

Influence of Temperature and Wetness Duration on Infection of Pearl Millet by *Claviceps fusiformis*

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Journal article 1112 of ICRISAT.

Accepted for publication 24 January 1991 (submitted for electronic processing).

ABSTRACT

Thakur, R. P., Rao, V. P., and King, S. B. 1991. Influence of temperature and wetness duration on infection of pearl millet by *Claviceps fusiformis*. Phytopathology 81:835-838.

The influence of temperature and wetness duration on infection of susceptible, moderately resistant, and resistant genotypes of pearl millet by *Claviceps fusiformis* was studied. In greenhouse and field experiments, panicles of pearl millet inoculated with *C. fusiformis* were subjected to various regimes of temperature (14–35 C) and wetness (95% relative humidity) duration (0–64 h) for a 15-day period. These environmental conditions influenced the level of ergot severity, but the relative ergot reactions of the genotypes were not changed. In susceptible genotypes, temperatures in the range of 14–35 C, with 8 h/day at less than 20 C and 4.6 h/day at greater than 30 C, were more favorable to ergot infection (70–72% severity; 32–49% sclerotial formation) than a temperature range

of 21–35 C, with 6.4 h/day at greater than 30 C (53% severity; 23% sclerotial formation). Results indicated that the minimum temperature is more critical for ergot infection than the maximum temperature. In both greenhouse and field experiments, panicle wetness duration of 16–24 h favored ergot infection. In controlled environment experiments, temperature and the interaction of temperature and wetness duration were significant for latent period ($P = 0.001$). Wetness duration and the interaction of temperature and wetness duration were significant for ergot severity ($P = 0.05$). Maximum ergot severity (60.5%) and minimum latent period (117 h) were obtained at a 30 C day/25 C night temperature regimes with 24–96 h of panicle wetness.

Additional keywords: *Pennisetum glaucum*, epidemiology.

Among the weather factors influencing plant diseases, moisture often is the most important, followed by temperature (5). Moisture can be measured in terms of both quantity and duration for successful establishment of infection by most fungal and bacterial pathogens. High relative humidity (RH) or surface wetness is essential, but the duration of this condition may be critical to infection. Similarly, a suitable temperature for a certain minimal length of time is critical for infection and disease development. Temperatures that are either too high or too low have adverse effects on the pathogen and disease development.

Ergot, caused by *Claviceps fusiformis* Loveless, is an important disease in the major pearl millet (*Pennisetum glaucum* (L.) R. Br.) growing areas of the world (6,10). Infection of pearl millet florets by *C. fusiformis* spores occurs through young, fresh stigmas, and infection is prevented or reduced after the stigmas wither either subsequent to pollination or during aging (15,17). Field screening of pearl millet lines for ergot resistance is done by spray-inoculating panicles with a conidial suspension of *C. fusiformis* at the full protogyny stage, protecting the panicles from cross-pollination by covering them before and after inoculation, and providing high humidity (>80%) by overhead sprinkler irrigation (16). Ergot infection becomes visible within 4–6 days after inoculation (13), and infection is favored by high RH (>80%) and moderate temperature (18–32 C) (2,3,7,9). However, there is no information available about the optimal duration of high humidity and temperature for ergot infection in pearl millet. Our objective was to determine the optimal postinoculation panicle wetness duration and temperature for ergot infection in pearl millet.

MATERIALS AND METHODS

Panicle wetness. In field and greenhouse experiments, panicle wetness was created by covering the panicles with polyethylene bags prewetted inside by spraying with tap water. RH was

measured inside the polyethylene bag with electronic humidity sensors connected to a data logger (21× Micrologger, Campbell Scientific Co., Logan, UT). Measurements were always RH>95% and free water droplets were deposited on the stigmas. We considered this condition as “panicle wetness,” and use the term interchangeably with high RH, unless otherwise mentioned.

Inoculum, inoculation, and ergot assessment. An aqueous conidial suspension of *C. fusiformis* (isolate ICR 1), grown on Kirchoff's medium at 25 C for 10 days, was used to inoculate protogynous panicles of a highly susceptible cultivar (BJ 104), grown in pots in a greenhouse. Honeydew, containing conidia, produced from these panicles was used as inoculum for subsequent experiments. A 5-ml honeydew conidial suspension in tap water (1×10^6 conidia per milliliter) was used to spray each protogynous panicle (with >75% emerged stigmas). The panicles were protected from cross-pollination by covering with parchment paper selfing bags or polyethylene bags before and after inoculation (16). Panicles were assessed for ergot severity (percentage of infected florets showing blasting and sclerotial formation) and sclerotial formation (percentage of florets bearing visible sclerotia) 15 days after inoculation, using an ergot severity assessment key (16).

Influence of temperature under field conditions. A set of six ergot susceptible (>20% severity) genotypes (BJ 104, BK 560, 841A, 841B, 842A, 842B) were sequentially planted in the field at ICRISAT Center on four dates, 16 November, 12 and 22 December 1988, and 16 January 1989. Each genotype was grown in a 2-row plot, 4 m long with two replications in a randomized block design. Rows were spaced 75 cm apart, and plants within rows were 15 cm apart. The field was irrigated by overhead sprinklers twice a day, 30 min each at noon and in the late afternoon during inoculation and ergot development.

One panicle of each of 10 plants, randomly selected, in a plot was inoculated and covered with a polyethylene bag. The polyethylene bags were replaced with parchment bags 24 h after inoculation. Ergot inoculation was started on 18 January, 30 January, 21 February, and 16 March for the first, second, third, and fourth plantings, respectively, and was completed within a week for each planting. The bags were removed 15 days after

inoculation and panicles were assessed for ergot severity and sclerotial formation. During this 15-day period, inoculated panicles in each set were exposed to variable prevailing temperature regimes in the field. Temperature was recorded, using a hygrothermograph (British Rototherm Co. Ltd., Margam, Port Talbot, West Glamorgan, South Wales, England) placed 1 m above ground in the field. Temperatures (minimum and maximum) were averaged for a 15-day period during inoculation until ergot assessment and were computed for each planting. Considering earlier reports (1,2,7,9), we found the most favorable temperature range for ergot infection to be 20–30 C, and determined the length of time of low temperatures (<20 C) and high temperatures (>30 C) for each set for a 15-day period.

Data on ergot severity and sclerotial formation were pooled over six genotypes and were subjected to analysis of variance (ANOVA) to determine significant effects of temperature regimes on ergot infection. A least significant difference was calculated to compare treatments.

Influence of wetness period in a greenhouse experiment. Pot-grown plants of two ergot-susceptible genotypes (BK 560, WC-C75), two moderately resistant (11–20% severity) genotypes (ICMPES 8, ICMPES 9), and two resistant (<10% severity) genotypes (ICMPES 29, ICMPES 34) were used. Overhead mist irrigation was provided to increase the ambient humidity and to reduce the ambient temperature. Ergot-inoculated panicles were

TABLE 1. Effects of temperature on ergot severity, and sclerotial development on six pearl millet genotypes grown sequentially and inoculated with *Claviceps fusiformis* in a field experiment

Average temperature (C) ^a	Hours per day temperature (C) ^a		Ergot severity (%) ^b	Sclerotial formation (%) ^b
	<20	>30		
15–32	7.5	3.1	72	42
14–34	9.4	4.6	71	49
16–35	6.7	6.2	70	32
21–35	0.0	6.4	53	23
LSD ^c			7.8	9.9

^a Average temperature over 15-day period during inoculation to ergot scoring. Minimum to maximum.

^b Mean of 10 inoculated panicles in each of two replications (five panicles per row) in each of six ergot-susceptible genotypes (BJ 104, BK 560, 841A, 841B, 842A, 842B).

^c Least significant difference at $P = 0.05$.

subjected to wetness periods of 0, 16, 24, 32, 40, 48, 56, and 64 h by covering them with polyethylene bags as in the field inoculation. The experiment was conducted in a completely randomized design with eight treatments \times two replications with five plants per replication. Polyethylene bags were replaced with parchment paper selfing bags after the required wet exposure periods, and plants were moved to another greenhouse without mist. Observations were recorded for latent period (time from inoculation to honeydew appearance), ergot severity, sclerotial formation, and seed set.

Effect of wetness duration under field conditions. The same set of genotypes that were grown in the greenhouse were also grown in the field during January–March 1990. The experiment was conducted in a randomized block design with eight treatments \times three replications with 10 plants in a row per replication. The other details were similar to those described for the greenhouse experiment above, except that sprinkler irrigation provided moisture twice daily, 30 min each at noon and in the late afternoon, to increase the ambient humidity.

Data from field and greenhouse experiments were averaged over groups of susceptible, moderately resistant, and resistant genotypes and were subjected to ANOVA to determine if treatment effects were significant. Least significant differences were calculated when an effect was significant.

Effect of temperature and wetness duration. In a controlled environment chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada), pot-grown plants of an ergot-susceptible male-sterile line 842A were subjected to postinoculation day/night (12 h/12 h) temperatures of 35/25, 30/25, 25/20, and 20/15 C and panicle wetness periods of 24, 48, 72, and 96 h. Panicle wetness was created by operating humidifiers (Defensor Aktiengesellschaft 8045 Zurich Binzstrasse 18, West Germany), and polyethylene bags were not used to cover the panicles. The experiment consisted of four temperature regimes and four wetness periods with four replicates in a completely randomized design. Each replicate consisted of at least four pots, one plant per pot. The plants were moved to a greenhouse at 30 C day/25 C night temperatures after the required wetness periods in the growth chamber. To determine the latent period the panicles were observed daily at 9:00 a.m. and 4:00 p.m., starting the fourth day after inoculation. Ergot severity was scored 15 days after inoculation. Data were subjected to ANOVA to determine effects of treatments and compute the least significant difference.

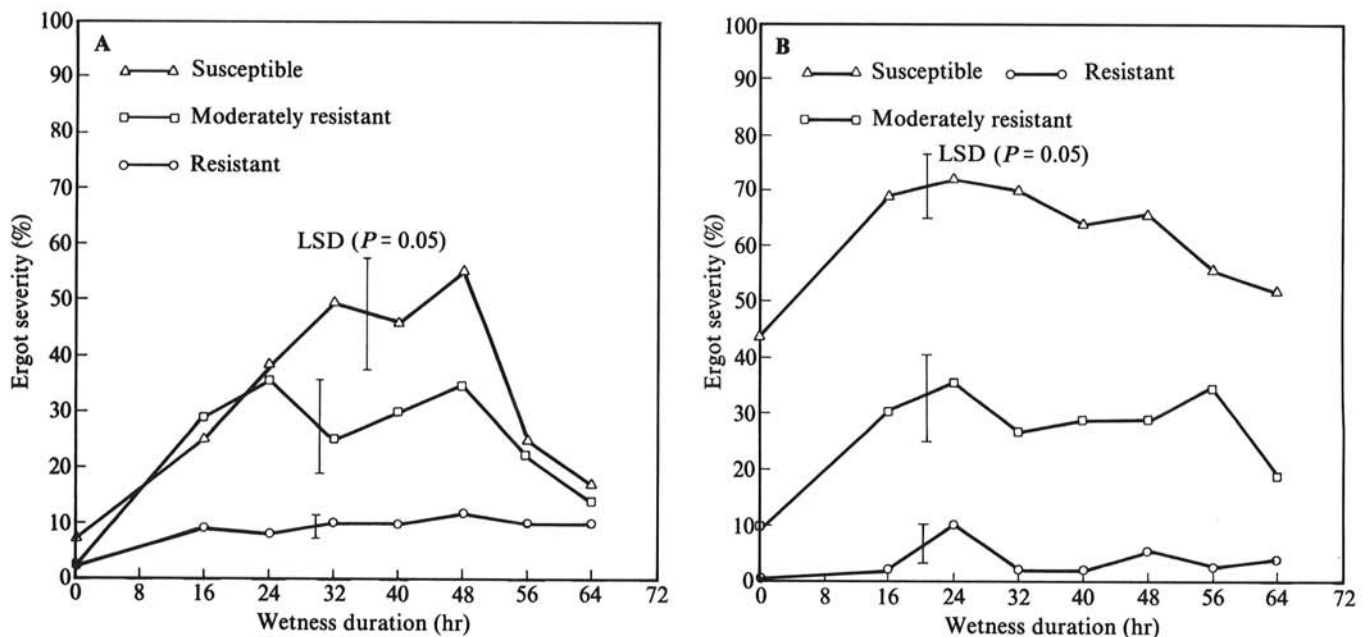


Fig. 1. Influence of panicle wetness duration on ergot severity (percentage of infected florets) in ergot susceptible (BK 560, WC-C75), moderately resistant (ICMPES 8, ICMPES 9), and resistant (ICMPES 29, ICMPES 34) genotypes of pearl millet. A, greenhouse experiment; B, field experiment.

RESULTS

Effect of temperature under field conditions. Three temperature regimes (15–32, 14–34, and 16–35 C) generally favored more ergot infection (70–72% severity) and sclerotial formation (32–49%) than a 21–35 C regime (53% severity; 23% sclerotial formation) (Table 1). During the first three temperature regimes, 6.7–9.4 h/day were at less than 20 C and 3.1–6.2 h/day were at greater than 30 C. The temperature regime of 21–35 C with the temperature above 30 C for 6.4 h/day and a minimum temperature of 21 C appeared detrimental for ergot development.

Effect of wetness duration. In the greenhouse experiment, 16 h of panicle wetness of moderately resistant and resistant genotypes, and 24 h of wetness of susceptible genotypes produced significantly higher ergot severity than 0 h of panicle wetness (Fig. 1A). In susceptible genotypes, a maximum severity of 55% was observed after 48 h of wetness. This was a significantly greater severity than that at 16 h, but not different than for 24 h (Fig. 1A). In both susceptible and moderately resistant genotypes there was a significant decline in ergot severity from 48- to 64-h wetness periods. In the field, significantly higher ergot severities were observed after a 16-h wetness period than the control (0 h) in susceptible and moderately resistant genotypes, but in resistant genotypes the maximum severity occurred only after 24 h of wetness (Fig. 1B). With further increases in the wetness period there was no increase in ergot severity, and these trends were similar both for greenhouse and field conditions.

Significant effects of panicle wetness duration were observed for latent period both in greenhouse and field experiments for susceptible (BK 560, WC-C75) and resistant (ICMPES 29, ICMPES 34) genotypes, for ergot severity and seed set in all three groups of genotypes, and for sclerotial formation in susceptible and moderately resistant (ICMPES 8, ICMPES 9) genotypes (Table 2). Seed set across genotypes, both in greenhouse and field, was greater at 0 h (18–73%) of panicle wetness than at 16–64 h of panicle wetness (0–43%). At 16–64 h of panicle wetness, all genotypes had poor seed set under greenhouse compared to field conditions. Under field conditions, at 16–64 h of panicle wetness, moderately resistant and resistant genotypes had seed set in the range of 13–34% and 29–43%, respectively, compared with 0–1% in the susceptible genotypes.

Effect of temperature and wetness duration. Under a controlled environment, the main effect of temperature and the wetness duration \times temperature interaction were significant for latent period ($P = 0.001$); wetness duration and wetness duration \times temperature interaction effects were significant for ergot severity ($P = 0.05$) (Table 3). The minimum latent period of 117 h occurred at the 30 C day/25 C night regime and the maximum of 139 h occurred at 20 C day/15 C night regime (Table 4). The rela-

TABLE 2. Variance ratios (F values) for variables as influenced by panicle wetness on ergot-susceptible (S), moderately resistant (MR), and resistant (R) genotypes of pearl millet in greenhouse and field experiments

Variable	Genotype ^a	F	
		Greenhouse experiment	Field experiment
Latent period	S	4.69** ^b	3.79**
	MR	1.37	9.61**
	R	11.56**	3.23**
Ergot severity	S	5.41**	7.14**
	MR	2.95*	3.74**
	R	4.00**	2.41*
Sclerotial formation	S	2.81*	5.81**
	MR	1.81	3.29*
	R	1.99	3.55**
Seed set	S	5.43**	6.55**
	MR	58.70**	12.76**
	R	17.09**	2.41*

^aS = BK 560, WC-C75; MR = ICMPES 8, ICMPES 9; and R = ICMPES 29, ICMPES 34.

^b* = Significant at $P = 0.05$; ** = significant at $P = 0.01$.

tionships between temperature and latent periods were more consistent than between wetness duration and ergot severity.

DISCUSSION

The results from these experiments provide some useful information on the influences of temperature and wetness on infection of pearl millet by *C. fusiformis*. The effects of temperature and panicle wetness duration were more evident in susceptible genotypes than in moderately resistant and resistant genotypes. The results are in accordance with resistance stability of ergot-resistant genotypes under highly favorable epidemic conditions (11,12). The influences of panicle wetness duration on ergot infection were similar both in greenhouse and field experiments. However, the response of wetness to ergot infection was more distinct in the greenhouse than in the field, particularly in susceptible genotypes. Higher ergot severity at 0 h of wetness in susceptible genotypes in the field experiment was probably due to prevailing higher humidity on panicles inside the parchment paper bags, where sprinklers were in operation, than in the greenhouse, in which the plants were moved to a different greenhouse with relatively drier conditions. The susceptible genotypes had higher ergot levels and lower seed set than the moderately resistant and resistant genotypes. Under conditions of prolonged panicle wetness in the field, even in the absence of ergot, susceptible genotypes had poor seed set compared with moderately resistant and resistant genotypes (R. P. Thakur, unpublished). This could be because of better pollen viability and extended pollen production and stigma receptivity in resistant than in susceptible genotypes. In pearl millet-growing areas where rain continues during the flowering period and conditions become favorable for ergot epidemic, the ergot-resistant genotypes may have significant

TABLE 3. Variance ratios (F values) for latent period and ergot severity in pearl millet, infected by *Claviceps fusiformis*, as influenced by temperature and wetness periods in a controlled environment experiment

Source	df	Latent period (h)	Ergot severity (%)
Replication	3	1.35	1.56
Temperature	3	42.87*** ^a	0.65
Wetness duration (WD)	3	1.97	3.21*
Temperature \times WD	9	7.70***	2.09*

*** = Significant at $P = 0.001$; * = significant at $P = 0.05$.

TABLE 4. Effect of temperature and panicle wetness duration on infection by *Claviceps fusiformis* in a pearl millet male sterile line 842A, in a controlled environment

Temperature (day/night) (C)	Wetness duration (h)	Latent period (h) ^a	Ergot severity (%) ^a
35/25	24	137	42
	48	130	57
	72	125	55
	96	125	64
30/25	24	122	57
	48	116	67
	72	115	63
	96	116	55
25/20	24	126	57
	48	126	47
	72	130	53
	96	127	69
20/15	24	128	61
	48	138	44
	72	134	52
	96	155	73
LSD ^b		7.5	17.2

^aBased on mean of four inoculated panicles, with one panicle per replication.

^bLeast significant difference at $P = 0.05$.

advantage over the susceptible genotypes in terms of better seed set and higher yield. Ergot-resistant genotypes that have desirable agronomic traits and reasonably good yield potential, and also possess resistance to downy mildew (*Sclerospora graminicola*) and smut (*Tolyposporium penicillariae*) could be useful for cultivation by farmers (4,10,14).

Temperature influences the processes of infection and disease development (8). In a sequentially inoculated field experiment, in which ergot-inoculated panicles of pearl millet were exposed to the prevailing temperature regimes, significant differences in ergot severity and sclerotial formation were detected. Although the daily mean and maximum temperatures did not vary much, the duration of low temperature (<20 C) for 6.7–9.4 h/day seemed to favor ergot infection more than the duration of high temperature (>30 C) for 6.4 h/day. The results clearly suggest that a daily temperature range that includes higher minimum temperature (>20 C) and longer duration of >30 C is less conducive for ergot development. This generally happens during March when temperature goes up, and dew formation is minimal. This information has important epidemiological significance and could be utilized in developing a predictive model for occurrence of ergot in pearl millet. Such information could also provide a better insight for demarcating the pearl millet-growing areas into low and high ergot-risk areas, as has been attempted in India (3).

In a controlled environment experiment, in which ergot-inoculated panicles were exposed to various periods of wetness at different day/night temperature regimes, the lowest latent period (117 h) and maximum ergot severity (60.5%) were obtained at 30 C day/25 C night temperatures. This confirms the field results in which ergot severity was high at 14–35 C, but when the minimum temperature rose to 21 C, ergot severity was significantly reduced. These results are also in agreement with the finding that the optimal temperature for growth of *C. fusiformis* on artificial media is 25 C (1), and that favorable temperatures for ergot development are in the range of 18–32 C (2,7,9). The maximum latent period of 139 h at the lowest temperature regime 20/15 C could be due to delayed infection process at cooler temperatures. Further investigations are needed in the controlled environment to understand more precisely the influence of varying levels of RH and their interaction with temperatures in relation to pollination biology and ergot infection in pearl millet.

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