Pollination Effects on Pearl Millet Ergot

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ABSTRACT

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Pollination of inflorescences of three pearl millet male-sterile lines before or at the same time as inoculation with *Claviceps fusiformis* conidia reduced ergot to less than 3% infected florets. Pollination 16 hr after inoculation reduced ergot to 7–21% compared with 60–86% for inoculated, nonpollinated checks. Pearl millet pollen germinated in 1 hr when incubated in a 10% sucrose solution, whereas *C. fusiformis* conidia did not germinate in less than 16 hr of incubation. Results from inoculation of inflorescences of male-sterile lines at different stages of flowering in the absence of pollen, along with observations on the effects of pollination on stigma morphology and longevity, lead us to conclude that ergot infection in pearl millet occurs mainly through the stigmas, and that pollination protects against infection probably because it induces rapid withering of stigmas. The results help explain the increased ergot susceptibility of pearl millet F_1 hybrids compared with traditional cultivars, and have important implications for ergot-resistance screening methods and for the development of cultural measures to control this disease.

Additional key words: Pennisetum americanum, honeydew, protogyny, pollen-based escape mechanism.

Pearl millet (*Pennisetum americanum* [L.] Leeke) provides the staple food for millions of people in the semiarid tropics. The average yields of traditional cultivars are estimated to be 468 kg/ha in India and 650 kg/ha in Africa (8). In the last 10 years considerable increases in pearl millet yield potentials have been demonstrated in India through the development of F_1 hybrids. Unfortunately these hybrids have been highly susceptible to one or more fungal diseases, including downy mildew (*Sclerospora graminicola*) and ergot (*Claviceps fusiformis* Loveless) (2,18,21). Ergot is important not only because it reduces yield but also because alkaloids in the fungal sclerotia are toxic to humans and animals that eat millet products prepared from sclerotium-contaminated grain (3,5,9,11,15). Measures suggested for control of ergot in pearl millet include removal of sclerotia from seed prior to planting by flotation in 10% salt solution (14), postharvest deep plowing to bury sclerotia (21), adjustment of date of planting to avoid conditions conducive to infection (19), adjustment of fertility levels in the crop (10), and the use of fungicides directly on the inflorescences (1,5,17,20,21). The effectiveness of these control methods is questionable; some are not based on experimentation or on a clear understanding of the epidemiology of the disease, and others are not likely to be economically or technically feasible for peasant farmers.

During the past 3 yr we have attempted to identify sources of resistance to ergot in pearl millet and have examined several aspects of the biology and epidemiology of the disease. This paper deals with investigations of the effect of pollination on pearl millet ergot, and we discuss the implications of the results and the possible control measures indicated by them. Part of this work has been published as an abstract (22).

MATERIALS AND METHODS

Flowering in pearl millet. Pearl millet flowering is protogynous. The long feathery stigmas emerge between the glumes 2–5 days before the onset of anthesis (Fig. 1,2). Stigma emergence begins near the tip and progresses toward the tip and the base. The time of commencement and duration of flowering events varies with cultivar and environment.

The effect of pollination. The experiments were conducted in the fields of ICRISAT Center, during the monsoon (rainy) seasons of 1976 and 1977.

Inoculation and pollination treatments were made in three male-sterile (ms) pearl millet lines and pollen was provided by the corresponding male-fertile lines. Individual tillers were bagged with white parchment-paper bags at the boot-leaf stage so that the inflorescences emerged inside the bags and thus were isolated from pollen and inoculum.

Inflorescences were inoculated at the protogyny stage by dipping them into a *C. fusiformis* conidial suspension of approximately 1×10^4 macroconidia per milliliter, prepared as a water dilution of fresh honeydew from previously inoculated inflorescences. The bags were removed immediately prior to inoculation and replaced immediately following inoculation.

The standard pollination method used by ICRISAT pearl millet breeders was used in this study. Bags covering inflorescences of ms lines were removed and replaced with pollen-containing bags from bagged inflorescences of the corresponding male-fertile lines. The bagged heads were pollinated by holding them horizontally and tapping the bags to disperse the pollen over the stigmas.

Each of the three ms lines was subjected to seven pollination and/or inoculation treatments (Table 1). The single operation treatments were carried out at the maximum fresh stigma stage. In the other treatments, the first operation (pollination or inoculation) was carried out at the maximum fresh-stigma stage. In the treatment "pollination immediately after inoculation", the inflorescences were first dip-inoculated and then immediately rebagged with bags containing pollen. Twenty inflorescences from each ms line were used for each treatment.

The degree of ergot infection was estimated at crop maturity by comparing individual inflorescences with a set of standard drawings of various degrees of ergot sclerotial development (Fig. 3).

The effect of flowering stage at inoculation. Tillers of two ms lines were bagged at the boot-leaf stage and the inflorescences were inoculated at several flowering stages from preprotogyny to postanthesis (Table 2). Ten inflorescences were inoculated at each flowering stage of each ms line. Pollination was not performed.

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The effect of preinoculation bagging. Several rows of pearl millet hybrid ICH 105 were grown among rows of many pearl millet genotypes during the rainy season in 1978. At intervals throughout six rows of ICH 105, tillers of randomly selected plants were bagged with parchment-paper bags at the boot-leaf stage. When the inflorescences were at the protogyny stage of development, 20 bagged inflorescences and 20 nonbagged inflorescences in each of six rows were dip-inoculated. All inflorescences were bagged immediately after inoculation. The inoculations were conducted over a 5-day period.

Relative germination rates of pollen and conidia. Pollen and fresh honeydew conidia were collected from five pearl millet breeding lines in the field between 0630 and 0700 hr. At 10-min intervals the pollen grains and the conidia from one cultivar after another were suspended in 10% sucrose solution in cavity slides (10% sucrose promotes maximum pollen germination and has no effect of conidial germination [R. P. Thakur, *unpublished*]). The slides containing the pollen and the conidial suspensions were maintained in moist chambers at 25 C. At fixed intervals after placement in the sucrose solution, the pollen and honeydew conidia were observed microscopically for germination.

The effects of pollination and inoculation on stigma morphology. Plants in seven pearl millet breeding lines were bagged at the boot-leaf stage, and the inflorescences at protogyny were either pollinated, inoculated, or sprayed with distilled water. Inflorescences were immediately rebagged following the treatments. At intervals following the treatments, the stigmas were examined for morphological changes.

RESULTS

The effect of pollination. Inoculated nonpollinated inflorescences developed 60–86% ergot (Table 1). Pollination prior to or at the same time as inoculation reduced ergot to a low level (overall means of < 3% florets infected). Inflorescences pollinated 16 hr after inoculation developed more infection than those pollinated earlier, but the infection was significantly less than that in inoculated, nonpollinated inflorescences.

The effect of flowering stage at inoculation. Inoculation of inflorescences when they had maximal numbers of fresh stigmas resulted in maximum (up to 80%) ergot incidence. Infection was significantly lower in inflorescences inoculated at earlier flowering stages and was less than 2% in inflorescences inoculated after all stigmas had withered (Table 2).

The effect of preinoculation bagging. The mean ergot incidence in preinoculation-bagged inflorescences was 76%, which is significantly greater than the mean incidence (34%) in inflorescences not bagged prior to inoculation. Means per row varied from 67–81% for preinoculation-bagged inflorescences and from 17–46% from inflorescences not bagged before inoculation. The lower infection in the inflorescences not bagged prior to inoculation was probably due to the protective effects of pollination from surrounding cultivars.

Relative germination rates of pollen and conidia. Pollen germination 1 hr after incubation varied from 7–20% and showed no increase in subsequent observations (Table 3). Conidial germination was not observed before 16 hr of incubation, and it steadily increased up to the final observation at 32 hr (Table 3). Germination of pollen and conidia separately in 10% sucrose solution may not be the same as when they are together on the stigmatic surfaces, but the much more rapid germination of pollen compared with conidia and the effect of pollination on stigma withering is consistent with the observed inhibition of ergot development by pollination.

The effects of pollination and inoculation on stigma

TABLE 1. Percent ergot-infected florets in inflorescences of three male-sterile pearl millet lines subjected to various pollination (poll) and/or inoculation (inoc) treatments in two experiments

Treatment[a]	Infected florets[b] (%)			
	Experiment 1		Experiment 2	
	5141-A	111-A	111-A	5054-A
No inoc, no poll	< 1	< 1	0	0
Inoc, no poll	86	65	60	60
Poll, no inoc	0	< 1	0	0
Poll 24 hr before inoc	2	3	2	3
Poll 8 hr before inoc	< 1	< 1	3	8
Poll immediately after inoc	3	4	3	1
Poll 16 hr after inoc	8	21	7	11
LSD ($P \leq 0.05$)	4	9	6	7

[a] Single operation treatments and the first of double operation treatments were done at the maximum-fresh-stigma flowering stage.
[b] Mean of 20 inflorescences per datum.

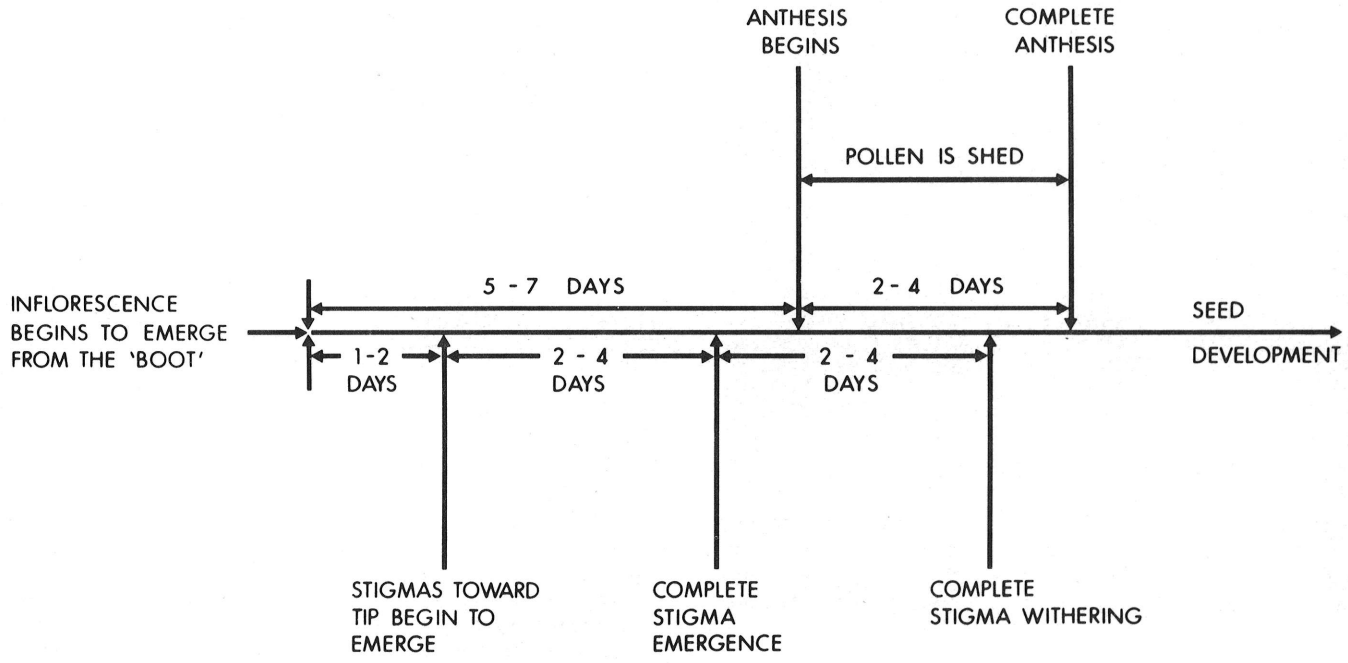

Fig. 1. Time-course of flowering events in pearl millet. Cultivar and environment can affect duration of events within the range indicated.

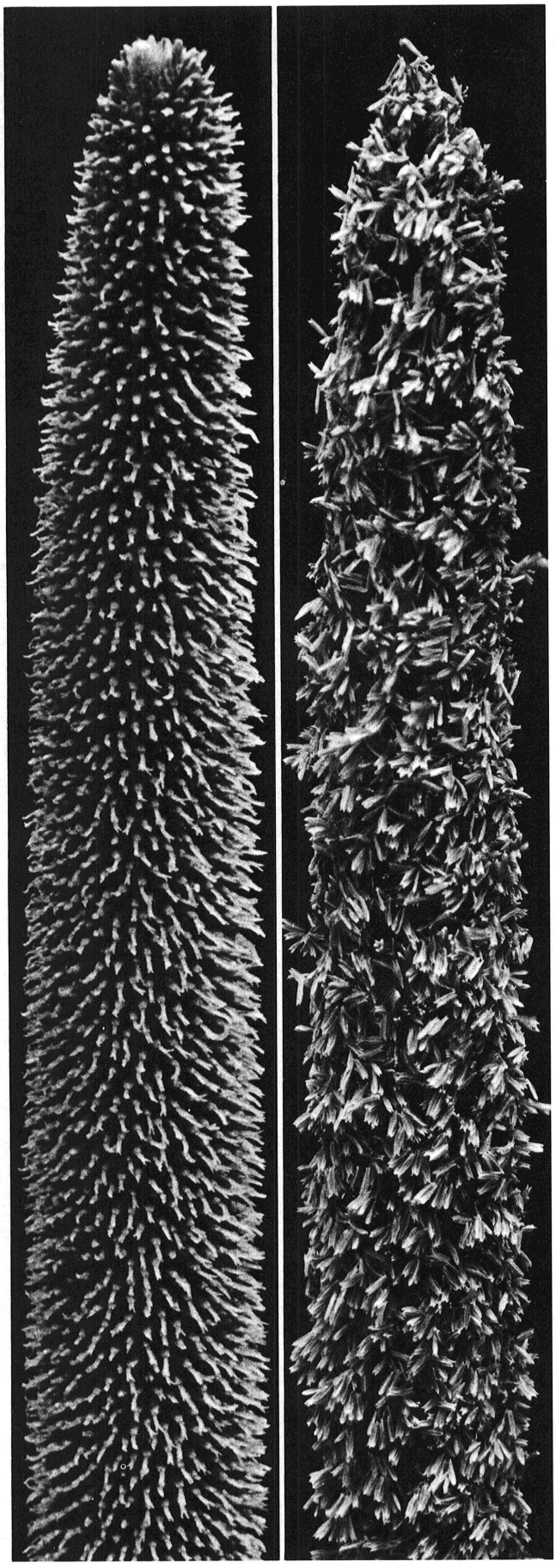

Fig. 2. Pearl millet inflorescences at full stigma emergence (left) and later at anthesis (right).

morphology. Stigmas on inoculated inflorescences and check inflorescences sprayed with water remained long, feathery, turgid, and generally white, with slight tip browing on the "sunny" side of the inflorescence, until the final observation 24 hr after treatment. The stigmas on pollinated inflorescences contracted considerably and curled within 3 hr after pollination (Fig. 4); 8 hr after pollination they were completely brown and withered. Inoculated and water-treated inflorescences cut from field-grown plants and kept at 25 C for 54 hr in an incubator maintained completely fresh long feathery turgid white stigmas, whereas pollinated inflorescences kept in the incubator for the same time had completely brown and withered stigmas (Fig. 5).

DISCUSSION

The major factor affecting successful infection of pearl millet florets by *C. fusiformis* conidia appears to be the availability of fresh receptive stigmas. In the ms lines, ergot infection was greatest in inflorescences inoculated when maximum fresh stigmas occurred and was reduced to a trace in inflorescences inoculated when the stigmas had withered even in the absence of pollination. Reddy et al (17) obtained similar results with pearl millet hybrid HB-1, but they concluded that the effect was due to aging of the ovaries. Our results on the effects of pollination on infection, and the effects of pollination on stigma morphology and longevity indicate that availability of fresh stigmas, and not age of the ovules, is the major factor that determines the success of inoculation. From this, we conclude that the stigmas provide the major infection route for the pathogen. The low levels of ergot on inflorescences inoculated after stigmas had withered suggest that there is another infection route but that it is comparatively unimportant.

There are conflicting reports on the infection path of *C. fusiformis* in pearl millet and other cereals. Reddy et al (17) concluded that ergot infection in pearl millet takes place mainly through ovary walls and rarely through stigmas or styles. Sundaram (21) stated that *C. fusiformis* enters millet ovules through the stigmas and styles, and that direct infection through the ovary walls also is common. Luttrell (13) concluded on the basis of direct histological observation and inoculation studies, that ergot infection of dallisgrass occurs through the stigmas and styles. He cited several conflicting reports on the infection path of *C. purpurea* in cereals and concluded that the early stages in invasion of the ovary have not been adequately documented (13). Pearl millet flowering, which is highly protogynous with large feathery stigmas pushed out between tight glumes, is quite different from flowering in wheat and barley, and thus the ergot infection process for pearl millet also may be quite different.

Other workers have observed that pollination and/or fertilization reduce ergot susceptibility in other cereals, and have presented various hypotheses to explain the interactions (6,7,12,16,23). Although histological studies are needed to test our hypothesis for the mechanism of the interaction of pollination and ergot susceptibility in pearl millet, our results help explain many observations on the disease, and they have important implications

TABLE 2. Percent ergot-infected florets in inflorescences of two male-sterile (ms) pearl millet lines inoculated with a *Claviceps fusiformis* conidial suspension at six flowering stages

	Infected florets[a] on indicated ms line (%)	
Flowering stage at inoculation	111-A	5141-A
Inflorescence emerging from boot	36	32
Stigmas begin to emerge	41	31
Maximum fresh stigmas	80	78
Anthers begin to emerge and stigmas begin withering	15	6
Stigmas withered and anther emergence complete	<1	<1
All stigmas and anthers withered	<1	2
LSD ($P \leq 0.05$)	12	14

[a] Mean of 10 inflorescences per datum.

for resistance screening and control of ergot in pearl millet.

The greater ergot susceptibility of pearl millet F_1 hybrids compared with traditional cultivars is probably caused by delayed pollination. Unlike the traditional cultivars, pearl millet F_1 hybrids have highly synchronous flowering, both in terms of tillers on a plant and plants in a crop. Each inflorescence is protogynous for 2–5 days, so a period occurs when pollen is scarce and there are many inflorescences at the fresh-stigma stage. In addition, the F_1 hybrids generally flower earlier than traditional cultivars and thus more frequently flower during rainy weather which further reduces pollen availability and provides favorable conditions for infection by the ergot pathogen. Incomplete fertility restoration can occur in F_1 hybrids, and is another factor that would lead to decreased effective pollen availability. To save money, farmers in India often replant seed saved from the F_1 hybrid crop. Male sterile plants appear as segregants in the progeny and thus pollen is further reduced.

In screening for ergot resistance the pollen-based escape mechanism must be avoided. In the past, resistance screening has been attempted by either spray or dip-inoculating *open* inflorescences at the fresh-stigma stage. Because flowering in all the genotypes in the screening test is not likely to be synchronous, all but the first flowering inflorescences could be pollinated before or at about the same time as they are inoculated. Thus, escapes may be selected as apparently resistant. We believe that this is why little progress has been made in attempts to select for ergot resistance. In our screening program we now bag all plants at the boot-leaf stage so that inflorescences to be inoculated emerge inside the bags; ie, in a pollen-free environment. The bags are removed briefly during inoculation and immediately replaced.

At least two approaches for ergot control in F_1 hybrids are indicated by these results. Variability exists in the germplasm for protogyny duration. It is therefore possible that a plant with much reduced protogyny, or with overlapping stigma emergence and anthesis, could be produced through crossing and selection. This should increase the probability of rapid pollination of fresh stigmas. The second possibility for control is through the use of a pollen donor. This would be an early-flowering cultivar, whose

TABLE 3. Percent germination over time of pollen and *Claviceps fusiformis* conidia, taken from inflorescences of five pearl millet breeding lines (L1 to L5)[a], and incubated in 10% sucrose in cavity slides maintained in moist chambers at 25 C

Incubation time (hr)	Germination[b] (%)											
	Pollen						Conidia					
	L1	L2	L3	L4	L5	Mean	L1	L2	L3	L4	L5	Mean
1	20	14	12	7	18	14.2	0	0	0	0	0	0
2	20	12	13	8	20	14.6	0	0	0	0	0	0
4	21	12	12	8	17	14.0	0	0	0	0	0	0
8	21	13	11	7	17	13.8	0	0	0	0	0	0
16	...[c]	...	...	...	...	...	1	4	4	3	6	3.6
24	22	13	12	7	16	14.0	3	8	5	21	6	8.6
32	...	...	...	...	...	...	1	10	33	30	23	19.4

[a] L1 = 700130-S-1-1; L2 = J-1623 × WC-6-S-1; L3 = EB-237-3-1-S-2; L4 = EB-137-1-1-S-8; L5 = EB-137-1-S-1.
[b] Each datum is based on examination of 100 pollen grains or conidia.
[c] No observation made.

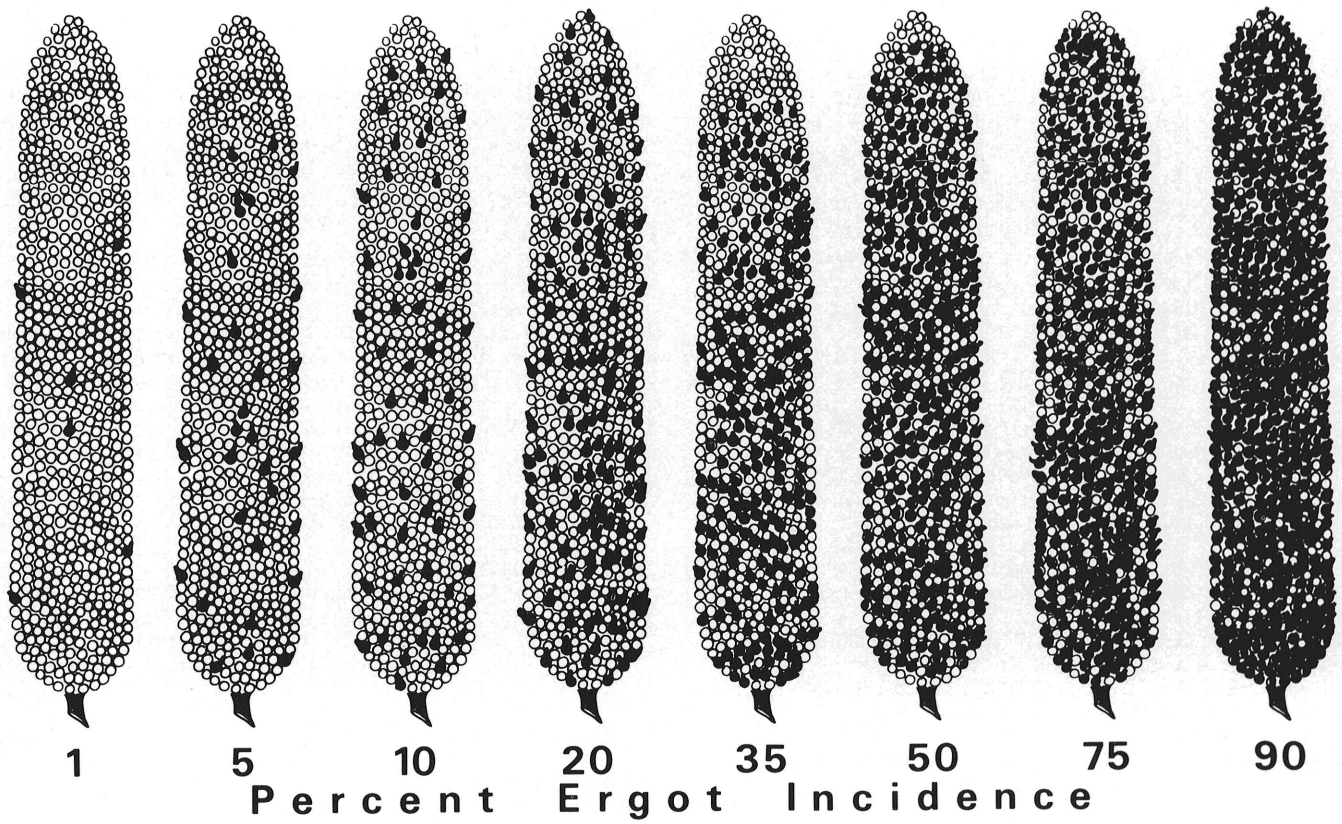

Fig. 3. Standard drawings used to aid estimation of ergot incidence in pearl millet inflorescences.

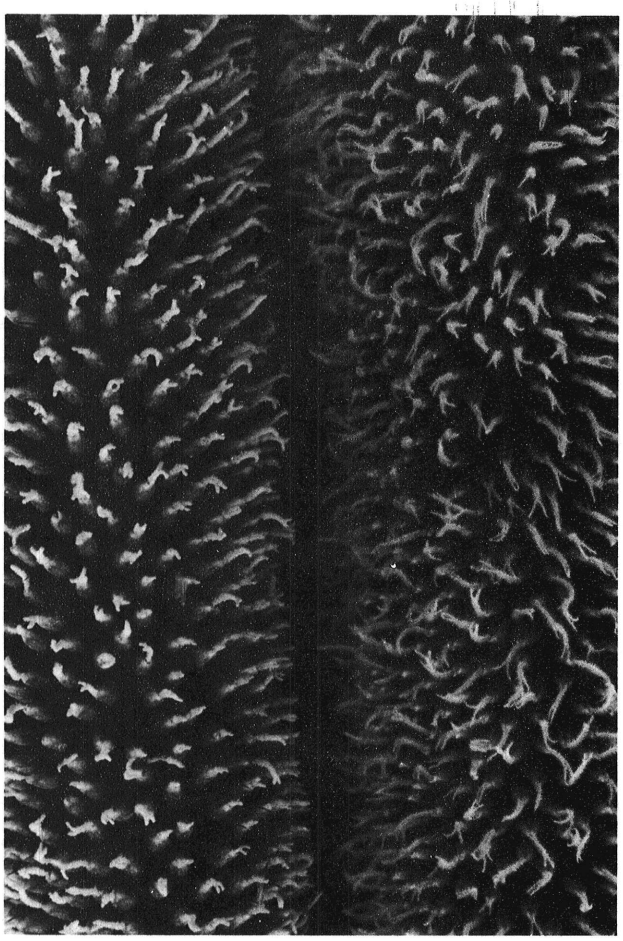

Fig. 4. Pearl millet inflorescences 3 hr after pollination (left) and water spray (right). Note contracted and curled stigmas on the pollinated inflorescence.

major period of anthesis would occur at the time of maximum fresh stigmas of the hybrid, used in a mixture with the hybrid. The variety would have to be resistant to ergot, or be able to escape ergot by means of asynchronous flowering, in order not to also act as an inoculum source. In nonreplicated isolation plots at ICRISAT Center, we have obtained considerable reduction in ergot incidence in a hybrid mixed with an early-flowering cultivar (Williams and Thakur, *unpublished*), but the results need confirmation in replicated trials. If flowering occurs during rainy weather, pollen availability may still be inadequate. Thus, true resistance is needed to assure control of this disease.

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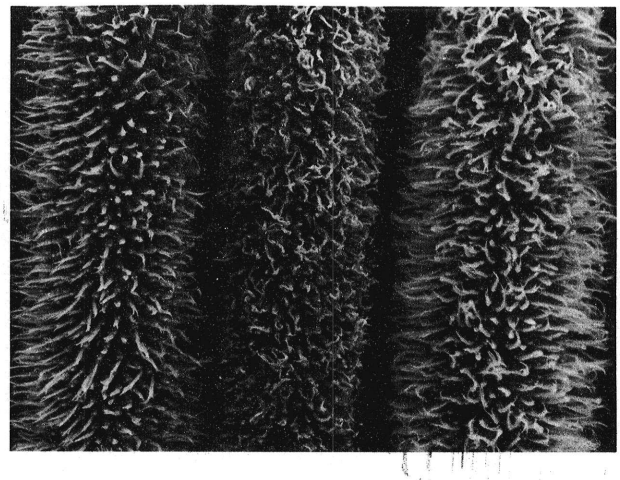

Fig. 5. Pearl millet inflorescences 54 hr after inoculation with *Claviceps fusiformis* conidia (left), pollination (center), and water spray (right). The inflorescences were cut from field-grown plants shortly after treatment and were maintained in the laboratory at 25 C enclosed in parchment paper bags until photographed. Note the long feathery turgid stigmas on the nonpollinated inflorescences and the withered stigmas on the pollinated inflorescence.

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