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Effectiveness of Ethrel as a Male Gametocide in Pearl Millet and its Influence on Ergot

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With 5 tables

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Abstract

In field and greenhouse experiments Ethrel (2-chloroethyl phosphonic acid) was tested for its male gametocidal effects on pearl millet (*Pennisetum americanum*) and its subsequent effects on ergot development. Application of Ethrel at 2000 ppm at late boot or early protogyny was the most effective for inducing male sterility in the hybrid, BJ 104. Female fertility in a male sterile line, however, was not affected by Ethrel treatment. Ethrel at 2000 ppm applied at the late boot stage resulted in partial panicle exsertion, and reduced plant height and panicle length. *In vitro* Ethrel (2000 ppm) completely inhibited pollen germination but did not affect germination of conidia of *Claviceps fusiformis*, the causal agent of ergot of pearl millet. Ergot resistance or susceptibility in pearl millet lines was not affected, probably because Ethrel could not induce complete male sterility.

Key words: *Pennisetum americanum* — *Claviceps fusiformis* — Ethrel — gametocide — induced male sterility — ergot

Ethrel (2-chloroethyl phosphonic acid) has been used as a male-gametocide since 1969 when foliar-applied liquid ethephon increased the number of pistillate flowers in monoecious cucumbers (MCMURRAY and MILLER 1969, ROBINSON et al. 1969). Experimental use of Ethrel to induce male sterility in hybrid breeding has been reported in several crops (ROWELL and MILLER 1971, STOSKOPF and LAW 1972, BROWN and EARLEY 1973, HUGHES et al. 1974, FAIREY and STOSKOPF 1975, VERMA and KUMAR 1978, DOTLACIL and APLTAUEROVA 1978, BANGA and LABANA 1984). There is, however, no report of use of Ethrel in pearl millet, *Pennisetum americanum* L. Leeke, probably because pearl millet is a highly cross-pollinated crop and cytoplasmic-genetic male sterility is readily available for hybrid production.

Ergot, caused by *Claviceps fusiformis* Loveless, is an important disease of pearl millet. Infection occurs through fresh stigmas by germinating conidia, and ovaries are colonized to produce sclerotia in place of seed. There is a strong competition between pearl millet pollen and *C. fusiformis* conidia on the stigmatic surface for entry into the ovary (THAKUR and WILLIAMS 1980, WILLINGALE et al. 1986). Under normal conditions pollination has often been more successful than ergot infection, and pollination even 16 h after inoculation has been effective in significantly reducing ergot infection (THAKUR and WILLIAMS 1980). Most of the ergot-resistant lines that have been developed by pedigree breeding (THAKUR et al. 1982) exhibit short protogyny, i.e., the time interval between stigma emergence and initiation of anthesis on a given panicle, < 48 h in resistant lines compared with > 48 h in susceptible lines (WILLINGALE et al. 1986). This suggests that resistance to ergot in individual panicles is more likely to be self-pollination induced under bagged conditions. If Ethrel could induce complete male sterility, then the effect of self-pollination in relation to ergot resistance could be determined.

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In this paper we report the results of several field and greenhouse experiments on the effectiveness of Ethrel as a male gametocide and its influence on ergot severity in pearl millet.

Materials and Methods

Effect of Ethrel concentrations at different stages of flowering on male fertility: In a greenhouse, panicles of pot-grown plants of a hybrid, BJ 104, were treated with six concentrations of Ethrel, 0, 1000, 1500, 2000, 2500, and 3000 ppm at five different stages of flowering, early boot (panicle enclosed in leaf sheath), late boot (initiation of panicle emergence from the leaf sheath), early protogyny (stigma initiation stage), full protogyny (> 75% stigma emergence), and early anthesis (initiation of anther emergence). The experiment was conducted in a completely randomized design (CRD) with three replications. Five panicles were treated for each concentration at each flowering stage. Ethrel or water (as control) were injected into the boot at the early or late boot stage (5 ml plant^{-1}), and sprayed uniformly onto exserted panicles at other stages of flowering. Panicles were protected from cross-pollination and other contamination by bagging them at the boot stage with parchment selfing bags and rebagging immediately after the treatment.

Observations were recorded for per cent sterile florets on individual panicles 15—20 days after Ethrel application. Data were analyzed to determine separately the treatment effects and their interaction.

Effect on pollen viability: In a greenhouse experiment, panicles of pot-grown plants of two pearl millet genotypes, ergot-resistant ICMPES 1 and ergot-susceptible 5141 B, were treated with 2000 ppm Ethrel at late boot and early protogyny stages as described above. Ten panicles were treated in each genotype with untreated controls. At anthesis pollen was collected from each panicle by tapping, and protogynous panicles of a pot grown male sterile line, 5141 A, were pollinated and bagged. Pollen collected from one panicle was used to pollinate a single panicle of the male sterile line. Pollination was done during the morning (8.00—10.00 h) when most pollen grains are believed to be viable (AYYANGAR et al. 1933). Observations were recorded on per cent seed set 20 days after pollination.

Effect on female fertility: Panicles of pot-grown plants of 5141 A were treated with 2000 ppm Ethrel at late boot, early protogyny, and full protogyny as described before. At each stage, 15 panicles were treated with 15 untreated as controls. Pollen from the corresponding maintainer line, 5141 B, was used to pollinate the treated and untreated panicles at full protogyny. Observations were recorded on per cent seed set 20 days after pollination.

Effects on pollen and conidial germination: Pollen from ICMPES 1 and conidia of *C. fusiformis* were subjected to 2000 ppm Ethrel, 10% (w/v) sucrose in water, and 10% (w/v) sucrose in 2000 ppm Ethrel, in cavity slides. For each treatment, five cavity slides were used to germinate pollen and conidia. Slides were incubated at 25°C and germination of pollen after 7 h of incubation and of conidia after 24 h of incubation were observed on twenty microscopic fields.

Effect on ergot development: In a field experiment panicles of two ergot-susceptible genotypes, 5141 A and 5141 B, and two ergot-resistant genotypes, ICMPE 13-6-30 and ICMPE 134-6-34 were treated with 2000 ppm Ethrel at late boot, early protogyny, and full protogyny, as described before. Ten panicles were treated at each stage. At the full protogyny stage Ethrel-treated panicles were spray-inoculated with *C. fusiformis* spore suspension (10^6 conidia ml^{-1}) by briefly opening the bags. Ethrel-sprayed and uninoculated panicles were maintained as controls. In the second field experiment, Ethrel (2000 ppm) treated panicles of two ergot-susceptible (BJ 104, 5141 B) and two ergot-resistant (same as above) lines at the early protogyny stage, were either inoculated with ergot at the full protogyny stage or left uninoculated. In each treatment 60—90 panicles were used. High humidity was maintained by periodically providing overhead sprinkler irrigation to promote ergot infection. Observations were recorded on ergot severity (THAKUR and WILLIAMS 1980) and per cent sterile florets 20 days after inoculation.

Effect on plant growth: In a field experiment, 20 panicles each of ICMPE 13-6-30 and BJ 104 were treated with Ethrel (2000 ppm) at the late boot stage and bagged with parchment selfing bags. Water-injected and bagged panicles were maintained as controls. Observations were recorded for plant height, panicle length, and panicle exsertion 20 days after treatment.

Statistical analyses were performed on arcsin transformed values of per cent data.

Results

Effect of Ethrel concentration at different stages of flowering on male fertility

There were significant differences ($P \leq 0.01$) for induced sterility at different Ethrel concentrations and stages of flowering, but the interactions were not significant. Application of Ethrel at 2000 ppm at late boot and early protogyny produced sterility in 95—99% of the

Table 1. Effect of Ethrel concentrations at different stages of flowering on male fertility in a pearl millet hybrid, BJ 104

Ethrel concentration (ppm)	Sterile florets (%) at flowering stages					
	Early boot	Late boot	Early protogyny	Full protogyny	Early anthesis	Mean
0	15	34	34	15	15	23
	(22)[a]	(35)	(35)	(22)	(22)	(27)
1000	67	50	56	51	74	60
	(57)	(45)	(48)	(46)	(59)	(51)
1500	76	72	71	56	42	63
	(65)	(58)	(57)	(48)	(40)	(54)
2000	76	95	99	83	40	79
	(65)	(80)	(85)	(66)	(39)	(66)
2500	74	90	66	62	29	64
	(64)	(77)	(57)	(52)	(33)	(57)
3000	52	67	68	63	16	53
	(46)	(57)	(62)	(53)	(23)	(48)
Mean	60	68	66	55	36	
	(53)	(59)	(57)	(48)	(36)	

SE conc. ± 5.3 (3.5); SE stages ± 6.9 (5.1)
[a] = Arcsin transformed values

florets (Table 1). In general, Ethrel was less effective at early boot, full protogyny, and early anthesis than at late boot and early protogyny. Application of Ethrel at concentrations higher than 2000 ppm did not significantly increase the degree of male sterility.

Effect on pollen viability

Hand pollination with pollen from plants of both genotypes, ICMPES 1 and 5141 B, treated with Ethrel at late boot, produced very little seed (0—1 %) on 5141 A compared with those treated at early protogyny and the untreated control (Table 2). Ethrel application at early protogyny proved less effective than at late boot stage.

Effect on female fertility

Application of Ethrel (2000 ppm) at three different stages of flowering, late boot, early protogyny, and full protogyny in 5141 A and followed by pollination with pollen from untreated 5141 B line produced 37—68 % seed set, results that were not significantly different from the control.

Table 2. Extent of seed set in a male sterile line 5141 A hand-pollinated with pollen from two male fertile lines treated with Ethrel (2000 ppm) at two stages of flowering

Ethrel application at flowering stage	Seed set (%)			
	ICMPES 1		5141 B	
	Treated	Control	Treated	Control
Late boot	0 (0)[a]	84 (68)	1 (2)	74 (62)
SE		± 3.8 (3.1)		± 8.4 (6.5)
Early protogyny	44 (39)	77 (63)	60 (51)	69 (58)
SE		± 13.4 (10.0)		± 14.5 (11.0)

[a] = See Table 1

Table 3. Effect of Ethrel (2000 ppm) treatment at different stages of flowering on ergot development in four pearl millet genotypes, under field conditions

Ethrel treatment at the flowering stage	Mean ergot severity (%) 5141 A	5141 B	ICMPE 13-6-30	ICMPE 134-6-34
Late boot	69	75	0	0
	(58)[a]	(60)	(0)	(0)
Early protogyny	83	81	1	5
	(70)	(69)	(5)	(10)
Full protogyny	62	80	2	0
	(53)	(65)	(6)	(0)
Control	87	64	5	2
	(71)	(55)	(10)	(6)
SE	± 12.7	± 12.6	± 1.1	± 1.3
	± (9.4)	± (10.0)	± (2.1)	± (2.2)

[a] = See Table 1

Effects on pollen and conidial germination

Ethrel (2000 ppm) in water or in 10% sucrose solution inhibited pollen germination completely compared with 46% germination in 10% sucrose solution. Germination of *C. fusiformis* conidia in Ethrel was not significantly different from that in 10% sucrose solution, although germination in Ethrel in 10% sucrose was significantly less than in Ethrel alone.

Effect on ergot development

Ethrel did not affect ergot development in either susceptible or resistant genotypes. The two susceptible genotypes, 5141 A and 5141 B treated with Ethrel (2000 ppm) showed ergot severity levels comparable to the control. Similarly Ethrel-treated panicles of two resistant genotypes, ICMPE 13-6-30 and ICMPE 134-6-34, showed the ergot severity levels similar to the control (Table 3). In another experi-

Table 4. Effect of Ethrel (2000 ppm) treatment and ergot inoculation at different stages of flowering on induction of male sterility and ergot severity in two ergot susceptible (BJ 104, 5141 B) and two ergot resistant (ICMPE 134-6-34, ICMPE 13-6-30) lines of pearl millet

Treatment	Sterile florets (%) BJ 104	5141 B	ICMPE 134-6-34	ICMPE 13-6-30	Ergot severity (%) BJ 104	5141 B	ICMPE 134-6-34	ICMPE 13-6-30
Ethrel at early protogyny	85	61	75	84	<1	<1	0	0
	(67)[a]	(51)	(60)	(67)	(2)	(3)	(0)	(0)
Ethrel at early protogyny and ergot at full protogyny	7	4	75	75	87	82	<1	1
	(16)	(11)	(60)	(60)	(69)	(65)	(4)	(5)
Ergot at full protogyny	6	2	20	19	91	78	1	1
	(15)	(8)	(26)	(26)	(73)	(62)	(5)	(6)
Control (selfing)	54	3	12	10	<1	<1	<1	<1
	(47)	(9)	(20)	(19)	(1)	(1)	(1)	(1)
SE	± 2.1	± 1.4	± 4.2	± 2.2	± 0.9	± 0.6	± 0.05	± 0.3
	±(1.5)	±(2.5)	±(3.1)	±(1.6)	±(0.4)	±(0.3)	±(0.2)	±(1.0)

[a] = See Table 1

Table 5. Effect of Ethrel (2000 ppm) application at late boot on plant growth on two cultivars of pearl millet under field conditions

Cultivar		Plant height (cm)[a]	Panicle length (cm)[a]	Panicle exsertion
ICMPE 13-6-30	Treated	70	21	partial
	Control	99	22	complete
	SE	± 2.0	± 0.4	
BJ 104	Treated	45	13	partial
	Control	64	14	complete
	SE	± 1.5	± 0.3	

[a] = Mean of 20 plants/treatment

ment, when Ethrel-treated panicles (at early protogyny) were inoculated with ergot (at full protogyny), high levels of ergot developed in two susceptible genotypes (82—87% severity), but the resistant genotypes had only ≤ 1% ergot, although they showed 75% male sterility (Table 4).

Effect on plant growth

Ethrel-treated panicles (at late boot) of ICMPE 13-6-30 and BJ 104 showed significant reduction in plant height, panicle length, and panicle exsertion from the boot-leaf sheath compared to the untreated plants. In both genotypes there were reductions of about 30% in plant height, and 5% in panicle length, with partial panicle exsertion (Table 5).

Discussion

The results have clearly demonstrated the potential of Ethrel as a male gametocide in pearl millet, although complete sterility could not be achieved. The chemical behaved as a true male gametocide and did not affect female fertility. The minimum effective concentration of Ethrel was 2000 ppm and the critical flowering stage of application was late boot or early protogyny. Since Ethrel application at late boot resulted in partial panicle exsertion, the early protogyny stage (which succeeds the late boot stage) seems to be the most appropriate time for Ethrel application to induce maximum male sterility in pearl millet. The inconsistency in the levels of induced male sterility in different genotypes in different experiments (Tables 1, 2, and 4) may be attributed to several factors, including differential genotypic responses to Ethrel and variations in the age of the florets in different parts of the same panicle at the time of Ethrel application. Incomplete sterility from Ethrel application was also reported in wheat (*Triticum aestivum* L.) (Lucas 1972) and barley (*Hordeum vulgare* L.) (Nicholls and May 1963), and this effect was attributed to the differences in the age of the florets in the spikelets and among the tillers of the same plant. To obtain maximum male gametocidal response, Ethrel should be sprayed before meiosis is initiated in the oldest florets of the panicles (Bennett and Hughes 1972, Hughes et al. 1974). In our study application of Ethrel at late boot had negative effects on plant growth in terms of height, panicle exsertion and its length. Similar results were reported on corn (*Zea mays* L.) (Earley and Slife 1969) and soybean (*Glycine max.* L.) (Slife and Earley 1970).

Ethrel application in both ergot resistant and susceptible genotypes induced male sterility by affecting pollen viability, but did not influence the ergot severity levels. This result was confirmed by *in vitro* experiments on germination of pollen and conidia, wherein pollen germination was completely inhibited but conidial germination was not affected. In an ergot-susceptible genotype wherein anthesis occurs much after inoculation, self-pollination does not interfere with ergot infection. In an ergot-resistant genotype, on the other hand, where rapid self-pollination induces stigmatic constriction and prevents ergot infection (Willingale and Mantle 1985, Willingale et al. 1986), Ethrel treatment would be expected to

prevent self-pollination completely (by rendering pollen nonviable) and subsequently to increase ergot severity; but these results were not obtained. In ergot-resistant lines where maximum sterility was 75—84% (Table 4), 16—25% florets were still fertile and there were sufficient pollen grains from these fertile florets to self-pollinate the florets and reduce ergot severity. In this case, therefore, it was not possible to eliminate completely the effect of self-pollination on imparting ergot resistance in the test lines. Two recently developed ergot-resistant male sterile lines, however, show complete sterility (no evidence of fertile pollen) and have given consistently high levels of ergot resistance (RAI and THAKUR, unpublished data).

Although Ethrel could not induce complete male sterility in pearl millet lines tested in this study, its potential of inducing male sterility could be realized in the production of pearl millet hybrid seed. One of the major problems in hybrid seed production in pearl millet is pollen-shedding, which impairs the purity of male sterile lines. A few fertile sectors in some panicles of male sterile lines produce pollen, the amount of which varies in different environments. This variability causes a serious problem in producing true hybrid seed where a few promising, high yielding F_1 hybrids can be produced on male sterile lines that have marginally more pollen shedders. Application of Ethrel at 2000 ppm at early protogyny could help overcome this problem, and complete male sterility could be obtained by affecting the fertile sectors in the male sterile lines. The potential and economic viability of this method, however, remains to be determined.

Zusammenfassung

Die Wirkung von Ethrel als Gametozid bei der Perlhirse und sein Einfluß auf die Mutterkornbildung

In Feld- und Gewächshausversuchen wurde die gametozide Wirkung von Ethrel (2-Ethylchlorid-Phosphonsäure) auf die Perlhirse (*Pennisetum americanum*) und sein Einfluß auf die Entwicklung von Mutterkorn geprüft. Bei Anwendung von 2000 ppm Ethrel zu einem Zeitpunkt, wenn sich die Rispen noch in den Blattscheiden befinden oder die Narben beginnen, empfängnisbereit zu sein, konnte bei der Hybride BJ 104 ein Höchstmaß an männlicher Sterilität erzeugt werden. Durch die Behandlung mit Ethrel wurde die weibliche Fertilität in einer männlich sterilen Linie nicht berührt. Die Anwendung von 2000 ppm Ethrel zum Zeitpunkt des Rispenschiebens hatte zur Folge, daß sich die Rispen nur zu einem Teil entfalteten und die Länge der Pflanzen und Rispen verringert wurde. *In vitro* angewendet verhinderte Ethrel (2000 ppm) die Keimung der Pollen vollständig; aber es vermochte nicht, das Auskeimen der Konidien von *Claviceps fusiformis*, der die Mutterkornbildung bei Perlhirse verursacht, zu beeinträchtigen. Durch die Anwendung von Ethrel konnte weder die Resistenz noch die Anfälligkeit gegenüber Mutterkorn verändert werden, was wahrscheinlich darauf zurückzuführen ist, daß mit Ethrel keine vollständige männliche Sterilität erreicht werden kann.

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