Postpollination Stigmatic Constriction, the Basis of Ergot Resistance in Selected Lines of Pearl Millet

J. Willingale, P. G. Mantle, and R. P. Thakur

First and second authors, Department of Biochemistry, Imperial College of Science and Technology, London, U.K.; third author, Pearl Millet Improvement Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., A.P. 502324, India.

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ABSTRACT

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Five ergot-resistant pearl millet lines, developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and showing varying degrees of protogyny, and two highly susceptible hybrids were tested for ergot resistance. Where protogyny of florets of individual inflorescences lasted for 48 hr or less, the lines were resistant to ergot infection. Disease escape was mediated through the development of a localized stigmatic constriction that occurred 6 hr after self-pollination. In contrast, stigmas of highly susceptible hybrids remained receptive for up to 6 days, when an aging constriction occurred, located similarly at the pollination-induced abscission site. Development of an aging constriction in stigmas of susceptible lines, prior to self-anthesis, also resulted in self-incompatibility. Extension of the period between stigma emergence and anthesis allows establishment of the pathogen within the unfertilized ovary. Where protogyny lasted for more than 48 hr, resistance could be conferred only by cross-pollination prior to gynoecial aging. It appears that ergot resistance is based on a pollen escape phenomenon linked to normal events occurring during pollination. Breeding at ICRISAT for ergot resistance under high inoculum pressure has resulted in selection of individuals in which stigmas emerge only a few hours before self-pollen is shed. Consequently, stigmatic constriction is rapidly self-induced.

Additional key words: Claviceps fusiformis, Pennisetum americanum.

Infection of the ovary of pearl millet (*Pennisetum americanum* (L.) Leeke) by the ergot fungus, *Claviceps fusiformis* Loveless, occurs through the stigma (6). Subsequent development of ergot sclerotia in place of the seed reduces grain yield and contaminates the cereal with a toxic component. Ergot disease is significant in millet in both India and Africa, and a search for effective control has therefore been an objective in tropical agricultural research.

Although *Claviceps* spp. cause ergot diseases in diverse crops such as wheat, barley, rye, triticale, maize, sorghum, and millet, there is no consistent evidence of genetic sources of somatic resistance within any of the Gramineae that ensures protection against the particular *Claviceps* spp. or biotype that normally parasitizes that crop plant. Therefore, at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the systematic selection of pearl millet genotypes that show markedly reduced susceptibility to *C. fusiformis* (3) offers notable progress in the search for effective control of ergot. The strategy employed had been the repeated empirical process of interbreeding between relatively less susceptible plants and screening each new generation under high disease pressure.

Subsequent experiments at ICRISAT have shown that cross-pollination prior to inoculation with the ergot pathogen substantially reduces the severity of ergot disease both in male-sterile lines (2) and in hybrids (4). However, the exact nature of this escape mechanism is unclear.

The first evidence that ergot resistance in selected pearl millet lines was based solely on a pollination-induced stigmatic constriction phenomenon, and not a specific gene-for-gene interaction between host and pathogen, came from experiments conducted in the United Kingdom in 1983. The resistant lines ICMPE 13-6-30 and 134-6-9, grown in open plots, became as heavily infected as the susceptible hybrid BJ104 under the cooler conditions that delayed anthesis.

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Subsequent formulation of a rational explanation that integrates the various findings at ICRISAT with the observations in the United Kingdom was initially impeded by the absence of any study in depth of the infection process of *C. fusiformis* in millet. In the course of correcting this deficiency (6), a stigmatic constriction, occurring in pearl millet within a few hours after pollination, was recognized for the first time (5). The constriction also occurs in aging unpollinated stigmas and shortly after infection by *C. fusiformis* in the absence of pollination (6). At the time, the discovery of postpollination stigmatic constriction appeared to reveal a unique feature, but an analogous occurrence has recently been described in maize (1). These novel findings allow the present explanation of the mechanism operating in the selected pearl millet cultivars that show resistance to ergot. However, in order to integrate the constriction phenomenon into ovary disease biology and host resistance, it was necessary first to make a critical reappraisal of the time course of flowering of pearl millets.

MATERIALS AND METHODS

Ergot-resistant cultivars of pearl millet (ICMPE 13-6-30, 134-6-9, 134-6-34, 13-6-27, 37, 71) were grown in open plots at ICRISAT Centre, Hyderabad, India, and variously studied during the rainy season in 1983 and as part of the dry season crops in 1984 and 1985. Comparisons were made with the susceptible hybrids BJ 104 and BK 560 and the male-sterile line 5141 A.

Plant inoculation. Individual tillers were bagged at the boot stage, while the inflorescence was enclosed by the flag-leaf sheath, to ensure protection from both foreign pollen and ergot spores when they finally emerged. Inflorescences were then inoculated when most of the stigmas were exserted (termed "full protogyny" [5]) by spraying with a conidial suspension prepared from honeydew taken from ergot-infected inflorescences. Inoculated inflorescences were rebagged immediately to prevent cross-pollination (2,4), except in a proportion of individuals left unbagged to demonstrate the effect of this treatment. In hybrids, full inflorescence protogyny was not reached until 2–3 days after first stigma emergence. The anthers emerged 4–5 days later. In contrast, full inflorescence protogyny was never reached in some

resistant lines where anthers emerged at the top of the inflorescence soon after stigmas had just emerged in the middle region. Therefore, to facilitate infection by allowing the maximum time for the inoculum to reside on stigmas before self-pollination within the inflorescence, inoculum was applied when the first stigmas emerged.

Observation of flowering biology and pathology. The dynamics of flowering biology in the various pearl millet cultivars was studied by frequent observation and regular sampling (12, 16, 24, 36, 48, 72, and 96 hr after inoculation) for laboratory dissection, so that the times of first stigma emergence, full protogyny, self-anthesis, and stigmatic constriction could be recorded for individual inflorescences of all the cultivars. Since bagging of the inflorescence, being an integral part of the routine procedure, restricted observation, the flowering sequence in this situation was seen as a series of "frames," and not as a continual process. Consequently, a parallel series of unbagged ears were monitored to confirm that a closed environment did not significantly affect the relative timings of stigma emergence and anthesis. The term "inflorescence protogyny" has been introduced to describe the predominant emergence of stigmas in hermaphroditic florets throughout the inflorescence prior to the appearance of anthers from both hermaphroditic and staminate florets.

Some floret samples taken for laboratory investigation were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 8% (w/v) sucrose, for 2 hr at 23 C or for 24 hr at 4 C. The relative positions of pollen tubes and hyphae of *C. fusiformis* within the gynoecium were determined by light and fluorescence microscopy of appropriate sections or whole-mount preparations (5).

RESULTS

Comparison of the flowering sequences of resistant and susceptible cultivars. Thakur and Williams (2) previously illustrated the generalized flowering sequence in pearl millet (Fig. 1A). However, there is considerable variation in the timing of flowering events between different cultivars, even under the same environmental conditions. The male-sterile line 5141 A and the ergot-susceptible hybrid BJ 104 have a protracted temporal separation between stigma emergence and anthesis. In BJ 104, for example, the stage at which 75% of the florets have stigmas extruded was not reached until 4 days after the first stigmas emerged (Fig. 1B). In the absence of cross-pollination, the exserted stigmas remained receptive to pollen for a further 2–3 days. Five or 6 days after the first stigmas of an inflorescence emerged, gynoecial aging became evident, marked by a localized constriction in the fused portion of the stigmas (5). The protruding stylodia distal to the constriction remained white and turgid throughout the development of the constriction and for a few hours thereafter. Anthesis occurred only after aging stigma constriction had been initiated in BJ 104, and so, in practice, this cultivar is self-incompatible (5), relying on cross-pollination, as does male-sterile 5141 A. Duration of protogyny was recorded for 20 ears of each resistant line.

In contrast to susceptible hybrids, all the ergot-resistant lines possessed very short periods of protogyny (Fig. 2A–D). The maximum mean time between first stigma emergence and self-anthesis in the resistant inflorescences was 56 hr. The period was usually much shorter, and in some cases (e.g., ICMPE 13-6-30 and 134-6-9), plants were even protandrous by about 1 hr (Fig. 2E). The maximum mean time might appear long and give a misleading idea of the duration of protogyny that affects resistance. There is a wide range of periods of protogyny (0–96 hr) within a single inbred line of pearl millet. Similarly, there is a wide range of ergot severity (0–85%). These two means are calculated from populations and cannot be applied to individuals. Stigmatic constriction due to aging never occurred in resistant cultivars, because constriction was routinely induced by self-pollination and was evident after 6 hr. Similarly, aging stigma constriction was never seen in unbagged ears of both resistant and susceptible cultivars because of the abundance of airborne pollen that gave rise to stigma constriction within 8 hr of stigma emergence, withering being prominent after 12 hr.

Summary of the time course of ergot infection in the absence of pollination in male-sterile 5141 A and BJ 104. Ergot honeydew conidia germinated on the stigma surface within 12–16 hr after inoculation. After 24 hr, the stylodia were withered from the tip down and hyphae were evident within the transmission tracts of the fused stigmas. After 30 hr, there was complete collapse of cells in

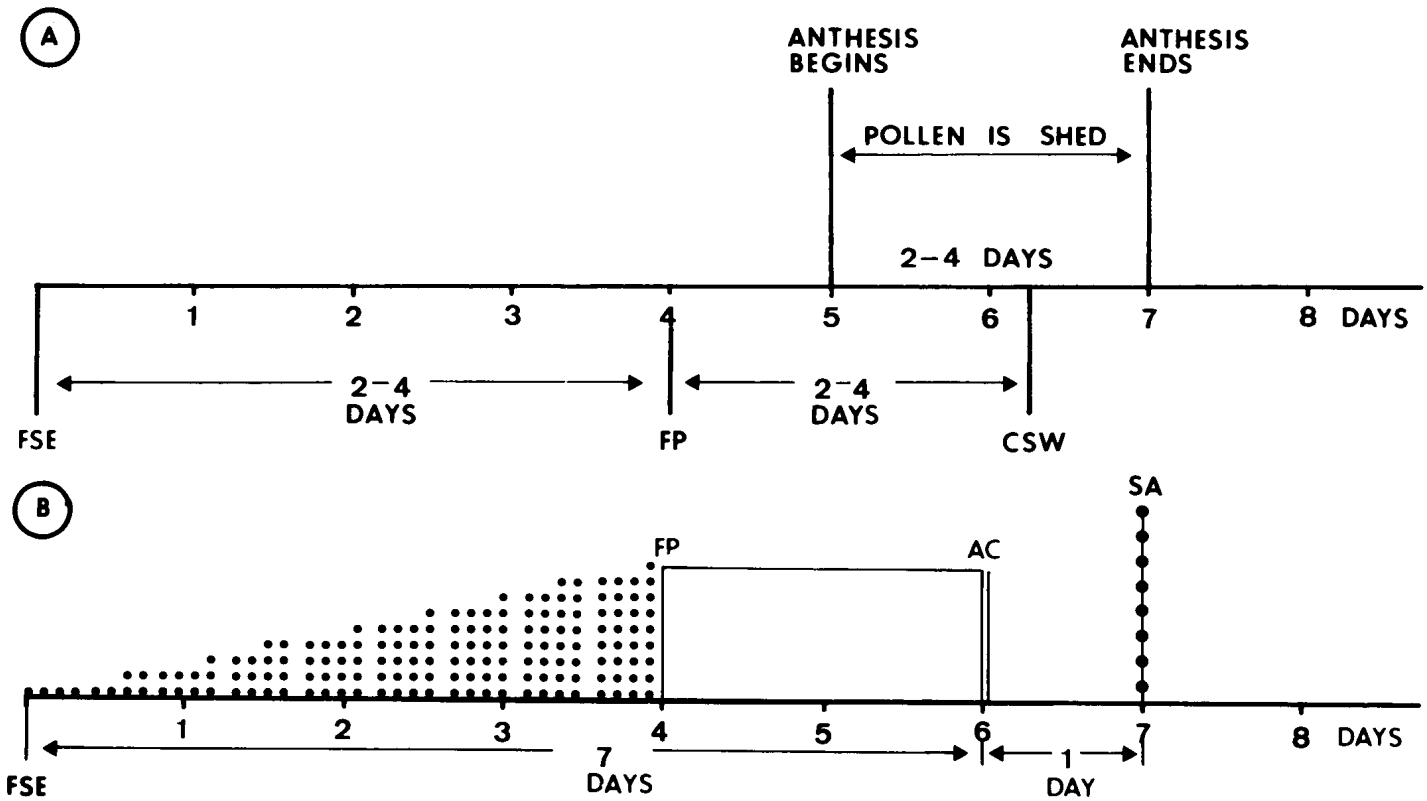

Fig. 1. A, A generalized scheme for the time course of flowering events in pearl millet (after Thakur and Williams [2]). **B,** The flowering sequence of the ergot-susceptible hybrid BJ 104. Abbreviations: FSE, first stigma emergence; FP, full protogyny; CSW, complete system withering; AC, constriction due to stigma aging; and SA (dotted line), self-anthesis. Dotted areas represent cumulative stigma protrusion.

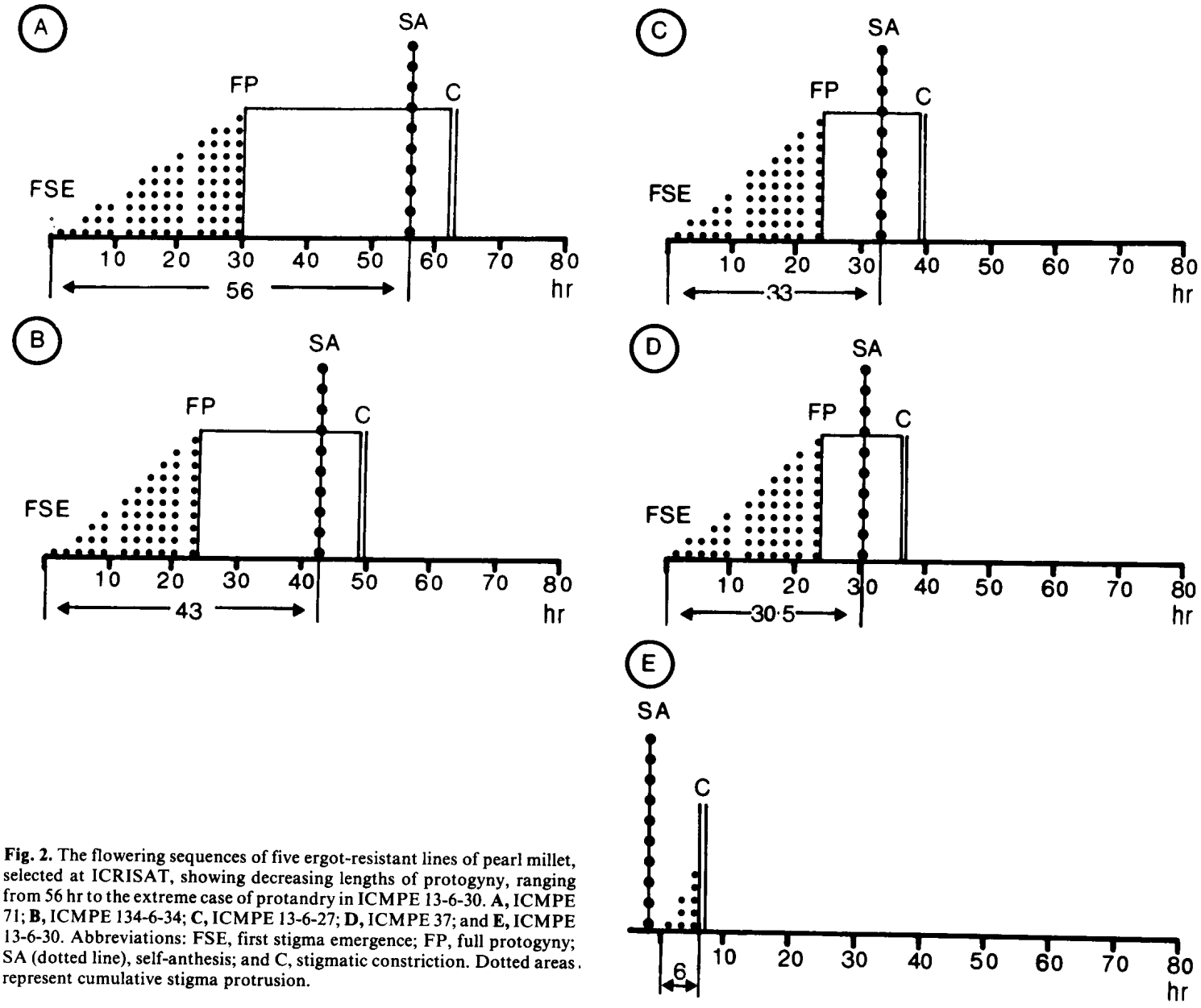

Fig. 2. The flowering sequences of five ergot-resistant lines of pearl millet, selected at ICRISAT, showing decreasing lengths of protogyny, ranging from 56 hr to the extreme case of protandry in ICMPE 13-6-30. **A**, ICMPE 71; **B**, ICMPE 134-6-34; **C**, ICMPE 13-6-27; **D**, ICMPE 37; and **E**, ICMPE 13-6-30. Abbreviations: FSE, first stigma emergence; FP, full protogyny; SA (dotted line), self-anthesis; and C, stigmatic constriction. Dotted areas represent cumulative stigma protrusion.

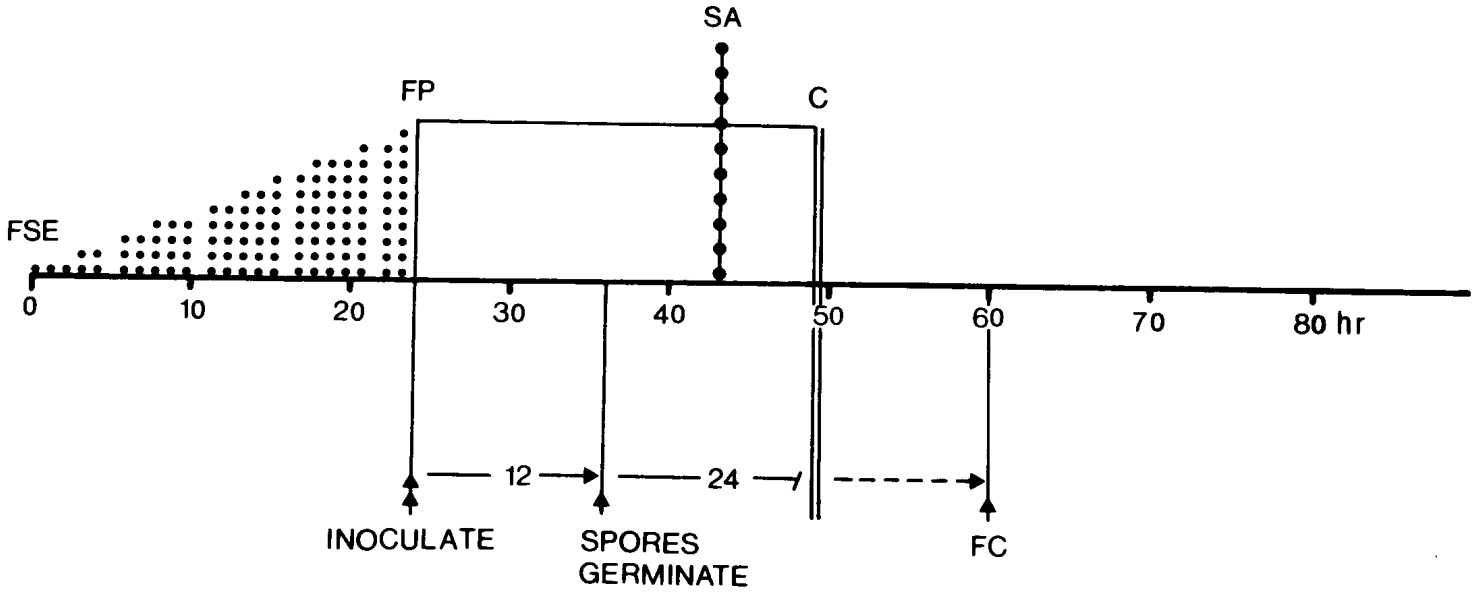

Fig. 3. The window of ergot infection for pearl millet is shown superimposed on the flowering sequence of ICMPE 134-6-34. Even though the period of protogyny is 43 hr, there is insufficient time to allow successful ergot infection before self-induced stigmatic constriction occurs. Abbreviations: FSE, first stigma emergence; FP, full protogyny; SA (dotted line), self-anthesis; C, stigmatic constriction; and FC, constriction due to fungal infection. Dotted areas represent cumulative stigma protrusion.

the constriction region and fungal hyphae had reached the outer integuments of the ovary. By 36 hr, infection had reached the base of the ovary, still confined to the integuments, but particularly congregated around the main vascular tracts. Honeydew production commenced 4–5 days after inoculation, marking the full establishment of ovary infection and the beginning of sphacelial fructification.

Influence of self-pollination on infection by *C. fusiformis*. When millet cultivars were inoculated at either first stigma emergence or full protogyny and rebagged immediately, resistant cultivars developed ergot infection in $<3\%$ of the florets, whereas at least 80% of the florets of susceptible cultivars were infected.

DISCUSSION

In pearl millet, extension of the feathery stigmas beyond the confines of the glumes provides the surface for capture of both pollen and fungal spores. The primary path of entry for ergot is directly down the stylodia, mimicking pollination (6). Invading hyphae and pollen tubes are, therefore, in direct competition for penetration points on the stigma surface and for the specialized tracts forming the transmission tissue.

Ergot infection can occur only if the stigmas remain fresh long enough to enable ergot conidia to germinate and the penetrating hyphae to pass down the stylodia. Once stigmatic constriction due to the fungal infection has been initiated 36 hr after inoculation (6), the stigma is completely unreceptive to pollen and the ovary is, in effect, isolated from the stigma.

Highly ergot-susceptible pearl millet hybrids show prolonged periods of protogyny, and if they are protected from cross-pollination, their stigmas remain fresh until aging constriction develops prior to self-anthesis. In these plants, escape from applied ergot inoculum can be mediated only by prior cross-pollination.

The period required for a stigma to be infected by *C. fusiformis* is usually 36–48 hr in the tropics (6). This period is the "window of infection." Stigmas that remain fresh in the absence of cross- or self-pollination for 48 hr or more are potentially at risk from ergot. When the "window" is shortened, escape from ergot becomes likely (Fig. 3).

The phenomenon of postpollination stigmatic constriction seems to be ubiquitous among pearl millets. It provides a unique mechanical barrier to invasion of the fertilized ovary by fungal pathogens. In contrast, wheat or rye ovaries cease to be susceptible to their ergot pathogen (*C. purpurea*) several days after fertilization, probably as a result of induced changes in ovary structure. The 6-hr postpollination stigmatic constriction in millet preempts dependence on gradual postfertilization changes in the ovary and provides a rapid and efficient mechanical barrier to ovary pathogens for this tropical cereal. It is, therefore, important to exploit this natural feature in the breeding of ergot-resistant cultivars for commercial use.

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