr after inoculation at 16 C (Fig. 3). The 80% level was reached within 12 hr. However, at 3 hr after inoculation, when 50% of the germinated spores had formed appressoria, 10% of those also had formed substomatal vesicles. At 14, 18, and 20 C, development of substomatal vesicles was much slower. At 12 C, less than 50% of urediniospores with appressoria had formed substomatal vesicles within the 16-hr limit of the experiment (Fig. 3).

Infection hyphae with their first

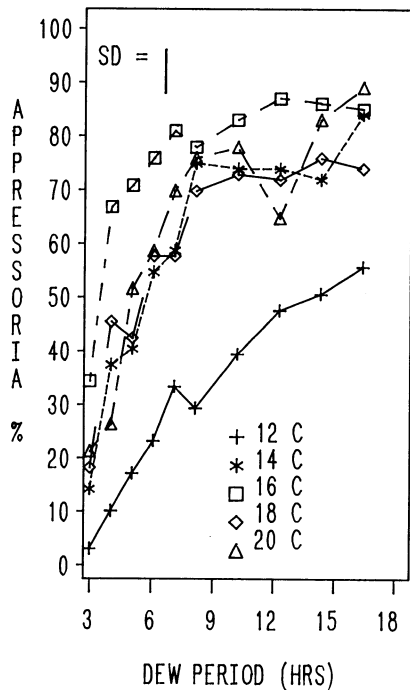


Fig. 2. Appressorium formation as a percentage of germinating *Puccinia recondita* urediniospores (ATCC PR76) over a range of temperatures and various dew periods in the dark. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

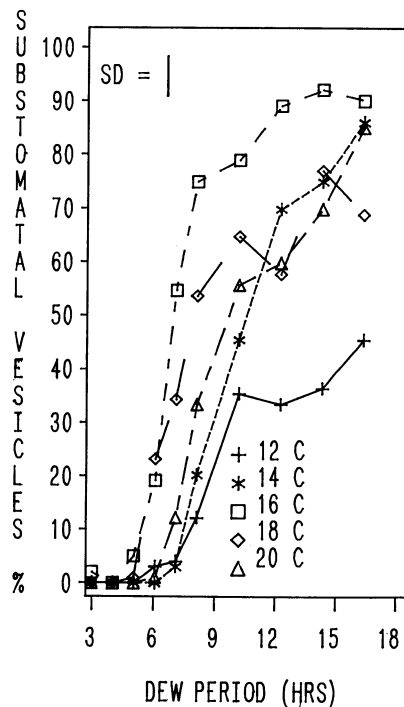


Fig. 3. Substomatal vesicle formation as a percentage of germinating *Puccinia recondita* urediniospores (ATCC PR76) that formed appressoria over a range of temperatures and various dew periods in the dark. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

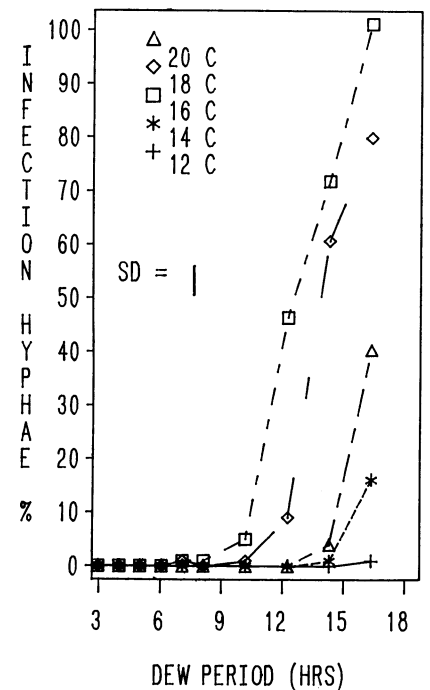


Fig. 4. Infection hyphae formation as a percentage of substomatal vesicles formed over a range of temperatures and various dew periods in the dark. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

haustorium mother cells were formed on 50% of the substomatal vesicles within approximately 12 hr after inoculation and first appeared approximately 8 hr after inoculation at 16 C and 10 hr at 18 C (Fig. 4). The lower and higher temperatures delayed initiation of the formation of infection hyphae to 13–14 hr after inoculation. At 12 C, less than 1% of the substomatal vesicles had formed infection hyphae by the end of the 16-hr experimental period (Fig. 4).

The number of uredinia per leaf that appeared 6 days after inoculation increased with increased dew period (Fig. 5). A trace infection (one uredinium per leaf) was observed after a 3-hr dew period at the 16-C optimum temperature. The increase in uredinium formation with duration of dew was greater at 16 C than at 12 C. With a 16-hr dew period, 5.7 times more uredinia were formed per leaf at 16 C than at 12 C (Fig. 5).

Effects of light and length of dew period on development of infection structures. Urediniospore germination reached 80–90% within 3 hr after inoculation with all combinations of light/dark and agar/leaf-surface treatments at 16 C (Fig. 6). During the first 3 hr, germination was slightly higher on water agar incubated in either light or dark than on the surface of wheat leaves. After 7 hr, however, the level of germination was higher on leaf surfaces with light than with any other treatment combination (Fig. 6).

In both light and dark at 16 C,

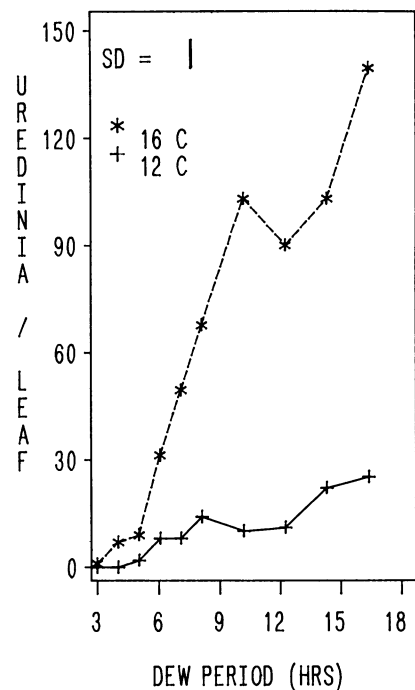


Fig. 5. Uredinia formation of *Puccinia recondita* (ATCC PR76) at 16 and 12 C under various dew periods in the dark. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

appressorium development was initiated within 2 hr after inoculation (Fig. 7). The rate of increase was slightly greater in the dark than in the light, however. In the dark, 50% of the germinated spores

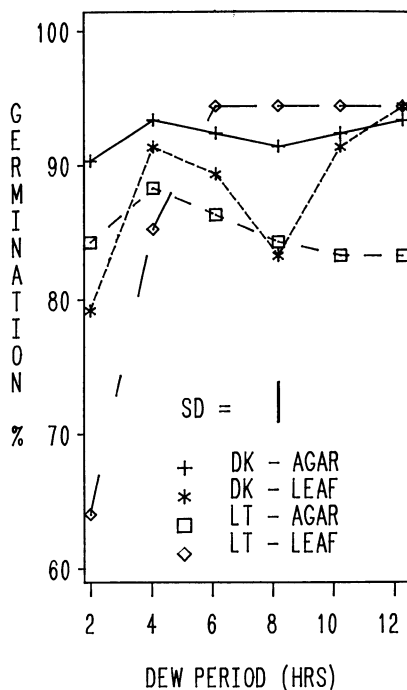


Fig. 6. Percentage germination of *Puccinia recondita* urediniospores (ATCC PR76) on water agar and leaf surface at 16 C under various dew periods in the dark (DK) and in the light (LT). Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

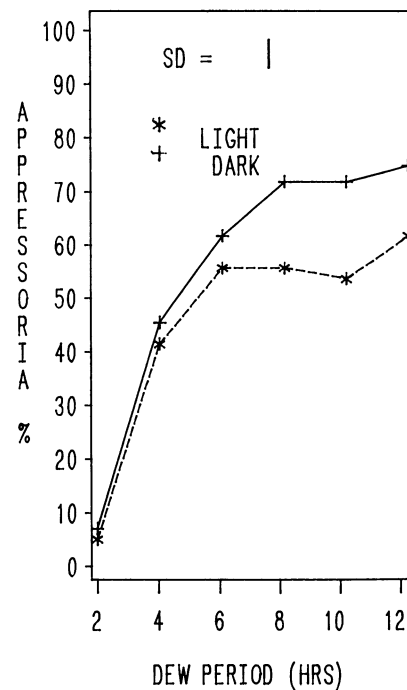


Fig. 7. Appressorium formation as a percentage of germinating *Puccinia recondita* urediniospores (ATCC PR76) at 16 C in various dew periods in the dark and in the light. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

formed appressoria in approximately 5 hr, whereas approximately 6 hr were required to reach that level in the light.

Similar trends occurred in the formation of substomatal vesicles (Fig. 8) and

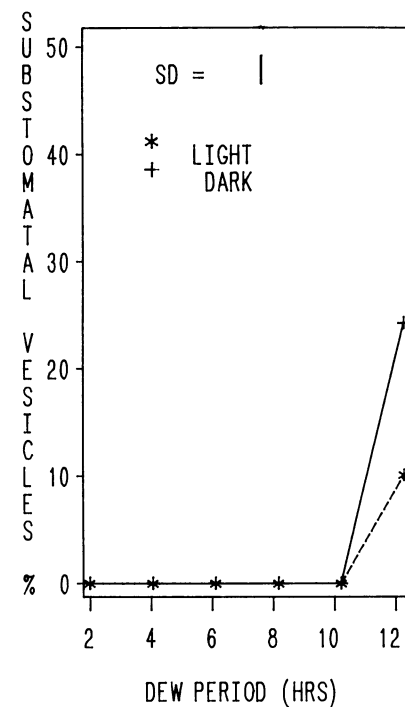


Fig. 8. Substomatal vesicle formation as a percentage of germinating *Puccinia recondita* urediniospores (ATCC PR76) at 16 C in various dew periods in the dark and in the light. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

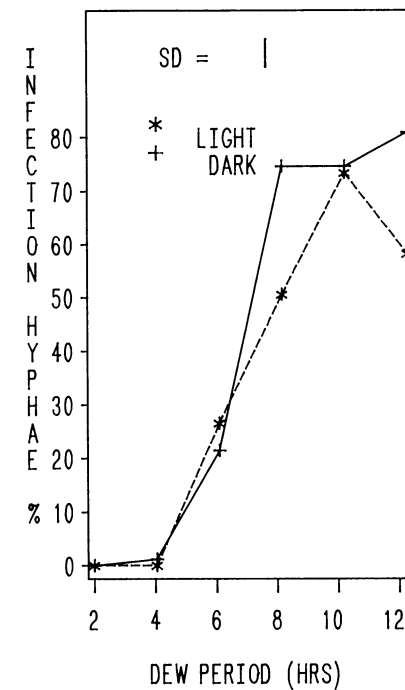


Fig. 9. Infection hyphae formation as a percentage of germinating *Puccinia recondita* urediniospores (ATCC PR76) at 16 C in various dew periods in the dark and in the light. Vertical bar (SD) represents value for comparison of means ($P \leq 0.05$).

infection hyphae (Fig. 9). In the dark at 16 C, 50% of the substomatal vesicles were formed approximately 8 hr after inoculation, whereas in the light that level was reached at 10 hr (Fig. 8). Likewise, development of infection hyphae began earlier and progressed faster in the dark than in the light (Fig. 9).

DISCUSSION

Two of the primary environmental factors that influence urediniospore germination and the development of infection structures in *P. recondita* are temperature and free moisture, usually in the form of dew on the leaf surface. Although germination and the development of appressoria occurred over a wide temperature range, the optimum was 16 C. Rates of development were somewhat lower, but similar, at 14, 18, and 20 C and notably lower at 12 C. Clifford and Harris (2) obtained similar results, indicating that 15 C was the optimum temperature for germination and appressorium formation. However, they reported a slightly higher optimum temperature range for penetration as measured by "ghost" appressoria. In contrast, the present study suggests a single optimum temperature (16 C) for the complete infection cycle. At all temperatures studied between 12 and 20 C, germination and appressorium formation increased with length of dew period. Free moisture was necessary for both spore germination and appressorium formation but was not necessary for formation of substomatal vesicles and infection hyphae. Appressoria remained viable and capable of penetration

through stomatal openings to form substomatal vesicles, even after leaf surfaces had dried. The longer the dew period and the closer the temperatures are to the optimum (16 C), the greater the percentage of deposited urediniospores that complete the infection process. Trace amounts of infection (one uredinium per leaf) were observed with a minimum of 3 hr of free moisture at 16 C and 4 hr at 12, 14, 18, or 20 C.

Chang et al (1) have reported that light inhibits urediniospore germination and that the degree of inhibition is proportional to light intensity. Although we were unable to cause complete inhibition of germination with light, germination during the initial 3 hr after inoculation was reduced, as also reported by Givan and Bromfield (4) and Chang et al (1) for hydrated urediniospores of *P. recondita*.

Knights and Lucas (5) also reported a significant difference in germination of *P. graminis* when incubated in light and dark on water agar and leaf surfaces. They found that spores germinated more rapidly on agar in either light or dark than they did on leaf surfaces. This apparently was due to more efficient water absorption from the agar surface than from the leaf surface, where coverage may not be complete. On both surfaces, however, light caused a slight reduction in germination. Givan and Bromfield (4) and Chang et al (1) suggested that hydration initiates a series of reactions that temporarily render spores light-sensitive.

Under field conditions, the effect of light on urediniospore germination is negligible, since high light intensity and

leaf surface wetness usually do not coincide. Thus, leaf wetness and temperature are the limiting factors under field conditions. In the field, all sources of leaf wetness (dew, rain, and fog) must be considered, since they may serve equally to provide the free moisture required for germination and appressorium formation. Epidemiologically, the source of moisture is important in that temperatures favorable for dew during a clear, calm night are likely to be lower than on a cloudy night. Leaf temperatures well below the ambient air temperature may not be optimum for spore germination and infection.

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