Antibiosis component of resistance in sorghum to sorghum midge, *Contarinia sorghicola*

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Summary

Sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) is an important pest of grain sorghum, and host-plant resistance is one of the most effective means of controlling this pest. We studied the antibiosis mechanism of resistance in sorghum to *C. sorghicola* in a diverse array of midge-resistant and midge-susceptible genotypes. Data were recorded on adult emergence, postembryonic developmental period, number of mature eggs in the ovary, fecundity, larval survival from artificially implanted eggs; and the tannins, soluble sugars, and protein content of 10-day old and mature grains during the 1982–91 rainy and post-rainy seasons.

Adult emergence was significantly lower in the midge-resistant genotypes compared with the susceptible controls. Initiation of adult emergence was delayed by 4–8 days on DJ 6514, IS 8571, IS 9807, IS 10712, IS 19474, IS 19512, ICSV 830 and ICSV 197. Postembryonic developmental period was prolonged on DJ 6514, IS 15107, IS 3461, IS 7005, IS 19474, ICSV 831 and ICSV 197. However, the delay in adult emergence or the extended developmental period was not observed during the post-rainy season in some genotypes. These differences in the expression of antibiosis to midge in resistant genotypes over seasons may be attributed to the effect of environmental conditions on the insect development and chemical composition of sorghum grain. Amounts of tannins and proteins were generally greater in the midge-resistant lines compared with the susceptible ones (except tannins in DJ 6514) while the soluble sugars were low in the midge-resistant lines (except TAM 2566). These differences in chemical composition of the grain between genotypes and variations over seasons have been discussed in relation to the expression of antibiosis mechanism of resistance to the sorghum midge. Antibiosis to sorghum midge was also evident in terms of smaller size of larvae, lower number of eggs in the ovary, reduced fecundity, and larval survival. Midge-resistant lines have diverse effects on the biology of this insect. Antibiosis along with other components of resistance can be used to develop cultivars with stable resistance to *C. sorghicola*.

Key words: Sorghum, *Contarinia sorghicola*, antibiosis, plant resistance, tannins, sugars, proteins

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops in the semi-arid tropics. Grain yields on peasant farms are generally low, and insect pests are one of the major constraints in increasing sorghum production. Over 150 insect species

damage this crop in different sorghum-growing regions, of which sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) is the most destructive pest on a worldwide basis (Harris, 1976; Sharma, 1985*a,b*). Host-plant resistance is the most effective and economic means of controlling sorghum midge (Sharma, 1985*a,b*). Considerable progress has been made in screening and breeding for host-plant resistance to this insect (Johnson, Rosenow & Teetes, 1973; Wiseman, McMillian & Widstrom, 1973; Wiseman, Duncan & Widstrom, 1988; Sharma, 1985*b*; Peterson, Johnson, Teetes & Rosenow, 1988; Sharma, Taneja, Leuschner & Nwanze, 1992*b*; Sharma *et al.*, 1992*a*).

Midge-resistant germplasm sources have different combinations of the factors associated with midge resistance (Sharma, Vidyasagar & Leuschner, 1988*a*, 1990*b*, 1991). Cultivar non-preference to visiting adults (Wiseman & McMillian, 1968; Sharma, 1985*b*; Sharma, Leuschner & Vidyasagar, 1990*a*), morphological barriers to oviposition (Bergquist, Rotar & Mitchell, 1974; Rossetto, Goncalves & Diniz, 1975; Sharma, 1985*b*; Waquil, Teetes & Peterson, 1986*a*; Sharma *et al.*, 1990*b*), and possibly antibiosis (Rossetto, 1977, 1985; Teetes & Johnson, 1978; Wuensche, 1980; Waquil, Teetes & Peterson, 1986*b*; Sharma *et al.*, 1990*a,b*) are the major components of resistance to sorghum midge. However, it has been difficult to separate the role of oviposition nonpreference and antibiosis components in host-plant resistance to this insect, and hence, most of the reports are based on indirect inferences (Rossetto, 1985). However, there is no clear evidence on the nature and extent of antibiosis to this insect in sorghum. This paper reports the extent and nature of antibiosis in different sources of resistance to sorghum midge on insect biology: adult emergence, postembryonic development, fecundity, and the possible influence of chemical factors in the grain on these parameters.

Materials and Methods

Studies were conducted on postembryonic developmental period and adult emergence in relation to tannins, soluble sugars and protein content in midge-resistant and midge-susceptible genotypes, and the effect of midge-resistant cultivars on the number of mature eggs in the ovary, fecundity, larval mass and adult emergence from eggs implanted artificially in the spikelets. These studies were carried out between 1982–91 at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.

Crop

The crop was raised under rainfed conditions (June-October) during the rainy season, and under irrigated conditions during the post-rainy season (November-April). Test cultivars were planted in a randomised block design with three replications. Each plot measured 12 m^2, and consisted of four ridges, 4 m long and 75 cm apart. Carbofuran 3G was applied to the soil at the rate of 1.2 kg a.i. ha^{-1} at sowing to protect the crop against the sorghum shoot fly, *Atherigona soccata* Rondani. The plants were thinned to a spacing of 15 cm between the plants 15 days after crop emergence. No insecticide was applied during the reproductive phase of the crop. During the post-rainy season, overhead sprinkler irrigation was used to increase the relative humidity to increase midge infestation (Sharma, Vidyasagar & Leuschner, 1988*b*).

Insects

Midge adults emerge in the morning hours (0700–1030), mate, and the females proceed in search of sorghum panicles at anthesis for oviposition. Midge females visiting flowering

sorghum panicles for oviposition were collected in plastic bottle aspirators for infestation. Only freshly emerged females were collected and used for infestation in the headcages between 0800–1000 h (Sharma *et al.*, 1988*a*). The headcage consisted of a wire-framed cage (16 cm diameter, 20 cm long) tied around the sorghum panicle at the half-anthesis stage. The cage was covered with a blue cloth bag to confine the midge females with the sorghum panicles for oviposition. The number of midges released inside the cages are indicated in each experiment.

Adult emergence and postembryonic development

Adult emergence pattern and postembryonic developmental period were studied on four midge-resistant (DJ 6514, AF 28, TAM 2566 and IS 15107) and two midge-susceptible (CSH 1 and Swarna) cultivars during 1982–1984. Sixty female midges were released on each panicle at the half-anthesis stage using the headcage technique (Sharma *et al.*, 1988*a*). Three randomly tagged panicles were caged in each plot. Midge emergence in the infested panicles was recorded daily between 15 and 30 days after infestation. From the headcages, the midges were collected with an aspirator and counted. Postembryonic developmental period was computed from the total number of emerging adults on each genotype. During the 1986/87 post-rainy season, midge emergence and postembryonic development were studied on 14 resistant (IS 3461, IS 7005, IS 8571, IS 8721, IS 9807, IS 10712, IS 19474, IS 19512, ICSV 830, ICSV 831, ICSV 832, ICSV 197, DJ 6514 and TAM 2566) and one susceptible (CSH 1) cultivar. Other experimental details were similar to those described above.

Estimation of tannins, soluble sugars and protein content

The grain samples were collected from three uninfested panicles of each cultivar at 10 days after anthesis and at maturity. The samples were drawn from the middle portion of respective genotypes. Grains were separated from the spikelets, and oven dried at 80°C and used for chemical analysis. Tannin content of the grain was determined by vanillin assay as described by Price, Van Scoyoc & Butler (1978), and expressed in catechin equivalents as a percentage of grain mass. Proteins were estimated by the ninhydrin method of Jambunathan, Rao & Gurtu (1983), and the soluble sugars by anthrone reagent method of Clegg (1956) and Dubois *et al.* (1956).

Effect of midge resistant genotypes on fecundity

The effect of midge-resistant genotypes on egg numbers was studied on 15 genotypes (IS 3461, IS 9807, IS 10712, IS 8721, TAM 2566, PM 9250, IS 19512, IS 7005, PM 10291, IS 19474, DJ 6514, PM 10750, CSH 1, ICSV 197 and Swarna) during the 1987/88 post-rainy season, and on nine genotypes (CSH 1, CSH 11, ICSV 112, ICSV 197, ICSV 745, AF 28, DJ 6514, IS 3461 and IS 10712) during the 1988/89 post-rainy season. Five particles were infested with 40 midges/panicle under headcages in each plot. Ten females were collected at random from infested panicles 18 days after infestation in each genotype. Each female was dissected under binocular microscope, and the number of mature eggs in the ovary were counted.

The effect of different sorghum genotypes on fecundity was also studied by rearing midges on resistant and susceptible genotypes. Midges reared on different genotypes were released in pairs of five on CSH 1 (a susceptible cultivar) under headcage. Five panicles of CSH 1 were infested with midges reared on each genotype. Number of emerging midges on CSH 1 was used to measure fecundity of midges reared on different genotypes. CSH 1 is a susceptible genotype and has no physical barriers to oviposition or antibiotic effects on sorghum midge (Sharma *et al.*, 1990*b*).
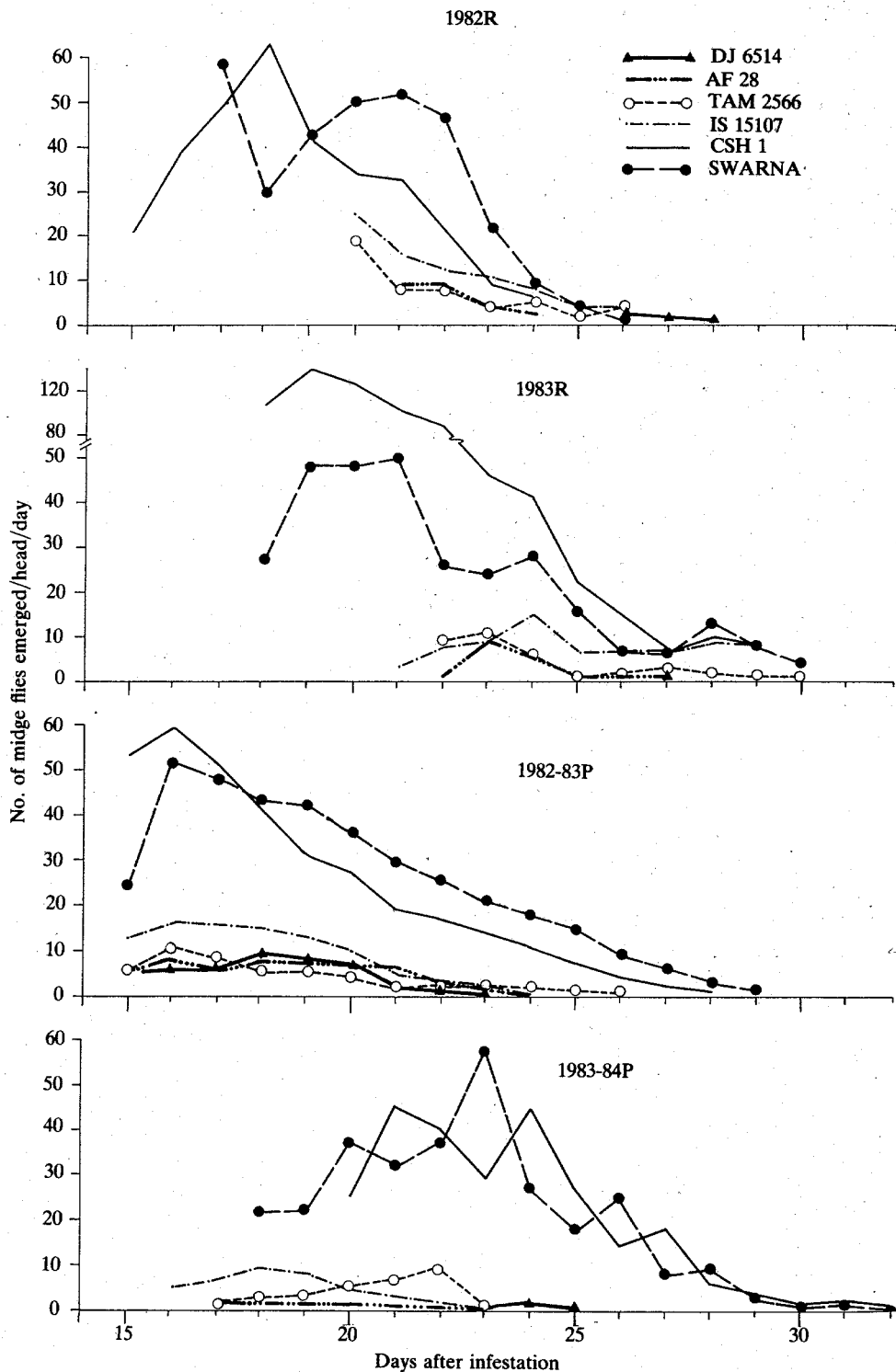
Fig. 1. Adult emergence pattern of sorghum midge, *C. sorghicola* in six sorghum cultivars over four seasons (60 midges/panicle) (ICRISAT Centre, 1982–84).

Midge emergence from implanted eggs

The effect of resistant genotypes on larval mortality was studied by artificially implanting eggs inside the spikelets during the 1986 rainy season. This procedure was used to overcome cultivar non-preference to adults and oviposition. Forty midges were caged with CSH 1 panicles for one day at anthesis under the headcage. On the following day, the spikelets were dissected individually under a microscope. The eggs were gently taken out with a needle, and placed inside the spikelets of different genotypes individually. The spikelets were opened up with forceps for placing the eggs. Twenty-five spikelets were infested artificially in each panicle, and three panicles were infested in each genotype. Numbers of adults emerging in each genotype were recorded 20 days after infestation.

Larval size and weight

Size and weight of the midge larvae on 10 sorghum genotypes were recorded during the 1991 rainy season. Panicles at the half-anthesis stage were infested with 40 midges/panicle under a headcage as described before. Ten days after infestation, the panicles were excised and brought to the laboratory. Infested spikelets were dissected under a 10× lens, and the larvae were taken out with forceps and placed on a moist filter paper in a Petri-dish. Size and mass of 50 larvae was recorded in each genotype.

Statistical analysis

Data were subjected to analysis of variance to determine the standard error of mean, and the significance of differences between treatment means. Differences between variety means were compared using the least significant difference (LSD) at $P = 0.05$. Correlation and regression coefficients of the chemical constituents of the sorghum grain with the biological parameters of the sorghum midge were also computed.

Results

Adult emergence and postembryonic development

The pattern of adult emergence was different between midge-resistant and midge-susceptible genotypes (Fig. 1). Fewer adults emerged from the infested panicles of midge-resistant cultivars, DJ 6514, AF 28, TAM 2566 and IS 15107 as compared with the midge-susceptible cultivars CSH 1 and Swarna. Low adult emergence in the midge-resistant lines was largely because of physical barriers to oviposition (Sharma *et al.*, 1990a), and/or antibiosis to larvae. Postembryonic developmental period was prolonged by 4 and 7 days on IS 15107 and DJ 6514, respectively; by 3 days on AF 28 and TAM 2566 during the 1982 rainy season, and by 1 to 2 days during the 1983 rainy season compared with the susceptible control CSH 1 (Table 1). However, such a prolongation of the developmental period was not observed during the post-rainy seasons of 1982/83 and 1983/84. Adult emergence began 5 to 11 days later on DJ 6514, AF 28, TAM 2566 and IS 15107 compared with CSH 1 during the 1982 rainy season, and 3–6 days later during the 1983 rainy season. However, such a delay in adult emergence was not observed during the 1982/83 and 1983/84 post-rainy season (except on DJ 6514 during the 1983/84 post-rainy season). Thus, growing season appears to influence the nature of antibiosis in midge-resistant genotypes.

Midge emergence was lower in IS 3461, IS 9807, IS 10712, IS 19474, IS 19512, ICSV 830, ICSV 831, ICSV 832, ICSV 197, DJ 6514 and TAM 2566 (< 150 midges/panicle) compared with the susceptible control, CSH 1 (640 midges/panicle) (Table 2). Postembryonic development was prolonged by > 3 days on IS 3461, IS 7005, IS 19474, ICSV 831 and ICSV 197

Table 1. *Mean postembryonic developmental and period of adult emergence of sorghum midge in six sorghum genotypes (ICRISAT Centre, 1982–84)*

| | Mean postembryonic period (days) (Mean ± SE) | | | | Period of adult emergence after infestation (days) | | | |
| | Rainy season | | Post-rainy season | | Rainy season | | Post-rainy season | |
Genotype	1982	1983	1982/83	1983/84	1982	1983	1982/83	1983/84
DJ 6514	26 ± 0.6	—	18 ± 1.1	24 ± 0.3	26–27	—	24–23	15–22
AF 28	22 ± 1.8	24 ± 1.4	19 ± 0.9	20 ± 0.1	21–24	22–27	18–21	15–23
TAM 2566	22 ± 2.1	24 ± 1.3	19 ± 0.8	21 ± 1.2	20–25	22–29	18–23	15–25
IS 15107	23 ± 2.8	25 ± 1.1	18 ± 1.9	19 ± 1.9	20–26	21–29	17–22	15–23
Susceptible control								
CSH 1	19 ± 5.8	23 ± 14.5	18 ± 5.3	23 ± 4.3	15–23	18–29	21–29	15–27
SWARNA	20 ± 6.9	24 ± 4.6	20 ± 4.2	23 ± 4.3	17–25	18–30	20–29	15–27

during the 1986–87 rainy and post-rainy seasons. Postembryonic developmental period was shorter on IS 8571, IS 9807, IS 10712, IS 19512, ICSV 830, TAM 2566 and CSH 1 during the rainy season compared with the post-rainy season, while the reverse was true in the case of ICSV 832. Adult emergence was delayed by > 5 days on IS 3461, IS 7005, and IS 19574 during the 1986 rainy season. During the 1986/87 post-rainy season, adult emergence was delayed by > 4 days on IS 3461, IS 7005, IS 8571, IS 9807, IS 10712, IS 19474, IS 19512, ICSV 830, ICSV 197 and TAM 2566.

Effect of midge-resistant genotypes on fecundity

The number of mature eggs was significantly lower in adult females reared on IS 3461, IS 7005, IS 8571, IS 8721, IS 9807, IS 10712, IS 19474, IS 19512, ICSV 830 and TAM 2566

Table 2. *Postembryonic developmental period and adult emergence in 15 sorghum genotypes (ICRISAT Centre, 1986–87)*

| | Postembryonic period (days) (Mean ± SE) | | Period of adult emergence after infestation (days) | | No. of adults emerged/panicle | |
| | 1986 | 1986/87 | 1986 | 1986/87 | 1986 | 1986/87 |
Genotype	R	PR	R	PR	R	PR
IS 3461	25 ± 0.9	26 ± 1.1	22–30	25–30	29	128
IS 7005	24 ± 1.5	26 ± 1.3	28–28	25–31	86	198
IS 8571	20 ± 8.9	26 ± 1.6	17–26	25–32	294	160
IS 8721	—	24 ± 2.3	—	21–29	—	450
IS 9807	18 ± 12.1	26 ± 1.5	17–22	25–30	103	60
IS 10712	17 ± 2.7	27 ± 1.9	15–19	25–34	54	118
IS 19474	24 ± 5.1	27 ± 2.3	22–30	26–34	127	98
IS 19512	19 ± 1.9	27 ± 1.2	15–24	27–33	109	135
ICSV 830	21 ± 0.2	27 ± 2.0	18–22	26–32	2	27
ICSV 831	22 ± 0.2	30 ± 2.5	19–25	21–34	3	20
ICSV 832	25 ± 3.9	18 ± 2.0	19–28	17–23	26	44
ICSV 197	24 ± 0.3	31 ± 2.3	20–26	28–34	3	26
DJ 6514	21 ± 0.2	23 ± 2.0	19–25	20–26	3	23
TAM 2566	19 ± 2.1	28 ± 2.4	16–21	24–30	44	148
CSH 1	18 ± 12.9	22 ± 2.4	17–29	20–33	328	640

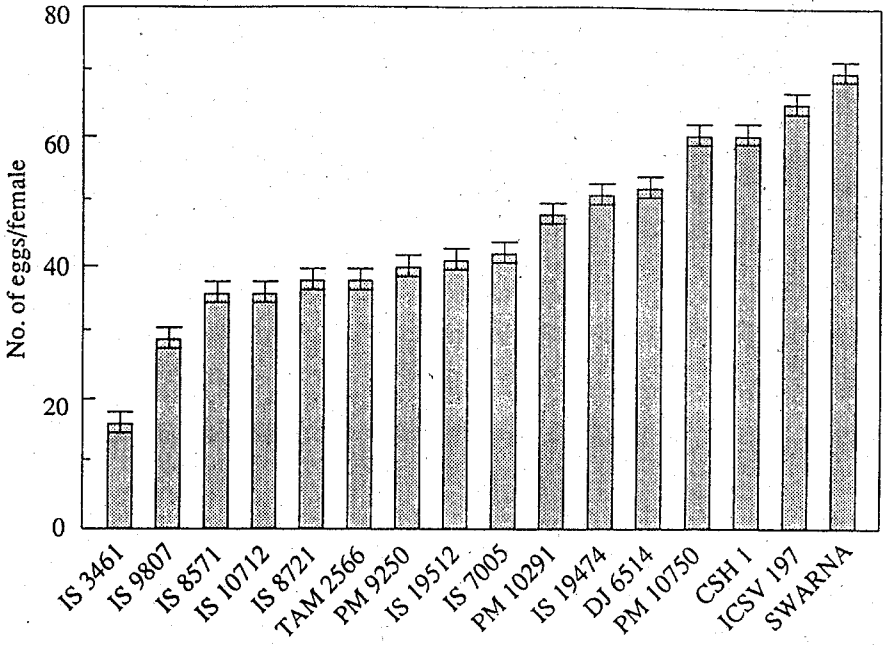R = Rainy season, PR = Post-rainy season.

Fig. 2. Egg numbers in the ovaries of midge females reared on different sorghum genotypes (ICRISAT Centre, 1987/88 post-rainy season).
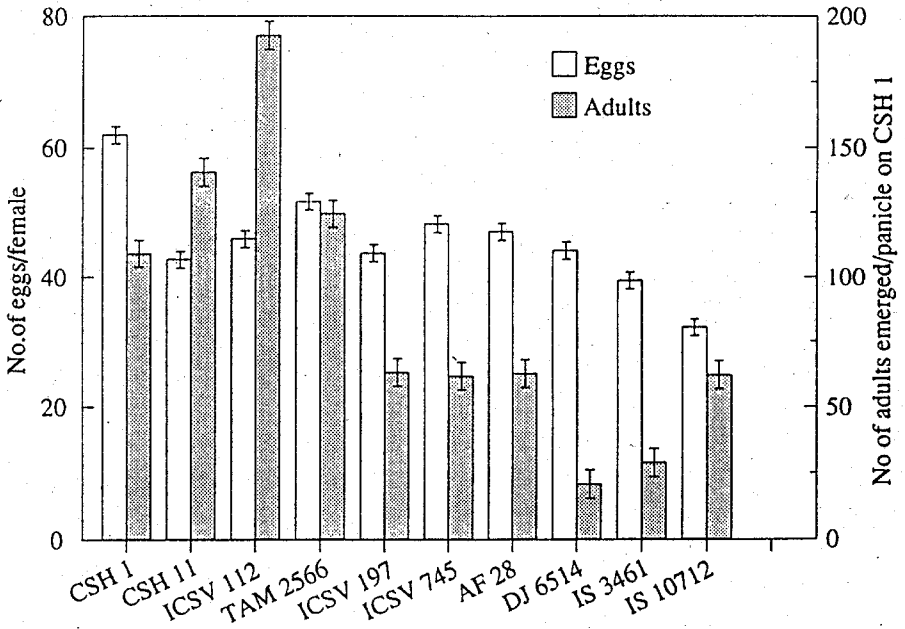

Fig. 3. Egg numbers in the ovaries and progeny production on CSH 1 in midge females reared on different sorghum genotypes (ICRISAT Centre, 1988/89 post-rainy season).

(< 48 eggs/female) compared with CSH 1 and Swarna (60 and 70 eggs/female, respectively) during the 1987/88 post-rainy season (Fig. 2). During the 1988/89 post-rainy season, a slight reduction in egg numbers was recorded in females reared on IS 3461 and IS 10712 (< 45 eggs/females) compared with 62 eggs/female on CSH 1 (Fig. 3). Number of adults emerging on panicles of CSH 1 infested with females reared on IS 3461, IS 10712, DJ 6514, AF 28, ICSV 197 and ICSV 745 were < 63 midges/five females compared with 109 to 192 midges/five females on the susceptible controls (ICSV 112, CSH 1 and CSH 11). Genotypic effects on fecundity (both for eggs and adult emergence) was consistent over seasons for IS 3461, DJ 6514 and IS 10712. In some genotypes, there was a variation in egg numbers over seasons.

Adult emergence from implanted eggs

Less than two adults emerged from 25 eggs implanted in the spikelets of AF 28, TAM 2566, IS 15107 and DJ 6514 compared with 11 adults from Swarna, the susceptible control (Fig. 4).
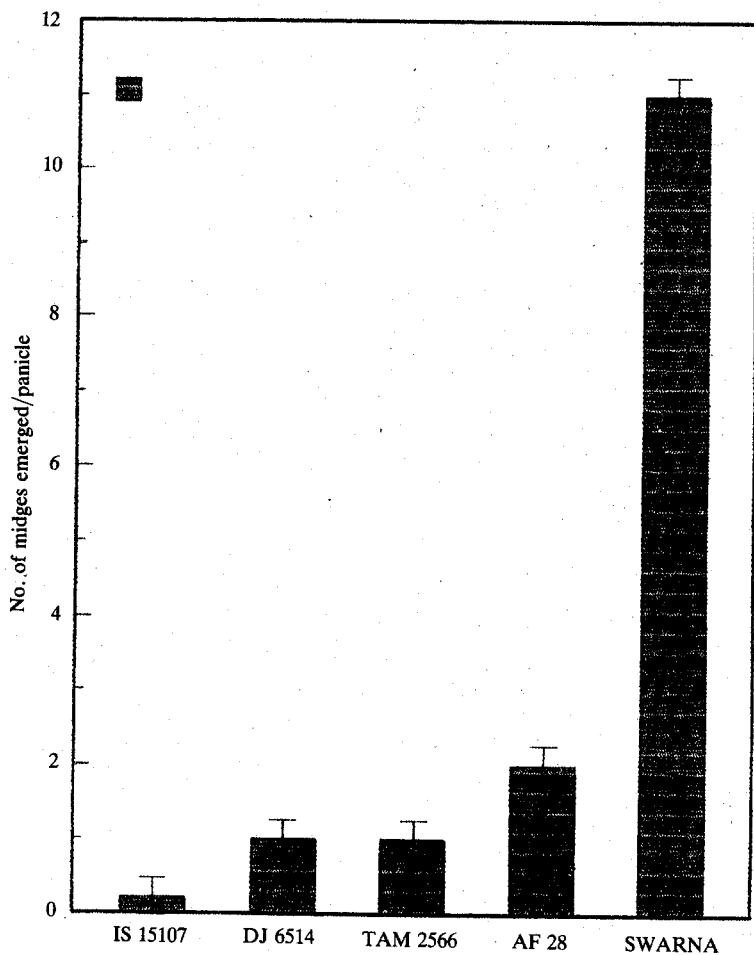

Fig. 4. Emergence of sorghum midge adults from artificially implanted eggs in five sorghum genotypes (ICRISAT Centre, 1986 rainy season).

Table 3. *Size (length and breadth) and weight of 10-day-old larvae of sorghum midge,*
C. sorghicola *on nine sorghum genotypes (ICRISAT Centre, 1991/92 post-rainy season)*

Genotype	Length[1]	Breadth[1]	Weight (mg/10 larvae)
AF 28	25.76 ± 0.71	13.08 ± 0.23	4.49
TAM 2566	18.27 ± 0.91	9.00 ± 0.34	1.34
IS 10712	18.67 ± 1.07	10.32 ± 0.61	2.61
ICSV 112	28.86 ± 0.58	13.28 ± 0.18	5.08
ICSV 197	24.99 ± 0.52	12.67 ± 0.21	3.86
ICSV 743	24.30 ± 1.45	11.05 ± 0.68	—
ICSV 745	23.39 ± 1.85	12.46 ± 0.74	2.42
CSH 1	21.33 ± 1.04	12.29 ± 0.53	2.90
CSH 11	28.98 ± 0.45	16.33 ± 0.82	5.34
SED	—	—	0.210

[1]Ocular units (40 ocular units = 1 mm).

Larval size and weight

Larval size and weight were greater on the susceptible cultivar CSH 11 and ICSV 112 compared with the midge-resistant cultivars TAM 2566 and IS 10712 (Table 3). Size of the larvae was also smaller on CSH 1. At a second date of infestation, the larvae were smaller on TAM 2566 compared with the larvae reared on CSH 1 and AF 28.

Tannins, soluble sugars and proteins

The tannin content of 10-day-old and mature grains was greater in the midge-resistant lines (AF 28, TAM 2566 and IS 15107) compared with the susceptible controls CSH 1 and Swarna (Table 4). However, tannin content in DJ 6514, which is highly resistant to the sorghum midge, was also low. Across seasons, tannin content of the 10-day old grain was greater (6.35%) than the mature grain (2.02%). Tannin content varied from 0.03% to 17.16% for the 10-day grain and 0.02% to 7.81% for the mature grain in different genotypes and across seasons. Generally, tannin content was greater during the rainy season compared with the post-rainy season (except the tannin content of the mature grain during the 1983/84 post-rainy season). However, differences in the tannin content of the mature grain were not evident across seasons.

Table 4. *Tannin content (%) of ten-day-old and matured grain in six sorghum genotypes (ICRISAT Centre, 1982–84)*

	Ten-days-after anthesis					Matured grain				
	Rainy season		Post-rainy season			Rainy season		Post-rainy season		
Genotype	1982	1983	1982/83	1983/84	Mean	1982	1983	1982/83	1983/84	Mean
DJ 6514	0.1	0.0	0.0	0.0	0.03[a]	0.0	0.0	0.03	0.03	0.02[a]
AF 28	26.32	24.71	10.04	7.55	17.16[c]	9.9	7.01	6.16	8.2	7.81[d]
TAM 25646	11.11	13.44	8.0	—	10.34[b]	0.0	1.15	1.26	1.25	0.92[b]
IS 15107	13.85	10.94	5.98	—	10.25[b]	4.1	2.89	2.95	3.32	3.32[c]
CSH 1	0.4	0.0	0.15	0.0	0.14[a]	0.0	0.0	0.06	0.02	0.02[a]
SWARNA	0.36	0.22	0.15	0.0	0.18[a]	0.0	0.0	0.06	0.02	0.02[a]
Mean	8.69	8.22	4.05	1.89	6.42	2.33	1.84	1.75	2.14	2.02
SED					2.82					0.54

Figures followed by the same letter within a column are not significantly different at $P = 0.05$.

Table 5. *Total soluble sugars (%) in grain of six sorghum genotypes (ICRISAT Centre, 1982–84)*

| Genotype | Ten-days-after anthesis | | | | | Matured grain | | | | |
| | Rainy season | | Post-rainy season | | | Rainy season | | Post-rainy season | | |
	1982	1983	1982–1983	1983–1984	Mean 1982	1982	1983	1982–1983	1983–1984	Mean
DJ 6514	1.6	2.8	2.9	4.65	3.0[a]	1.3	1.4	1.4	1.5	1.4[ab]
AF 28	1.9	1.8	2.9	4.85	2.8[a]	1.8	1.4	1.9	1.5	1.6[bc]
TAM 2566	4.6	4.8	3.1	4.2	4.2[b]	1.2	1.1	1.7	1.3	1.4[a]
IS 15107	2.9	1.7	2.8	2.5	2.5[a]	1.9	1.5	2.5	1.7	1.9[c]
CSH 1	2.8	4.5	2.9	9.8	5.0[ab]	1.3	0.9	1.2	1.3	1.1[a]
SWARNA	3.5	4.3	5.4	9.55	5.7[b]	1.3	1.1	1.2	1.2	1.2[a]
Mean	2.9	3.3	3.3	7.21	3.2	1.4	1.2	1.7	1.4	1.4
SED					0.49					0.13

Figures followed by the same letter within a column are not significantly different at $P = 0.05$.

Soluble sugars varied from 2.5% to 5.7% in the 10-day grain and 1.1% to 1.9% in the mature grain in different genotypes (Table 5). Mean sugar content across seasons was lower in the midge-resistant lines for the 10-day grain (2.5% to 4.2% *vs* 5.0% to 5.7% in CSH 1 and Swarna). However, this trend was reversed for the mature grain where midge-resistant lines had greater soluble sugars (1.3% to 1.9%) compared with the susceptible controls (1.1% to 1.2%). There was a considerable variation in the amounts of soluble sugars over seasons. Soluble sugars in TAM 2566 were similar to or greater than those in CSH 1 and Swarna in some seasons. Generally, soluble sugars in the mature grain of midge-resistant lines was greater than in CSH 1 and Swarna (except TAM 2566 in the 1983 rainy season). Protein content of the 10-day grain was greater (14.5% to 16.8%) for the midge-resistant lines compared with the susceptible controls (12.4% – 13.9%) (Table 6). However, such differences were less pronounced for the mature grain. There was some variation in protein content across seasons.

Table 6. *Protein content (%) of grain in six sorghum genotypes (ICRISAT Centre, 1982–84)*

| Genotype | Ten-days-after anthesis | | | | | Matured grain | | | | |
| | Rainy season | | Post-rainy season | | | Rainy season | | Post-rainy season | | |
	1982	1983	1982–1983	1983–1984	Mean	1982	1983	1982–1983	1983–1984	Mean
DJ 6514	16.1	12.7	14.6	14.65	14.5[abc]	16.5	12.8	11.4	10.3	12.8[c]
AF 28	15.9	16.0	12.9	14.95	14.9[abc]	11.2	12.3	11.3	10.0	11.2[abc]
TAM 2566	18.4	17.0	15.0	16.6	16.8[c]	11.4	12.3	11.8	10.3	11.5[bc]
IS 15107	16.6	16.3	14.9	15.9	15.9[bc]	11.3	11.3	10.2	12.5	11.3[abc]
CSH 1	17.2	10.5	17.3	14.55	12.4[a]	9.6	12.5	7.0	9.4	9.6[a]
SWARNA	16.9	13.0	15.1	15.0	13.9[ab]	11.2	11.3	10.9	10.5	11.0[ab]
Mean	16.9	14.2	12.9	15.3	15.3	11.9	12.2	10.4	10.5	11.0
SED	0.36	0.18	0.57	0.32	0.77	0.12	0.21	0.72	0.15	0.50

Figures followed by the same letter within a column are not significantly different at $P = 0.05$.

Table 7. *Correlation and regression coefficients of tannins, proteins, and soluble sugars with biological parameters of sorghum midge*

Chemical component	Post embryonic development period		Adult emergence		Fecundity		No of eggs/ panicle	
	r	b	r	b	r	b	r	b
Tannins 10-day grain	0.19	0.06	−0.48*	−10.88*	−0.05	−0.76	−0.04	−0.01
Tannins mature grain	0.05	−0.04	−0.43*	−25.40*	−0.28	−5.40	−0.17	−0.50
Sugars 10-day grain	0.11	0.13	0.35	29.20	0.60*	15.80	0.34	1.12
Sugars mature grain	−0.37	−2.61	−0.50*	−258.50*	0.02	6.00	0.14	7.40
Proteins 10-day grain	−0.04	−0.06	−0.36	−36.50	0.26	17.00	0.67*	6.30
Proteins mature grain	0.49*	0.72*	−0.24	−25.70	0.28	7.90	0.13	0.50

r = Correlation coefficient and b = regression coefficient.
* = Significant at $P = 0.05$.

Tannin content of the sorghum grain showed significant and negative effect on adult emergence (Table 7). However, there was no relationship with the postembryonic developmental period. Tannin content of mature grain showed negative correlation and regression coefficients with the fecundity of midge females and the number of eggs/female. The coefficients were not significant at $P = 0.05$. Soluble sugar content of the mature grain was negatively associated with number of adults emerged. However, soluble sugar content of the 10-day grain was positively associated with midge fecundity and the number of eggs/ female. Protein content of the mature grain was positively associated with postembryonic development, fecundity and the number of eggs/females, but negatively associated with adult emergence. Some of these correlation and regression coefficients were not significant at $P = 0.05$. Negative association between soluble sugars and the protein contents of sorghum grain and number of adults emerged may be because of indirect influence of tannins and other chemical constituents of the grain on the biology of sorghum midge.

Discussion

Initiation of adult emergence was delayed by 4–5 days when the midges were reared on DJ 6514, TAM 2566 and IS 15107 compared with CSH 1 and/or Swarna during the 1982–83 rainy seasons. Mean postembryonic developmental period was also prolonged by 3–5 days on DJ 6514 and IS 15107. However, adult emergence continued for a longer period on the susceptible cultivars, CSH 1 and Swarna, possibly because of the large number of insects that emerged from these cultivars, and the possibility of having more than one larva in each spikelet (due to heavy oviposition), which may lead to delayed development because of competition. Delay in adult emergence or prolonged postembryonic development was not observed during the 1982/83 and 1983/84 post-rainy seasons. These differences in the expression of antibiosis may be attributed to the environmental effects on the production and accumulation of secondary plant substances, and the nutritional quality of crop plants.

Variation in temperature (25°C during the rainy season *vs* 35°C during the post-rainy season), photoperiod (10 h during the rainy season *vs* 14 h during the post-rainy season), and relative humidity (> 60% during the rainy season *vs* < 30% during the post-rainy season) may account for the environmental conditions affecting chemical composition of the grain, and the nature and extent of antibiosis to sorghum midge.

There were considerable differences in the relative amounts of tannins, soluble sugars, and protein contents of the grain over seasons. Tannins and protein contents were greater during the rainy season in the 10-day-old grain (on which the midge larva feeds) compared with the post-rainy season, while the reverse was true in case of soluble sugars. However, there are some exceptions. Greater amounts of tannins in the grain may result in slower growth of the larvae because of their antifeedant and/or antibiotic effects on the larvae (Sharma & Agarwal, 1982a,b; Rossetto, 1985; Sharma & Norris, 1991). In DJ 6514, which has a low tannin content, some other factors may be responsible for the antibiotic effects on the larvae.

Sorghum lines with high levels of midge resistance have testa (Johnson, 1977), and such lines have a high tannin content (Kofoid, Maranville & Ross, 1982). The major site occurrence of sorghum polyphenols is in the testa of the seed coat (Rooney & Miller, 1982). Polyphenols vary widely between genotypes. Factors such as grain maturity, environmental conditions and location strongly influence the polyphenol composition of the sorghum grain (Butler, 1982a,b; Price, Stremberg & Butler, 1979). Even single grains from the same source differ 10-fold in the amount of tannins present (Price, Scoyoc & Butler, 1978). Thus, these changes in the chemical composition of the sorghum grain are likely to influence the nature and extent of antibiosis to sorghum midge.

A correlation between tannin content of grain and midge resistance has been suggested by Santos & Carmo (1974) and Sharma *et al.* (1990a). In the present studies, considerable variation was recorded in the tannin content of the grain across stages of grain development, between genotypes, and across seasons. Midge-resistant lines showed higher tannin content, although there were distinct exceptions, e.g. DJ 6514. Since there is considerable variation in the quality and quantity of polyphenols and tannins in the sorghum grain during grain development, some specific compounds may be responsible for insect resistance while others may be products of the general phenylpolypropanoid biosynthetic pathway. Resistance to midge is largely influenced by the extent of oviposition, and hence, a realistic association between tannin content of grain and midge resistance may be difficult to establish (Rossetto, 1985; Sharma *et al.*, 1990a). Further studies using a set of isolines for tannin content and midge resistance are required to elucidate this hypothesis.

Phenolic compounds are responsible for antifeedant and/or antibiotic effects on insects in soybean (Sharma & Norris, 1991) and cotton (Sharma & Agarwal, 1982a,b). Insect feeding on sorghum is deterred by phenolic acids (Woodhead & Cooper-Driver, 1979; Woodhead, Padgham & Bernays, 1980), cyanogenic glycosides and some components in the surface wax (Woodhead, 1983). Flavan-4-ols (procyanidins) are also reported to be a deterrent to aphid feeding (Dreyer, Reese & Jones, 1982). Some of these polyphenols may act as antifeedant and/or antibiosis towards the larvae of the sorghum midge. Butler (1989) indicated that there is no indication of significant effects of tannins on resistance to insects in sorghum. However, earlier reports and the results of the present studies indicate that these compounds might act as antifeedant and/or antibiotic to a number of insects feeding on sorghum.

Lower amounts of soluble sugars in midge-resistant cultivars (except in TAM 2566) may have a direct bearing on the nutritional value of these genotypes to the midge larvae, and it may partly account for the slow development observed on these lines. It is difficult to link greater amounts of proteins in the midge-resistant lines (during the rainy season), with

midge development. Polyphenols have an affinity for binding with proteins. Tannins in sorghum do not interact equally with all proteins, but bind strongly with hydrophobic proteins (Hagerman & Butler, 1981) such as prolamines (Butler, 1989). High tannin sorghums are known to suppress growth and increase the level of faecal nitrogen by affecting digestion, with major effects after the digestion possibly by interacting with bone development and liver enzymes in higher animals (Butler, Rogler, Mehansho & Carlson, 1986), and by interactions with several metabolic reactions. Polyphenols also affect the utilisation of carbohydrates by increasing the formation of resistant starch after cooking (Knudsen, Kirleis, Eggum & Munck, 1988). Such adverse effects of tannins on food utilisation by insects need to be studied in detail.

Antibiotic effects of resistant genotypes on sorghum midge were also apparent in terms of smaller size of the larvae, number of mature eggs in the ovary, and fecundity. However, there were variations in such effects between seasons. These differences could be linked to changes in production and accumulation of tannins, and the nutritional quality of the grain as influenced by temperature, photoperiod and other factors affecting the chemical composition of the sorghum grain. Midge-resistant lines have diverse antibiotic effects on the biology of this insect. Genotypes with diverse effects on insect biology can be used to develop cultivars with stable resistance to *C. sorghicola*.

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