Evaluation of a Laboratory Bioassay for Determining Resistance Levels to Sorghum Midge *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in Grain Sorghum

B. A. FRANZMANN

Department of Primary Industries, P.O. Box 102, Toowoomba, Qld 4350.

ABSTRACT The level of resistance to the sorghum midge, *Contarinia sorghicola*, in a range of grain sorghum hybrids was assessed: in the laboratory, by measuring oviposition (eggs/spikelet and % spikelets infested); in the glasshouse, by measuring oviposition and seed set; and in the field, by measuring panicle weight loss per ovipositing female per day. The levels of oviposition and seed set determined in the laboratory and glasshouse trials were significantly correlated with the field parameter. Number of eggs per spikelet was the laboratory parameter most highly correlated with the field result ($r = 0.93$, $P < 0.01$). Studies on the rate of oviposition under the conditions of the bioassay confirmed 6 h as an appropriate interval to expose panicles to midges. A laboratory method based on estimating the number of eggs produced by 5 midge females on 50 flowering spikelets in 6 h at 25 °C and 75%rh is suggested as a practical method of testing for level of resistance in those sorghum hybrids showing ovipositional antixenosis.

Introduction

The sorghum midge, *Contarinia sorghicola* (Coquillett) is the main pest of sorghum in Australia (Passlow *et al.* 1985) and probably the most important insect pest of the crop worldwide (Young and Teetes 1977). Females oviposit into spikelets, usually at flowering, and during the following 2-3 weeks the larvae feed on the ovary, preventing kernel development. The sorghum midge costs Australian producers an estimated $8m per year in chemical control and loss of grain yield (Henzell *et al.* 1993). Commercial grain sorghum hybrids with a degree of resistance to sorghum midge are now being grown by Australian farmers to minimise midge damage. An economic injury level is required for each of these hybrids, because host plant resistance offers only partial protection and some insecticide use may be necessary. These levels are calculated from an assessment of the number of ovipositing females present in the crop (Franzmann *et al.* 1992).

The most appropriate measure of the level of resistance to sorghum midge is the amount of yield lost when subject to a standard midge infestation. Franzmann *et al.* (1986) reported that the average weight loss per panicle per ovipositing midge per day for a range of commercial, midge-susceptible sorghum hybrids was 1.4 g. The figure for a midge resistant hybrid ATx2755/RTx2767 was 0.4 g. The field trials used to develop these figures were very labour intensive and expensive. Experimental variability was high and there were significant interactions with environmental factors. The resistance in available breeding material is due largely, if not completely, to ovipositional antixenosis (Franzmann 1993). An obvious extension of this was to investigate whether the level of resistance could be assessed by measuring oviposition only, and whether this could be done reliably in the laboratory. Levels of midge resistance were compared when measured in a laboratory, glasshouse and a field test with the objective of developing a relatively simple and inexpensive assessment method.

Materials and methods

Laboratory bioassay. The nine hybrids to be evaluated (Table 1) were grown in the field. As the panicles (heads) began to emerge they were covered with fine nylon gauze bags to exclude ovipositing females. Panicles flowered a few days later. Early in the morning of the test flowering rachis branches bearing spikelets were collected and taken to the laboratory. Treatment plots consisted of 50 flowering spikelets covered with a 180 mL ventilated plastic container. A small amount of water was also provided by dabbing the branches with a moist brush. Females emerging with males early in the morning from field-collected panicles were collected by allowing them to walk into glass vials and five females were introduced into each container about mid-morning. Five replications were prepared for each treatment. After 6 h in a constant temperature room (25 °C:75%rh), branches were removed and frozen for subsequent dissection of spikelets to count eggs under a microscope.

Glasshouse trial. Experimental design was randomised blocks with single plant plots of each of seven hybrids (Table 2) replicated five times. When panicles were in flower, rachis branches were removed leaving 250 flowering spikelets per panicle. Each panicle was caged in a cylindrical wire frame (length 25 cm; diam. 15 cm) covered with fine nylon gauze. About mid-morning 25 females (procured as above) were introduced into each cage. Females were left to oviposit for 6 h, after which 50 spikelets from each panicle were collected at random and frozen for later dissection for eggs. When the first adult emerged, 50 further

spikelets were sampled and examined to determine numbers of pupae and the percentage seed set. The numbers of adults emerging per day from the remaining 150 spikelets were recorded. After completion of adult emergence, 25 spikelets from each panicle were dissected for counting of diapausing larvae.

Table 1. Oviposition by 5 sorghum midge females caged on 50 flowering spikelets of different sorghum hybrids in the laboratory for 6 h at 25 °C and 75%rh.

Hybrid		Eggs/spikelet	% spikelets infested
ATx3197/RQL20	(S)	5.74a*	90.4a
ATx378/RQL12	(S)	3.18b	80.8ab
Image T2	(R)	3.38b	76.8ab
MR40	(R)	2.81bc	76.0ab
Barrier	(R)	2.46bcd	68.8bc
MR Sputnik	(R)	1.75cd	58.4cd
AQL38/RQL36	(R)	1.74cd	53.6cd
AQL39/RQL36	(R)	1.66cd	51.2d
AQL29/RQL36	(R)	1.37d	47.2d

(S) = susceptible; (R) = resistant
* Means within columns not followed by the same letter differ significantly ($P < 0.05$).

Field trial. A field trial was carried out at Gatton in southern Queensland to compare nine hybrids (Table 3) under insecticide treated and untreated conditions. A randomised block layout with four replications was used and included a sprayed and an unsprayed plot of each hybrid in each block. Individual plots comprised 4 rows each 12 m long. During flowering counts were made of the number of ovipositing female midges on seven panicles in each unsprayed plot (between 9 and 11 am each day) and the number of flowering panicles in 10 m of row. Sprayed plots were treated every 3-4 d with 0.002% fenvalerate using a knapsack sprayer.

After completion of flowering percentage seed set was visually estimated on 10 panicles in each plot. Grain produced along 10 m of the two middle rows in each plot was harvested with a header and the seed weighed. The weight loss per panicle per ovipositing midge per day was calculated from the midge and panicle counts and the difference in grain weight between sprayed and unsprayed plots.

Laboratory bioassay—Exposure period. The duration of midge oviposition in the field approximates 6 h (Modini *et al.* 1987), a period initially chosen to compare resistance levels amongst hybrids. Two later trials examined oviposition on hybrids with different levels of resistance, at various time intervals over an 8-h period.

Three hybrids were used in each trial, ATx378/RQL12 (susceptible), AQL41/RQL36 (low resistance), AQL39/RQL36 (moderate resistance). The trials were set up as for the 6-h laboratory bioassay described earlier, except that oviposition was allowed for 0.5, 1, 2, 4 or 8 h. There were 5 and 6 replications in trials 1 and 2, respectively. In trial 1, old rachis branches were removed and new branches supplied after each specified time interval. In trial 2, branches were not changed, with oviposition being allowed on

Table 2. Oviposition and infestation by 25 sorghum midge females caged on 250 flowering spikelets of different sorghum hybrids for 6 h under ambient conditions in the glasshouse.

Hybrid		No./spikelet		% spikelets infested		% seed set	Adults per 100 spikelets	Time for develop-ment (d)
		eggs	pupae	eggs	pupae			
ATx3197/RQL20	(S)	1.96a*	1.24a	70a	68a	32a	85a	19.7c
ATx378/RQL12	(S)	2.19a	0.95ab	65a	52b	47ab	78a	19.7c
Barrier	(R)	1.62ab	0.72bc	52ab	44bc	56bcd	46b	20.8ab
MR Sputnik	(R)	0.97bc	0.68bc	39bc	45b	53bc	19c	20.9a
MR40	(R)	0.78bc	0.59c	34bc	38bcd	61bcd	51b	19.8bc
AQL38/RQL36	(R)	0.50c	0.38cd	33c	29cd	67cd	17c	20.1abc
AQL39/RQL36	(R)	0.26c	0.30d	14d	24d	74d	17c	19.2c

(S) = susceptible; (R) = resistant
* Means within columns not followed by the same letter differ significantly ($P < 0.05$).

Table 3. Response of grain sorghum hybrids to sorghum midge attack—field trial.

Hybrid		Midge × panicles#	Seed set ratings$		Yield (t/ha)		Weight loss (g)⁹
			sp	us	sp	us	
ATx3197/RQL20	(S)	2427ab*	6.6c	2.4a	7.5de	3.3a	2.55a
ATx378/RQL12	(S)	2396ab	6.6c	4.6b	6.8c	4.5b	1.30b
Image T2	(R)	1896bc	8.7ef	7.1cd	8.1ef	6.9c	0.95bc
Barrier	(R)	2678a	8.9fg	6.8c	8.1ef	7.6de	0.87bc
AQL29/RQL36	(R)	2591ab	9.2fg	7.0cd	8.1ef	6.9c	0.62bc
MR40	(R)	957d	9.4fg	8.6ef	8.6f	8.1ef	0.50bc
AQL38/RQL36	(R)	2143abc	9.0fg	7.8de	7.6de	7.3cd	0.28c
AQL39/RQL36	(R)	1479cd	9.8g	9.1fg	8.4f	8.2ef	0.27c
MR Sputnik	(R)	2340ab	9.5fg	8.0de	8.1ef	6.8c	0.15c

sp = sprayed; us = unsprayed—(S) = susceptible; (R) = resistant
# Sum of daily (ovipositing females per panicle × flowering panicles in 10 m).
$ Seed set ratings—1 = 10%, 2 = 20%, etc.
⁹ Weight loss (g) per ovipositing female per flowering panicle per day.
* Means within columns (columns and rows for seed set and yield) not followed by the same letter differ significantly ($P < 0.05$).

the original branch for the specified time, after which it was removed. Branches were immediately stored in a freezer for later dissection of spikelets for egg counts.

Simple linear regression analyses were carried out to relate the number of eggs per 25 spikelets (y-axis) to hours for oviposition (x-axis).

Results

Laboratory bioassay. Significantly ($P < 0.05$) more eggs were laid per spikelet on the susceptible hybrid ATx3197/RQL20 than on any of the others (Table 1). Numbers of eggs per spikelet on the other susceptible hybrid were not significantly different to Image T2, MR40 and Barrier. These three resistant hybrids and the two susceptible hybrids had the highest percentage of spikelets infested. The most susceptible hybrid carried more than four times the number of eggs/spikelet and had about double the number of spikelets infested than the most resistant hybrid.

Glasshouse trial. Significantly ($P < 0.05$) more eggs per spikelet were found in the susceptible hybrids and Barrier than in AQL38/RQL36 and AQL39/RQL36 (Table 2). A similar pattern was evident with number of pupae per spikelet and percentage infestation with eggs and pupae, percentage seed set and number of emerging adults. There were slight but significant ($P < 0.05$) differences in the number of days for immature development which did not follow the patterns of resistance as indicated from the other parameters. Very low numbers of diapausing larvae (1.03/100 spikelets) were found in spikelets after completion of adult emergence.

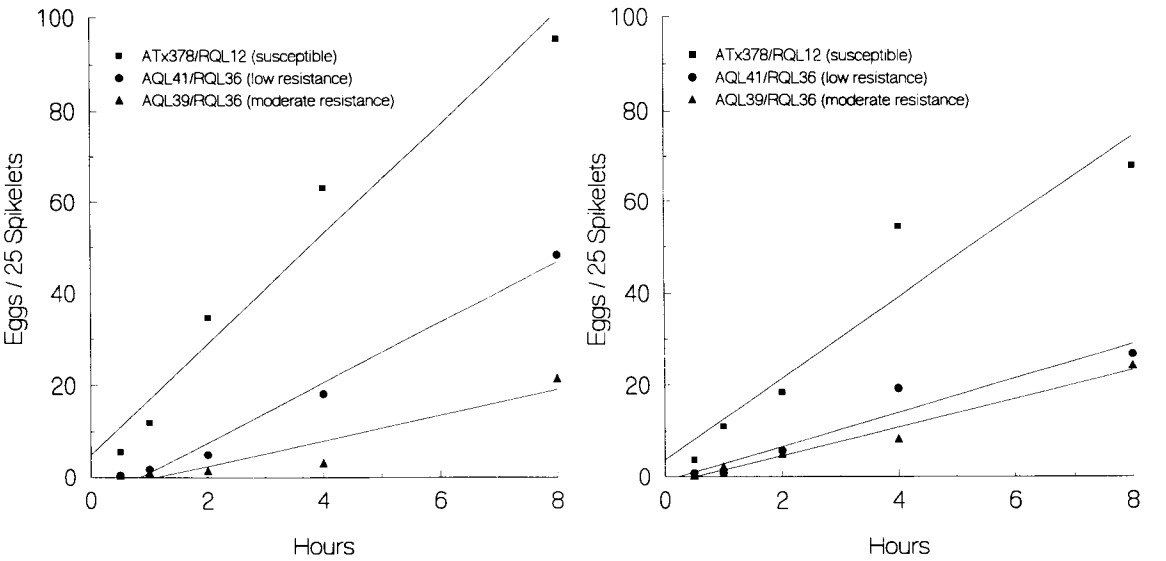
Field trial. Within the total trial flowering period of 18 d individual plots flowered for 11-14 d. The sum of the daily product of females per panicle and panicles in flower was significantly different ($P < 0.05$) between hybrids (Table 3). Midge infestation was particularly low for MR40 which demonstrated a considerable amount of antixenosis to visitation by ovipositing females.

There was a significnt ($P < 0.05$) interaction between hybrids and insecticide spraying for the seed set ratings. Spraying did not significantly increase seed set on MR40 and AQL39/RQL36.

Table 4. Correlation coefficients between six parameters of sorghum midge resistance measured in the laboratory, glasshouse and field.

		Eggs/spikelet		% infested		% seed set	Weight loss#
		lab	g'house	lab	g'house	g'house	field
Eggs/spikelet	lab	1.0					
	g'house	0.70	1.0				
% infested	lab	0.91**	0.83*	1.0			
	g'house	0.78**	0.97**	0.85*	1.0		
% seed set	g'house	−0.87*	−0.86*	−0.86**	−0.93**	1.0	
Weight loss#	field	0.93**	0.79*	0.80*	0.83*	−0.87*	1.0

Weight loss per ovipositing female per flowering panicle per day.
* ($P < 0.05$)
** ($P < 0.01$)



Figs 1, 2. Eggs per 25 spikelets in relation to number of hours exposed to oviposition in the laboratory: **(1)** new spikelets supplied after each time interval; **(2)** spikelets left for the entire oviposition interval.

Overall, the effect of spraying on seed set was highly significant ($P < 0.01$). For yield there was a significant ($P < 0.05$) interaction between hybrids and spraying. Spraying did not significantly affect the yield of Barrier, MR40, AQL38/RQL36 or AQL39/RQL36. However the effect of spraying on yield was highly significant ($P < 0.01$) overall. The weight loss per panicle per ovipositing midge per day was highest ($P < 0.05$) on the susceptible hybrid ATx3197/RQL12, however no significant differences were found between any of the resistant hybrids. Although the range of values for the resistant hybrids was large the variation was high (CV = 75.8%).

All parameters of resistance measurement were significantly correlated ($P < 0.05$) except eggs per spikelet in the laboratory and glasshouse trials ($r = 0.70$, $P = 0.08$) (Table 4). Eggs per spikelet was the laboratory parameter most highly correlated with the field measurement of resistance ($r = 0.93$, $P < 0.01$).

Laboratory bioassay—Exposure period. Results of the first laboratory trial indicated that the number of eggs deposited on regularly renewed branches increased linearly with time. The rate of deposition was negatively correlated with the level of resistance. All eggs had not been deposited on the resistant hybrids after 8 h but the data suggest that after 4 h the oviposition rate slowed down on the susceptible hybrid (Fig. 1). The rate of egg deposition was initially high and declined rapidly between 2 and 4 h on the susceptible branches whereas on the hybrid with low resistance a slow decline began after 4 h and on the hybrid with moderate resistance the rate was still increasing after this time. The slopes of the regression lines for each of the hybrids which were fitted to test the average rate of increase, were significantly different ($P < 0.01$).

A similar pattern of oviposition was recorded in trial 2 where midges were exposed to unchanged branches through the test (Fig. 2). The response on the susceptible hybrid differed significantly ($P < 0.05$) from the response on the two resistant hybrids. However, the two resistant hybrids did not differ significantly from each other.

Discussion

The high correlations between laboratory, glasshouse and field measurements of resistance parameters indicate that the laboratory bioassay may be used to assess the level of midge resistance due to ovipositional antixenosis. The overall result from the laboratory oviposition rate studies suggests that there would be no advantage to use other than a 6-hour exposure period.

The glasshouse trial result shows in general that the mechanism of resistance in all the hybrids is ovipositional antixenosis. There was no evidence of antibiosis or tolerance as initial differences in numbers of eggs were generally maintained through the pupal stage and were broadly reflected in percentage seed set and numbers of adults emerging. Only very slight differences were found in the number of days required to complete development and the pattern of these differences was not related to the level of resistance. These results substantiate the results of previous studies of midge-resistant genotypes in Australia (Franzmann 1993).

The seed set ratings in insecticide sprayed plots showed that the spray treatment failed to provide complete control, therefore potential yields in sprayed plots, particularly in the susceptible hybrids, should have been slightly higher than those obtained. Consequently, calculation of weight loss per ovipositing female was probably slightly underestimated.

Although results of field trials are potentially the best indicators of the level of midge resistance, the conduct of such trials are beset with a number of difficulties. Firstly, field trials are subject to environmental hazard. Secondly, the certainty of gaining a precise single final measurement of the resistance is jeopardised by the variability associated with each of the many measurements made during the course of the trial. Thirdly, the generation and interpretation of data in the field trials as described is based on a number of assumptions which may not strictly be justified; for example, damage per midge may not be independent of amount of flowering of individual panicles and daily midge population; spraying may not give 100% control; other pests may be controlled by spraying; spraying may directly affect the plant. Fourthly, the costs are high; the conduct of a field trial as described in this paper costs three times as much as a laboratory test. On the other hand, the conduct of the laboratory test has comparatively few difficulties and separation of resistance levels is more precise. An economic injury level can be calculated for each new hybrid by correlating its bioassay with that of another hybrid for which field data are available. In conclusion, the level of midge resistance in sorghum hybrids showing ovipositional antixenosis can be determined using a precise, quick, simple and inexpensive laboratory bioassay.

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References

FRANZMANN, B. A. (1993). Ovipositional antixenosis to *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in grain sorghum. J. Aust. ent. Soc. 32: 59-64.

FRANZMANN, B. A., ELDER, R. J., MURRAY, D. A. H., ROBERTSON, L. N., ROSSITER, P. D. and SIMPSON, G. B.

(1992). Sorghum. *In* Elder, R. J., Brough, E. J. and Beavis, C. H. S. (Eds). Managing insects and mites in field crops, forage crops and pastures, pp. 99-108. Information Series Q192004. QDPI: Brisbane.

FRANZMANN, B. A., PAGE, F. D. and MODINI, M. P. (1986). Evaluating midge resistance in grain sorghum. Proc. 1st. Aust. Sorghum Conf., Gatton, pp. 3.20-3.26.

HENZELL, R. G., BRENGMAN, R. L. and FRANZMANN, B. A. (1993). Advances in breeding for host plant resistance to the sorghum midge and for staygreen in grain sorghum. *In* Focused Plant Improvement: towards responsible and sustainable agriculture. Proceedings of the 10th Australian Plant Breeding Conference, Gold Coast, pp. 163-167.

MODINI, M. P., PAGE, F. D. and FRANZMANN, B. A. (1987). Diurnal oviposition activity in grain sorghum by *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae). J. Aust. ent. Soc. 26: 293-294.

PASSLOW, T., FRANZMANN, B. A. and ALLSOPP, P. G. (1985). Sorghum insect problems in Australia. *In* Proceedings of the International Workshop on Sorghum Insects Pests. Texas A&M University/ICRISAT: Patancheru, India. pp. 65-72.

YOUNG, W. R. and TEETES, G. L. (1977). Sorghum entomology. Ann. Rev. Ent. 22: 193-218.

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