Euphytica **122:** 391–395, 2001.

Cytoplasmic male-sterility and source of pollen influence the expression of resistance to sorghum midge, *Stenodiplosis sorghicola*

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Received 18 August 2000; accepted 12 February 2001

Key words: plant resistance, pollen effects, resistance mechanisms, *Sorghum bicolor*, *Stenodiplosis sorghicola*, sorghum midge

Summary

Sorghum midge, *Stenodiplosis* (*Contarinia*) *sorghicola* (Coquillett), is an important pest of grain sorghum, and host plant resistance is an important aspect of control of this pest. This research investigated how cytoplasmic male-sterility and source of pollen influence the expression of resistance to sorghum midge. Sorghum midge emergence was significantly lower in panicles of midge-resistant and midge-susceptible cytoplasmic male-sterile lines when pollinated with AF 28 – a midge-resistant restorer line, than those pollinated with Swarna – a midge susceptible restorer line, indicating the presence of xenia effects. Maintainer lines (B-lines) of midge-resistant parents had significantly lower numbers of eggs and larvae than the B-lines of midge-susceptible parents. Male-sterile lines of the both midge-resistant and midge-susceptible lines were equally susceptible, indicating that resistance to sorghum midge is influenced by factors in the cytoplasm of the B-line. These findings will have an important bearing on the production of hybrids with resistance to insects.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in the semi-arid tropics. It is damaged by over 150 insect species, of which sorghum midge [*Stenodiplosis* (*Contarinia*) *sorghicola* (Coquillett)] (Diptera: Cecidomyiidae) is the most destructive pest worldwide (Harris, 1976). Host plant resistance is the most effective and economic means of controlling sorghum midge (Sharma et al., 1993). Considerable progress has been made in screening and breeding for resistance to this insect (Johnson et al., 1973; Peterson et al., 1988; Wiseman et al. 1988; Sharma et al., 1993). Efforts have also been made to transfer midge-resistance into male-sterile lines (Sharma et al., 1993) to increase the production and productivity of sorghum. Resistance to sorghum midge is governed largely by additive gene action (Sharma et al., 1996), and resistance is needed in both parents to produce midge resistant hybrids. Sorghum midge damage is lower in sorghum hybrids based on midge-resistant females than those based on midge-susceptible ones, and male-sterile lines of the midge-resistant parents are as susceptible as the male-sterile or maintainer lines of the midge-susceptible parents (Sharma et al., 1994). This may have an important bearing for the development and deployment of insect-resistant hybrids for sustainability of crop production and environment protection. This paper reports the effects of pollen from midge-resistant (AF 28) and midge-susceptible (Swarna) restorer lines on expression of resistance to sorghum midge in cytoplasmic male-sterile lines in sorghum.

Materials and methods

The test material consisted of two midge-resistant (ICSA 88019 and ICSA 88020) and two midge-susceptible (296A and ICSA 42) cytoplasmic male-sterile lines (A-lines), and their corresponding maintainer lines (B-lines). The A-lines were pollinated with a midge-resistant (AF 28) or a midge-susceptible (Swarna) restorer line (R-line). The experiment was planted in a randomized complete block design and there were three replications.

At panicle emergence, nine panicles selected at random in each replication were covered with muslin cloth bags before flowering to prevent natural infestation by the sorghum midge. At the half-anthesis stage, the panicles were infested with 40 midges per panicle under the no-choice headcage technique (Sharma et al., 1988). In each A-line, three panicles each were dusted with pollen from Swarna (midge-susceptible) and AF 28 (midge-resistant). Three panicles were infested without pollination in each male-sterile line. In the B-lines (with fertile pollen), three panicles were similarly infested with sorghum midge females inside a headcage as described above. Observations were recorded on the numbers of spikelets with eggs and larvae per 100 spikelets drawn at random from the midge infested panicles, and adult emergence per panicle. One hundred spikelets were drawn at random from the infested panicle in each replication 24 h after infestation to record the number of spikelets with eggs. The spikelets were dissected under a binocular microscope (40X) to observe the midge eggs. Similarly, one hundred spikelets were taken 10 days after infestation to record the number of spikelets with midge larvae. The number of midges that emerged from each infested panicle was counted 30 days after infestation. Data on chaffy spikelets was recorded in the B-lines at maturity.

Data on percentage spikelets with eggs and larvae was subjected to angular transformation and those on midge numbers to square root transformation before analysis of variance. Significance of differences between treatments was established by the F-test, and treatment means were compared using least significant difference (LSD) at $p = 0.05$.

Table 1. Egg laying by the sorghum midge, *Stenodiplosis sorghicola,* females in panicles of four cytoplasmic male-sterile lines of sorghum dusted with pollen from a midge-susceptible (Swarna) and a midge-resistant (AF 28) line under no-choice conditions in the headcage (40 midges per panicle)

Female	Pollination treatments			
	No-pollen	Swarna pollen	AF 28 pollen	B-line
ICSA 88019	22.2^{bc}	15.5^{ab}	22.4^{bc}	7.8^{a}
ICSA 88020	39.1^{d}	26.8^{c}	22.0^{bc}	12.3^{a}
296A	60.1^{e}	69.1^{f}	62.7^{ef}	36.9^{d}
ICSA 42	41.6^{d}	65.2^{ef}	61.3^{ef}	24.8^{c}
Mean	40.8^{B}	44.1^{B}	42.1^{B}	20.4^{A}

Figures followed by the same letter are not significantly different at $p < 0.05$. B-line = maintainer (fertile) line.

Table 2. Percentage spikelets with sorghum midge, *Stenodiplosis sorghicola,* larvae in panicles of four cytoplasmic male-sterile lines of sorghum dusted with pollen from a midge-susceptible (Swarna) and a midge-resistant (AF 28) line under no-choice conditions in the headcage (40 midges per panicle)

Female	Pollination treatments			
	No-pollen	Swarna pollen	AF 28 pollen	B-line
ICSA 88019	10.5^{a}	19.3^{a}	14.9^{a}	14.6^{a}
ICSA 88020	14.4^{a}	10.8^{a}	11.9^{a}	12.0^{a}
296A	23.2^{b}	20.7^{a}	33.9^{b}	28.5^{b}
ICSA 42	22.0^{a}	36.9^{b}	21.3^{a}	23.3^{b}
Mean	17.5^{A}	21.9^{A}	20.5^{A}	19.6^{A}

Figures followed by the same letter are not significantly different at $p < 0.05$. B-line = maintainer (fertile) line.

Results and discussion

When the panicles of male-sterile lines were infested with 40 sorghum midge females at flowering under no-choice headcage conditions without pollination or dusted with pollen from Swarna or AF 28, the differences in percentage of spikelets with eggs were not significant (40.8–44.1% spikelets with eggs) (Table 1). In ICSA 42, percentage spikelets with eggs were lower in panicles without pollination than those pollinated with pollen from AF 28 or Swarna, suggesting that pollen may elicit oviposition response by the midge females. Percentages of spikelets with eggs were lower (20.4%) in the B-lines than their respective A-lines (40.8 to 44.1% spikelets with eggs). B-lines of the midge-resistant parents had significantly lower oviposition (7.8 to 12.3% spikelets with eggs) than the B-lines of the midge-susceptible parents (24.8 to 36.9%). Across pollination treatments, egg laying in the spikelets of ICSA 88019 and ICSA 88020 was significantly lower (20.0–29.3%) than in the midge-susceptible lines 296A and ICSA 42 (56.0–64.0% (Figure 1). Pollination with a resistant or a susceptible restorer did not influence the egg laying by the sorghum midge females. There were no differences in percentage of spikelets with larvae within a male-sterile line across pollination treatments (Table 2). However, percentage

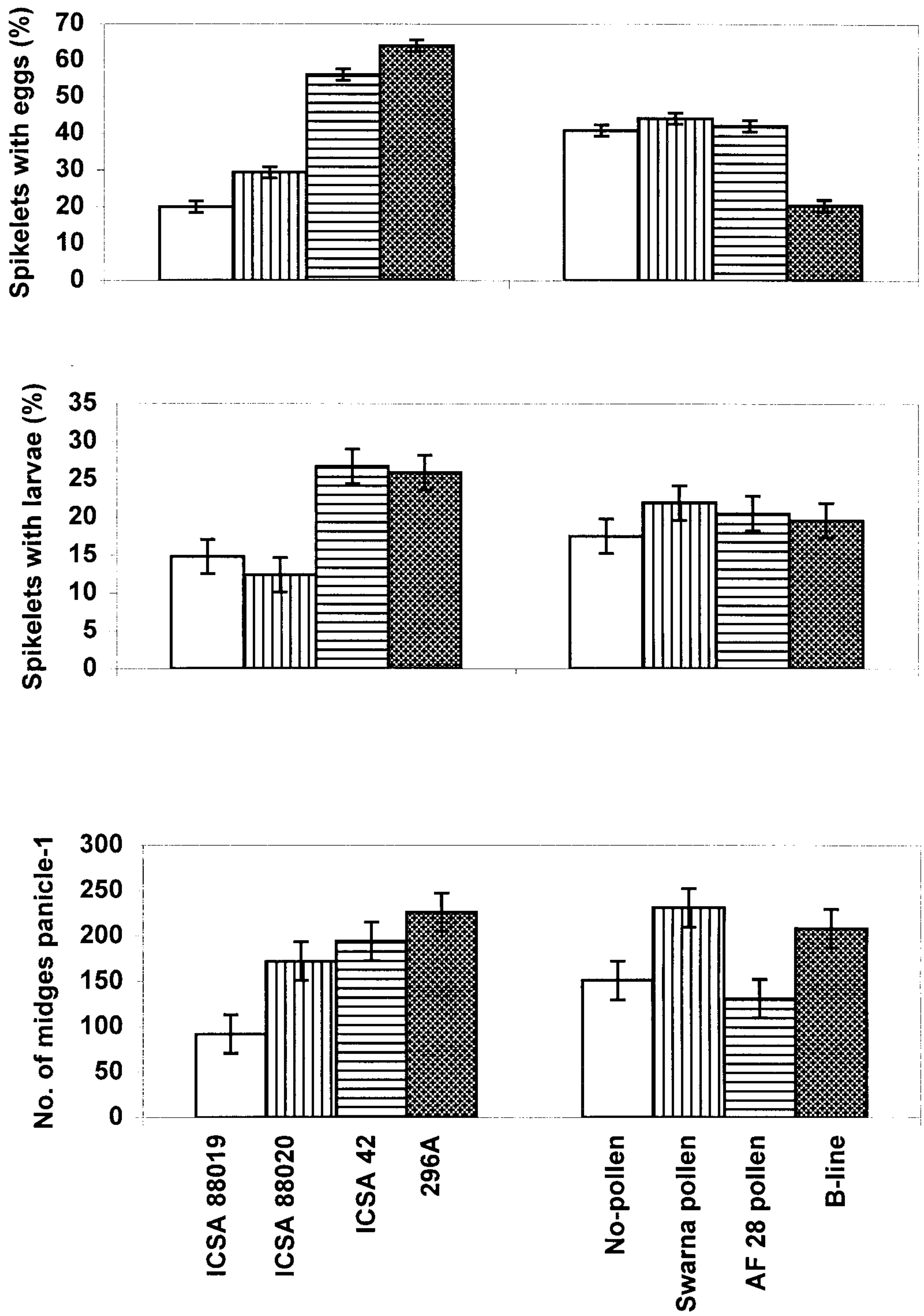

Figure 1. Egg laying (% spikelets with eggs), spikelets with larvae (%), and adult emergence per panicle in panicles of four cytoplasmic male-sterile lines of sorghum dusted with pollen from a midge-susceptible (Swarna) and a midge-resistant (AF 28) line under no-choice conditions in the headcage (40 midges per panicle).

Table 3. Sorghum midge, *Stenodiplosis sorghicola,* emergence in panicles of four cytoplasmic male-sterile lines of sorghum dusted with pollen from a midge-susceptible (Swarna) and a midge-resistant (AF 28) line under no-choice conditions in the headcage (40 midges per panicle)

Female	Pollination treatments			
	No-pollen	Swarna pollen	AF 28 pollen	B-line
ICSA 88019	28^{a}	155^{bcde}	94^{abc}	129^{abcd}
ICSA 88020	219^{defg}	204^{cde}	94^{abc}	36^{ab}
296A	250^{efg}	251^{efg}	178^{cdef}	292^{f}
ICSA 42	109^{abcd}	315^{gh}	158^{cde}	374^{h}
Mean	151^{A}	231^{B}	131^{A}	208^{B}

Figures followed by the same letter are not significantly different at $p = 0.05$. B-line = maintainer (fertile) line.

spikelets with midge larvae were significantly lower in the panicles of midge-resistant A-lines than in the panicles of midge-susceptible A-lines (Figure 1). Also, B-lines of the sorghum midge-resistant parents had significantly lower numbers of larvae in the spikelets than the B-lines of the midge-susceptible parents.

Sorghum midge emergence was significantly lower in ICSA 88019 and ICSA 88020 than in ICSA 42 and 296A across pollination treatments (Figure 1). Sorghum midge emergence was significantly lower in panicles pollinated with the midge-resistant line, AF 28 than in panicles pollinated with the midge-susceptible line, Swarna (Table 3, Figure 1). There was no trend in adult emergence in the panicles without pollination between midge-resistant and midge susceptible A-lines. However, adult emergence was significantly lower in the B-lines of the midge-resistant parents than in the B-lines of the midge-susceptible parents, suggesting that factors in the cytoplasm of the B-line and the source of pollen influence the expression of resistance to sorghum midge. Grain of the F_1 hybrids based on Swarna, as well as the parental lines, was white, while the grain color of the hybrids involving AF 28 was red-brown. Perhaps the induction of tannin production by AF28 pollen may affect the survival and development of the midge larvae. Numbers of midge-damaged spikelets were significantly lower (14 to 19% spikelets with midge damage) in the B-lines of midge-resistant parents than in the B-lines of midge susceptible parents (58 to 93% midge damaged spikelets).

Short and tight glumes, tannins, and a faster rate of grain development immediately after pollination are associated with resistance to sorghum midge (Sharma et al., 1990). B-lines of the midge-resistant genotypes with small glumes and normal ovaries had low oviposition perhaps due to short and tight glumes and less space available for oviposition and larval development. Because of shrunken pollen tubes in the male-sterile lines, the spikelets are quite loose/soft, and hence it is easier for the midge females to lay eggs in the A-lines than in the B-lines. Adult emergence was significantly lower in panicles of A-lines pollinated with AF 28 than those pollinated with Swarna, indicating the presence of xenia effects. AF 28 has a chalky-gray grain with high tannin content, and the tannin content of sorghum grain is associated with resistance to sorghum midge (Sharma et al., 1990). All the hybrids based on AF 28 had a red-brown-grain (with high tannin content), whereas those based on Swarna had white grain (little or no tannins). Therefore, induction of tannin production by the AF 28 pollen may be responsible for low midge emergence. Cytoplasmic male sterility in sorghum is due to interaction between milo-cytoplasm and *Kafir* nuclear genes (Stephens & Holland, 1954), and is controlled by two pairs of ‘fr’ genes in association with S-cytoplasm of milo. For inducing sterility in *Kafir* cytoplasm, genes at both loci are required, whereas only one gene is required for inducing sterility in milo-cytoplasm. Intra- and interallelic interaction and complementation influence the fertility restoration. There are at least three different types of cytoplasms in sorghum (Schertz & Pring, 1982), and mt-DNA restriction patterns are correlated with observed differences in fertility restoration, indicating that mt-DNA is the carrier of the c-gene, which may be associated with expression of resistance to sorghum midge. Midge-resistant A-lines were more susceptible to midge than the corresponding B-lines, and this may be due to factors associated with male-sterility or fertility restoration cytoplasm in sorghum. Reduction in sorghum midge emergence on the midge-resistant and midge-susceptible females as a result of pollination with the midge-resistant line AF 28 may be because of xenia and pollen effects. Levels of resistance to sorghum midge increased when pollinated with AF 28 compared with Swarna. Since resistance to sorghum midge is largely governed by the additive gene action (Sharma et al., 1996) such interactions can be used advantageously for developing hybrids with resistance to this insect.

Acknowledgements

I thank Dr Rodomiro Ortiz for his constructive comments, and Mr D. Krishna and Mr V.V. Rao for their help in carrying out these experiments.

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