Effect of Temperature, Light, and Dew Duration on Relative Numbers of Infection Structures of Puccinia coronata avenae

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ABSTRACT

In a cam-programmable dew-deposition environment chamber, essentially all germination by *Puccinia coronata avenae* uredospores occurred on oat leaves within the first 2 hours of a 21.0 C dew period. A few appressoria formed within 2 hours, but no penetration occurred until after 4 hours of dew. Five hours of dew at 21.0 C was the practical minimum for infection. After 16 hours of dew at 21.0 C, there was no further increase in numbers of germ tubes, appressoria, penetration tubes, or pustules. Temperatures of 15.5, 21.0, and 26.5 C favored germination equally, but 10 C was less favorable. Once germination occurred, appressoria formed equally well from 10.0 - 26.5 C. Optimum dew period temperatures for penetration and pustule formation were 15.5 and 21.0 C. An initial 12-hour dew period at 21.0 C followed by 4 more hours of dew at 21.0 C resulted in the same number of pustules as one followed by 4 hours of dew at 26.5 C, and more pustules than one followed by 4 hours of dew at 17.0 C. A variable temperature regime that simulated natural night-time dew conditions resulted in fewer pustules than constant 15.5 or 21.0 C dew periods. Light (8,600 lux) near the end of the dew period did not enhance penetration or pustule formation by *P. coronata,* but it did for *P. graminis tritici* and *P. graminis avenae*.

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The relation of climate to plant disease development frequently is based on meteorological records that may not adequately represent the climate near the plant. Yet the microclimate may be all important in the establishment of a pathogen and in its subsequent build-up and spread (15). Further, each pathogen, and sometimes each stage of each pathogen, may have its own climatic requirements for development. For the wheat stem rust fungus, for example, the optimum conditions for spore germination and appressorium formation are not optimum for substomatal vesicle formation (2, 11, 13, 16, 17). We undertook this study to obtain information on climatic requirements, under precisely controlled conditions, for optimum development of infection structures of the oat (*Avena sativa* L.) crown rust fungus (*Puccinia coronata* Cda. var. *avenae* Fraser & Led.). The results should be helpful in understanding crown rust epidemiology. An abstract has been published (9).

MATERIALS AND METHODS.—*Collection and storage of spores.*—To assure a supply of uniform inoculum, we increased uredospores of a culture (that was collected in 1957 and that since has been purified and increased repeatedly in the greenhouse) of *P. coronata* race 290 on adult oat plants of the cultivar Markton in a 26 C growth chamber, collected them 8, 10, and 12 days after inoculation by shaking them onto aluminum foil, mixed and screened them through a 149-μm (100-mesh) screen, sealed them in 5-mm glass vials, and stored them in a liquid nitrogen cryostat (6). Also, we used uredospores of *P. graminis tritici* race 15B-2 and of *P. graminis avenae* race 8.

Host.—For oat rust experiments, we planted six 10-cm

diameter pots with the susceptible oat cultivar Markton. We planted 15 seeds per pot in a circle with the embryo end down and the groove toward the pot center so that leaves would grow with the abaxial side out. We grew plants in a growth chamber at an 18-21: 13-16 C day (14 hours):night regime. After nonuniform seedlings had been removed, the four pots with the most uniform plants were inoculated one week after planting. In experiments involving wheat stem rust, we used the susceptible wheat cultivar Little Club.

Inoculation.—For each experiment, we removed a vial of uredospores from liquid nitrogen and thawed them in a 45 C water bath (5). *P. coronata* and *P. graminis avenae* uredospores were used at 1.5 mg of spores per milliliter of Mobilsol 100, a nonphytotoxic isoparaffinic oil (10). For *P. graminis tritici,* we used 3 mg of spores/ml of oil. The seedlings were inoculated quantitatively using an aliquot inoculator attached to the side of a spore-settling turntable-tower (J. A. Browning, M. D. Simons, and G. D. Booth, *unpublished*). This device consisted of a mechanism for continuous agitation of spores in oil, an atomizer, and a timer to allow delivery of an aliquot of inoculum in a desired amount of time. The plants revolved at 20 rpm 60 cm from the atomizer that was set to spray for 9 seconds. When the oil had evaporated, the plants were placed dry in the dew chamber.

Incubation.—A cam-programmable, dew-deposition environment chamber of new design (3) that maintains temperature within ± 0.5 C was used in all experiments for the early stages of the infection process. In most experiments, constant dew-period temperatures were used. Plants were allowed to dry in the dew chamber by subjecting them to a drying cycle which was initiated by draining water from the bottom pan, increasing air temperature gradually to approximately 28 C, having a light intensity of approximately 1,600 lux (150 ft-c), and moving air through the chamber with an exhaust fan. In a few experiments, the wall temperature was cam-programmed to vary continuously from approximately 19 C at 1600 hours when the cycle started, dropping gradually to 13.5 C at 0500 hours when incandescent lights came on and water drained from the bottom pan to start the drying cycle. At 0700 hours the fluorescent lights, air heater, and exhaust fan came on. The air temperature rose gradually from 13.5 C at 0500 hours to approximately 25 C at 1000 hours when the cycle ended. Plants were not placed in the chamber until the bottom pan had filled with water and the chamber had equilibrated to the desired temperature. After the desired experimental treatment, plants were removed to a 26 C growth chamber (14-hour day) until pustules formed.

Microscopic examination and pustule counts.—When plants were removed from the dew chamber, we took one leaf at random from each of four pots and taped it to a slide, abaxial side up, for microscopic examination. Using an artists's airbrush, we then sprayed the leaves with a mixture (1) that stained the cytoplasm of the fungus red and the cell walls blue. We counted spores, germ tubes, appressoria, and penetrations on 0.2×3.8 cm of leaf surface at ×110. If an appressorium was full, its content was red. If an appressorium had emptied, all that could be seen was the blue-stained wall of the appressorium, which meant penetration had taken place. Counts for each leaf were recorded separately. The next day we repeated this procedure for another leaf. About 1 week after inoculation, we counted pustules on five leaves from each of the four pots. Averages were obtained for each pot.

Statistical analysis.—Because of uniformity of inoculum, plants, inoculation, and environmental conditions, trials on different days were regarded as different treatments of the same experiment. Analysis of covariance was used for the germ tube, appressorium, and penetration counts, and the adjusted means were obtained. Spores, germ tubes, and appressoria were the covariates for germ tubes, appressoria, and penetrations, respectively. Each pot was considered a replication. Counts within pots were designated readings. Analysis of variance was used for pustule counts. The average for each pot was used in the analysis, each pot being considered a replication.

RESULTS.—All experiments are with crown rust unless stated otherwise. Germination of uredospores of *P. coronata, P. graminis tritici,* and *P. graminis avenae* on water agar was 90% or more at room temperature.

Development of infection structures at intervals during the dew period.—During a 21.0 C dew period, we removed four leaves, one from each pot, at 2-hour intervals for 12 hours and examined them microscopically. Nearly all spores (90%) had germinated within 2 hours. A few appressoria had formed at this time, but the number increased steadily during the next 10 hours. No penetration occurred within the first 4 hours of dew, but after 12 hours, 74% of the germ tubes had formed appressoria and 63% of the appressoria had formed penetration tubes.

Length of the dew period.—We studied the minimum length of a 21.0 C dew period required for pustule formation by inoculating 40 pots of oats and placing them in the dew chamber. Four pots were removed at 30-minute intervals from 2.0-6.5 hours and the plants dried immediately with an electric fan and taken to a growth chamber. Four hours of dew at 21.0 C was minimal for trace infection (one pustule per leaf), and the number of

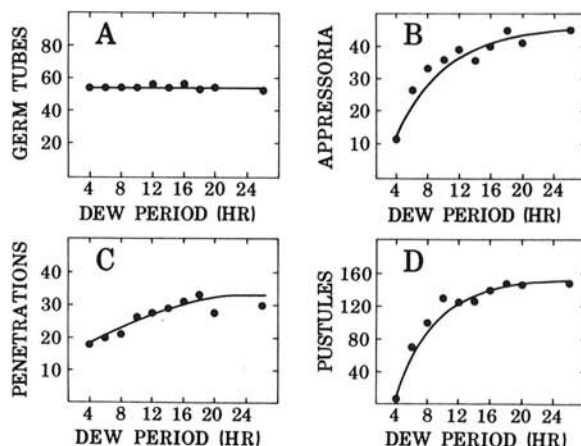

Fig. 1-(A to D). Mean numbers of **A)** germ tubes; **B)** appressoria; **C)** penetrations; and **D)** pustules of *P. coronata* for ten dew periods (hours). Numbers of germ tubes, appressoria, and penetrations are relative to the numbers of spores, germ tubes, and appressoria, respectively.

pustules increased with longer dew periods (55 pustules per leaf for 6.5 hours). For practical purposes, 5 hours was the minimum for pustule formation (15 pustules per leaf).

We determined the effect of dew duration at 21.0 C on the formation of germ tubes, appressoria, penetrations, and pustules by conducting a series of experiments involving ten dew periods (4, 6, 8, 10, 12, 14, 16, 18, 20 and 26 hours). There was no significant difference in the number of germ tubes per leaf. There were, however, significant differences ($P = 0.01$) for the adjusted means of appressoria and penetrations, and for the mean number of pustules per leaf (Fig. 1). After 16 hours of dew at 21.0 C, there was no further increase in numbers of germ tubes, appressoria, penetration tubes, or pustules.

Temperature during the dew period.—We used four temperatures (10.0, 15.5, 21.0, and 26.5 C) in a series of experiments involving different temperatures during a 12-hour dew period. A test also was run at 4.5 C, but no germination was observed on water agar or on the plants. There was a significant difference ($P = 0.01$) in the number of germ tubes formed at the four temperatures (Fig. 2). The least favorable temperature for germination was 10.0 C; the other three gave about equally good results. There was no significant difference in the number of appressoria; once germination had taken place, appressoria formed equally well from 10.0 - 26.5 C. There were, however, significant differences ($P=0.01$) in the numbers of penetrations and pustules formed, and the most favorable temperatures for both were 15.5 and 21.0 C.

In all experiments, the percentage germination, germ tubes forming appressoria, and appressoria forming penetration tubes did not differ significantly between counts on the first and second days after inoculation. This indicates that, once dew had dried from the plants, no more germ tubes, appressoria, or penetration tubes were formed on the leaf surface.

Therefore, to determine the effect of temperature on penetration and pustule formation after germination and

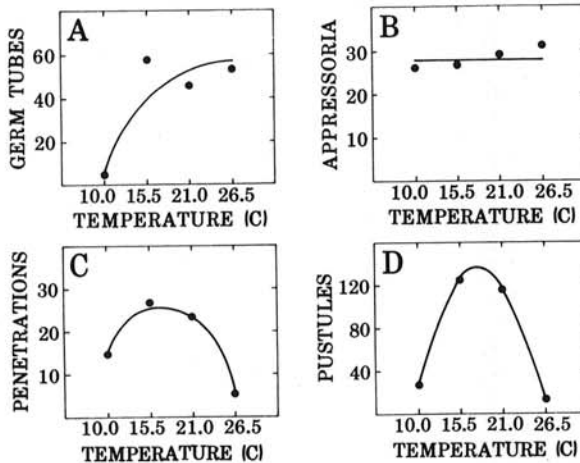

Fig. 2-(A to D). Mean numbers of **A)** germ tubes; **B)** appressoria; **C)** penetrations; and **D)** pustules of *P. coronata* for four temperatures (Celsius) during the dew period. Numbers of germ tubes, appressoria, and penetrations are relative to the numbers of spores, germ tubes, and appressoria, respectively.

essentially all appressorium formation had taken place, we varied temperature the last 4 hours of the dew period. A 12-hour dew period at 21.0 C was followed by 4 hours of dew at 17.0, 21.0, and 26.5 C. The plants were dried with a fan as soon as they were taken from the dew chamber. There was a significant difference ($P=0.10$) in the number of penetrations, with a few more penetrations occurring at the higher temperatures, but the number of pustules differed ($P=0.05$). The most favorable temperatures during the last 4 hours of the dew period for pustule formation were 21.0 and 26.5 C. At these two temperatures, 74 pustules per leaf formed, while at 17.0 C only 56 pustules formed.

Variable versus uniform temperatures during the dew period.—We attempted to determine if a cam-controlled varying temperature regime that simulated the changing night-time temperatures in nature was better for crown rust development than a constant temperature regime of 15.5 or 21.0 C during the major part of the dew period. No significant difference was observed for numbers of germ tubes and appressoria, but there was a significant difference ($P = 0.05$) in the number of penetrations, with a few more penetrations occurring at the constant temperatures. The number of pustules, however, was significantly different ($P=0.01$). The cam program resulted in 88 pustules per leaf, and constant 15.5 and 21.0 C resulted in 134 and 126 pustules, respectively. This suggests that a constant temperature is superior for pustule formation, but it is possible that the single cam-controlled regime selected was not the best choice.

Light during the dew period.—Wheat inoculated with *P. graminis tritici* race 15B-2 was used as a control because light enhances penetration of wheat by this fungus (2, 11, 13). Wheat and oats were inoculated and placed in the dew chamber for a 12-hour dew period at 21.0 C. For the dark treatment, one half of the plants, still wet with dew, were moved to the bottom shelf and enclosed with a cardboard box on three sides and the top. Then lights in the dew chamber were turned on and a reflector-photoflood lamp (General Electric No. PH/RFL2) was made to shine through the window port of the dew chamber. The light intensity was approximately 8,600 lux (800 ft-c) on the plants. Distilled water from the bottom pan was atomized onto the plants exposed to light for 4 hours so that heat from the lamps would not dry them and so that the temperature could be kept at 21.0 C the whole time. Plants from both light and dark treatments were dried with a fan immediately on being removed from the dew chamber.

There was no significant difference in numbers of penetrations and pustules for *P. coronata* in light and in darkness. For *P. graminis tritici,* however, the mean number of penetrations was 11 in light and 4 in darkness, a difference significant at $P = 0.10$, and the mean number of pustules was 16 in light and 1 in darkness, a difference significant at $P = 0.05$. Light near the end of the dew period did not enhance penetration or pustule formation by *P. coronata,* but it did, as reported previously (2, 11, 13), for *P. graminis tritici.*

The effect of light on penetration and pustule formation on oats by *P. graminis avenae* was studied next to see whether the effect of light on this host-parasite interaction was like that for oats and *P. coronata* or that for wheat and *P. graminis tritici*. Again, significantly

more penetrations and pustules occurred in light for the *P. graminis tritici* controls. For *P. graminis avenae* the mean number of penetrations was nine in light and six in darkness, a difference significant at $P = 0.15$, and the mean number of pustules was 50 in light and 39 in darkness, a difference significant at $P = 0.01$. Although the effect was not as great as for *P. graminis tritici,* light near the end of the dew period clearly enhanced penetration and pustule formation by *P. graminis avenae*.

DISCUSSION.—Our study indicates that spores collected at one time and stored in liquid nitrogen allow experiments over time with uniform inoculum. Slight variation may be introduced in the spore concentration of the suspension, in settings of the aliquot inoculator, and among plants. These factors could affect only the number of pustules per leaf, however, because counts of the infection structures were adjusted by their covariates. Possibly the number of pustules should have been adjusted for the length of the inoculated leaf area, or only a portion of the leaf counted—practices we follow now.

Leaf wetness is the most critical factor affecting the development of crown rust, assuming a susceptible cultivar and a favorable temperature. Free moisture is essential for spore germination and, therefore, infection (8). The minimum wet period for pustule formation varies with temperature (7). We obtained trace infection with a wet period of 4 h hours at 21.0 C; with a longer wet period, more infection structures developed. After the maximum number of germ tubes, appressoria, and penetration tubes have formed, the free moisture may stabilize temperature until substomatal vesicles have developed. Development of the fungus within the leaf depends mainly on host vigor. Because we found no further increase in numbers of germ tubes, appressoria, penetration tubes, or pustules after 16 hours of dew at 21.0 C, we assume that this is the time needed for the fungus to become established within the host, or become independent (except for temperature) from the external environment. This may be related to the time it takes for the septum to form between the appressorium and the substomatal vesicle (12).

Optimum dew period temperatures range from 17-22 C (8), and our constant temperatures (15.5 or 21.0 C) through the dew period were favorable for germ tube, appressorium, penetration tube, and pustule development by *P. coronata*. This cannot be related directly to what happens in the field because temperatures usually decrease gradually during the dark period, especially on clear nights favorable for dew. We simulated this decrease in the dew chamber by use of a cam program. The variable temperature regime resulted in good infection, although not as good as those with constant temperatures.

On a typical night in June and July when oat crown rust epidemics occur in Iowa, the dew period may start in early evening when the temperature is between 15 and 20 C. During the night the temperature may drop to 10 C, but maximum germination will already have taken place because it occurs within 4 hours. As indicated in our study, temperatures over the range 10.0 - 26.5 C do not affect the number of appressoria formed. By sunrise, most appressoria that will form will have done so. The temperature gradually increases as the sun rises, reaching that for penetration after most appressoria have formed. Dew may remain until midmorning while temperatures remain favorable for penetration. In the field, all sources of leaf wetness; i.e., dew, rain, and fog, must be considered, as they serve equally the requirements for development of infection structures. Epidemiologically, the source of moisture is important in that temperatures remain mild and uniform during a night with rain or fog while temperatures during a clear, calm night favorable for dew are likely to be lower, with leaf temperature well below ambient. Also, a day following a rainy night is likely to be cloudy, cool, and humid while a day following a dewy night is likely to be clear, warm, and dry (15).

Light near the end of the wet period favored development of *P. graminis tritici,* corroborating others (2, 11, 13). It also favored *P. graminis avenae,* but did not favor *P. coronata*. The ability of *P. graminis tritici* to penetrate in light or in carbon dioxide-free air in darkness is probably not due to stomatal opening because stomata that were closed when occupied by appressoria of *P. graminis tritici* in darkness did not open when exposed to light (16). *P. coronata* penetrates very well in darkness when presumably the stomates are closed, and thus seems to act in a manner similar to *P. recondita* in that light does not enhance penetration (16, 17).

Higher temperature and light near the end of the dew period may affect mainly the host, the fungus, or the interaction between them. Emge (4) showed that substomatal-vesicle-like structures form on artificial membranes exposed to temperature and light conditions similar to those required for leaf penetration by *P. graminis tritici*. This, and our results with *P. graminis avenae* and *P. coronata,* suggest that these factors have their major effect on the pathogen.

Our results should enable oat crown rust researchers to optimize conditions for infection by *P. coronata* in routine tests for resistance, race identification, etc., as well as for more sophisticated studies, and may aid in developing a rust forecasting system. In the past, chemical control of crown rust has not been economical, but with a reliable forecasting system, one application of a fungicide at the right time might be sufficient (14). Of course, many other factors would have to be considered, such as the amount of inoculum, races of *P. coronata* present, oat cultivars grown, growth stage of the host, and the potential value of a given field for seed, feed grain, or human consumption.

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