

Host-plant preference and oviposition responses of the sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Dipt., Cecidomyiidae) towards wild relatives of sorghum

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Abstract: Sorghum midge, *Stenodiplosis (Contarinia) sorghicola* (Coquillett) is an important pest of grain sorghum world-wide. Considerable progress has been made in screening and breeding for resistance to sorghum midge. However, some of the sources of resistance have become susceptible to sorghum midge in Kenya, in eastern Africa. Therefore, the wild relatives of *Sorghum bicolor* were studied as a possible source of new genes conferring resistance to sorghum midge. Midge females did not lay eggs in the spikelets of *Sorghum amylum*, *Sorghum bulbosum*, and *Sorghum angustum* compared to 30% spikelets with eggs in *Sorghum halepense* when infested with five midge females per panicle under no-choice conditions. However, one egg was laid in *S. amylum* when infested with 50 midges per panicle. A larger number of midges were attracted to the odours from the panicles of *S. halepense* than to the panicles of *Sorghum stipoides*, *Sorghum brachypodium*, *S. angustum*, *Sorghum macrospermum*, *Sorghum nitidum*, *Sorghum laxiflorum*, and *S. amylum* in dual-choice olfactometer tests. The differences in midge response to the odours from *S. halepense* and *Sorghum intrans* were not significant. Under multi-choice conditions, when the females were also allowed a contact with the host, more sorghum midge females were attracted to the panicles of *S. bicolor* compared with *S. amylum*, *S. angustum*, and *S. halepense*. In another test, numerically more midges responded to the panicles of IS 10712 compared with *S. halepense*, whereas the differences in midge response to the panicles of ICSV 197 (*S. bicolor*) and *S. halepense* were not apparent, indicating that *S. halepense* is as attractive to sorghum midge females as *S. bicolor*. The wild relatives of sorghum (except *S. halepense*) were not preferred for oviposition, and they were also less attractive to the sorghum midge females. Thus, wild relatives of sorghum can prove to be an alternative source of genes for resistance to sorghum midge.

1 Introduction

Sorghum, *Sorghum bicolor* (L.) Moench is an important cereal crop in the semi-arid tropics (SAT). It provides food, feed and forage, but grain yields on peasant farms are generally low, due partly to insect pest damage. Nearly 150 species of insects have been recorded as pests of sorghum (JOTWANI et al., 1980), of which sorghum midge, *Stenodiplosis (Contarinia) sorghicola* (Coquillett) is the most important pest world-wide (HARRIS, 1976). Host-plant resistance is an effective means of keeping midge populations below economic threshold levels (SHARMA, 1993), and breeding for resistance to midge is considered an integral part of sorghum improvement programmes.

Sources of resistance to sorghum midge have been identified by several workers (JOHNSON et al., 1973; WISEMAN et al., 1973; ROSSETTO et al., 1975; SHYAM-SUNDER et al., 1975; PAGE, 1979; FARIS et al., 1979; SHARMA et al., 1993). Most of the high-yielding breeding lines developed at ICRISAT Center, India, have been derived from DJ 6514 (SHARMA et al., 1993). However, DJ 6514 and the breeding lines derived from it have shown high levels of susceptibility to sorghum

midge at Alupe, Busia, Kenya, indicating the possibility of the occurrence of a new biotype of midge in this region or the environment-induced breakdown of resistance mechanisms (SHARMA et al., 1999).

In Australia, sorghum hybrids with a range of resistance levels to sorghum midge are being grown by farmers (HENZELL et al., 1994; FRANZMANN, 1996). Most of these hybrids derive the midge resistance genes from a single source of resistance (JORDAN et al., 1996). Resistance to insects and diseases often breaks down when a cultivar is grown over large areas or cultivated continuously for a long period of time. This requires deployment of cultivars with newer genes for resistance. Incorporation of additional genes imparting resistance can also increase the levels, and longevity of resistance to crop pests. The wild relatives of sorghum can prove to be a useful source of resistance to sorghum midge. Seventeen species of indigenous sorghums have been collected from Australia (LAZARIDES et al., 1991). The wild relatives of sorghum are not a natural host to the sorghum midge (HARRIS, 1979), and midge females do not lay in several wild relatives of sorghum (FRANZMANN and HARDY, 1996).

In the present studies, we examined a few more wild relatives of sorghum for their acceptance as oviposition sites by the midge females, and compared the response of midge females to odours emanating from different sorghum species to those from *S. bicolor* and *Sorghum halepense*, the preferred hosts of this insect.

2 Materials and methods

Eight species of wild relatives of sorghum were evaluated for resistance to sorghum midge, *S. sorghicola*. These species normally grow under wild conditions, and are used as a fodder for cattle. However, they can be used as a source of useful genes for conferring resistance to sorghum midge. This is useful to broaden the basis and increase the levels of resistance to this insect.

2.1 Plants

Plants were grown in the greenhouse in plastic pots (20 cm diameter, 30 cm high) containing a mixture of soil (black vertisols) : sand : peat (3 : 2 : 1). The soil mixture was thoroughly mixed in a rotary mixture, and 0.02 kg lime was added to each pot to adjust the pH of the potting mixture. The plants were watered regularly, as needed. A slow-release fertilizer (Osmocote Plus® (Scotts Europe, Heerlen, The Netherlands); N 15%, P 4.8%, K 10.8%, S 3.6%, Mg 1.2%, Mn 0.06%, Cu 0.05%, Mo 0.02%, B 0.02%, Zn 0.015%, and Ca 3%) was applied to the plants 15 days after germination (at a rate of 10 g per pot). Plants were grown as individual seedlings in each pot, which developed into clumps of 5–20 flowering tillers at maturity.

2.2 Insects

Sorghum midge females were obtained from sorghum panicles collected from a field in the Darling Downs, Queensland, Australia and kept in 30 cm × 45 cm brown paper bags in the laboratory at 27 ± 2°C, 60 ± 5% relative humidity, and 12 h photoperiod. An inverted transparent plastic jar (21.5 cm long, 10.5 cm diameter), with three wire-mesh-screened windows (4 cm diameter), two on the sides and one at the top was placed over the paper bag, and tied to it using a 1-cm-wide rubber band. The rubber band was twisted at the rim of the jar and pulled onto the upper end of the handle of the jar to keep the jar upright without support. Upon emergence, sorghum midges moved upward into the plastic jar because of their positive phototactic behaviour. The sorghum midges were allowed to mate in the plastic jar until 1000 h. Each jar containing sorghum midges was covered on the sides with a sheet of black polyethylene. Sorghum midges

were collected in a 20 ml glass vial attached to an opening (2.5 cm diameter) in the lid of the jar. A small piece of clay was used to hold the vial to the lid. The sorghum midges moved into the glass vial as a result of attraction to light. Vials containing 40–50 sorghum midges were removed from the jar and replaced with a new one. Twenty sorghum midge females were collected from each vial and the males were allowed to fly away.

2.3 Antixenosis to oviposition

At flowering, five plants at anthesis were caged with midge females during the morning hours. Wire-framed cages (20 cm diameter, 30 cm long) were placed around the panicles and supported on a wooden stick. The position of the head-cage was adjusted so that the panicles were placed in the centre of the cage. Wire-framed cages were covered with a white nylon cloth bag (20 cm diameter, 40 cm long). Twenty-five spikelets at anthesis were retained on each panicle, and 5, 10 or 50 midge flies were released inside each cage in the morning hours. The infested panicles were detached the following day, and kept in the deep freeze till observation. The spikelets were dissected under a binocular microscope (50×), and observations were recorded on percentage spikelets with eggs, and number of eggs per 25 spikelets.

2.4 Response of sorghum midge females to the odours from panicle of different *Sorghum* species

Relative preference of sorghum midge females to odours emanating from the flowering panicles of eight sorghum species in relation to *S. halepense* was studied under dual-

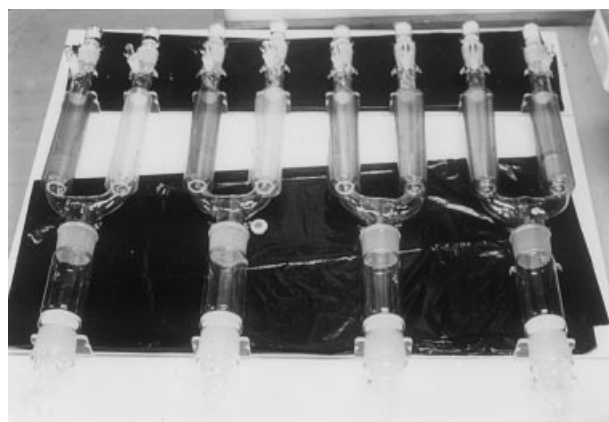


Fig. 1. Glass olfactometer

Sorghum species	Spikelets with eggs (%)	No. eggs per 100 spikelets
5 midge females per panicle		
<i>Sorghum halepense</i>	30.7 (5.4)	66.8 (8.17)
<i>Sorghum amplum</i>	00.0 (1.0)	0.0 (1.00)
<i>Sorghum bulbosum</i>	00.0 (1.0)	0.0 (1.00)
<i>Sorghum angustum</i>	00.0 (1.0)	0.0 (1.00)
10 midge females per panicle		
<i>Sorghum halepense</i>	66.7 (8.1)	216.0 (14.69)
<i>Sorghum amplum</i>	0.0 (1.0)	0.0 (1.00)
SE +	7.2 (0.55)	27.6 (2.32)
LSD at 5% t	22.8 (1.74)	21.7 (6.96)

Figures in parentheses are /N + 1 square-root transformed values.

Table. Oviposition by the sorghum midge, *Stenodiplosis sorghicola* on four wild relatives of sorghum under no-choice headcage conditions (Queensland Department of Primary Industries, Toowoomba, 1996)

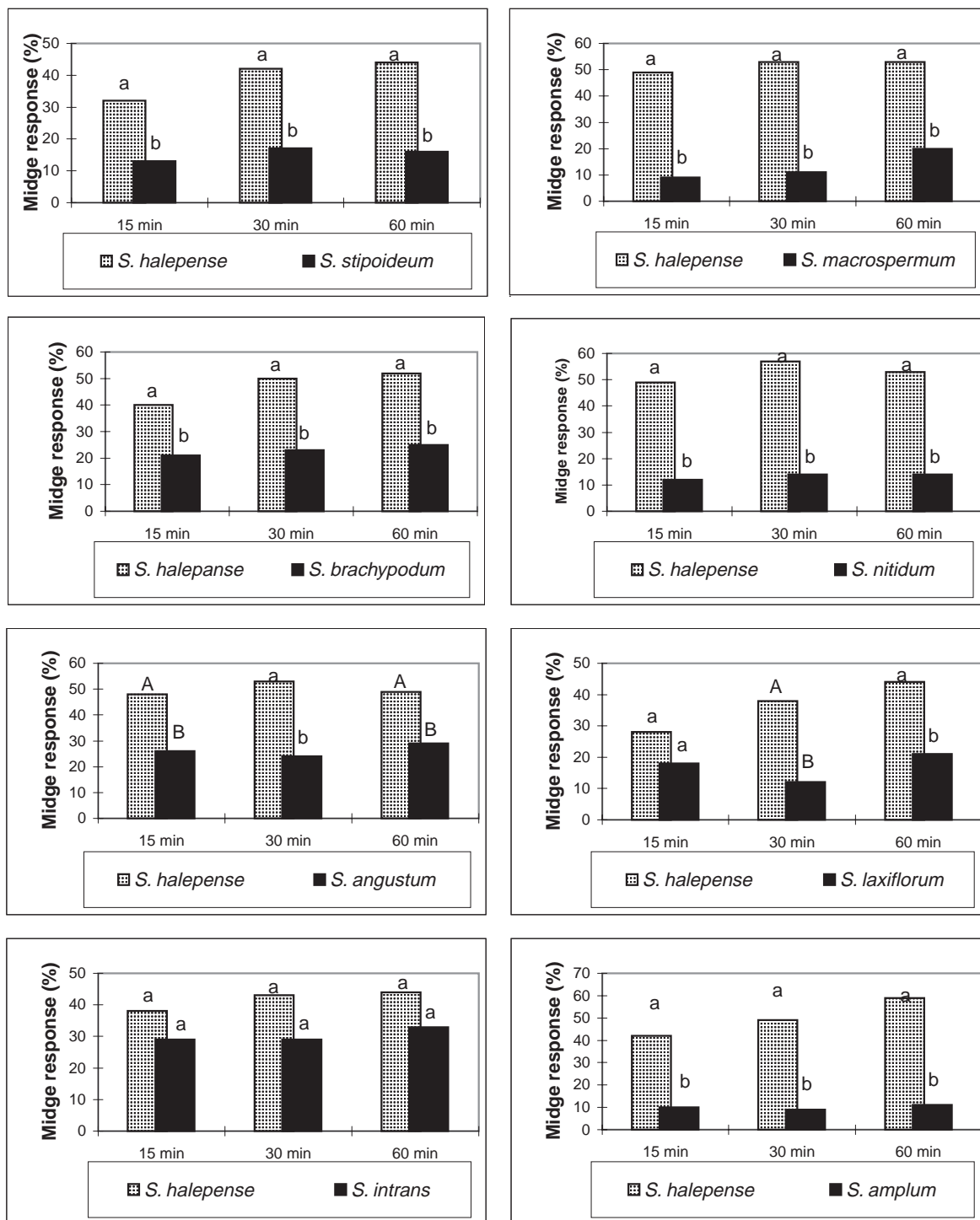


Fig. 2. Relative attraction to odours from the flowering panicles of wild relatives of sorghum to the sorghum midge, *Stenodiplosis sorghicola* females in dual-choice olfactometer tests (Toowoomba, QDPI 1996). Bars followed by the same letter at an observation period within a comparison are not significantly different at $P < 0.05$ (small letters), and $P < 0.07$ (capital letters)

choice conditions using a glass olfactometer having an insect-holding chamber (6 cm diameter, 23 cm long) (fig. 1). One end of the glass chamber was blocked with sintered glass, followed by an 18-cm-long glass joint tapering into a 2-cm-diameter tube. A plastic tube (1.8 cm diameter, 15 cm long) was connected to the glass tube. The other end of the plastic tube was connected by a T-joint to an airtight vacuum chamber (17 cm diameter, 15 cm height). A plastic hose (1.5 cm diameter, 3 m long) was connected to the vacuum chamber at one end and to a vacuum pipe inlet (connected to

the central vacuum system) at the other. The knob of the vacuum inlet was adjusted carefully to create a steady air flow through the glass apparatus. A bifurcated T-joint to which two glass arms (3.5 cm diameter, 30 cm long) were attached was connected to the insect-holding chamber at the other end. The glass arms were blocked with sintered glass at 18 cm, leaving an 11 cm portion to hold flowering branches of sorghum panicle (the stage at which sorghum midge females lay eggs in sorghum panicles). Five rachis branches from a flowering sorghum panicle were placed in this section

and used to measure the attraction of sorghum midge females to odour alone or in combination with visual stimuli. A 9 cm glass tube containing charcoal and cotton wool to filter the incoming air was attached to the end of this section. The glass units were placed horizontally on a wooden board placed on a table in the laboratory. Four glass units were placed on the wooden board and used either as replicates or for comparing different treatments as described for each experiment. A black polyethylene sheet was placed under the glass apparatus to avoid light from the white wooden board influencing the orientation behaviour of sorghum midge females.

Twenty sorghum midge females were released into the holding chamber, and the sections of the glass apparatus were joined together immediately. The insect-holding chamber and 5 cm portions of the glass arms were covered with a black polyethylene sheet to provide a directional visual stimuli to the sorghum midge females. The glass apparatus was cleaned with soap, rinsed with alcohol, and dried at 105°C after each experiment. In the first experiment, eight wild relatives of sorghum were compared with *S. halepense* for their preference to the midge females in dual-choice tests. The number of midges moving to the end of the olfactometer arm, near to the source of odour stimuli, was recorded 15, 30 and 60 min after initiating the experiment. In the second experiment, relative preference of midge females to the panicles of *S. halepense* was compared with *S. bicolor* (IS 10712 – red grain, and ICSV 197 – white grain). The glass apparatus was cleaned with soap, rinsed with alcohol, and dried at 105°C after each experiment.

In multi-choice tests, the response of midge females to four sorghum species was observed under multi-choice conditions in a 30 cm × 30 cm × 30 cm cage. The cage was made of aluminium frame, and the four sides and the top were covered with a thin transparent polyethylene sheet. The top of the cage was covered with a black polyethylene sheet to block the effect of tube lights in the laboratory. Twenty midge females were released in the centre of the cage, and the numbers settling on the panicles of different sorghum species were recorded at different intervals. The position of different species was changed in each replication to avoid the directional effect of natural light.

Data were transformed to square-root values, and subjected to analysis of variance. Significance of difference between treatments was judged by *F*-test, and the differences between treatment means were compared by least significant difference (LSD) at $P < 0.05$.

3 Results

3.1 Antixenosis to oviposition

Stenodiplosis (Contarinia) sorghicola females did not lay eggs in the spikelets of *Sorghum amylum*, *Sorghum bulbosum* and *Sorghum angustum*, whereas 31% of *Sorghum halepense* spikelets contained eggs when infested with five midges per panicle (table 1). There were 67 eggs per 100 spikelets in *S. halepense* compared with none in the wild relatives such as *S. amylum*, *S. bulbosum*, and *S. angustum*. At 10 midges per panicle, *S. halepense* had 67% spikelets with eggs and 216 eggs per 100 spikelets, whereas no eggs were laid in *S. amylum*. At 50 midges per panicle, one egg was laid in the spikelets of *S. amylum*. Thus, the wild relatives of sorghum were not accepted as hosts for egg laying by the sorghum midge females.

3.2 Attraction of sorghum midge females to the odours from panicles of different *Sorghum* species

Midge response to odours emanating from *S. halepense* panicles was greater than to the odours from *Sorghum stipoides*, *Sorghum brachypodium*, *S. angustum*, *Sorghum macrospermum*, *Sorghum nitidum*, *Sorghum laxiflorum* and *S. amylum* at 15, 30 and 60 min after releasing the insects in the olfactometer (fig. 2). The differences in midge response to the odours from *S. halepense* and *Sorghum intrans* were not significant. Response of sorghum midge females to the odours from the panicles of *S. amylum*, *S. nitidum* and *S. macrospermum* was quite low. Under multi-choice cage conditions, more midges settled initially on the panicles of *S. amylum*, which later moved to the panicles of other species (fig. 3). Number of midges attracted to the panicles of *S. bicolor* increased over time. Least number of midges were recorded on the panicles of *S. angustum*. Numerically, more midges responded to the panicles of *S. bicolor* (IS 10712) than to *S. halepense*, whereas the differences in midge response to ICSV 197 (*S. bicolor*) and *S. halepense* were not significant (fig. 4). These observations suggested that *S. halepense* is as much a preferred host of sorghum midge as *S. bicolor*.

4 Discussion

Sorghum bicolor is the most important host of sorghum midge world-wide. It has also been recorded from other species of *Sorghum* subsection *Sorghum* (*Sorghum dochna*, *Sorghum sudanense*, *Sorghum arundina-*

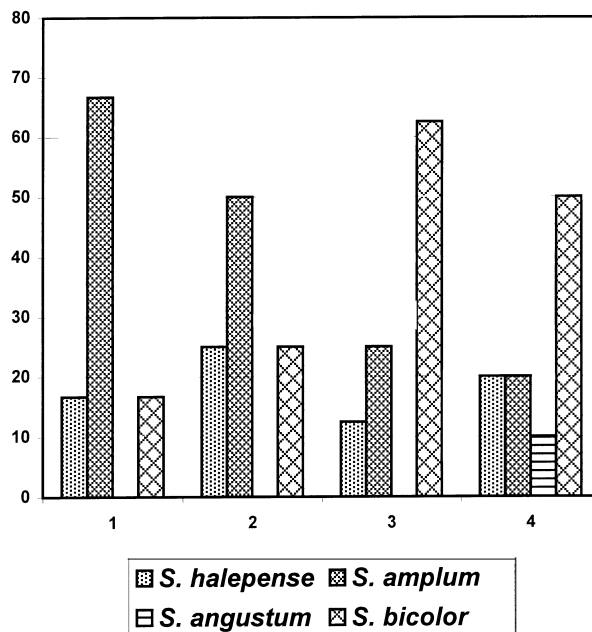


Fig. 3. Attraction of the sorghum midge, *Stenodiplosis sorghicola* females to the flowering panicles of four sorghum species under multi-choice conditions in a cage (Toowoomba, QDPI 1996). Bars followed by the same letter at an observation period within a comparison are not significantly different at $P < 0.05$

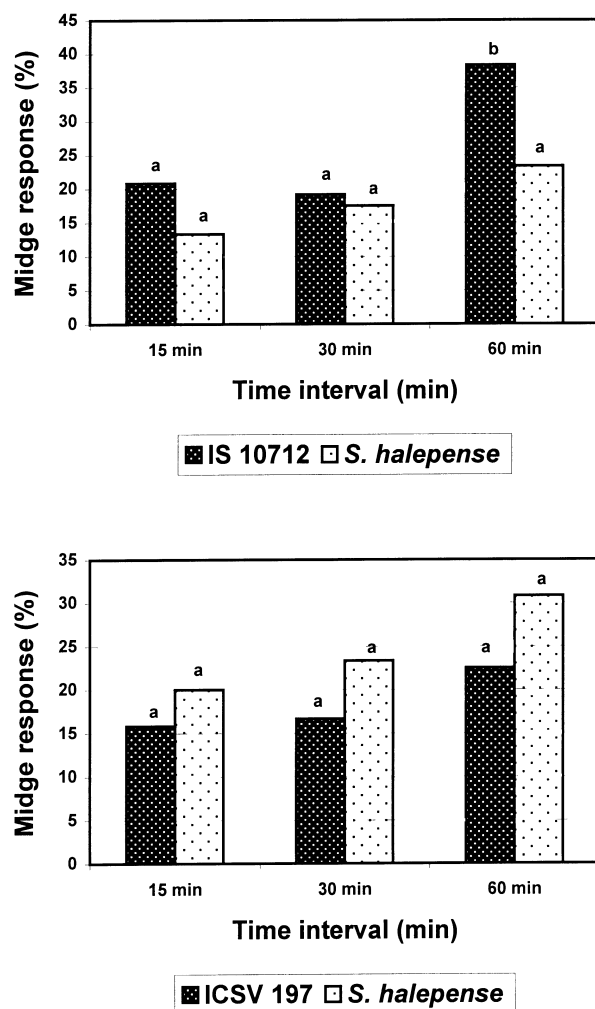


Fig. 4. Relative attraction of sorghum midge, *Stenodiplosis sorghicola* females to *Sorghum halepense* and *Sorghum bicolor* panicles at flowering in dual-choice olfactometer tests (Toowoomba, QDPI 1996). Bars followed by the same letter at an observation period within a comparison are not significantly different at $P < 0.05$

ceum, *S. halepense* and *Sorghum verticilliflorum*). HARRIS (1979) examined a wide array of midge specimens collected from sorghum, closely related wild species of sorghum, and some wild Gramineae and Cyperaceae from Australia, and concluded that *S. sorghicola* is restricted to *Sorghum* subsection *Sorghum*. Other species of *Contarinia* have evolved as specific pests of *Parasorghums* (HARRIS, 1979). *Contarinia plumosi* and *Contarinia roperi* have been described from *Sorghum plumosum*, and *Contarinia intrans* from *S. intrans* and *S. stipoideum*. The other species of Gramineae are infested by distinct species of midges (HARRIS, 1979). Thus, species of *Parasorghum* and *Stiposorghum* are not the hosts of sorghum midge. This has also been confirmed by earlier observations of FRANZMANN and HARDY (1996), who reported that sorghum midge females failed to lay eggs in the spikelets of 13 indigenous sorghums from Australia. A few eggs were observed in the spikelets of *S. bulbosum*, *Sorghum leiocladum*, *S. macrospermum*, *S. plumosum*, and *S. stipoideum*.

Difficulty in oviposition or oviposition non-preference is the principal mechanism of resistance to sorghum midge (SHARMA et al., 1990; FRANZMANN, 1993). This characteristic is also evident in the wild relatives of sorghum that are resistant to sorghum midge, where either the midges do not lay any eggs, or only a few eggs are laid in the spikelets. The non-host status of these sorghum species is also evident in terms of low response of midge females to the odours emanating from these sorghums. Differences in attraction to *S. sorghicola* females have also been observed in different *S. bicolor* genotypes (SHARMA and VIDYASAGAR, 1994). That only a few or no eggs are laid by *S. sorghicola* in wild relatives of sorghum, and the occurrence of species-specific midges in *Sorghum* indicates a close co-evolution of various *Sorghum* species and the *Stenodiplosis/Contarinia* species.

It has been suggested that wild relatives of sorghum can be a useful source of resistance to insects in sorghum (NWANZE et al., 1995). Attempts to cross any of the wild sorghums with *S. bicolor* have not been successful (HUEL GAS et al., 1996). Some success has already been achieved in transferring resistance to shoot fly, *Atherigona soccata* Rondani from *S. versicolor* to the cultivated sorghum (NWANZE et al., 1995). Various biotechnological approaches such as embryo rescue may be used to transfer the genes for insect resistance into the cultivated sorghums. However, it is not clear whether the mechanisms of resistance to sorghum midge in the wild sorghum species are different from those of the cultivated species. Further work is needed on the characterization of the mechanisms of resistance to sorghum midge in the wild relatives, and the development of techniques for introgression of useful genes from the wild relatives into the cultivated sorghums.

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