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INFLUENCE OF TEMPERATURE AND HIGH HUMIDITY DURATION ON SMUT INFECTION IN PEARL MILLET

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ABSTRACT

Pearl Millet (*Pennisetum glaucum*) panicles inoculated with smut (*Tolyposporium penicillariae*) and maintained at high relative humidity (>95% RH), when exposed to post-inoculation temperatures of <20°C for 8 h and >30°C for 3.5 h day^{-1} (range 15-32°C), were not infected, those exposed to <20°C for 0.0 h and >30°C for 7.7 h day^{-1} (range 20-38°C) and 30% smut-infected florets, while those exposed to <20° for 0.0 h and >30°C for 5.4 h day^{-1} (range 26-33°C) had 76% smut-infected florets. Of the temperatures tested, minimum temperatures inhibited smut infection more than the maximum temperatures. With the decreasing postinoculation exposure time from 6.7 to 0.0 h day^{-1} at <20°C there was a significant reduction in latent period from 23 to 11 days. Smut inoculated panicles exposed to high RH for 168 to 192 h produced maximum smut (>50%). The results thus indicate that high humidity periods of 168-192 h and the daily mean air temperature of about 30°C (range 25-35°C) may be most congenial for smut infection, and that temperatures below 20°C, even for few hours a day, greatly reduce smut infection. Implications of these findings in screening pearl millet for smut resistance are discussed.

KEY WORDS: *Pennisetum glaucum*, pearl millet, *Tolyposporium penicillariae*, Smut, High RH duration, Temperature, Resistance screening, Epidemiology.

Among the environmental factors, for most diseases, moisture is the most important factor followed by temperature (Jones, 1986). Moisture is measured in terms of relative humidity (RH), and high RH duration is often critical for infection. Temperature influences the process of infection, disease development and progress of a disease epidemic (Rotem, 1978). Although the daily mean, maximum and minimum temperatures are critical, it is often the duration of the low and high temperatures that influence most the pathogen reproduction, infection and disease development.

Smut, caused by *Tolyposporium penicillariae* Bref. is an important floral disease of pearl millet [*Pennisetum glaucum* (L.) R. Br. syn. *Pennisetum americanum* (L.) Leeke] (Rachie and Majmudar, 1980; Thakur and King, 1988). The disease is generally more severe when weather at flowering is warm and humid rather than when it is moderate or cool. In India, for example, smut is more severe in part of north India where average temperatures during July-September are 24-37°C compared with 19-31°C in central and south India (R. P. Thakur, unpublished). Infection of pearl millet florets by *T. penicillariae* sporidia occurs through young emerging stigmas (Bhatt,

1946). Inoculation of pearl millet panicles at the boot-leaf stage produces more infection than inoculation at other stages of flowering (Thakur *et al.*, 1983 b), and pollination reduces or prevents smut infection (Thakur *et al*, 1983 a).

The major screening for smut resistance in pearl millet at ICRISAT Center is done during the rainy season, June-September when the daily mean air temperatures are 27-36°C and RH above 80% (maintained by supplemental sprinkler irrigations). During the past few years our attempts to screen for smut resistance during the dry season, January-April, when the air temperatures are 15-38°C and RH below 60% (despite frequent sprinkler irrigations), have been less successful. We attributed this to the possible effects of temperature and RH during the dry period. In the present studies attempt has been made to determine the optimal temperature range and the postinoculation high RH exposure duration to obtain maximum smut infection in pearl millet.

MATERIALS AND METHODS

Smut inoculum and inoculation

An ICRISAT field isolate of *T. penicillariae* grown on potato agar for 3-5 days at 30°C (Subba Rao and Thakur, 1983) was used as inoculum source. Inoculations were done by injecting 5 ml aqueous suspension (10^6 sporidia/ml) into boot leaf of a plant and covering it by parchment or polyethylene selling bag (Thakur *et al.*, 1983 b). Bags were removed 20 days after inoculation and smut scores were taken with reference to a smut severity rating scale (Thakur and King, 1988).

Monitoring temperature and relative humidity (RH)

In both field and greenhouse experiments, temperature and RH were monitored, using thermohygrograph (British Rototherm Co. Ltd., England) during the period from inoculation to disease assessment. The thermohygrograph readings were checked against psychrometer readings (for wet and dry bulb temperatures and the corresponding RH) once every two days, and the thermohygrograph was calibrated when required. We also monitored simultaneously the temperatures inside the polyethylene bag, parchment bag and of the air, using electronic thermometer (Cole Parmar, Chicago, Illinois). Since there was no difference in the three readings, we used the temperature readings from the thermohygrograph for the studies. Covering panicles with polyethylene bags provided enough condensation water to create high humidity. The RH readings taken inside the polyethylene bags near the panicles using humidity censor (Campbell Scientific Inc. USA) were always >95%.

Effect of temperature

Field experiment. A set of two pearl millet hybrids BJ 104 and BK 560, and two inbred lines 5141 A and 5141 B, all susceptible to smut, were sequentially planted on

four dates : 16 November, 12 December, 22 December, 1988, and 16 January, 1989 at ICRISAT Center research farm. Each entry was grown in 2-row plot, 4 m long with two replications, in a randomized block design. Rows were spaced at 75 cm and plants at 15 cm within a row.

Inoculations were begun on 18 January, 3 February, 21 February and 13 March in the first, second, third and the fourth sets of planting, respectively, and each was completed within a week. Ten plants in each row were inoculated and covered with polyethylene bags. In addition, sprinkler irrigations were provided twice a day, 30 min each during noon and evening to maintain high field humidity (not <80%). The polyethylene bags were replaced with parchment bags seven days (168 h) later. To determine the latent period (the time between inoculation and appearance of sori), each inoculated panicle was observed every day for smut appearance, by briefly openings the bags, starting 10th day after inoculation.

Controlled temperature experiment—Pot-grown plants of a pearl millet hybrid BK 560, were inoculated, covered with polyethylene bags and exposed to postinoculation day (12 h)/night (12 h) temperatures of 30/30, 30/20 and 30/15°C for seven days in an incubator (Percival, Boone, Iowa, USA), and at approximately 30°C day/25°C night in a greenhouse. The polyethylene bags were replaced with parchment bags and the plants were moved from incubator to the greenhouse. Two sets of plants, with at least 10 plants in each set, were used in this experiment. The inoculated panicles were observed for smut infection and latent period as described before.

Effect of high RH duration

In experiment 1, during the rainy season (June-September) 1987 field grown plants of two pearl millet cultivars, BJ 104 and BK 560 were inoculated and covered with polyethylene bags. In each cultivar 10 plants were inoculated. The polyethlene bags were removed at 0, 24, 48 7 , 96, 168 and 360 h after inoculation, and the panicles were left uncovered until observations were recorded for smut severity and seed set. The open panicles were thus subjected to natural cross-pollination.

In experiment 2, during the dry season (January-April) 1988 field grown plants of four pearl millet cultivars/lines, BJ 104, ICMH 423 and 81 A the polyethylene covers of smut-inoculaled panicles were inoculated and covered with polyethylene bags. The polyethylene bags were replaced by parchment selfing bags at 24, 48, 72, 96, 120, 144, 168 and 192 h. In each cultivar 5 to 15 panicles were inoculated. High field RH was maintained by providing sprinkler irrigations as described before. In this experiment the inoculated panicles were not left open and thus not exposed to cross-pollination.

Data computation and analysis

From the thermohygrograph readings, we computed the minimum, maximum and the mean temperatures, and hours of low (<20°C) and high (>30°C) temperatures day^{-1}, for a 20-day period during the time from inoculation to smut assessment, for each set of inoculation experiment. Although several pearl millet genotypes, all susceptible to smut, were used in various experiments, data were presented as means of all genotypes for convenience of presentation of results. The data were subjected to ANOVA test to determine significant differences.

RESULTS

Effect of temperature on smut development

In the field experiment, in the first set of inoculation during January when the mean air temperature was 24°C (range 15-32°C), with <20°C for 8 h day^{-1} and >30°C for 3.5 h day^{-1} during the 20-day period, there was no smut infection in any of the four cultivars/lines (Table 1). In the second set of inoculation, during February, when the temperatures were similar (mean 24.°C, and range 15-33°C) and the duration of low (<20°C) temperatures changed to 6.7 h and 5.3 h day^{-1}, respectively, very low level of smut (1% severity) appeared on all four genotypes. As the temperatures increased, mean 28°C (range 20-38°C), <20°C for 0.0 h and >30°C for 7.7 h day^{-1} in the fourth set of inoculation during March-April, the smut severity increased to 30%. In contrast, during the rainy season 1988 when the mean temperature was mean 29°C (range 26-34°C), with no exposure to low temperature (<20°C) and >30°C for 5.4 h day^{-1}, smut was 76%; 841 B had much less smut than over three genotypes.

TABLE 1. Effect of diurnal temperature on smut infection and latent period in pearl millet cultivars/lines in a field experiment at ICRISAT Center.

Experimental set[a]	Temperature (°C)				Smut (%)[b]	Latent period (day)[b]
			Hours day^{-1}			
	Mean	Range	< 20	> 30		
I	24.0	15-32	8.0	3.5	0	—
II	24.5	15-33	6.7	5.3	1	22.5
III	26.0	17-35	5.0	6.3	19	15.0
IV	28.2	20-38	0.0	7.7	30	15.3
V	29.0	26-33	0.0	5.4	76	11.0
LSD	(P<0.05)				16.4	1.7

a. Set I-IV were conducted during the dry season (January-April) 1989, and set V in the rainy season (June-September) 1988.

b. Mean of 20 inoculated panicles in each of four cultivars/lines (BJ 104, BK 560, 5141 A, 5141 B) from each of two replications.

In the controlled-temperature experiment at day/night temperatures of 30/30°C there was poor exsertion of panicles from the boots and no smut development. There were only 1 and 2% smut at 30/15 and 30/20°C, respectively, compared with 51% smut at 30/25°C (Table 2).

TABLE 2. Effect of temperature on smut infection and latent period controlled temperature experiment at ICRISAT Center.

Day/night temperature regime (°C)[a]	Smut severity (%)[b]	Latent period (day)[b]
30/30	0	—
30/15	1	13.4
30/20	2	11.7
30/25	51	11.7
LSD ($P \leq 0.05$)	11.6	0.25

a. Smut inoculated polyethylene covered panicles of pearl millet hybrid BK 560 were incubated in percival incubators. The polyethylene covers were replaced by parchment bags seven days after incubation at different temperatures and plants were moved to greenhouse at 30/25 C.

b. Mean of 7-10 incubated panicles,

Effect of temperature on latent period

In the field experiment during the dry season when the daily mean temperatures were <20°C for 6.7 h and >30°C for 5.3 h, (range (15-33°C) the mean latent period was 22.5 days with very low level of smut infection but during the rainy season when the daily mean temperature was 29°C with >30°C for 5.4 h day^{-1} (range (26-33°C) the mean latent period was reduced to 11 days with high levels of smut severity (Table 1). In controlled-temperature experiment, the latent period (11.7 days) at 30/25°C day / night temperature was significantly lower than at 30/15°C (Table 2).

Effect of high RH duration on smut

In experiment 1, the highest smut severity (8\ %) was recorded after 360 h exposure to high RH but it was not significantly different from that recorded (69%) after 198 h of high RH (Table 3). In experiment 2, the highest mean smut severity (57%) was recorded after 192 h of high RH exposure period, although this was not significantly different from smut severities recorded at 144 and 168 h of high RH (Table 4). Although there were some variations in smut severity levels among and within genotypes at different high RH periods, generally exposure of 192 h was mos effective.

Effect of high RH on seed set

In the field experiment, where panicles were left uncovered after the required period of high humidity, in both cultivars, BJ 104 and BK 560, >80% seed set occurred up to 96 h of high humidity exposure period but seed set was drastically reduced to <20% at 168 and 360 h of high humidity periods (Table 3).

TABLE 3. Effect of post-inoculation high RH (> 95%) duration on smut development and seed set in pearl millet hybrid in a field experiment during the 1988 rainy season at ICRISAT Center.

High RH duration (hour)[a]	Smut severity (%)[b]	Seed set (%)[b]
0	5	90
24	5	90
48	7	87
72	8	84
96	12	80
168	69	13
360	80	12
LSD ($P \leq 0.05$)	12.1	19.9

a. Inoculated panicles were left uncovered after the required period of exposure to high RH; cross-pollination occurred.

b. Mean of 10 inoculated panicles in each of two pearl millet hybrids BJ 104 and BK 560.

DISCUSSION

Both temperature and high humidity were found to have signifieant effects on smut development in pearl millet. In field experiments with sequential planting, smut-inoculated panicles were naturally exposed to various temperature ranges during January to April. Although the daily mean and range of temperatures were not much different, the duration of low temperature <20°C appeared inhibitory for smut development. The minimum temperatures of 15 and 17°C in the II and III sets of experiment where smut severities were 1 and 19%, respectively. appear more critical for smut infection. Although the minimum temperature seems to influence smut infection more than the mean or the maximum temperatures, we, however, chose the lower (<20°C) and the upper (>30°C) limits within the available temperature range for the convenience of interperating the results meaningfully.

TABLE 4. Effect of post-inoculation high RH (> 95%) duration on smut infection in pearl millet cultivars/lines in a field experiment during the 1988 dry season (January-April) at ICRISAT Center.

High RH duration (hours)[a]	Smut severity (%)[b]
0	11
24	13
48	18
72	33
96	40
120	41
144	49
168	56
192	57
LSD ($P \leq 0.05$)	11.6

a. The polyethylene bags on the inoculated panicles were replaced with parchment bags: cross-pollination prevented.

b. Mean of 5-15 inoculated panicles in each of four pearl millet cultivars/lines (BJ 104, ICMH 451, ICMH 423, MS 81 A).

The results of the controlled-temperature experiment clearly indicated that exposure of smut-inoculated panicles at 15-20°C for 12 h was inhibitory to smut-infection. Subba Rao and Thakur (1983) have shown that culture of *T. penicillariae* had slower growth at 20°C than at 25-35°C, and teliospore germination was minimum at 15°C and maximum at 30°C. In a preliminary experiment when a *T. penicillariae* culture on potato agar plates was incubated at 15°C for 2 days its growth was completely inhibited, and the growth resumed when the plates were transferred at 30°C.

Our failure to successfully screen breeding lines for smut resistance during February-March 1989 at ICRISAT Center, when the night temperatures were generally <20°C, could be attributed to the low temperature effects on smut development. This information is also important in explaining the natural occurrence and variable severity of smut in different pearl millet growing areas of India. Smut is generally more severe in the north Indian states of Haryana, Punjab, Delhi, Uttar Pradesh, and Rajasthan than in the central and southern states of Maharashtra, Karnataka, Andra Pradesh, and Tamil Nadu. This can now be well explained on the basis of prevailing

coolar air temperature in the lattar states (range 19-31° C) than in the formers (range 24-37° C) during the crop season, July-September (Huda and Thakur, 1988).

In both field experiments, postinoculation exposure to high RH for 168-192h (7-8 days) was generally sufficient to induce high smut infection in most pearl millet genotypes tested. Significant linear relationships ($R^2=0.96$ at $P<0.05$) were obtained between high humidity duration and smut severity levels. Use of pre-wetted polyethylene bags to cover smut-inoculated panicles for about 16 h was found effective to provide high RH favourable for smut development under conditions of Tifton, Georgia. In this experiment, exposure to high humidity even upto 144 h, however was not very effective. Prolonged covering with polyethylene bag beyond 7-8 days after inoculation affected panicle exsertion and this resulted in poor seed set. Replacement of polyethylene bags by parchment bags 6-8 days after inoculation, by which time infection has already taken place. was useful for sorus development and pollen production, self fertilization and seed formation.

The results suggest that for a successful smut screening in the field temperatures of <30°C should be avoided, and diurnal temperatures of 25-35°C and high RH for 7-8 days are optimal for maximum smut infection. Locations, such as Delhi, Hisar, Ludhiana and Jaipur in north India provide congenial temperatures during the rainy season for smut development and these locations can effectively be used with supplemental sprinkler irrigations for smut screening. Further studies in controlled environment are necessary to understand the effects of interaction of temperature and high humidity on smut development and the disease epidemiology. Such information would also be useful in refining smut screening technique and developing a predictive model for smut appearance as has been successfully done with other plant diseases (Bulger *et al.*, 1987).

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