

**University of Southern Queensland**

**New Midge Resistance for  
Australian Grain Sorghum**

A Dissertation Submitted by

Adam Hardy

Bachelor of Applied Science

For the Award of

Doctor of Philosophy

2007

## **Abstract**

The development and commercial release of midge resistant grain sorghum hybrids in Australia has been one of the real success stories of the sorghum industry. Almost all the current commercial sorghum hybrids grown contain a significant baseline of midge resistance that has greatly reduced that pest status of this insect. . However while breeding efforts have been successful, it seems only one mechanism of resistance remains present in commercial hybrids. This mechanism of resistance known as ‘ovipositional-antixenosis’ is polygenic in nature and has contributed to the gradual reduction in the genetic base of commercial sorghum hybrids, potentially limiting the advancement of other agronomic traits.

In order to expand both the level of resistance and the genetic diversity within Australian commercial sorghum hybrids, research was undertaken to capture new sources of midge resistance. Initially the goal of this work was to isolate and characterise the most promising new antibiosis sources of midge resistance previously documented within international breeding lines. However as the work progressed the discovery of a previously undescribed tolerance source of midge resistance led to more detailed studies of midge larval biology within a narrow range of sorghum germplasm lines to better characterise the resistance mechanism.

To complete this work a new water-injection technique was developed to allow more precise studies of midge larval biology where previous traditional screening methods were deemed inadequate. Using this method several antibiotic lines were shown to contain diverse modes of action, while the tolerance mechanism of resistance was confirmed and more accurately characterised. In all cases plant characters within the developing spikelet were associated with each resistance mechanism.

Overall, one source of resistance, found in lines derived from the Indian land race line DJ6514, was identified as superior to the others tested. Germplasm derived from this source was found to confer inter-related antibiosis and tolerance mechanisms. Both mechanisms were indirectly shown to be caused by the unique

antibiotic properties of the developing caryopsis. Larval mortality (antibiosis) increased in this line, in line with increased feeding against the developing caryopsis. A second tolerance mode of action was also linked to anti-feeding properties of the developing caryopsis. Larvae were recorded at higher than normal rates feeding away from the caryopsis resulting in the survival of both larvae and grain in a significant proportion of spikelets. This escape 'tolerance' mechanism of resistance was confirmed indirectly in several studies and could be artificially induced in other genotypes when larval feeding against the caryopsis was delayed. As such the resistance is better described as antixenosis to caryopsis feeding.

When both resistance mechanisms are present, the resistance isolated from DJ6514 was found to cause a three to four fold increase in seed set in resistant lines. This source of resistance has been directly selected for incorporation into the Australian breeding program and has been shown to be inherited simply as a single gene that needs to be deployed on both sides of the breeding program to ensure stable expression. When breeding efforts are complete a new suite of highly resistant and genetically diverse midge resistant sorghum hybrids will emerge into the marketplace.

**Declaration**

I certify that the ideas, experimental work, results, analyses, software and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.

.....

Date:

## Previously Published Material

Some parts of this work have been previously published and presented at several scientific conferences.

All the work in Chapter 5 was published in:

Hardy, A.T., Franzmann, B.A. & Sutherland, M. W. (2001) ‘Artificial infestation of sorghum spikelets with eggs of *Stenodiplosis sorghicola* (Coquillett) (Diptera: Cecidomyiidae) by water injection’, *Australian Journal of Entomology*, Vol. 40, pp. 380-385.

Some of the screening results in Chapter 7 were used in:

Hardy, A., Tao, Y. & Franzmann, B. 2001, ‘New Host-Plant Resistance to the Sorghum Midge and the Use of Molecular Marker Mediated Selection’, *Proceedings of the Fourth Australian Sorghum Conference*, Kooralbyn – Gold Coast Hinterland, CD Rom.

Various parts of this research have also been presented in various forms at the following conferences:

2000 31st Annual Scientific Conference of the Australian Entomological Society

2001 Fourth Australian Sorghum Conference

2006 Fifth Australian Sorghum Conference

## Acknowledgements

Many have contributed to the work in this thesis over nearly a decade. Thanks to you all.

Casual staff on university breaks have spent countless hours in the field and lab during summer. Lawrie Smith, Ken Duzza, Andrea Gerard, Petro Ioannis, Ashley Moore, Tammy Bull and James Mclean.

QDPI&F biometricians Dave Butler, Kerry Bell and Alison Kelly gave me invaluable advice and assistance with statistical analyses.

Nat Parker and Chris Freebairn took some of the great midge photos in this thesis.

Thanks also to the sorghum breeding team at Hermitage for all their help in developing material for testing and for advice and assistance in the finer points of plant breeding.

To my supervisors Dr Mark Sutherland, Dr Dave Jordan and Dr Bernie Franzmann, and my friends Cam and Heather Fletcher, I am deeply grateful for the long hours spent reviewing manuscripts and discussing the best way to write this thing. In particular I would like to thank Bernie for the many years of support and gentle guidance. Bernie you have always treated me more like a son than a student.

This research was funded by GRDC and QDPI&F.

To my beautiful wife Rosita, who kept me going to get this thing finished. You are also a great editor and a most cherished love of my life, thank you my darling.

Finally I would like to dedicate this thesis to my parents, who over many years have sacrificed much for their kids. Even though you are highly unlikely to ever fully read this thing, mum and dad, this one's for you!

# Table of Contents

<b>CHAPTER 1 Review of Literature</b> .....	<b>1</b>
<b>Introduction</b> .....	<b>1</b>
<b>Sorghum Midge Biology</b> .....	<b>2</b>
<b>Grain Sorghum Biology</b> .....	<b>7</b>
<b>Integrated Pest Management</b> .....	<b>8</b>
<i>IPM of sorghum midge</i> .....	8
<i>The contribution of midge resistant sorghum to IPM</i> .....	9
<i>A rating scheme for midge resistant hybrids in Australia</i> .....	10
<b>Economic Impact on Industry</b> .....	<b>11</b>
<i>Benefits of resistant hybrids to farmers</i> .....	13
<i>The emerging success of IPM in Australian grain sorghum</i> .....	15
<b>Host Plant Resistance Theory</b> .....	<b>17</b>
<i>Durability of resistance</i> .....	17
<i>Resistance mechanisms and components</i> .....	18
<i>Ovipositional antixenosis</i> .....	20
<i>Antibiosis</i> .....	21
<i>Tolerance</i> .....	22
<b>Midge Resistant Germplasm</b> .....	<b>23</b>
<b>Rationale and Structure</b> .....	<b>24</b>
Chapter 2 – A search for new sources of midge resistance .....	24
Chapter 3 – A closer examination of larval antibiosis .....	25
Chapter 4 – The water injection technique .....	25
Chapter 5 – Using the water injection method to study larval antibiosis .....	25
Chapter 6 – Is there a link between antibiosis and tolerance? .....	26
Chapter 7 – The inheritance of antibiosis .....	26
Chapter 8 – General discussion and future developments .....	26
<b>CHAPTER 2 A Search for New Sources of Midge Resistance</b> .....	<b>27</b>
<b>Introduction</b> .....	<b>27</b>
<b>Materials and Methods</b> .....	<b>28</b>
<i>Stage 1 – Screening for antibiosis</i> .....	28
Glasshouse trial procedure .....	28
Collation of results .....	30
<i>Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance</i> ...	31
Glasshouse screening procedure .....	32
Collation of results .....	33
Antixenosis and antibiosis .....	33
Tolerance.....	33
<b>Results</b> .....	<b>35</b>
<i>Stage 1 – Screening for antibiosis</i> .....	35
Contribution of pedicellate spikelets to midge resistance.....	36
Screening for antibiosis.....	37
Larval antibiosis.....	38

Pupal antibiosis .....	41
Adult development times .....	41
<i>Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance...</i>	41
Contribution of resistance mechanisms to seed set.....	41
Antibiosis and tolerance within infested spikelets.....	42
<b>Discussion.....</b>	<b>45</b>
<i>Stage 1 – Screenings for antibiosis .....</i>	45
Larval antibiosis .....	45
Pupal antibiosis .....	47
Adult development times .....	47
<i>Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance...</i>	48
<b>Summary and Conclusion .....</b>	<b>50</b>
<b>CHAPTER 3 A Closer Examination of Larval Antibiosis .....</b>	<b>51</b>
<b>Introduction.....</b>	<b>51</b>
<b>Material and Methods .....</b>	<b>52</b>
<i>Developing a sampling regime .....</i>	52
<i>Glasshouse cage trial procedure .....</i>	53
<i>Collation of results.....</i>	54
<i>Statistical analyses.....</i>	56
<b>Results .....</b>	<b>57</b>
<i>Predicted verses actual immature developmental timelines.....</i>	57
Midge infestation and mortality within immature development stages.....	59
Number of eggs/spikelet .....	59
Percentage of spikelets infested with eggs.....	60
Midge immature feeding position and size .....	62
Midge damage, antibiosis and final seed set.....	67
<b>Discussion.....</b>	<b>69</b>
<i>Midge survival and development .....</i>	69
<i>Antibiosis components in each line.....</i>	70
<b>Conclusions .....</b>	<b>71</b>
<b>CHAPTER 4 The Water Injection Technique .....</b>	<b>72</b>
<b>Introduction.....</b>	<b>72</b>
<b>Materials and Methods.....</b>	<b>73</b>
<i>Water injection technique .....</i>	73
Collection of midge eggs .....	73
<i>Water injection procedure .....</i>	73
<i>Water injection technique verses natural cage method.....</i>	73
<i>Water injection trial.....</i>	74
Standard cage trial: natural egg lay.....	74
Water storage bioassay of midge eggs.....	74
<b>Results .....</b>	<b>75</b>
<i>Water injection technique verses natural cage method.....</i>	75
Eggs per spikelet.....	75
Percentage of spikelets infested.....	76



<i>Relative egg position within the spikelet</i> .....	77
<i>Water storage bioassay of midge eggs</i> .....	78
<i>Neonate movement</i> .....	80
<b>Discussion</b> .....	<b>82</b>
<b>Summary and Conclusions</b> .....	<b>84</b>

## **CHAPTER 5 Using the Water Injection Technique to Study Larval Antibiosis ..... 85**

<b>Introduction</b> .....	<b>85</b>
<b>Methods</b> .....	<b>85</b>
<b>Results</b> .....	<b>86</b>
<i>Midge infestation and mortality over the larval period</i> .....	86
Eggs per spikelet.....	86
Percentage of spikelets infested with eggs.....	89
<i>Midge immature feeding position and size</i> .....	90
<i>Midge damage and larval antibiosis</i> .....	94
<b>Discussion</b> .....	<b>95</b>
<i>Midge survival and development</i> .....	96
Egg eclosion rates.....	96
Larval size and movement.....	96
Larval mortality.....	97
<b>Summary and Conclusions</b> .....	<b>98</b>

## **CHAPTER 6 Is There a Link Between Antibiosis and Tolerance? ..... 100**

<b>Introduction</b> .....	<b>100</b>
<b>Methods</b> .....	<b>102</b>
<i>Isolating larval feeding to the developing caryopsis</i> .....	102
<i>Larval feeding with the caryopsis absent</i> .....	103
Developing the procedure.....	103
Trial on four lines.....	103
Collation of results.....	104
Statistical analyses.....	104
<i>Artificially Delayed Feeding against the Caryopsis</i> .....	105
( <i>Egg deposition delayed four days</i> ).....	105
Testing procedure.....	105
Collation of results.....	106
Egg infestation figures.....	106
Larval infestation, midge damage and record of tolerance.....	106
Statistical analysis.....	107
<b>Results</b> .....	<b>108</b>
<i>Isolating larval feeding to the developing caryopsis</i> .....	108
<i>Larval feeding with the caryopsis absent</i> .....	108
<i>Artificially Delayed Feeding against the Caryopsis</i> .....	109
( <i>Egg deposition delayed four days</i> ).....	109
<b>Discussion</b> .....	<b>110</b>

Summary and Conclusions.....	111
<b>CHAPTER 7 The Inheritance of Antibiosis .....</b>	<b>113</b>
<b>Introduction .....</b>	<b>113</b>
<b>Methods.....</b>	<b>113</b>
<i>Population development and selection .....</i>	<i>113</i>
<i>F5/6 Recombinant Inbred Lines (ICSV745 x 90562).....</i>	<i>114</i>
Screening Method .....	114
Data collection and analysis.....	115
<i>F1 and F2 populations.....</i>	<i>115</i>
Screening method.....	116
Data collection and analysis.....	116
<b>Results .....</b>	<b>117</b>
<i>F5/6 Recombinant Inbred Lines (ICSV745 x 90562).....</i>	<i>117</i>
Antibiosis scores in parent lines and repeatedly tested RILs.....	118
Segregation of antibiosis scores in RIL population .....	121
<i>F1 and F2 populations.....</i>	<i>122</i>
Midge oviposition across populations .....	122
Inheritance of antibiosis in the F1 hybrids.....	125
<b>Discussion.....</b>	<b>127</b>
<b>Summary and Conclusions.....</b>	<b>128</b>
<b>CHAPTER 8 General Discussion and Future Directions.....</b>	<b>129</b>
<b>Introduction .....</b>	<b>129</b>
<b>Different Mechanisms of Antibiosis .....</b>	<b>129</b>
<i>Antibiosis during larval establishment .....</i>	<i>130</i>
<i>Antibiosis caused by the developing caryopsis.....</i>	<i>130</i>
<b>Value of Antibiosis Mechanisms .....</b>	<b>131</b>
<i>Antibiosis during larval establishment .....</i>	<i>131</i>
<i>Antibiosis caused by developing caryopsis.....</i>	<i>131</i>
<i>The issue of resistance durability .....</i>	<i>132</i>
<b>Future Directions .....</b>	<b>134</b>
<i>Breeding for antibiosis using marker-assisted selection .....</i>	<i>134</i>
<i>Cloning the antibiosis gene.....</i>	<i>135</i>
<b>Summary and Conclusions.....</b>	<b>136</b>
<b>List of References .....</b>	<b>137</b>

## List of Figures

- Figure 2.1** Percentage of total midge immatures pupated within aborted and tolerant spikelets in seven lines sampled 17 days post egg lay. Analysis between lines was not possible due to insufficient larvae. .... **45**
- Figure 3.1** Sorghum flower – a diagrammatic and actual view of the parts of a spikelet
- (a) Drawing of a sessile spikelet consisting of: two outer glumes; two papery thin lemmas; one papery thin palea (often reduced/absent); and a split fleshy lodicule covering the base of the caryopsis. When spikelets were dissected, midge immatures were recorded at one of three positions indicated by the following numbering:
1. outside the spikelet against either glume
  2. between either glume and palea/lemmas
  3. against the caryopsis (including between caryopsis and lemmas/lodicule/palea)
- (b) Bottom three attached sorghum spikelets separated, two pedicellate spikelets and one sessile spikelet (all intact). Above (middle row) a sessile spikelet (flower) dissected to show either glume surrounding the caryopsis containing attached styles and stigmas. Above (top row), outmost the papery thin lemmas surrounding three anthers. .... **54**
- Figure 3.2** Formula used to calculate larval size. .... **55**

- Figure 3.3** Midge survival over time in glasshouse cage trials as a percentage of the original egg infestation across seven lines.  
 (a) Midge number per spikelet as a percentage of egg count (angular equivalent means).  
 (b) Percentage of spikelets infested as a percentage of egg count (angular equivalent means). Significant differences between lines on each day were recorded in both (a) and (b) above indicated by line LSD ( $p < 0.05$ ). ..... **61**
- Figure 3.4** Percentage of midge present against the caryopsis within each line at egg lay, and over the larval period. Bars indicate least significant difference between lines at any day (line.day LSD;  $p = 0.05$ ). ..... **64**
- Figure 3.5** Larval size (log transformed ocular units) of midge feeding against the caryopsis/lopicules within seven sorghum lines over the larval period. Differences between lines at any day (line.day LSD) indicated by line LSD bar after REML analysis at  $p < 0.05$ . ..... **66**
- Figure 3.6** Percentage of infested spikelets aborted due to midge feeding over the larval/pupal period. Differences between lines ( $p < 0.05$ ) across any day indicated (line.day LSD). ..... **68**
- Figure 4.1** Densities of midge eggs in five sorghum lines one day after egg insertion using three methods: natural caging of mated female midge adults (black); water injection, two eggs per spikelet (white); water injection, 4-6 eggs per spikelet (grey). Columns that have the same letter do not differ significantly from one another ( $p \geq 0.05$ ). ..... **77**

- Figure 4.2** Relative egg position within the spikelet [glumes (black); palea/lemma (light); ovary (grey)] of five lines using three methods of egg insertion [n – natural caging of mated female midge adults; w2 – water injection, two eggs per spikelet; w4 – water injection, 4-6 eggs per spikelet]. Statistical analysis was performed on one position of egg lay only (glumes). Columns that have the same letter do not differ significantly from one another on percentage of eggs at the glumes ( $p>0.05$ )..... **79**
- Figure 5.1** Midge survival over time as a percentage of the original egg infestation in eight lines after water injection of 4-6 eggs in each spikelet.  
 (a) Midge number/spikelet  
 (b) Percentage of spikelets infested  
 For both (a) and (b) above LSD between lines at each day shown. .... **88**
- Figure 5.2** Percentage of infested spikelets aborted due to midge feeding over the first three-quarters of the larval period (seven out of an estimated average of ten days larval development). Differences between lines at each day indicated by LSD bar ( $p=0.05$ ). ..... **94**
- Figure 7.1** Distribution of individual antibiosis scores in three antibiotic F5 recombinant inbred lines repeatedly tested over three years compared to those recorded in the antibiotic parent line ICSV745.... **119**
- Figure 7.2** Distribution of individual antibiosis scores in two antibiosis susceptible F5 recombinant inbred lines repeatedly tested over three years compared to those recorded in the susceptible (antixenotic) parent line 90562..... **120**

- Figure 7.3** Frequency distribution of mean antibiosis scores on a total of 122 F5 RILs from the cross ICSV745 x 90562. Scores are averages from 3-6 replicated testings of each RIL over one to three years testing. Grey bars indicate the range of scores recorded in the resistant antibiotic parent ICSV745 over 3-5 replications in all trials, black bars likewise for the susceptible (non-antibiotic parent) 90562, and white bars indicate scores not recorded in either parent. . **122**
- Figure 7.4** Frequency distribution of mean antibiosis scores on a total of 78 F2 lines from the cross B35 x 62191. Scores are averages from 2 replicated testings on one panicle of each line (top and bottom of the panicle). Grey bars indicate the range of scores recorded in the resistant antibiotic parent 62191 over 8 panicles, black bars likewise for the susceptible parent line B35 over 6 panicles, and white bars indicate scores not recorded in either parent..... **124**
- Figure 7.5** Distribution of individual antibiosis scores (calculated from the average of two scores at the top and bottom of each panicle) in the antibiotic parent line 62191, the susceptible (non-antibiotic) parent lines (B35, 31945) and three F1 hybrids developed from these lines..... **126**

## List of Tables

<b>Table 1.1</b>	Economics of midge resistance in Australian conditions without chemical control. ....	<b>14</b>
<b>Table 2.1</b>	Midge infestation within sessile and associated pedicellate spikelets in five lines under a glasshouse trial. Each plant was caged with a midge density of one mated female to ten sessile spikelets. ....	<b>37</b>
<b>Table 2.2</b>	Summary of ovipositional-antixenosis and larval antibiosis for 32 lines screened in 18 no-choice glasshouse cage trials under a midge density of 1 mated female midge to ten sessile spikelets. ....	<b>39</b>
<b>Table 2.3</b>	Summary of adult emergence and development in 32 lines over 18 no-choice glasshouse caged head trials under a midge density of 1 mated female midge to ten sessile spikelets. ....	<b>40</b>
<b>Table 2.4</b>	Summary of midge infestation and levels of antibiosis and tolerance within 18 sorghum genotypes over four years testing in glasshouse cage trials. ....	Error! Bookmark not defined.
<b>Table 3.1</b>	Two measures of midge infestation over six sampling intervals from egg lay to pupation in sessile spikelets of eight sorghum lines. Differences between lines on each day (*) and on all lines across days (#) are shown. Where appropriate log transformed (lg) and back transformed equivalent means (eq. m) were used to meet normality assumptions. ....	<b>58</b>
<b>Table 3.2</b>	Movement of midge larvae over time within the sessile spikelets of seven sorghum lines after natural egg lay in a glasshouse cage trial. Figures for each line are a percentage of the total larvae present at all three positions on that sampling day. Differences between lines on each day at each spikelet position (1-3) are indicated (*) along with differences across days for all lines (#). ....	<b>63</b>

<b>Table 3.3</b>	Larval size of midge (ocular units; 500 units = 1 mm) at three positions within the spikelet of seven sorghum lines over 12-14 days of larval growth. Log (lg) and back transformed equivalent means (eq. m) are shown after differences between lines on each day (*) and differences across days for all lines (#) were determined under REML analysis at $p < 0.05$ .....	<b>65</b>
<b>Table 3.4</b>	Pupal size (ocular units) of midge within seven sorghum lines at two sample dates (fifteen days after egg lay, and again at 17-19 days upon the emergence of the first adult from each panicle of each line). Significant differences in pupal size between lines at each sampling period(*), and between sampling periods for all lines (#) were recorded on log transformed data (lg) and back transformed equivalent means (eq. m) after REML analysis at $p < 0.05$ .....	<b>67</b>
<b>Table 3.5</b>	Summary of midge infestation, antibiosis and seed set within seven sorghum lines after natural cage infestation.....	<b>69</b>
<b>Table 4.1</b>	Survival, development and fitness parameters of midge neonates after eggs were stored for different periods in water at 4°C (Conditions of 25°C, 75% humidity were maintained after storage).....	<b>81</b>
<b>Table 5.1</b>	Midge egg and larval density on two measures of midge infestation in the sessile spikelets of eight sorghum lines after water injection of 4-6 eggs per spikelet. Differences between lines on each day (*) and between all lines across days (#) were recorded by ANOVA at $p < 0.05$ .....	<b>87</b>
<b>Table 5.2</b>	Movement of midge within the sessile spikelets of eight sorghum lines over the larval period after water injection of eggs. Figures for each line are a percentage of the total midge present at all three positions on that sampling day.....	<b>91</b>



<b>Table 5.3</b>	Larval size (ocular units; 500 units = 1 mm) of midge at three positions within the spikelet of seven sorghum lines over seven days of larval growth. Log (lg) and back transformed equivalent (eq. m) means are shown after differences between lines on each day (*) and between days for all lines (#) are recorded under REML analysis at $p < 0.05$ .....	<b>93</b>
<b>Table 5.4</b>	Summary of midge infestation, antibiosis and seed set within eight sorghum lines after spikelets were water injected with eggs at flowering and sampled nine days later.....	<b>95</b>
<b>Table 6.1</b>	Percentage of spikelets infested with larvae 15 days post water injection on four lines where the caryopses of all spikelets were artificially removed prior to egg infestation (Initial egg infestation estimates of 90-100%).....	<b>108</b>
<b>Table 6.2</b>	Midge larval infestation, damage and tolerance within seven lines infested with midge eggs four days post-anthesis.....	<b>109</b>

## List of Plates

<b>Plate 1.1</b> Adult female midge probing inside a flowering sorghum spikelet. ....	<b>1</b>
<b>Plate 1.2</b> Outer glume of a grain sorghum spikelet showing two freshly laid eggs inside (circled).....	<b>3</b>
<b>Plate 1.3</b> Aborted grain sorghum caryopsis and third instar larvae (excised from spikelet).....	<b>4</b>
<b>Plate 1.4</b> Close up of a sorghum panicle three weeks into grain fill showing an aborted (midge damaged) spikelet with an empty white pupal case at spikelet apex. ....	<b>5</b>
<b>Plate 1.5</b> Affect of midge resistance rating on seed set after exposure to high midge pressures of 50-100 midge per panicle at flowering. Seed set on panicles with increasing levels of midge resistance from left to right is approximately 1%, 50%, 75% and 90%.....	<b>11</b>
<b>Plate 2.1</b> Glasshouse grown sorghum lines at various stages of growth, several caged in one trial. Also shown is a close up of trimmed panicles inside cages.....	<b>29</b>
<b>Plate 2.2</b> Damage on sorghum caryopsis (kernels) after midge larval feeding 14 days post-anthesis. (a) midge pupae with aborted caryopsis; (b) 50% damage with large larva; (c) 25% damage - recorded as tolerance (large larva placed away from indented caryopsis feeding site); (d) un-infested caryopsis. ....	<b>34</b>
<b>Plate 3.1</b> Two dissected spikelets eight days post-anthesis showing caryopsis damage under midge larval feeding. Caryopses approximately 60% (a) and 100% (b) reduced in size by midge larvae. Caryopses in both (a) and (b) classified as irreparably damaged (aborted).....	<b>56</b>

# CHAPTER 1

## Review of Literature

### Introduction

Sorghum midge, *Stenodiplosis sorghicola* (Coquillett 1898), is the most ubiquitous and damaging pest of grain sorghum, *sorghum bicolor* (Monech) throughout the world (Young & Teetes 1977). This insect is a common panicle pest of grain sorghum almost everywhere the crop is grown and has been the subject of much research in Australia since its first discovery on sorghum in Queensland (Tyron, 1894). Globally, losses due to sorghum midge are known to vary over seasons and locations, but are thought to approximate 10-15% of the world sorghum crop (Sharma & Teetes, 1995). Recent estimates of annual costs to production are \$US28M in Texas (Peterson *et al.* 1994), \$US294M in the semi-arid tropics (ICRISAT 1992) and \$US7.9M in Australia (Henzell *et al.* 1996).



**Plate 1.1** Adult female midge probing inside a flowering sorghum spikelet.

## **Sorghum Midge Biology**

The sorghum midge is a fly of the family Cecidomyiidae. Adult flies are approximately 2 mm long and have a bright red abdomen making them relatively easy to see despite their small size (Plate 1.1). In spring midge populations begin to build up slowly from surviving diapaused larvae present in sorghum trash within the soil. Upon increasing soil temperatures and spring rains the diapaused larvae develop through to adults which fly to infest nearby flowering Johnson grass spikelets (*Sorghum halepense* L). Johnson Grass acts as a primary host crop for the midge population over a couple of generations before flowering grain sorghum and forage sorghum crops begin to flower in summer. (Teetes 1985; Franzmann *et al.* 2006). Both male and female adult midge are short-lived insects. Emergence of the male imago (adult) from the pupa begins at dawn under favourable temperatures 30-45 minutes before emergence of the female, and peaks one to two hours later (Fisher & Teetes 1982). Both Summers (1975), and Fisher *et al.* (1982) found that delayed emergence occurs at temperatures below 23°C and ceases altogether below temperatures of 13-16°C for males, and 18-21°C for females. Additionally Fisher and Teetes (1982) showed that rainfall resulted in decreased adult emergence, and increased mortality of pupae. After emergence, male midge swarm around the panicles from which they have emerged and mate with one or more females before the later-emerging females can expand their wings and leave the spikelet surface (Summers 1975, Taley *et al.* 1971). Females generally mate only once, and each female is capable of laying between 20-150 eggs (Hallman *et al.* 1984; Passlow 1965; Teetes 1985; Walter 1941). Egg lay occurs at temperatures between 20-40°C at 8-11 am on sunny days with a peak period of oviposition usually occurring around 10 am (Fisher 1981, Modini *et al.* 1987). Females will probe a suitable spikelet before remaining very still for approximately two seconds while an egg is laid. This procedure is repeated until the female is exhausted and the full quota of eggs is seldom deposited before the death of the female (Passlow 1965, Walter 1941).



**Plate 1.2** Outer glume of a grain sorghum spikelet showing two freshly laid eggs inside (circled).

Walter (1941) found that both males and females seldom lived for more than 24 hours, however this may be extended to a couple of days in field cage conditions when given water or dilute honey. Each female produces unisexual progeny at a ratio of near 1:1 female to male broods (Baxendale & Teetes 1981; Franzmann & Lloyd 1995).

Under sub-tropical conditions in Texas (USA) and Queensland (Australia) at mean daily temperatures of approximately 24°C, both Passlow (1965) and Walter (1941) recorded egg hatch after 2-3 days followed by larval periods of 10-12 days. The orange sausage shaped eggs (Plate 1.2) hatch into transparent white first instar larvae which begin feeding on the glume, or lemmas, and later move down to lie lengthwise against the developing caryopsis with their heads towards the base of the caryopsis. Larval feeding on the caryopsis creates a large depression in the normally plump filling caryopsis in which a fully fed larvae reverses itself and pupates (Walter, 1941). One larva feeding on the developing caryopsis is sufficient to prevent seed kernel development (Plate 1.3). Within each single seeded spikelet it is common for more than one egg, and subsequently more than one larvae to develop. Franzmann *et al.* (1989) found that an average of 2.3 larvae would survive to the pupal stage under

higher initial egg densities, and concluded that larval competition while feeding on the developing caryopsis is a significant mortality factor. Pupation occurs in the aborted spikelet and is followed in approximately 3-5 days by adult emergence (Plate 1.4)



**Plate 1.3** Aborted grain sorghum caryopsis and third instar larvae (excised from spikelet).

The total lifecycle of midge varies according to temperature. In central Queensland under field screen temperatures of 18-29°C and average soil temperatures of 32°C Passlow (1965) recorded a total lifecycle of 16-20 days. In glasshouse trials in Southern Queensland during summer, (Franzmann 1993a) recorded average development times of 19-20 days in two lines, while this increased to 23-27 days in a separate study in the autumn of the same year (Franzmann 1993b). This short generation time of 2-4 weeks allows many generations of midge to occur each season, which accounts for the build up of extremely high midge densities where the flowering period of sorghum is extended by successive planting dates.

A portion of late instar sorghum midge larvae construct silken cocoons and over winter in a state of facultative diapause within the aborted spikelets of their host plant (Dean 1911; Harding 1965; Passlow 1965; Walter 1941). In this way midges are able to avoid times of adversity when environmental conditions are unsuitable for both midge and sorghum development. Harding (1965) in Texas, and Passlow (1965) in Queensland, both found that the percentage of midges entering diapause increased rapidly as the end of the season approached, and that diapausing larvae become physiologically mature over an optimum period of 7.5 months. Forrester (1987) later showed that the rate of diapause development was temperature dependent, and that once diapausing larvae become physiologically mature diapause termination occurs after wetting at optimum temperatures between 20-30°C. Forrester (1987) concludes that these conditions of high humidity and high soil moisture coincide with the onset of the first spring rains.



**Plate 1.4** Close up of a sorghum panicle three weeks into grain fill showing an aborted (midge damaged) sessile spikelet with an empty white pupal case at spikelet apex.

The host plants for sorghum midge have been reviewed by numerous workers (Doering & Randolph 1963; Harding 1965; Harris 1961; Passlow 1965). While

numerous wild non-sorghum grasses have been reported as hosts of the sorghum midge, both Harris (1979), and Franzmann and Hardy (1996) conclude that only members of the genus *Sorghum* are hosts for the sorghum midge. These include Johnson grass (*S. halapense*), Columbus grass (*S. almum*), and Sudan grass (*S. sudanense*).

Johnson grass has been recognised as important in the population dynamics of the sorghum midge. Harris (1961) in Nigeria (Africa), Teetes (1985) in Texas (USA), and Franzmann *et al.* (2006) in Queensland (Australia) report that Johnson grass acts as an early season host for midge. Peak adult emergence of overwintering midge coincides with the widespread flowering of Johnson grass in an area after spring rains. Johnson grass acts as a host for two to three generations, and then midge migrate to infest flowering grain sorghum panicles.

Flowering grain sorghum crops are most susceptible to midge egg lay during anthesis. Female midge are attracted to sorghum crops via a combination of visual and chemical stimuli. Both Wiseman *et al.* (1972) and Sharma *et al.* (1990a) found that midge were attracted to yellow and white traps compared to darker colours such as blue or green. The latter authors also demonstrated the chemical and physical role of flowers in attracting and stimulating oviposition by sorghum midge. Chemical stimuli from viable pollen and receptive stigmata were found to attract and direct sorghum midge oviposition. Sharma *et al.* (2002) also found in lab choice tests that sorghum midge females were more readily attracted to yellow, red and green colours combined with odours than by colour, or odour alone. Hallman *et al.* (1984) found that under natural conditions sorghum midge lay approximately 90% of their eggs in flowering spikelets, about 10% in post flowering spikelets, and less than 0.5% of eggs in pre-flowering spikelets. Franzmann and Vaschina (1989) found that certain varieties may be particularly susceptible to egg lay in pre-flowering spikelets. Franzmann (1990) also found that while female midge have great difficulty laying in panicles after anthesis, under caged conditions second generation midge may reinfest post flowered aborted sessile spikelets in small numbers.



## Grain Sorghum Biology

Grain sorghum is the fifth most important cereal in the world behind wheat, rice, maize and barley (FAO 1995). Sorghum production is well suited to developing nations of the world because of its sustainability in marginal conditions with limited inputs of water, fertilisers, and pesticide. It is widely grown in a variety of subtropical and tropical climates where its versatility and yield is superior to other cereal grains. Sorghum production is greatest in Africa and Asia where it competes with maize and rice as a major food crop, while in countries such as the USA and Australia it is grown almost entirely as a crop for animal feed.

The flowering process in grain sorghum has been studied by numerous workers who give variable reports of flowering times (Diarisso 1997; Sharma & Hariprasad, 2002). Doggett (1988) concluded that blooming occurs chiefly in the morning before or after dawn, influenced by darkness, temperature and humidity. Under warm dry summer conditions some lines flower as early as 10 pm at night, while under cool wet conditions flowering may continue as late as 4 pm the next day. The flowering process begins with the fanning and opening of the two glumes; followed by the emergence of the stigmas and anthers; and is completed with the closing of the glumes. The entire flowering period of each spikelet may take from as little as 20 minutes to more than three hours, dependant on variety and environmental conditions. Pollen dehisces during flowering and germinates on the same day leading to fertilisation of the caryopsis and subsequent kernel development. Depending on growing conditions maximum seed size occurs 10-20 days later, while maximum dry weight of the seed usually occurs 25-50 days after flowering, and grain is harvested when moisture content drops below 15 percent. When planted under subtropical conditions, under even planting densities and optimum soil conditions, sorghum hybrids usually take between 60-80 days to flower (Doggett 1988). Flowering proceeds basipetally on each panicle, and Pendleton *et al.* (1994) reported that in commercially grown hybrids individual panicles completed flowering in eight days, while whole fields took 13 days to flower.

## **Integrated Pest Management**

In the 1950's a new theory 'Integrated control' was developed to manage insects in response to control problems that arose from the complete reliance on the one chemical method of control (Stern *et al.* 1959). The term was later modified and described as Integrated Pest Management (IPM), an ecological approach to crop protection in which different control methods are used to enhance each other, and together contribute to sustainable insect management practices over the long term. The international lesson from the history of insect management is that no single technology or method of control is likely to be ideal, at least for long (Binns & Nyrop, 1992). One of the main aims of IPM is to reduce pesticide usage. IPM in practice relies on benign tactics such as biological control, plant resistance, and cultural practices to maintain fluctuating pest populations below economic injury levels.

### **IPM of sorghum midge**

Such an integrated management approach may be adopted to control midge damage in grain sorghum, including cultural, chemical, and biological means of control. Cultural practices include: the destruction of Johnson grass to eliminate this early season host; the destruction of aborted sorghum spikelets within the soil containing diapausing larvae; the planting of uniform varieties with adequate fertiliser and moisture to ensure even flowering; and the early planting of sorghum to escape high midge numbers. Of these practices the early, uniform planting of sorghum crops is the most effective method of control, but lack of soil moisture or excessive rain can often mean that this is not possible (Sharma 1985; Teetes 1985). Where environmental conditions cause delayed and successive planting of crops the flowering period is usually extended, resulting in large midge infestations (Baxendale *et al.* 1984). Under such conditions the use of chemical control may be required. Sprays are most effective on adult midge as sorghum midge larvae within sorghum spikelets are protected within the glumes. Insecticide applications are used to control ovipositing females, which emerge in the morning and die in the afternoon. Application over the entire flowering period is expensive for a low-input

crop such as sorghum, may be only moderately effective, and has negative effects on the environment (Young & Teetes 1977).

The presence of chemical sprays may adversely affect any biological control present. Franzmann *et al.* (1989) found that larval parasitoids are known to provide some control of sorghum midge larvae. Passlow (1958) after a seven year study found that parasitism of midge larvae in late maturing crops may be as high as 24.2%. However he found that the average of 14.1% parasitism is a level of little economic significance, and such parasitoid populations build up only after yield damage has occurred. While other predatory insects such as lacewings, pirate bugs and spiders have been commonly observed controlling midge, their economic value is difficult to measure and thought to be minor (Walter 1941).

Plant resistance to insects can be an important component in IPM strategies, and the tactic has wide applicability and function (Kennedy 1987). There has been much effort placed into the search for and development of midge-resistant hybrids over the last three decades. Host plant resistance has many advantages over other methods of insect control. These include simplified management, decreased chemical spray costs, and the added benefit of biological control under decreased chemical usage (Teetes 1985). The use of genetically incorporated midge resistance offers the grower potential control at the cost of the seed alone.

### **The contribution of midge resistant sorghum to IPM**

Numerous workers have pointed out the financial benefits of developing insect resistant varieties. Schalk and Ratcliffe (1976) estimated that world-wide usage of insecticides would fall by approximately 319,000 tonnes (37% of the total) if insect resistant cultivars of corn, barley, sorghum and alfalfa were grown.

Efficient management of sorghum requires combining several strategies that suppress midge damage and abundance. The addition of midge resistant varieties simplifies management, decreases costs and provides greater midge control, ultimately resulting in greater profitability (Cronholm *et al.* 1993). Teetes *et al.* (1986) found that midge resistant sorghum hybrids gave higher yields and greater returns than susceptible hybrids under the same insecticidal spray regime. Hallman *et al.* (1984), and Franzmann *et al.* (1986), determined that for a range of susceptible

sorghum hybrids, 1.4 to 1.5 grams of grain (42-48 kernels) were destroyed by the progeny produced by one female per panicle, approximately 4% of the total kernels in each panicle. Similarly, each insect infesting resistant sorghum hybrids destroyed 0.32 to 0.4 grams of grain (nine kernels), less than 1% of the total kernels in each panicle.

### **A rating scheme for midge resistant hybrids in Australia**

In Australia the level of midge resistance as defined by Franzmann *et al.* (1986) has steadily increased with breeding efforts over the last decade through the combined efforts of Queensland Department of Primary Industries and Fisheries (QDPI & F) breeders and commercial seed companies who have incorporated elite midge resistant germplasm into their commercial breeding programs. Since 1993, the level of resistance in commercial hybrids has been identified by an industry-backed independent testing scheme (Franzmann *et al.*, 1996). Under this scheme sorghum seed sold within Australia is given an official midge resistance rating of one to seven according to the level of midge resistance present. Sorghum hybrids have been tested in joint QDPI & F and Seed company trials to determine midge damage ratings of hybrids compared to seven standard control hybrids. A seven-rated hybrid when exposed to the same midge density as a susceptible hybrid (rated one) sustains seven times less damage.

By 1996 QDPI & F sorghum breeders reported the development of several advanced commercial standard hybrids that recorded little or no economic damage under Australian conditions (Henzell & Hare 1996). By the turn of the century several commercial sorghum hybrids were available in the marketplace that contained this level of resistance. As such in 2002 the midge rating system was officially expanded to incorporate the presence of commercial hybrids that contained very high levels of resistance above the top rating of seven (Hardy & Jordan 2006). While many hybrids tested in both field and glasshouse tests between 1998-2003 recorded resistance levels 10-30 times higher than that present in susceptible hybrids, industry representatives and QDPI & F researchers agreed to assign a new 8+ rating to any hybrids that rated equal to or above an eight rated standard hybrid selected in trial work (Hardy unpublished data; Plate 1.5). The impact of the objectively rated midge

resistant hybrids in the Australian grain sorghum industry is significant and widespread, but has not been analysed in any detail over the last decade.



**Plate 1.5** Effect of midge resistance rating on seed set after exposure to high midge pressures of 50-100 midge per panicle at flowering. Seed set on panicles with increasing levels of midge resistance from left to right is approximately 1%, 50%, 75% and 90%.

### **Economic Impact on Industry**

Prior to the introduction of midge resistant hybrids control costs, residual losses, and uncontrolled damage from sorghum midge in Queensland alone were estimated at \$6-10 million annually (Passlow *et al.* 1985). As Queensland commonly makes up approximately two-thirds to three quarters of the annual crop in Australia, total losses were likely to be in the order of \$9-15 million annually.

However by 1996 it was estimated that 80% of farmers throughout Australia grew hybrids with at least some level (>rating 2) of midge resistance, while the average level of resistance was estimated at a rating of three (Franzmann *et al.* 1996; Boucher & Muller pers. comm. 2000). Even so, Henzell *et al.* (1996) estimated losses to midge in that year to be \$7.9 million, approximately 4% of the value of production.

Since 2003 over 99% of commercial grain sorghum sold contains some level of midge resistance at an average midge resistance rating of five. In terms of volume of hybrid seed sold; 10-20% has a 2-3 rating; 60-80% has a 4-5 rating; and 20-30% has a 6-7 rating, and less than 5% has the new 8+ rating (unpublished commercial sorghum hybrid sales, 2003).

The impact of increased levels of midge resistance may then be calculated from differences in the increase in resistance ratings over the period 1996-2003. Based on the same production levels and midge pressures as those recorded in 1995/6, costs from sorghum midge damage would be reduced from \$7.9 to \$3.8 million, and less than 2% of the value of production.

However in reality, since midge resistant hybrids were introduced, the midge pressures experienced in farmers fields have declined greatly. The effect of increasing sorghum midge hybrid production on the build up of midge populations throughout the season was modelled by Franzmann and Zalucki in 1993. Using a mathematical model under current cropping scenarios, at initial midge densities of 1000 midge per hectare, susceptible (one rated) hybrids produced a 552 fold increase in midge numbers over one season, while this was reduced to 13 fold in moderately resistant hybrids (4-5 rating), and further reduced to only three fold in highly resistant hybrids (8+ rating). The results of this are seen today. After a decade of midge resistance, the midge pressures throughout all regions while highly variable, begin from a much lower population base each spring, and increase at dramatically reduced rates in each crop, the rate of increase being dependant on the level of resistance (Franzmann 2004). Over the next decade this situation should continue, and sorghum midge control, damage and associated residual costs are likely to continue the downward trend as midge pressures decline under increased widespread

plantings of higher rated midge resistant hybrids. Under this scenario it is likely that sorghum midge will cost the industry less than \$1 million annually, less than 1% the value of production.

### **Benefits of resistant hybrids to farmers**

Today sorghum is grown throughout the sorghum growing regions of Australia over a broad planting window and producers now rarely consider midge damage to be a factor in determining planting dates, and only occasionally resort to insecticides to control midge. Growers now often pay no attention to this insect and often place complete trust in the incomplete level of resistance present in midge resistant hybrids. This situation has similarly led to a reduction in careful and daily monitoring of midge infestation during flowering (Muller N & Boucher B pers. comm. 2003).

The question remains as to whether the low to moderate midge pressures now present in Australia may still be capable of significantly reducing yields on the range of moderate to highly resistant hybrids now commonly grown without chemical control. The impact of midge resistant hybrids relative to susceptible hybrids under unsprayed conditions is demonstrated in Table 1.1. The figures are based on a crop density of 75000 plants per hectare, where individual flowering panicles remain attractive to midge egg lay over five days. A susceptible hybrid on average is estimated to lose 1.4 grams per midge per day during flowering (Franzmann 2004). This amount is reduced proportionally by three, five, and seven times in the same rated hybrids, while the 8+ hybrids are known to contain resistance levels at least ten times those present in susceptible hybrids (unpublished data).

**Table 1.1** Economics of midge resistance in Australian conditions without chemical control.

midge rating of hybrid	yield (t/ha)	damage (\$/ha) @ selling price of:		
		\$120/t	\$160/t	\$190/t
<b>early-mid season</b> (0.5 midge per head per day)				
susceptible (rating 1)	0.26	31	42	49
3	0.09	10	14	16
5	0.05	6	8	10
7	0.04	4	6	7
8+ rating*	0.03	3	4	5
<b>late</b> (3 midge per head per day)				
susceptible (rating 1)	1.58	189	252	299
3	0.53	63	84	100
5	0.32	38	50	60
7	0.23	27	36	43
8+ rating*	0.16	18	24	30

\* All 8+ hybrids tested have resistance ratings of 10-30 times. 10 rating used in yield loss figures.

Under the low midge pressures of less than one midge per panicle per day experienced throughout most of the season, all midge resistant hybrids rated three and above provided good protection. In this situation at prices between \$120-190/tonne all midge resistant hybrids recorded \$3-16/ha damage compared to \$31-49 damage in susceptible hybrids.

The impact of higher resistance ratings is more obvious at the higher midge pressure of three midge per panicle per day. Under these conditions without chemical control, a susceptible hybrid will lose approximately half the crops yield, reducing profits by approximately \$200-300/ha. In most situations this would make the crop unprofitable. In contrast, all 3-8+ midge resistant hybrids recorded approximately \$18-100/ha damage.



Under the above situations, when midge pressures remain below 0.5 midge per panicle all of the 3-8+ rated hybrids provided a level of protection well above the cost of chemical control (synthetic pyrethroids @ \$10-16/ha). However when midge pressures increase to three midge per panicle per day, the value of the highest midge ratings become evident. In this situation at all prices, it is still economic to spray the three and five rated hybrids to prevent approximately \$27-100/ha damage. The economics of spraying are marginal in both the 7 and 8+ rated hybrids, where a well-timed spray may only save \$18-30/ha damage. This is particularly evident when we consider that the best chemical application is likely to only be 80% effective, so that the value of grain saved is less than or equal to the cost of application.

Therefore resistant hybrids with a rating of three or more are of great value under low midge pressures, reducing the need to spray for most of the season. However under higher midge pressures late in the season all other hybrids other than the 7 or 8+ rated hybrids remain economic to spray .

### **The emerging success of IPM in Australian grain sorghum**

It is also possible to view the benefits of midge resistant hybrids beyond the economic control of sorghum midge alone, as an integral part of an emerging Integrated Pest Management (IPM) program in grain sorghum. Over the last decade the management strategy to control the two major insect pests in sorghum has rapidly changed from a heavily dependent insecticidal regime to a classic IPM strategy (Franzmann, 2004). This could not have occurred without the initial adoption of midge resistant hybrids as the back bone of IPM in sorghum.

In the past Australian farmers used pyrethroid sprays at flowering to control both sorghum midge and a second major insect pest, the corn ear worm (*Helicoverpa armigera*) (Murray *et al.* 1995). However increasing development of *H. armigera* resistance to pyrethroids, endosulfan, and carbamates over the 1990's means that such chemical means of control is no longer possible (Forrester *et al.* 1993). Over the last decade the availability of midge resistant hybrids means that this pest has taken the place of midge as the most important pest of grain sorghum in Australia (Adamson *et al.* 1997). As a result there has been renewed interest in applying

previously documented effective bio-control methods to control this pest. Studies in the late 1970's showed that nuclear polyhedrosis virus (NPV) was an effective control agent against *H. armigera* in sorghum, however it was not until the failure of insecticides in the 90's that such agents gained serious consideration (Murray *et al.* 1995; Teakle *et al.* 1983). Subsequently, over the last few years various formulations of NPV have emerged on the commercial market-place where they have been in strong demand by farmers who have rapidly adopted the use of viral preparations on midge resistant sorghum, in place of traditional chemistry, to control *H. armigera*. The adoption of NPV as a biological insecticide over recent years has proven to be highly effective in grain sorghum crops particularly when the effects of parasites and predators of corn ear worm are included (Murray *et al.* 1996). With the move away from disruptive insecticides has come an appreciation of the contribution of beneficial insects, especially egg parasitoids like *Trichogramma* spp. and larval parasitoids like *Microplitis demolitor*. In many cases the activity of these parasitoids alone is sufficient to reduce infestations below economically damaging levels (Murray *et al.* 2001). In the past, under conditions of ineffective chemical controls which impacted on biological control by beneficial insects, *H. armigera* were considered to build up on grain sorghum and move onto other crops causing significant economic damage. However with the success of NPV for *H. armigera* control, there is huge potential for grain sorghum to act as both a sink (trap) for *H. armigera* and a nursery for beneficial insects which act at all life stages of this insect, dramatically reducing the number of insects surviving to the next generation (Murray & Zaluzki 1994; Murray *et al.* 1995). NPV sprays do not disrupt beneficial insects which can subsequently move to other nearby crops. Furthermore, NPV is not susceptible to current insecticide resistance mechanisms in its host, and so NPV may serve to prolong the useful life of some insecticide groups (Murray *et al.* 2001). These benefits are being exploited in tactics used in regional or area-wide management (AWM) of corn ear worm on the Darling Downs and elsewhere in Australia (Murray *et al.* 2000).

Another major pest of grain sorghum during grain fill and harvest is the corn-aphid (*Rhopalosiphum maidis*), which in the 1990's was estimated to cost the industry around \$11/ha or \$5-10 million per annum (Titmarsh, unpublished data). This pest is

a potentially major problem at harvest where honey-dew excreted from aphids was found to cause problems at harvest and result in additional post-harvest treatment costs in processing grain. In the past the use of pyrethroids and carbamates to control *H. armigera* was highly disruptive to beneficial insects which are known to effectively control aphid populations, resulting in secondary pest outbreaks of aphids (Murray *et al.* 2001). Recent studies indicate that under unsprayed conditions beneficial insects such as ladybirds, and parasitoids are extremely important in controlling the corn aphid in sorghum, keeping aphid populations well below economic thresholds (Franzmann, unpublished data).

The emerging success story of IPM in Australian grain sorghum has been made possible by the introduction of midge resistant hybrids into the commercial marketplace in the 1990's. Midge-resistant hybrids form an essential pre-cursor to successful IPM of all major insect pests in Australian grain sorghum (Franzmann, 2004).

## **Host Plant Resistance Theory**

### **Durability of resistance**

Van der Plank (1968), a plant pathologist, concluded that the number of genes involved in conferring resistance to a crop variety should influence the rate at which virulent biotypes of pathogens evolve. He maintained that monogenic sources of resistance would be more rapidly overcome than polygenic sources, and some entomologists (Maxwell & Jennings, 1980) have adopted this approach, referring to monogenic resistance as “temporary”, and polygenic as “permanent”. Alternatively, Russell (1978) maintained that the number and types of mechanisms involved in resistance to a pest significantly affects the stability or durability of resistance and were likely to be much more important than the number and nature of genes which control these mechanisms. He concludes that it is presumably more difficult for a pest to overcome several independent resistance mechanisms than one mechanism, and the presence of several mechanisms in a variety may thus delay or stop the

development of resistance-breaking biotypes. Kennedy *et al.* (1987 p. 332) subsequently supported this contention by stating that;

“Rather than emphasising the genetic nature of plant–resistance factors as a basis for determining the durability of resistance, more emphasis needs to be placed on understanding the direct biological effect, behavioural or physiological, that a resistance factor has on the pest insect. Such an understanding would provide a reasonable basis for attempting to predict whether the change required on the part of the pest to circumvent the biological effect of a plant defense would be simple or complex, and for computing the selection intensity operating on pest populations that contain a low frequency of genotypes that can circumvent the resistance.”

### **Resistance mechanisms and components**

Painter (1951) defined plant resistance to insects as ‘the heritable characteristics possessed by a plant which influence the ultimate degree of damage done by an insect’. He described three mechanisms of resistance: non-preference, antibiosis, and tolerance.

Kogan and Ortman (1978) subsequently argued that the term “non-preference” be replaced by “antixenosis”. Antixenosis detrimentally affects insects as they attempt to use plants for food, oviposition, or shelter. The resistant plant is then rejected by the pest as an unsuitable host. Antixenosis has been recorded in several genotypes of durum wheats that contain extended, tightly wrapped glumes that inhibit the initial larval feeding of the wheat midge (*Sitodiplosis mosellana*). This physical mechanism of resistance has been lost in most domesticated wheat varieties that contain free-threshing seeds, and compact spikes (Wise *et al.* 2001).

The antibiosis mechanism of resistance occurs after host plant infestation where the biology of the insect is affected detrimentally as it feeds on the plant. Antibiosis to wheat midge larval feeding is also described in wheat (Ding *et al.* 2000; Lamb *et al.*

2000). In this case young wheat midge larvae fail to grow and eventually die when they feed on young developing seeds that illicit a hypersensitive response near the seed surface associated with high levels of phenolic acids. This chemical mechanism of resistance is highly effective and kills nearly all larvae.

Plants with tolerance show the ability to repair injury, and/or grow and reproduce in spite of supporting an insect population that would damage a susceptible plant. Examples of this resistance mechanism are found in certain wheat genotypes in response to the hessian fly (*Mayetiola destructor*). While the larvae of this insect cause abortion of the developing shoot apex early in plant development, high tillering genotypes that are able to abort infested tillers and continue to produce new un-infested tillers are able to produce similar yields under low and moderate hessian fly infestations (Sosa & Forster 1976).

Resistant plants may contain one or a combination of the above mentioned three mechanisms that collectively contribute to the level of insect resistance. Each mechanism of resistance acts at some stage of the insect/plant relationship and is contributed to by physical or chemical plant characters that may be referred to as components of resistance. Farrell (1977) describes successive phases of the insect/plant relationship in which the plant is firstly colonised then utilised by the insect and finally emerging adults are dispersed. Non-preference or antixenosis mechanisms of resistance may occur during the colonisation phase as insects approach, make contact with, arrest, and oviposit on host plants. Antibiosis resistance occurs at the utilisation phase of insect/plant interaction where larval growth, survival and adult fecundity may be affected as the insect ingests, assimilates and converts food. Finally, as emerging adults disperse from their host plant they may either reinfest the crop, or emigrate to another distant host.

Midge resistant sorghum was first reported in 1953 in 'Nunaba' varieties from West Africa (Bowden & Neve, 1953). They attributed this resistance to the long glumes of these varieties that did not open during anthesis. However these varieties proved to be resistant only under choice conditions. Passlow (1965) reported in Australian field tests that 'Nunaba' varieties may be resistant under choice conditions in the

presence of an alternative host but susceptible in the absence of a more favourable host.

However several decades later, Franzmann (1988) and Waquil (1985) described an antixenosis resistance mechanism in genotypes that displayed similar levels of resistance under no-choice conditions in glasshouse and field trials. Such resistance has been described as ovipositional antixenosis.

### **Ovipositional antixenosis**

Ovipositional antixenosis or reduced egg lay has been recognised around the world as the major mechanism of resistance in midge resistant hybrids (Franzmann 1988; Rossetto *et al.* 1984; Sharma & Vidyasagar 1994; Waquil *et al.* 1986b). The exact causes of the antixenosis mechanism of resistance may be closely linked with the structural morphology of spikelets (Henzell *et al.* 1994). Various workers have found a positive relationship between midge resistance and small glume size, the extent of glume closure (Bergquist *et al.* 1974; Jadhav and Jadhav 1978; Rossetto *et al.* 1975). Sharma (1985, Sharma *et al.* 1990 a ,b ; Sharma *et al.* 2002) found that the midge susceptibility of a number of sorghum lines was positively and significantly correlated with glume, palea, lemma, anther and style length, while Diarisso (1997) and Waquil *et al.* (1986a), found that glumes of spikelets of resistant varieties were more tightly closed than those in susceptible varieties. Waquil *et al.* (1986a) found that while searching time was shorter (5.9 sec) for midge females on a resistant hybrid compared to a susceptible hybrid (7.2 sec), probing time and successful oviposition took four times as long in resistant hybrids.

In addition to spikelet morphology, Diarisso (1997) in Texas, USA recorded the presence of a mechanism of resistance related to the asynchronous flowering of florets in a few resistant lines and hybrids early in the morning before peak midge activity. However both Sharma *et al.* (2002), and Hardy and Franzmann (2001), did not observe the same effect on a wider range of resistant and susceptible hybrids and lines.

## **Antibiosis**

There is evidence for antibiosis to midge in sorghum germplasm, leading to decreased rates of postembryonic growth, survival, and adult fecundity. Sharma *et al.* (1993) and Wuensche (1980) found larvae reared on a number of resistant varieties were smaller in size and weight compared to larvae reared on susceptible varieties. Waquil *et al.* (1986c) found a greater proportion of larvae developing through to late instars on a susceptible hybrid compared to resistant hybrids and a greater proportion of larva positioned against the caryopsis in the susceptible hybrid. Kulkarni (1985) found evidence for delayed emergence of adults in resistant lines, while Sharma *et al.* (1993) found that in addition to delayed emergence there was decreased fecundity and lower rates of progeny production in females reared on midge resistant lines. Natarajan and Chelliah (1985) found that all sorghum midge postembryonic stages of life cycle were smaller, lighter, and took longer to complete development when reared on a number of midge resistant lines. There is also evidence for higher larval mortality in midge resistant lines from a number of worldwide studies (Rossetto 1977; Sharma 1985, Sharma *et al.* 1993; Teetes & Johnson 1978).

In contrast to ovipositional antixenosis, there is little clear evidence on the exact chemical or physical components that effect the antibiosis mechanism of resistance in sorghum. Santos and Carmo (1974) suggested a correlation between tannins and midge resistance. However Martins (1977) working with the same lines failed to obtain a correlation between midge resistance and tannin content. Sharma (1985, 1993) found a positive correlation between grain (caryopsis) growth rates, and midge resistance. While this correlation was true when comparing resistant lines to susceptible lines, there was no analysis of any difference between lines that contained different levels of antibiosis. He similarly found positive correlations between tannin contents and midge resistance, with the exception of the highly antibiotic line DJ6514.

## Tolerance

There is no consistent evidence for tolerance as a mechanism of resistance within midge resistant cultivars. Wuensche (1980) evaluated yield, adult emergence, and caryopsis development in a range of midge resistant cultivars, and failed to identify tolerance as a significant mechanism. Franzmann (1993a) is the only author to record a high percentage of pupae within spikelets that contained fully developed kernels in one midge resistant line. He concluded that these larva were not feeding on the developing kernel, but elsewhere in the spikelet, resulting in the presence of fully developed seed.

Increased yield compensation recorded as increased weight on undamaged grains within midge infested panicles has been proposed as a possible form of tolerance in resistant lines. However as yet there are no reports of increased yield in panicles of resistant lines above that present in susceptible lines with similar levels of midge damage. While various studies have shown that yield compensation occurs within panicles of all sorghum lines when spikelets or developing kernels are physically removed (Fisher & Wilson 1975; Hamilton *et al.* 1982; Henzell & Gillieron 1973), Franzmann and Butler (1993) showed that there is no difference between midge resistant and susceptible hybrids in the amount of grain compensation under similar levels of midge damage. Compensation was generally positive in both midge resistant and susceptible hybrids and greatest between 30 and 50% seed set. While Sharma *et al.* (2002) recorded variable but generally positive levels of yield compensation in a wide range of midge resistant lines compared to negative compensation in a susceptible line. However differences were recorded in both the levels of midge larval infestation across lines and in the amount of sterility recorded as 'chaffy' spikelets between the resistant and susceptible line(s). As such factors other than midge infestation, such as sterility and uneven midge infestation may have unduly influenced the results.



## **Midge Resistant Germplasm**

The identification of midge resistance and development of midge-resistant hybrids worldwide under both private and public breeding programs over the last 40 years has provided farmers with a number of hybrids that are higher yielding than susceptible hybrids under field conditions under moderate levels of midge activity. While much screening and breeding of midge resistant cultivars has occurred, little is known of the exact nature of the genetic factors involved. Researchers agree that the nature of midge resistance in current cultivars is largely polygenic (Henzell & Hare 1996). Midge resistance has been utilised in breeding programs in USA, Australia, India, Myanmar, Africa, Argentina, and El Salvador. (Bowden & Neve 1953; Faris 1979; Henzell *et al.* 1994; Johnson *et al.* 1973; Jotwani 1978; Page 1979; Rossetto *et al.* 1975; Sharma *et al.* 1994; Shyamsunder *et al.* 1975; Wiseman *et al.* 1988).

In Australia sorghum breeders within the public breeding program have used pedigree and limited backcross breeding methods for midge resistance (Henzell & Hare 1996). The breeding method includes cycles of crossing, evaluation and the selection of parents to commence a new cycle with the occasional infusion of new material. This new material has included regularly released material from the Sorghum Conversion Program at the Texas Agricultural Experiment Station, and from the ICRISAT breeding program in India.

The midge resistance present in Australian hybrids is largely drawn from North American sources (Henzell *et al.* 1994). The resistance genes within this material confer only one mechanism of resistance, ovipositional-antixenosis (Franzmann 1993a). In contrast the lines DJ6514 and its derivatives from India are known to contain both antixenotic and antibiotic mechanisms of resistance (Sharma 1985; Sharma *et al.* 2002). While midge resistance within Australian grown hybrids appears to be effective, it is only in the last few years that highly resistant sorghum crops have been planted over wide areas, and as a result the stability of individual mechanisms of midge resistance within these crops remains largely untested.

The selection of one mechanism of resistance as also resulted in a gradual reduction in the level of genetic diversity within sorghum germplasm due to the polygenic nature of resistance (Jordan *et al.* 1998; Tao *et al.* 1998).

In order to reduce the risk of resistance breakdown, to continue to increase levels of midge resistance and to halt the decrease in genetic diversity within Australian commercial sorghum hybrids, sorghum breeders need to have access to new sources of midge resistance that are not associated with antixenosis. In 1996, the Grains Research and Development Corporation and the Queensland Department of Primary Industries and Fisheries saw the need for such work and funded a project to isolate and evaluate new sources of midge resistance for Australian breeding programs.

## **Rationale and Structure**

This thesis represents a decade of sustained sorghum midge-resistance research. Initially the goal of this work was to simply isolate the most promising sources of antibiosis type resistance in a range of international sorghum lines for incorporation into the Australian breeding program. However as this screening work progressed I observed a potentially novel source of midge resistance previously undescribed that warranted further investigation. In order to confirm results, I developed a new screening technique that allowed a closer examination of potentially diverse and novel sources of midge resistance. I have now characterised in some detail several different sources of resistance, one of which holds great promise for the sorghum breeding program.

### **Chapter 2 – A search for new sources of midge resistance**

The work began by collecting and screening a diverse array of over 30 geographically diverse midge-resistance germplasm lines from around the world to identify new sources of midge resistance. From this work several promising sources of antibiosis were identified that warranted further investigation (Chapter 3). However while performing this work a potentially previously undescribed tolerance mechanism of resistance was discovered in several lines derived from the same genetic background. In order to confirm the mechanism of resistance in more detail

another three years screening work was conducted on a wider set of lines. From this result the new tolerance mechanism of resistance was confirmed in antibiotic lines derived from the Indian land race DJ6514. However the link between the antibiotic and tolerance mechanisms of resistance in this material was unclear and warranted further investigation (Chapter 6).

### **Chapter 3 – A closer examination of larval antibiosis**

The results from initial screenings lead me to further select a small, genetically diverse range of the most promising antibiotic lines for a more detailed study of the midge larval period of development. The aim was to sample the entire larval period in order to discern different patterns of larval growth and mortality associated with different mechanisms of antibiosis. While some differences were recorded, this method did not yield precise results due to difficulties in establishing similar egg densities across lines that contained variable levels of antixenosis. To overcome this problem a new method was developed (Chapter 4).

### **Chapter 4 – The water injection technique**

To enable a more precise study of larval development, I developed a new artificial water injection technique designed to deliver a precise and exact egg density across all lines to facilitate precise studies of larval mortality across lines that contain variable levels of antixenosis. The method is further evaluated by testing the effects of storing eggs in water for extended periods at 4°C on subsequent egg hatch and neonate fitness.

### **Chapter 5 – Using the water injection method to study larval antibiosis**

A second attempt was made to conduct a precise study of the early larval period in a range of potentially diverse antibiotic lines using the newly developed water injection technique. This study was designed to record any differences in larval development that may be caused by different antibiotic modes of action across genotypes. In particular this study focused on the period between egg eclosion and early larval establishment where a majority of larval mortality occurs across all lines.

Some clear differences in larval antibiosis between lines were observed in this study, indicating that different antibiosis mechanisms may be present across some lines.

### **Chapter 6 – Is there a link between antibiosis and tolerance?**

Previous screening results indicated that there may be a link between the antibiosis and newly characterised tolerance mechanisms of resistance present in lines derived from DJ6514. In this chapter I closely investigated this link in three studies designed to show that the unique antibiosis mechanism of action causes a change in larval feeding behaviour that results in the survival of both larvae and the developing caryopsis. Each trial was designed to focus on a separate part of the insect/plant interaction to directly or indirectly confirm this hypothesis.

### **Chapter 7 – The inheritance of antibiosis**

The highly useful source of antibiosis and related tolerance mechanisms of resistance recorded in the Indian land race line DJ6514 have been selected for incorporation into the Australian sorghum breeding program. As an integral part of this work I studied the inheritance of antibiosis in several populations to confirm the nature of inheritance.

### **Chapter 8 – General discussion and future developments**

Researchers continue to select, characterise and incorporate the highly promising DJ6514 source of antibiosis into the Australian sorghum-breeding program using new technologies. The timelines, potential benefits and pitfalls associated with this work are discussed.

## CHAPTER 2

### A Search for New Sources of Midge Resistance

#### Introduction

The first midge-resistance breeding program began thirty years ago at Texas A & M University (Johnson *et al.* 1973). Similar breeding programs commenced at the Queensland Department of Primary Industries (QDPI & F), Australia in 1975 (Henzell *et al.* 1980), and at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India in 1980 (Sharma *et al.* 1994). In both Texas and Australia, initial sources of midge resistance were obtained from the sorghum conversion program run at Texas (Henzell *et al.* 1994), while at ICRISAT a combination of locally sourced material and converted sorghum cultivars have been used to develop advanced midge-resistant sorghum for commercial use (Sharma pers. comm. 2002). In all cases two mechanisms of resistance have been consistently reported, ovipositional-antixenosis, and antibiosis.

The midge-resistant germplasm developed in Australia over the last 30 years has been selected from several sources and is reported to contain multiple genes for resistance (Henzell *et al.* 1996). However, while moderate to high levels of ovipositional-antixenosis have been incorporated into commercial hybrids in Australia, no consistent reports exist of useful levels of antibiosis within elite QDPI & F sorghum germplasm (Franzmann 1988; Henzell *et al.* 1996). Internationally antibiosis has been recorded in lines for many years. Antibiosis has been recorded as mortality of midge larvae (Waquil *et al.* 1986c, Rossetto 1977, Sharma 1985, Teetes & Johnson 1978), reduced larval size and weight (Sharma *et al.* 1993, Wuensche 1980), delayed development times from oviposition to adult emergence, (Kularni 1984, Natarajan & Chelliah 1985, Sharma 1985) and a reduction in the percentage of larvae feeding against the caryopsis (Waquil *et al.* 1986c).

The initial aim of this study was to identify lines with useful levels of antibiosis from range of international breeding lines previously reported to contain useful levels of midge resistance. While my initial screenings confirmed high levels of antibiosis in several international lines, repeated screenings of antibiotic lines from similar backgrounds revealed the presence of a previously unreported tolerance mechanism of resistance.

## **Materials and Methods**

Two stages of screening were employed over five years. A total of 32 lines were screened in multiple trials over one summer (year 1), while a smaller range of lines and hybrids were tested over the next four years (years 2-5), one trial per year. In all cases trial work was carried out between January-February each year to coincide with peak midge activity during late summer.

### **Stage 1 – Screening for antibiosis**

A total of 32 sorghum lines were collected for screening from the public sorghum breeding programs at ICRISAT in India, Texas A & M University and the associated sorghum conversion program, and from the QDPI & F sorghum breeding program in Australia.

#### **Glasshouse trial procedure**

No-choice glasshouse cage trials were conducted on all lines according to the method developed by Franzmann (1993a). Plants were grown in the glasshouse in pots and provided with adequate fertiliser and water.

A total of 18 trials were conducted in one summer. Each trial established on one day consisted of a randomised complete block design containing 2-5 panicles of each line to be tested along with at least one panicle of the midge susceptible line QL12 as a control. On each trial day flowering panicles were trimmed to contain 200 flowering sessile and associated pedicellate spikelets. Each panicle was caged with a white nylon screen supported by a wire frame approximately 20 cm in diameter and 30 cm long (Plate 2.1).



**Plate 2.1** Glasshouse grown sorghum lines at various stages of growth, several caged in one trial. Also shown is a close up of trimmed panicles inside cages.

Panicles infested with sorghum midge were collected from sorghum and Johnson grass panicles growing around the Darling Downs and Lockyer Valley. The cut panicles were placed in plastic bins, where mixed male and female midge emerged, and crawled up towards the light to mate in ventilated clear plastic jugs suspended over ventilated metal mesh cylinders attached to a 15 cm hole in the lids of the plastic bins. Midge were gathered into glass vials from the jugs from which twenty female midge were transferred into each cage via glass vials between 9-12 am to achieve a midge density of one midge to ten sessile spikelets on each panicle. After all panicles were infested with midge, cages were lightly sprayed with water and left for 24 h. At this time the midge were removed and an egg sample was taken by cutting 3-6 raceme sub-branches from each panicle collecting a total of 50 sessile spikelets. Upon the emergence of the first adult midge in each trial (2-3 weeks later), a final 50 sessile spikelet pupal sample was taken from each panicle. Both egg and pupal samples were stored in the freezer (-18°C) for later dissection. Numbers of adult midge emerging from each panicle were recorded daily until adult emergence was complete.

## Collation of results

Egg and pupal numbers for each line were assessed by dissection of frozen samples under a dissecting microscope. The number of eggs, and pupae per sessile spikelet were recorded, as well as % spikelets infested, and % seed set. In one trial, the pedicellate spikelets were also dissected in both the egg and pupal samples to determine the contribution of pedicellate spikelets to subsequent adult emergence.

In each of the 18 trials, differences between egg and pupal counts were used to calculate levels of antixenosis and larval antibiosis in each line. Percentage seed set was calculated directly from midge damage alone from sessile spikelets in the pupal sample. The number of sessile spikelets aborted under midge larval feeding was recorded in the pupal sample, and then directly used to calculate seed set. Any spikelets in the pupal sample that did not set seed due to other causes such as sterility were removed from both pupal infestation and seed set results. All midge pupal infestation and seed set data were calculated directly from the presence or absence of midge damage alone. Amounts of sterility and other sources of seed loss were only minor in all cases but varied slightly across lines and trials.

Larval antibiosis was recorded by differences between the initial egg counts and pupal/larval counts taken 15-20 days later after larval feeding was complete in each plant, at the emergence of the first adult in each cage

In all cases larval antibiosis was first calculated in each line, and then compared to the level of antibiosis present in the highly susceptible QL12 control line using the following formulae.

% larval antibiosis

$$= ((\text{number of eggs} - \text{number of pupa}) / \text{number of eggs}) * 100$$

% adjusted larval antibiosis

$$= \% \text{ difference} - \% \text{ larval antibiosis in QL12}$$



Pupal counts from each line were used to estimate the expected midge emergence from the remaining 100 sessile and associated pedicellate spikelets on each panicle. An expected emergence of 100% of the recorded surviving larvae/pupae counted in 50 sessile spikelets of each panicle was used to calculate the expected number of midge to emerge from the remaining 100 sessile spikelets. Actual midge emergence in each cage was then compared with the calculated expected emergence to record any death of midge between pupation and emergence of adults. Again all such pupal antibiosis was then adjusted relative to that present in QL12 control panicles using the following formulae.

percentage pupal antibiosis

$$= ((\text{expected emergence (em.)} - \text{actual em.}) / \text{expected em.}) * 100$$

adjusted % pupal antibiosis

$$= \% \text{ pupal antibiosis} - \% \text{ pupal antibiosis in QL12}$$

The number of adults emerging each day was used to calculate average development times for each line, and the total number of adults to emerge from each panicle was used to calculate the average number of midge to emerge from 100 sessile and associated pedicellate spikelets.

All trials were analysed separately, but presented collectively after normality assumptions were met upon square root transformation of egg and pupal counts. Equivalent means were then analysed using analysis of variance (ANOVA) and differences determined by least significant difference (LSD) at  $p < 0.05$ .

## **Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance**

Over four years a single glasshouse screening trial was conducted each year in late summer on a range of 18 lines. Seven of these lines were repeatedly tested from stage one including: QL12 and QL20 – susceptible controls; QL39 and 90562 – antixenotic controls; DJ6514, ICSV197 and ICSV745 – closely related antibiotic controls. One previously untested line AF28 was included in one year's testing,

while the remaining ten closely related breeding lines (including one hybrid) were obtained from Pioneer Overseas Breeding Corporation. All of these lines contained variable portions of DJ6514 co-ancestry over many generations.

### **Glasshouse screening procedure**

In all years except the third year of testing, replicates of all lines were tested simultaneously with panicles of the control lines on each day of testing. However in year three the asynchronous flowering of several lines apart from the controls made it impossible to maintain this trial procedure. Despite this anomaly in trial design, the results are in line with other year's data and have been presented but not statistically analysed due to the aberration in trial design.

A modified version of the cage technique described in stage one of testing was used to screen all lines. In contrast to stage one screenings however, only egg and larval/pupal samples were taken with no sampling of the pupal-adult development period.

Plants were grown in pots in the glasshouse. Panicles were trimmed back to contain 150 flowering sessile spikelets, and a total of 30 or 40 mated female midge per panicle were used on each panicle in an attempt to obtain high levels of egg infestation across all lines.

In year one, four lines (Pioneer 1-4) from Pioneer Overseas Corporation were tested along with QL12, QL39, ICSV197 and ICSV745. In this trial a total of four panicles of each line were caged for testing over two dates at a density of 40 mated female midge to 150 flowering sessile spikelets. Pupal samples were taken 17 days after trial commencement.

In year two, a total of seven breeding lines (Pioneer 1-7) from Pioneer Overseas Corporation were tested, along with the same checks tested in year one with the addition of the lines DJ6514 and 90562. A minimum of five panicles of each line (five replications) were caged over nine dates at the same midge density used in year

one testing. In this trial the pupal sample was taken at 14 days after trial commencement in an attempt to record any variation in larval size and position.

In year three, 3-4 panicles of four Pioneer breeding lines (Pioneer 1 & 1A; Pioneer 3 & 3B) and one hybrid (Pioneer 1 x 4) were tested at a cage density of 30 mated female midge to 150 flowering sessile spikelets. The larval sample was taken 14 days post-anthesis.

In year four, the line Pioneer 3 was re-tested along with all six lines tested in previous years using the same methods employed in year three. Also included in testing were the lines AF28 and QL20.

### **Collation of results**

#### *Antixenosis and antibiosis*

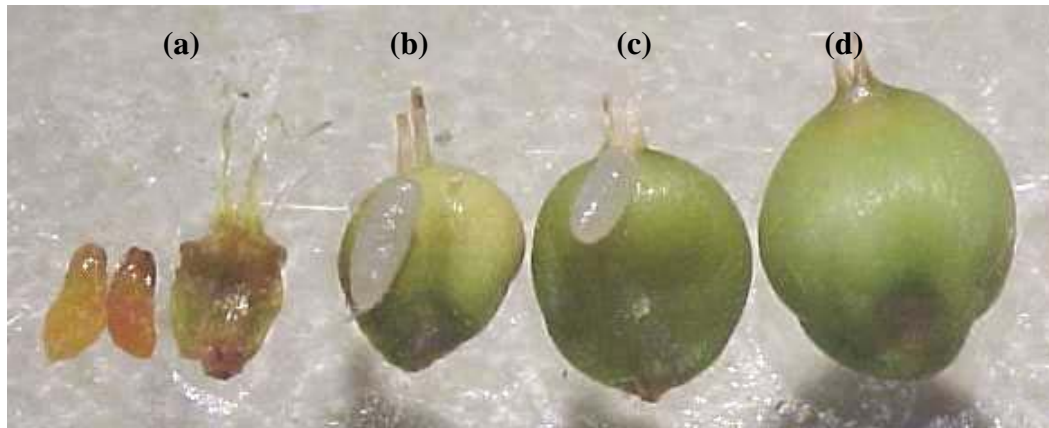
Initial egg densities and final pupal counts were used to measure antixenosis and antibiosis in exactly the same manner recorded in stage one of testing. The same methods of statistical analysis were also used to determine differences between lines as those recorded in stage one of testing.

#### *Tolerance*

In year one, tolerance was recorded in the pupal sample as the presence of large larvae/pupae next to filled caryopses that contained a small feeding indent. In future years the pupal sample was then taken earlier (14 days after trial commencement) in an attempt to capture larval feeding at an earlier stage of kernel development. Therefore two different definitions of tolerance were examined in year one (formula a) and years 2-4 (formula b) as follows:

formula (a) tolerance year one defined as: the number of sessile spikelets containing a filled caryopsis (kernel) and large larva(e) or pupa(e) with a small indent at the feeding site on one side of the kernel.

formula (b) tolerance years 2-4 defined as: the number of sessile spikelets containing a partially filled caryopsis (kernel) and larva(e) or pupa(e) where larval feeding did not reduce caryopsis size below 75% normal size (Plate 2.2).



**Plate 2.2** Damage on sorghum caryopses (kernels) after midge larval feeding 14 days post-anthesis. (a) midge pupae with aborted caryopsis; (b) 50% damage with large larva; (c) 25% damage - recorded as tolerance (large larva placed away from indented caryopsis feeding site); (d) un-infested caryopsis.

The definitions above were then used to determine the level of tolerance within midge infested spikelets using two formulae. The first formula records tolerance as increased seed set in each line regardless of the level of midge infestation, while the second formula records tolerance relative to the final larval/pupal infestation.

% tolerance

$$= [\text{formula (a) or (b) / total number of sessile spikelets dissected}] * 100$$

% tolerance in infested spikelets

$$= [(\% \text{ tolerance (from above)}) / \% \text{ pupal infestation}] * 100$$

In addition to the above, differences in larval size and feeding position within aborted or tolerant spikelets were also recorded in years two to four to coincide with an earlier larval sampling time.

Larval size and position was recorded as follows:

Larval size:

Small - larvae less than half the minimum size of the largest larvae feeding against aborted caryopses in QL12 spikelets. These larvae were deemed too small to cause abortion of the developing kernel.

Large - All other larvae (or pupae). These larvae were deemed to be capable of causing abortion of the developing caryopsis as seen in aborted spikelets of QL12.

Larval position was defined as:

Against the caryopsis.

Against the lemmas/glumes.

In all years statistical analysis and comparisons of relative midge larval size and position within tolerant spikelets were not appropriate or significant due to the record of very low sample sizes of tolerance in many cases causing large variable sampling errors. This occurred in the control lines QL12 and QL39 and several other lines where tolerance was recorded in only one or two spikelets in thousands of spikelets sampled over four years. Because of this the overall results of the last three years testing are presented together without any statistical analysis.

## **Results**

### **Stage 1 – Screening for antibiosis**

A total of 18 trials were conducted on 32 lines over one summer in the glasshouse. During these trials a total of 2-18 panicles of each line were screened, several lines tested in more than one trial according to availability of flowering plants. Individual trial results (each day's testing) were analysed separately to account for any differences in midge activity across days. All results were presented relative to those recorded in the susceptible QL12 line in each trial. However in order to simplify the

presentation of all trial data, I have combined some data and present the results in two parts:

Firstly the results of one trial result where extra data were collected on midge infestation within pedicellate spikelets;

Secondly a summary of all trial data combined across all trials.

### **Contribution of pedicellate spikelets to midge resistance**

In one trial on five lines a record of midge infestation was made in both sessile (seed producing) and pedicellate (non-seed producing) spikelets to determine the contribution of pedicellate spikelets to total oviposition, pupal survival and subsequent adult emergence. In each caged panicle a total of approximately 130 pedicellate and 100 sessile spikelets were present across all lines tested.

While egg lay was reduced ( $p < 0.05$ ) in the sessile spikelets of all four resistant lines relative to that in the susceptible control line QL12, there was no difference in oviposition within the pedicellate spikelets of all five lines (Table 2.1). Moderate but not statistically significant levels of larval mortality (antibiosis) were recorded between egg lay and the pupal sample within the sessile spikelets of all four resistant lines relative to the line QL12. Similar rates of larval mortality were observed across all five lines within pedicellate spikelets, even though no pupae were found the susceptible line QL12. Despite similarly low egg and subsequent pupal infestations within the pedicellate spikelets across all five lines tested, surviving adult midge emerging from pedicellate spikelets were estimated to contribute to 24-36% of the total adult emergence of both sessile and pedicellate spikelets in all four midge resistant lines compared to 0% in the susceptible line.

Despite this uneven contribution of pedicellate spikelets within resistant lines to the overall expected adult emergence in the cages of each line, there were no differences in pupal antibiosis between lines based on differences in expected versus actual adult emergence midge numbers within the cages of each line. Pupal antibiosis (mortality

between pupation and adult emergence) in all four resistant lines was calculated at 28-74% of the total pupal count in each caged panicle, while the susceptible line QL12 recorded a similarly high figure of 68% pupal mortality. .

Similarly, no differences in average development time from egg to adult were recorded between lines.

**Table 2.1** Midge infestation within sessile and associated pedicellate spikelets in five lines under a glasshouse trial. Each plant was caged with a midge density of one mated female to ten sessile spikelets.

<b>(a) sessile spikelets</b>		<b>no. per spikelet</b>		<b>% spikelets infested</b>		expected	% seed
genotype	panicles tested	eggs	pupae	eggs	pupae	emergence (a) <sup>^</sup>	set
QL12	2	2.50 a*	0.86 a	72 a	64 a	86 a	38 b
QL39	3	0.60 b	0.15 b	33 b	14 b	15 b	91 a
ICSV745	3	0.95 b	0.07 b	47 ab	7 b	7 b	93 a
DJ6514	3	0.48 b	0.09 b	33 b	7 b	9 b	93 a
ICSV197	3	0.25 b	0.13 b	20 b	12 b	13 b	88 a
<b>(b) pedicellate spikelets</b>		<b>no. per spikelet</b>		<b>% spikelets infested</b>		expected	% of total <sup>!</sup>
genotype	panicles tested	eggs	pupae	eggs	pupae	emergence (b) <sup>^</sup>	exp. emergence
QL12	2	0.11 a	0 a	8 a	0 a	0 a	0 a
QL39	3	0.29 a	0.05 a	19 a	5 a	7 a	32 b
ICSV745	3	0.24 a	0.03 a	20 a	3 a	4 a	36 b
DJ6514	3	0.27 a	0.04 a	20 a	4 a	5 a	36 b
ICSV197	3	0.20 a	0.03 a	17 a	3 a	4 a	24 b
<b>(c) total - sessile &amp; pedicellate</b>		<b>no. per spikelet</b>		expected	actual	% pupal	development
genotype	panicles tested	eggs	pupae	emergence (c) <sup>^</sup>	emergence <sup>"</sup>	antibiosis <sup>!</sup>	time (d)
QL12	2	1.31 a	0.43 a	86 a	48 a	68 a	22.56 a
QL39	3	0.45 b	0.10 b	22 b	6 b	74 a	22.62 a
ICSV745	3	0.60 b	0.07 b	11 b	1 b	62 a	23.67 a
DJ6514	3	0.38 b	0.07 b	14 b	6 b	67 a	24.78 a
ICSV197	3	0.23 b	0.08 b	17 b	8 b	28 a	22.43 a

\* Means within columns not followed by the same letter differ significantly ( $p < 0.05$ ).

<sup>^</sup> Numbers of adult midge expected to emerge based on pupal counts from (a) 100 sessile spikelets; (b) 130 pedicellate spikelets; and both (c).

<sup>!</sup> Contribution pedicellate reared midge make towards the expected total of midge that emerge in all spikelets. (exp. em. b/ exp. em.c \* 100).

<sup>"</sup> Actual emergence of adult midge from 100 sessile and associated pedicellate spikelets.

<sup>!</sup> Formula = (expected emergence - actual emergence) / expected emergence \* 100

## Screening for antibiosis

All 18 trials were analysed separately to account for some differences in midge oviposition and larval mortality across QL12 control panicles on each day of testing. However in order to simplify presentation all data are summarised together in Tables 2.2 and 2.3. While direct comparisons between lines in each table cannot always be made due to lines being tested on different days, differences between each line and the susceptible line QL12 are recorded in each table over all days testing.

#### *Larval antibiosis*

Summarised egg and pupal counts from each line are recorded in Table 2.2. Many lines produced significantly higher levels of larval mortality relative to that present in QL12. The Indian line DJ6514 and its derivative ICSV745 produced the highest levels of larval antibiosis of lines repeatedly tested over multiple trial days. Both these lines averaged moderate levels of antixenosis (23 and 26%) and high levels of antibiosis (54 and 74%) relative to QL12, resulting in approximately 95% seed set in both. These results were consistently recorded with little variation as evident by the low standard errors across all trial data.

Most other lines were only tested on one trial day and of these the highest levels of larval mortality were recorded in the lines IS10759, IS21883-1, PM7017 and SC62C, approximately 40-50% above that present in QL12.

The lines MB110, QL38, and TX2880 showed very high levels of antixenosis resulting in levels of seed set approaching 100% (Table 2.2). In these lines antibiosis was difficult to estimate accurately because of the low egg counts

The line QL39 recorded moderate levels of antixenosis and mixed levels of larval antibiosis across several trials. This line recorded low or insignificant levels of larval antibiosis in trials where the initial level of midge egg lay was greater than 1 egg per spikelet, and varying but significant antibiosis in all other trials under lower egg infestation.



**Table 2.2** Summary of ovipositional-antixenosis and larval antibiosis for 32 lines screened in 18 no-choice glasshouse cage trials under a midge density of 1 mated female midge to ten sessile spikelets.

line	no. trials	panicles tested	no. per sessile spikelet				% sessile spikelets infested				% larval antibiosis					
			eggs		pupae		eggs		pupae		% antibiosis <sup>^</sup>		adj. antibiosis <sup>''</sup>		% seed set	
			mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
<b>QL12</b>	<b>18</b>	<b>41</b>	<b>2.71</b>	<b>0.31</b>	<b>0.94</b>	<b>0.08</b>	<b>75</b>	<b>3</b>	<b>62</b>	<b>4</b>	<b>18</b>	<b>6</b>			<b>39</b>	<b>4</b>
DJ6514	3	9	0.44 *	0.07	0.06 *	0.02	23 *	4	5 *	1	78 *	5	54 *	15	95 *	1
ICSV197	3	11	0.58 *	0.10	0.11 *	0.04	26 *	4	10 *	3	62 *	17	17 *	27	90 *	3
ICSV745	3	9	0.48 *	0.17	0.03 *	0.01	26 *	8	3 *	1	88 *	3	74 *	7	97 *	1
IS10759	1	3	2.12	0.89	0.35 *	0.23	59	23	27	13	55 *	12	46 *	12	64 *	10
IS12608C	1	2	0.31 *	0.18	0.28	0.08	22 *	14	26	5	-	-	-	-	74 *	5
IS15107	1	3	0.71 *	0.35	39.30 *	0.21	20 *	7	46 *	9	-	-	-	-	56 *	10
IS18733	1	3	1.83 *	0.55	0.62 *	0.17	63	15	39 *	10	37 *	41	16 *	47	63 *	9
IS21871	1	4	1.36 *	0.26	0.74 *	0.17	64	7	48 *	9	25 *	23	21 *	18	52 *	9
IS21873	1	3	0.48	0.12	0.51 *	0.09	39	6	36 *	3	7	17	-	-	61 *	3
IS21881	1	3	0.09	0.06	0.20 *	0.10	7	4	20 *	9	-	-	-	-	74 *	8
IS21883-1	1	4	1.51 *	0.25	0.44 *	0.09	53 *	6	29 *	4	45 *	11	44 *	20	71 *	4
IS22806	1	2	0.36 *	0.24	0.18 *	0.02	22 *	10	15 *	3	33	54	20	65	86 *	2
IS2579C	1	2	1.58 *	0.26	0.58 *	0.06	68 *	16	47 *	7	31 *	28	18 *	29	53 *	6
IS26789	1	3	0.98 *	0.46	0.37 *	0.08	34 *	7	30 *	5	12	8	0	9	72 *	5
IS7005	1	3	0.44 *	0.14	0.16 *	0.03	18 *	2	15 *	1	22	1	11	2	86 *	2
IS8100C	2	5	0.53 '	0.28	0.25 *	0.14	30 *	12	19 *	10	36	19	11	25	81	10
IS8721	1	3	2.73	0.57	0.19	0.04	55	8	15	3	72	5	5	5	85 *	3
MB110	1	3	0.04 *	0.04	0.00 *	0.00	3 *	3	0 *	0	100 *	-	100 *	-	100 *	0
PM7017	1	3	3.03	0.29	0.61 *	0.05	88	2	44 *	3	50 *	4	48 *	12	58 *	1
PM8782-2	1	3	0.12 *	0.10	0.09 *	0.09	11 *	9	9 *	9	13	33	19	41	91 *	9
QL38	1	3	0.02 *	0.02	0.02 *	0.01	3 *	2	2 *	1	25	83	29	89	98 *	1
QL39	4	13	1.06 *	0.24	0.39 *	0.09	44 '	6	31 *	6	30	14	2	14	70	6
SC1088-8bk	1	4	1.93 *	0.51	1.05 *	0.17	67	7	54 *	7	20 *	7	21 *	15	48 *	8
SC1089-8bk	1	4	1.13 *	0.37	0.82 *	0.22	48 *	11	55 *	12	-	-	-	-	42 *	10
SC1218-8bk	1	3	0.60 *	0.31	0.23	0.12	32	16	19 *	10	40 *	5	18	22	81 *	10
SC1222-11ebk	1	3	0.11	0.01	0.30	0.13	9	1	25	10	-	-	-	-	72 *	10
SC423C''	2	6	1.99 *	0.41	0.52	5.90	67	8	47	5	30	15	0	11	55	4
SC512-14E	1	3	0.52 *	0.22	0.33 *	0.13	25 *	9	24 *	8	5	4	0	2	72 *	11
SC62C	1	3	0.47 *	0.14	0.12 *	0.04	24 *	6	10 *	2	58 *	7	49 *	6	90 *	4
SC846-14E	1	3	2.15	0.20	0.71	0.20	80	2	50	9	38	10	0	14	60 *	8
TX2880	2	6	0.08 *	0.05	0.06 *	0.03	8 *	5	6 *	4	21	14	7	25	94 *	4

<sup>^</sup> Overall percentage decline in midge infestation from egg to pupal stage (%egg - % pupae) as a percentage of initial egg count (%egg - % pupae/%egg \* 100).

<sup>''</sup> Adjusted antibiosis. Overall percentage decline in numbers from egg to pupal stage after difference in control is accounted for. i.e. The antibiosis after adjusting for that already present in QL12.

\* Significantly different from the QL12 control in individual trials tested at p<0.05.

' Variable results in multiple trials, some significantly different from QL12 at p<0.05.

**Table 2.3** Summary of adult emergence and development in 32 lines over 18 no-choice glasshouse caged head trials under a midge density of 1 mated female midge to ten sessile spikelets.

line	no. trials	panicles tested	adults/100 spikelets#			antibiosis of pupae		development time		
			actual	se	expected"	expected - actual <sup>^</sup>	% antibiosis!	around QL12 (d) <sup>'</sup>	se	% of QL12 <sup>'</sup>
<b>QL12</b>	<b>18</b>	<b>41</b>	<b>66</b>	<b>8</b>	<b>94</b>	<b>28</b>	<b>29</b>	<b>22.17 days</b>	<b>0.28</b>	
DJ6514	3	9	4	1	6	3	41	2.78 *	0.74	113
ICSV197	3	6	7	2	12	5	40	-0.23	0.64	99
ICSV745	3	9	2	1	3	1	29	1.45	3.25	107
IS10759	1	3	33	14	35	2	6	0.16	1.21	101
IS12608C	1	2	13	5	28	15	54	-1.23	0.23	94
IS15107	1	3	17	8	58	41	71	2.15	1.32	110
IS18733	1	3	52	13	63	11	18	2.03	0.98	109
IS21871	1	4	102	20	74	-28	-38	1.06	0.28	105
IS21873	1	3	5	2	51	46	88	1.55	0.05	107
IS21881	1	3	8	4	21	14	64	0.94	0.72	104
IS21883-1	1	4	55	20	44	-11	-24	1.03	0.41	105
IS22806	1	2	10	4	18	9	47	-3.02	0.38	86
IS2579C	1	2	53	8	58	5	9	-1.13	0.87	95
IS26789	1	3	12	5	37	25	67	-1	0.75	95
IS7005	1	3	7	1	16	9	56	0.24	0.83	101
IS8100C	2	5	14	5	25	11	44	0.31	0.61	101
IS8721	1	3	14	7	19	5	26	3	1.37	114
MB110	1	3	0	0	0	0	-	-	-	-
PM7017	1	3	36	13	61	25	41	1.18	0.22	105
PM8782-2	1	3	5	5	9	4	43	1.35	-	106
QL38	1	3	2	2	2	0	0	4.11 *	0.60	119
QL39	4	13	28	8	40	11	29	0.64	0.46	103
SC1088-8bk	1	4	42	14	105	63	60	0.44	0.70	102
SC1089-8bk	1	4	63	25	82	19	23	-1.23	0.35	94
SC1218-8bk	1	3	33	25	23	-10	-43	-0.47	0.69	98
SC1222-11ebk	1	3	14	11	30	16	54	0.79	0.66	104
SC423C	2	6	46	5	52	6	12	-1.52	0.32	93
SC512-14E	1	3	36	12	33	-2	-7	0.02	0.53	100
SC62C	1	3	7	3	12	5	45	0.53	0.94	102
SC846-14E	1	3	38	13	71	33	47	0.67	0.06	103
TX2880	2	6	1	1	6	5	81	0.89	-	104

# Adult midge emergence in cages from sessile and associated pedicellate spikelets.

<sup>^</sup> Difference between expected adult emergence (from sessile spikelet pupal counts) and actual emergence.

! Difference between expected adult emergence and actual emergence as a percent of expected emergence count (formula = (expected - actual) / expected \* 100).

" Expected adult emergence if 100% of pupa sample numbers emerge. Pupa per spikelet densities from Table 2.1 results.

' Mean increased or decreased development times of midge reared on each line compared to those reared on the QL12 control in each trial.

" Development time expressed as a percentage of the QL12 control (formula = line / QL12 \* 100).

\* Significantly different from the QL12 control in individual trial tested at p<0.05.

Another two measures of antibiosis were recorded. Adult emergence (number and time of development) was recorded in each line to determine whether some lines contained significant levels of antibiosis resulting in death of pupae, or extended development times relative to that present in QL12 (Table 2.3).

#### *Pupal antibiosis*

High and varying levels of pupal antibiosis were recorded across all lines including the QL12 control. It was not possible to demonstrate any significant increase in pupal mortality ( $p>0.05$ ) of midge reared on any of the resistant lines compared to those reared in the susceptible line QL12.

#### *Adult development times*

The susceptible line QL12 recorded an average midge development time from oviposition to adult emergence of 22.17 days over 19 trials (Table 2.3). In all lines the development times were not significantly different ( $p<0.05$ ) from QL12, with the exception of the lines QL38 and DJ6514 (Table 2.3). However in both these lines adult development times were recorded from the emergence of only 2-3 adults from each cage.

## **Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance**

Ten of the 14 sorghum genotypes screened were repeatedly tested giving multiple results for these lines. In all four years significant levels of three mechanisms of resistance were recorded between oviposition and pupation; antixenosis (reduced oviposition), antibiosis (midge immature mortality between oviposition and pupation) and tolerance (filled kernels with larvae/pupae present) (Table 2.4).

#### **Contribution of resistance mechanisms to seed set**

All lines tested except Pioneer 3B in one year (26%) recorded increased seed set (40-93%) relative to the QL12 (5-23%) and QL20 (33%) susceptible controls (Table 2.4).

The combined contribution of antibiosis and tolerance to increased seed set was significant ( $p < 0.05$ ) in most lines except the highly antixenotic lines AF28, QL39 and 90562. However the levels of antixenosis, antibiosis and tolerance varied within lines across years. Despite these differences, in eight of the ten Pioneer sorghum genotypes high seed set was recorded despite moderate to high levels of egg infestation. The repeatedly tested lines Pioneer 1, 3 and 4 over 2-4 years recorded similar, or significantly higher ( $p < 0.05$ ) seed set (61-92%) than that present in the QDPI & F bred antixenotic lines QL39 and 90562 (40-63%). While similar high levels of seed set were recorded in the Indian lines DJ6514, ICSV197 and ICSV745, variable levels of antixenosis, antibiosis and tolerance contributed differently to seed set in each.

### **Antibiosis and tolerance within infested spikelets**

In the first year of testing when sampling was latest (coinciding with pupation across all lines), most of the Pioneer and Indian genotypes tested recorded significant levels of tolerance and antibiosis relative to initial and final midge infestation respectively (Table 2.4). All four Pioneer lines recorded 28-58% of infested spikelets that contained midge pupae next to filled grains. In contrast the susceptible line QL12 and the antixenotic line QL39 recorded insignificant ( $p > 0.05$ ) levels of tolerance.

In all other years of testing when earlier sampling (at 14 days instead of 17 days) of the late-larval stage occurred, significant ( $p < 0.05$ ) individual and combined levels of antibiosis and tolerance were recorded in most Pioneer and Indian bred lines. Only two lines, ICSV745 and Pioneer 3 were screened over all four years. Of these, Pioneer 3 recorded variable levels of antibiosis (16-41%) and tolerance (24-62%) across years. In contrast, the line ICSV745 recorded consistently high levels of antibiosis (42-68%) and low levels of tolerance (5-21%). Several other lines were tested 2-3 times. Amongst these lines, only ICSV197 recorded similar levels of antibiosis (39-46%) and tolerance (40-44%) across years.

In the third year of testing a hybrid derived from Pioneer 4 x Pioneer 1 was tested and recorded high levels of antibiosis and tolerance similar to its parents. However, mixed results were recorded on the lines Pioneer 1A and 3B. Pioneer 1A recorded similar high levels of antibiosis and tolerance to Pioneer 1. In contrast, the line Pioneer 3B did not record any antibiosis or tolerance.

**Table 2.4** Summary of midge infestation and levels of antibiosis and tolerance within 18 sorghum genotypes over four years testing in glasshouse cage trials.

genotype	panicles tested	% spikelets infested		seed set (%)	seed set increase		resistance relative to infestation	
		eggs	larvae/pupae		antibiosis	tolerance	antibiosis <sup>^</sup>	tolerance <sup>''</sup>
<b>year 1 - midge density of 40 mated females to 150 sessile spikelets</b>								
QL12	5	94 c*	91 b	10 a	3 a	1 a	0 a	1 a
QL39	4	42 a	41 a	60 b	1 a	1 a	0 a	2 a
ICSV745	5	66 b	38 a	69 b	28 b	7 ab	42 b	21 ab
PIONEER 1	4	71 b	44 a	71 b	27 b	15 bc	39 b	28 bc
PIONEER 2	3	72 b	63 a	73 b	9 ab	36 d	13 ab	58 d
PIONEER 3	4	62 b	56 a	66 b	6 ab	22 c	18 ab	44 cd
PIONEER 4	4	71 b	61 a	64 b	10 ab	25 cd	9 a	43 cd
<b>year 2 - midge density of 40 mated females to 150 sessile spikelets</b>								
QL12	9	96 d	96 e	5 a	1 ab	0 a	1 ab	0 a
QL39	5	58 ab	62 cd	40 b	0 a	2 ab	0 a	2 ab
90562	6	51 a	53 bcd	47 bcd	0 ab	0 a	0 a	0 a
ICSV745	9	81 c	37 a	68 fg	44 f	5 abc	55 f	15 bc
DJ6514	4	76 c	56 bcd	52 bcde	20 cd	8 abc	27 cde	14 abc
PIONEER 1	5	81 c	51 abcd	61 defg	30 de	12 cd	37 def	23 cd
PIONEER 2	5	78 c	66 d	42 bc	11 abc	9 bc	12 abc	13 abc
PIONEER 3	5	81 c	68 d	58 cdef	13 bc	26 e	16 bcd	36 d
PIONEER 4	5	83 cd	44 abc	65 efg	39 ef	9 bc	46 ef	18 bc
PIONEER 5	5	71 bc	45 abc	74 g	26 cde	19 de	37 def	35 d
PIONEER 6	5	78 c	43 ab	64 efg	35 def	7 abc	44 ef	16 bc
PIONEER 7	5	70 bc	43 ab	61 defg	26 cde	4 abc	40 ef	7 ab
<b>year 3 - midge density of 30 mated females to 150 sessile spikelets (data not analysed+)</b>								
QL12	4	90	94	7	0	0	0	0
QL39	4	40	39	63	1	2	0	8
ICSV745	4	81	26	76	55	2	68	5
ICSV197	4	58	35	79	24	13	39	44
PIONEER 1	3	52	12	92	40	4	76	14
PIONEER 1A	4	56	26	79	30	5	53	17
PIONEER 3	4	74	51	73	24	24	32	62
PIONEER 3B	4	72	74	26	0	12	0	0
PIONEER 4 x 1	4	51	28	83	24	11	46	41
<b>year 4 - midge density of 30 mated females to 150 sessile spikelets</b>								
QL12	3	83 d	77 e	23 a	6 abc	0 ab	5 abc	0 ab
QL20	7	73 cd	68 de	33 ab	5 ab	0 a	7 ab	0 a
QL39	3	35 ab	45 bcd	56 bcd	0 a	1 ab	0 a	3 ab
90562	3	33 ab	41 bc	59 cde	0 a	0 ab	0 a	0 ab
ICSV745	5	55 bc	25 ab	77 def	30 d	2 abc	55 d	15 abc
ICSV197	4	61 bcd	33 ab	81 def	28 cd	14 d	46 cd	40 cd
DJ6514	5	50 b	11 a	93 f	39 d	4 abc	77 d	42 d
PIONEER 3	3	45 ab	25 ab	81 def	19 bcd	7 c	41 bcd	24 abcd
AF28	3	21 a	19 ab	86 ef	1 ab	5 bc	6 abc	28 bcd

\* Means within columns not followed by the same letter differ significantly ( $p < 0.05$ )

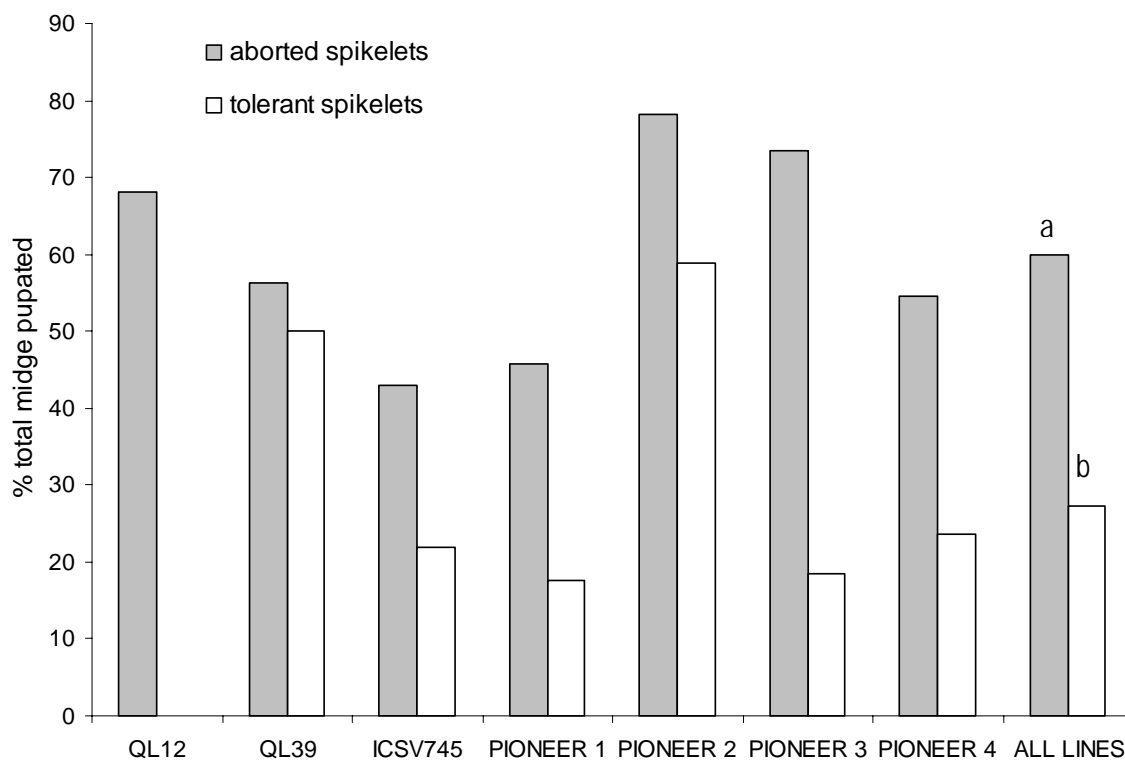
+ Data not analysed. In this year all lines were not tested on the same days as the susceptible check (QL12), in contrast to all other data presented.

<sup>^</sup> Antibiosis relative to initial egg infestation (antibiosis / egg \* 100).

<sup>''</sup> Tolerance relative to final pupal infestation (larvae/pupae - tolerance / larvae/pupae \* 100).

In the first year of testing a significantly ( $p < 0.05$ ) reduced rate of larval development through to pupation in tolerant spikelets (60%) relative to aborted spikelets (27%) was recorded across all lines (Figure 2.1). While there was variation across lines, in all lines a lower percentage of midge had pupated in tolerant spikelets relative to those in aborted spikelets. No record of tolerance was recorded in the susceptible line QL12. In the final three years of testing statistical comparison between lines across years was not appropriate (see methods). Even so in these trials sampling occurred earlier at 14 days post-anthesis resulting in lower midge pupation across all lines (52%) to that recorded in the first years testing (60%). Across the last three years testing, lines recorded a similar trend of reduced rates of pupation within tolerant spikelets relative to aborted spikelets. Under the slightly earlier sampling regime, an even higher reduced rate of larval development within aborted spikelets was recorded across all lines. In all three years across all lines, a total of only ten pupae (4%) were recorded within tolerant spikelets, compared to 243 larvae (96%). This was despite only modest decreases in overall pupation rates in aborted spikelets of all lines (52%) relative to those recorded in the first years testing (60%). During this time QL12 recorded very similar rates of pupation within aborted spikelets of 57-61%, suggesting that similar environmental conditions were present across trials each year within the glasshouse.

In these trials 75% of tolerant spikelets contained larvae too small to cause abortion of kernels. Of these, 66% were recorded feeding on the developing caryopsis (kernel). In the remaining 25% large larvae or pupae within tolerant spikelets, a total of 83% were recorded against the kernel.



**Figure 2.1** Percentage of total midge immatures pupated within aborted and tolerant spikelets in seven lines sampled 17 days post egg lay. Analysis between lines was not possible due to insufficient larvae.

## Discussion

### Stage 1 – Screenings for antibiosis

Three measures of antibiosis (larval antibiosis, pupal antibiosis, and extended development times) were recorded by sampling at different stages in the midge life cycle. Of these, the only useful levels of antibiosis that resulted in increased seed set were recorded between oviposition and pupation (larval antibiosis).

#### Larval antibiosis

Several lines contained significant levels of larval antibiosis that warrant further testing. DJ6514 and its derivatives ICSV745 and ICSV197 and the line PM7017 recorded high levels of larval antibiosis over repeated testings. These results are

consistent with those of Sharma *et al.* 2002. ICSV745 in particular showed very high levels of antibiosis to larvae under moderate egg infestation. This line is agronomically superior to DJ6514, less photo-period sensitive, higher yielding, and contains a large seed size. The line PM7017 also contains similarly useful agronomic characters (Sharma pers. comm. 2002). SC62C (IS12572) has loose panicles and glumes that do not tightly clasp the kernel (Henzell *et al.* 1996), and may prove to be a promising and possible diverse source of antibiosis caused by differences in spikelet morphology.

The lines MB110, QL38, and TX2880 contained very high levels of ovipositional antixenosis and need to be tested again under high midge pressures to obtain useful levels of egg infestation to accurately determine their relative amounts of antibiosis. MB110 and TX2880 have been used to produce highly-resistant hybrids through the Texas A & M breeding program (Peterson *et al.* 1985). TX2880 was selected in part from SC423C and TAM2566 pedigree (Henzell *et al.* 1996). Wuensche (1980) found that TAM2566 contained a marginally lower survival rate to a susceptible line from egg to adult, while both TAM2566 and SC423C produced smaller larvae than those reared on the same susceptible line. My results confirm similar levels of antibiosis within both IS2579 and its converted line SC423C. Based on pedigree, TX2880 may also contain significant larval antibiosis, however under repeated screenings (Table 2.2) I recorded no larval antibiosis in this line. MB110 is another advanced Texas breeding line derived from different sources of midge resistance, including the lines SC176-6 and SC175-9 and may be useful as a diverse source of resistance.

QL38 and QL39 are products of the QDPI & F breeding program, and both appear to contain high levels of antixenosis. While Franzmann (1988) reported high levels of antixenosis, and antibiosis to larvae in QL38, the low egg counts in this trial made it difficult to confirm these results. The mixed results for antibiosis in QL39 over four testings indicate that this line may contain a low level of antibiosis that is not expressed consistently under higher midge infestations.



### **Pupal antibiosis**

The high pupal antibiosis figures within QL12 may be normal occurrence, a result of inherent mortality at this stage of the midge life cycle. Evidence for this was similarly recorded by Wuensche (1980), who over three years testing recorded large midge mortality between pupation and adult emergence in both susceptible and resistant lines in the same trials. Consequently, it may be that if there are significant differences in pupal mortality between susceptible lines and resistant lines, they may be both difficult to record, and of little benefit.

### **Adult development times**

Midge development and emergence from pedicellate spikelets in resistant sorghum lines may be a possible cause of extended development times in this study, specifically when low sessile spikelet pupal infestations occurred. This result is supported by Franzmann (1988, 1993b) who also recorded significant midge infestation within pedicellate spikelets of resistant and susceptible sorghum lines, all of which produced extended midge development times in relation to similarly reared midge within sessile spikelets. As a result, the non-significant extended development times in a majority of resistant lines in this trial and other similar studies may be due to pedicellate-reared midge. This may also be the case in the two lines QL38 and DJ6514, which showed the most pronounced extended development times in relation to the susceptible control QL12. In both cases low adult emergence numbers of 2-3 midge per cage were recorded in these two lines in the same trial. QL38 was not tested again, while in two other trials DJ6514 did not produce extended development times. Similar no-choice trial work conducted by Sharma (1985) found DJ6514 to be highly resistant, producing approximately 10% larval infestation after high initial oviposition. In addition he found a delayed, and reduced adult emergence pattern in this line, and recorded adult emergence numbers of 2-3 midge per head. These results from three different authors highlight the possibility that both QL38 and DJ6514 emergence figures were influenced by pedicellate reared midge, particularly under low adult emergence numbers, and so reports of extended development times in these resistant hybrids should be treated with caution.

## **Stage 2 – Repeated screenings for antibiosis and the discovery of tolerance**

The repeated testing of the Indian lines DJ6514 and its derivatives ICSV197 and ICSV745 confirmed the presence of high levels of larval antibiosis within these lines. In addition most of the Pioneer Overseas Corporation advanced breeding lines tested lines contained similar levels of antibiosis to that present in the Indian lines. These lines were specifically tested for this mechanism of resistance as they contain various amounts of DJ6514 pedigree (Bruce Boucher pers. comm. 2002).

The new record of tolerance in several lines over four consecutive years confirms that tolerance is a consistently measurable and expressed resistance mechanism of significant value in several lines. In many cases the combined level of antibiosis and tolerance was greater than the maximum level of antixenosis recorded in QL39 or 90562. These results also show that QL39 does not contain significant levels of tolerance or antibiosis.

Several questions surround the newly described ‘tolerance’ mechanism of resistance. Firstly, why were significant rates of tolerance recorded in the lines ICSV197 and DJ6514 in stage two of testing in contrast to stage one? The reason for this may be a case of selective sampling error during dissection. During dissection of spikelets, those with set seed were dissected rapidly with little attention to detail, because in all other lines, and under previous trial work very few midge immatures were observed next to filled caryopses within sessile spikelets, and in all cases no evidence of feeding against the caryopsis was recorded. It was serendipitous that I observed several cases of distinct minor feeding damage against the caryopsis in one trial that led to a closer examination of all samples from this point forward. This result prompted a retest of this material under more detailed scrutiny to examine larvae of all sizes at all positions within aborted and filled spikelets.

Secondly why is it that the tolerance mechanism of resistance is restricted to lines that contain ICRISAT pedigree traced back to DJ6514? There are two possibilities that may explain this situation.

Firstly it may be that larval antibiosis and tolerance are separate, unrelated mechanisms of resistance that by chance are co-inherited by chance in these populations. Secondly, it may be that the antibiosis mechanism of resistance present in DJ6514 and its derivatives contributes to the record of tolerance in these lines.

While I find no evidence for the first scenario, several results in this study support the second scenario. It may be no coincidence that only lines that contain antibiosis also contain tolerance. The larval feeding results highlight that larval growth was retarded in tolerant spikelets relative to aborted spikelets. In the last three years testing 75% of the total larvae found within tolerant spikelets were small larvae. Of these, 66% were found feeding against the developing caryopsis, while the rest were feeding elsewhere within the spikelet. From this, I believe a further number of scenarios are possible.

Firstly, the developing kernels within ‘tolerant’ spikelets may escape immediate abortion because larvae in these lines are slow to move and feed against the developing caryopsis. It is also possible that larvae remove few overall caryopsis reserves by feeding periodically, or permanently elsewhere in the spikelet.

Franzmann (1993b) suggested one of these possibilities when he observed a similar occurrence in the line QL38 that was not observed again in future testings (Franzmann pers. comm 2003). He suggested the retarded larval development was the result of larvae feeding at positions away from the caryopsis. He further postulated that larval feeding away from the ovary may be the result of increased kernel development rate in this line, which may make it difficult for larvae to establish feeding, causing them to feed elsewhere in the spikelet.

Whatever the case, in all the above scenarios ‘tolerance’ is the result of a change in feeding behaviour of midge larvae within antibiotic genotypes and may also be described as ‘antixenosis to ovary (caryopsis) feeding’. If so the morphological, chemical, or physiological traits that cause larval antibiosis also contribute to ‘tolerance’.

## Summary and Conclusion

Antibiosis to larvae appears to be the most consistent and effective measure of antibiosis recorded in different lines from screening results. One clearly useful source of larval antibiosis was recorded in the Indian land race line DJ6514 and its derivatives ICSV197 and ICSV745. The levels of larval antibiosis remained consistent across 5 years testing and these lines additionally recorded a unique ‘tolerance’ mechanism of resistance. When both resistance mechanisms are viewed in combination these lines are clearly the most promising new source of resistance for the Australian breeding program. The line ICSV745 in particular may be the line of choice to breed from due to its high antibiosis, yield, large seed size, and general agronomic characters (Sharma pers. comm. 2002)

Several other potentially useful sources of antibiosis were also identified in screenings. The line PM7017 may prove to be a diverse source of antibiosis as it was similarly derived from the Indian breeding program from a wide genetic background. The converted antibiotic line SC62C, is another diverse source of resistance and may be a useful a source of antibiosis. The Texas bred commercially acceptable lines TX2880, and MB110 need to be tested further for their levels of antibiosis. The low initial egg lay in these lines made it difficult to accurately record larval antibiosis in these lines.

Stage two testing results open opportunities for further research. The results of this study highlight the presence of a potentially novel resistance mechanism present within lines related to DJ6514. There is opportunity to study this resistance mechanism in greater detail. This occurs in the following chapter where I report on a more detailed study of larval biology in a smaller range of susceptible, antibiotic and antixenotic lines.

## CHAPTER 3

### A Closer Examination of Larval Antibiosis

#### Introduction

Larval antibiosis has been identified as a useful source of resistance to the Australian breeding program in several lines (Chapter 2). While many researchers have reported decreased larval size, and increased mortality within the larval period (Melton & Teetes 1984, Sharma 1993, Wuensche 1980) there are no published reports that confirm when antibiosis occurs during the larval period, or if the pattern of mortality varies from line to line as a result of different antibiosis components.

In published reports of midge-resistant studies, sampling has been restricted to coincide with periods of egg lay, and late larval, or pupal development to record the final outcome of antibiosis within sorghum genotypes. There are no published reports of sampling at various stages of the larval period to record when antibiosis occurs over the entire larval period, or at what stage the developing caryopsis aborts.

This chapter examines the fate of larvae during the larval period on a range of midge resistant and susceptible lines in order to determine the time of larval mortality in these lines, and hence determine the “time of action” of the antibiosis. The pattern of larval feeding and mortality generated from these sampling data may also help provide clues to the variety of physical or chemical components that cause antibiosis in different genotypes.

## Material and Methods

### Developing a sampling regime

The sequential sampling of midge immatures *in situ* progressively over the entire larval period through to pupation has not been attempted in midge resistance research to my knowledge. Predicting the exact time periods for different stages of larval development during summer glasshouse conditions in order to study larval development during different growth periods is a precise task as larval development occurs over a relatively short period of less than two weeks and is largely influenced by temperature, larval density and sorghum genotype. The most relevant data on larval development in summer glasshouse conditions in Toowoomba (where the studies in this chapter were undertaken) were published by Franzmann (1993a). Under summer glasshouse conditions in Toowoomba, this author recorded an average midge lifecycle developmental period of 19-20 days. While the duration of egg, larval and pupal stages were not recorded, Passlow (1965) in central Queensland under similar temperatures recorded the duration of each stage as follows: egg 2-3 days; larval 10-12 days; pupal 3-5 days. Taley *et al.* (1971) recorded the duration of each larval instar within the larval period at screen temperatures of 10-33°C as follows: first instar larvae 2.7 days; second instar larvae 3.0 days; third instar larvae 2.6 days; fourth instar larvae 2.5 days. Each of the first three larval instars was distinguished by differences in the number of spiracles present, however no such differences were recorded between the third and fourth instars. It is likely that the two later instars recorded by Taley *et al.* (1971) were a third larval instar only (5.1 days in duration), as later authors consider that Cecidomyiidae have only three larval instars (Gagne 1989). Gagne reviewed the literature on all gall midges including sorghum midge and concluded that reports of four larval instars in Cecidomyiidae may arise from including the distinct change in shape, which occurs in some species when they undergo pre-pupal development, as a separate instar.

Combining the above data, the following average immature developmental periods were predicted under Toowoomba glasshouse summer conditions.

eggs	first instar larvae	second instar larvae	third instar larvae	pupae
(2-3 days)	(2-3 days)	(2-3 days)	(4-6 days)	(3-5 days)

The following study in this chapter used the above assumed time periods to sample and thus study the antibiosis in several diverse genotypes over the larval period.

### **Glasshouse cage trial procedure**

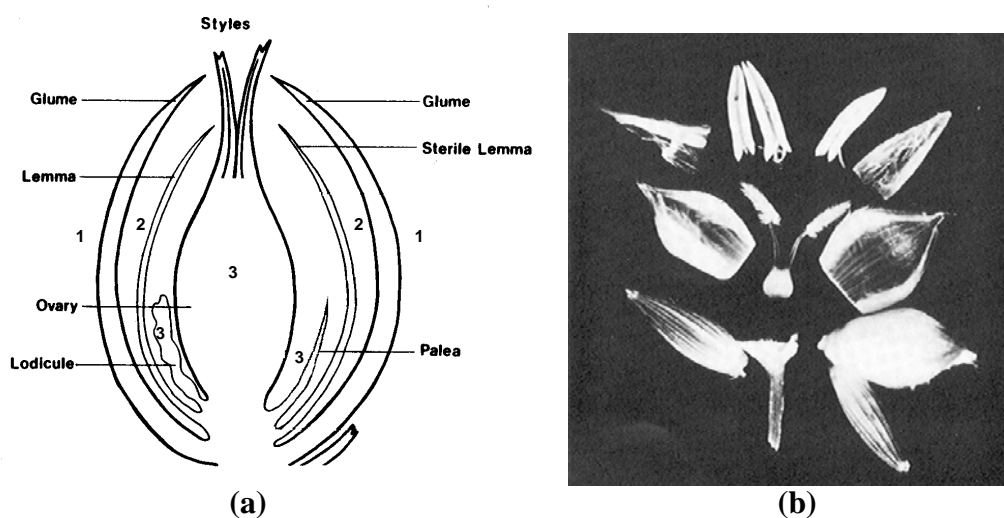
A high density cage method was used to establish natural egg lay within six resistant (ICSV745, MB110, QL39, PM7017, SC62C, TX2880) and one susceptible line (QL20). These lines were chosen from diverse geographical backgrounds, all of which may contain different antibiosis components.

The glasshouse cage method (described in Chapter 2) was used in a single trial to establish egg lay in 180-200 flowering spikelets of each line. A total of five panicles (replications) of each line were tested. Different numbers of mated females were introduced into each cage according to the estimated level of antixenosis present in each line (from Chapter 2 results) in an attempt to obtain similar levels of midge infestation in each line. Midge numbers were as follows: QL20 – 20; PM7017 – 30; ICSV745– 40; QL39 –40, SC62C – 50, MB110 – 70, and TX2880 – 70. A similar sampling procedure was used to that described in Chapter 2. Random samples of twenty sessile spikelets were taken from each panicle by cutting 2-3 small rachis branches off different parts of each panicle over the following six sample dates in line with the following predicted periods of immature development:

Day one (egg sample), day five (end first instar), day eight (end second instar), day 11 (third instar), 15 (pupation), and day 17-19 (end of pupation- taken at the emergence of first adult in each cage). In all cases samples were stored in the freezer immediately after collection for later dissection.

## Collation of results

Two measures were taken to estimate midge density over time: the number of midge per spikelet and percentage of spikelets infested. In addition the position of midge within each spikelet was recorded at each sample date at one of three positions (Figure 3.1).



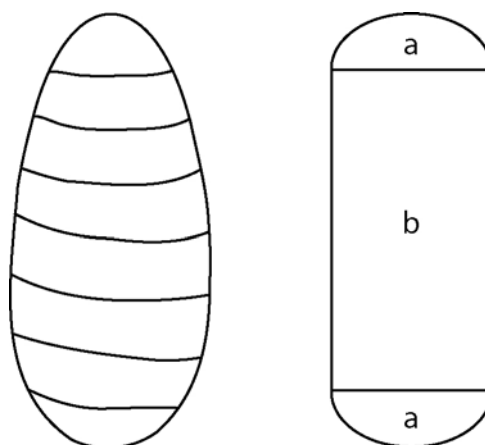
**Figure 3.1** Sorghum flower – a diagrammatic and actual view of the parts of a spikelet  
 (a) Drawing of a sessile spikelet consisting of: two outer glumes; two papery thin lemmas; one papery thin palea (often reduced/absent); and a split fleshy lodicule covering the base of the caryopsis. When spikelets were dissected, midge immatures were recorded at one of three positions indicated by the following numbering:

1. outside the spikelet against either glume
2. between either glume and palea/lemmas
3. against the caryopsis (including between caryopsis and lemmas/lodicule/palea)

(b) Bottom three attached sorghum spikelets separated, two pedicellate spikelets and one sessile spikelet (all intact). Above (middle row) a sessile spikelet (flower) dissected to show either glume surrounding the caryopsis containing attached styles and stigmas. Above (top row), outmost the papery thin lemmas surrounding three anthers.



The sizes of each larvae/pupae at each sample date were recorded by measuring length and width under a dissecting microscope using an ocular micrometre (0.01 mm units) at 50 times magnification (500 ocular units = 1 mm). The following formula was used to estimate the size, based on the oblong like larval/pupal shape (Figure 3.2).



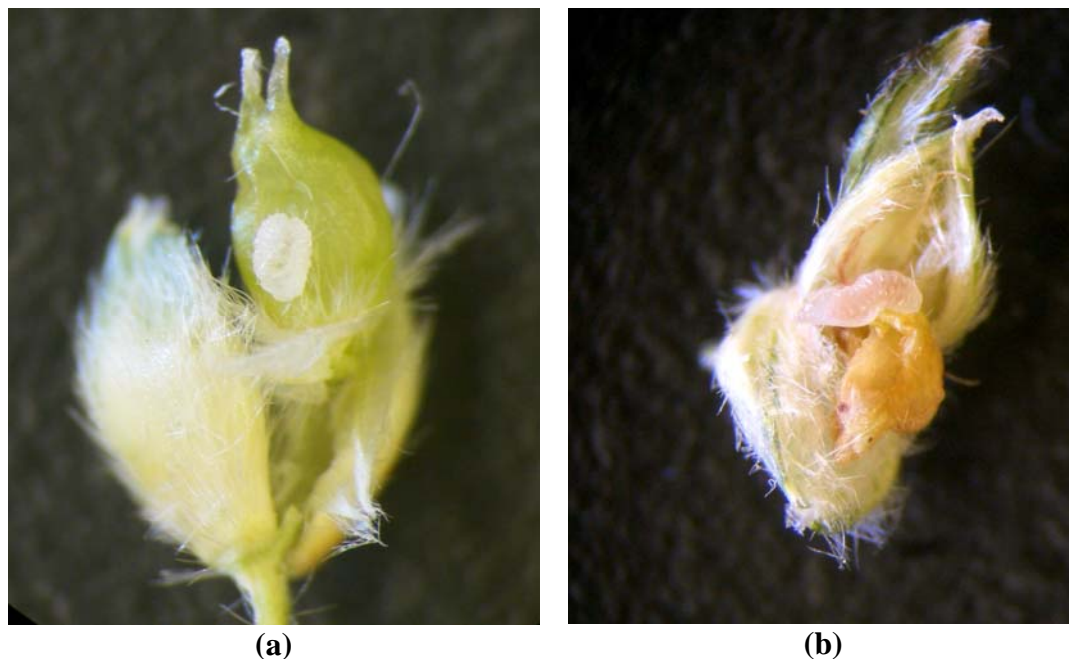
larval or pupal size = (a) + (b):

where: (a) =  $(\pi \times 0.5 \times \text{width})^2$  and (b) = length x width

**Figure 3.2** Formula used to measure larval size.

The first part of the formula calculates the area of a circle made up by the semicircular distal and proximal ends of each midge larva/pupa. The second part of this formula calculates the greater portion of each larva/pupa, the long rectangular shaped thorax and much of the abdomen. Length measurements of each larva/pupa were recorded without the anterior/posterior rounded ends.

In order to determine the point at which feeding larvae cause irreparable grain loss to developing sorghum caryopses within each spikelet, individual sorghum caryopsis were recorded as either set seed, or aborted according to the degree of midge damage. Midge infested sorghum caryopses less than 50% the size of un-infested caryopsis were recorded as aborted (Plate 3.1).



**Plate 3.1** Two dissected spikelets eight days post-anthesis showing caryopsis damage under midge larval feeding. Caryopses approximately 60% (a) and 100% (b) reduced in size by midge larvae. Caryopses in both (a) and (b) classified as irreparably damaged (aborted).

Results were recorded at each sampling date, for each of the above measures, while the pattern of midge movement over time, larval mortality, and increase in larval size, were recorded by a combined record of sample dates representing stages of midge development between egg lay and pupation.

### **Statistical analyses**

All data except size data were analysed by analysis of variance (ANOVA), while in some cases data were further transformed (log and angular transformations) to meet normality assumptions before statistical analysis using Genstat 4.1 software. Size data were analysed using residual maximum likelihood (REML) analysis also using Genstat 4.1 software. Significant differences for all data were estimated using least significant differences (LSD) to a 5% level of significance, incorporating all sampling days. For simplicity, any analyses where a complete data set across all sampling days was recorded were made as one two-way (line.day) analysis.

This also allowed the three following differences to be recorded across all sampling days:

1. Differences between lines on any sampling day (line LSD)
2. Differences between days for all lines (day LSD).
3. Differences in the patterns (slope) of midge movement, mortality and larval growth over time between sampling days (line.day LSD).

## **Results**

### **Predicted versus actual immature developmental timelines**

The estimated length of the different periods of immature development was similar to that predicted. The average length of the larval period could be estimated by viewing the results of day 15 samples, where an average of 50% of all midge were recorded as pupae, without any significant difference ( $p < 0.05$ ) across lines. This makes the average larval period somewhere between 12-13 days if a 2-3 day egg eclosion period is accounted for at trial commencement.

The pupal samples (designed to coincide with the end of the pupal period) in all cases were taken between day 17-19 at the emergence of the first adult in each panicle. In this sample an average of 86% of individuals recorded were pupae across all lines, again without any significant difference ( $p < 0.05$ ) between lines. The predicted rate of larval instar development from first to third instars was also confirmed in dissections where average larval size increases over the three instars were in line with those recorded by Taley *et al.* (1971). Consequently, the above actual average stages of midge immature development at each sampling timeframe have been directly correlated to each stage of midge immature development in line with that predicted and are quoted in all tables and figures throughout results.

**Table 3.1** Two measures of midge infestation over six sampling intervals from egg lay to pupation in sessile spikelets of eight sorghum lines. Differences between lines on each day (\*) and on all lines across days (#) are shown. Where appropriate log transformed (lg) and back transformed equivalent means (eq. m) were used to meet normality assumptions.

midge density	line	day 1 (egg)		day 5 (1st instar larvae)		day 8 (2nd instar larvae)		day 11 (3rd instar larvae)		day 15 (50% pupae)		day 17-19 (86% pupae)		
		eq.m	lg	eq.m	lg	eq.m	lg	eq.m	lg	eq.m	lg	eq.m	lg	
(a) number per spikelet	QL20	2.80	1.34 c*	2.28	1.19 e	1.49	0.91 c	1.51	0.92 d	1.38	0.87 d	1.25	0.81 d	
	QL39	1.06	0.72 a	0.72	0.54 b	0.55	0.44 a	0.25	0.22 a	0.20	0.18 a	0.28	0.25 ab	
	LSD (log) = 0.09*	TX2880	2.59	1.28 c	1.14	0.76 c	0.87	0.63 b	0.42	0.35 b	0.46	0.38 bc	0.38	0.32 bc
	MB110	1.81	1.03 b	0.56	0.44 a	0.49	0.40 a	0.26	0.23 a	0.29	0.25 ab	0.21	0.19 a	
	SC62C	1.13	0.76 a	0.98	0.68 c	0.94	0.66 b	0.61	0.48 c	0.37	0.31 b	0.31	0.27 b	
	PM7017	2.79	1.33 c	1.60	0.96 d	0.96	0.67 b	0.57	0.45 c	0.56	0.44 c	0.46	0.38 c	
	ICSV745	2.78	1.33 c	1.70	0.99 d	0.86	0.62 b	0.41	0.34 b	0.24	0.22 a	0.19	0.17 a	
	LSD (log) = 0.08#	all lines	1.99	1.10 d	1.07	0.73 c	0.74	0.55 b	0.43	0.36 a	0.39	0.33 a	0.34	0.29 a
(b) % spikelets infested		mean		mean		mean		mean		mean		mean		
	QL20	86 d*		86 d		78 e		86 c		77 e		78 e		
	QL39	45 a		41 a		34 b		16 a		17 a		21 a		
	LSD = 7*	TX2880	69 c		58 b		50 c		30 b		36 c		33 c	
	MB110	57 b		38 a		33 a		18 a		22 ab		18 a		
	SC62C	46 a		43 a		47 c		36 b		27 b		26 b		
	PM7017	78 c		76 c		60 d		37 b		48 d		41 d		
	ICSV745	72 c		60 b		45 c		33 b		21 ab		19 a		
LSD = 6#	all lines	65 d		57 c		50 b		37 a		35 a		34 a		

Means in columns (\*) or rows (#) followed by the same letter are not significantly different ( $p < 0.05$ ).

### **Midge infestation and mortality within immature development stages**

At each sample date two measures of midge density were recorded, the number of midge per spikelet, and the percentage of spikelets infested.

#### *Number of eggs/spikelet*

The number of eggs per spikelet was significantly different between lines ( $p < 0.05$ ), despite the adjusted midge pressure within the cage of each midge resistant line (Table 3.1a). In order to directly compare the pattern of mortality over time across lines with uneven starting egg densities the midge numbers per spikelet over time were recorded as a percentage of the original eggs per spikelet count in each line (line.day  $p = 0.322$ ; Figure 3.3a). Under this analysis a similar pattern of larval mortality was recorded in all lines between egg lay and pupation (line.day  $p = 0.322$  Figure 3.3a). All mortality occurred before pupation, between egg lay and day 11 and was greatest between egg lay and early larval feeding (day 1-5).

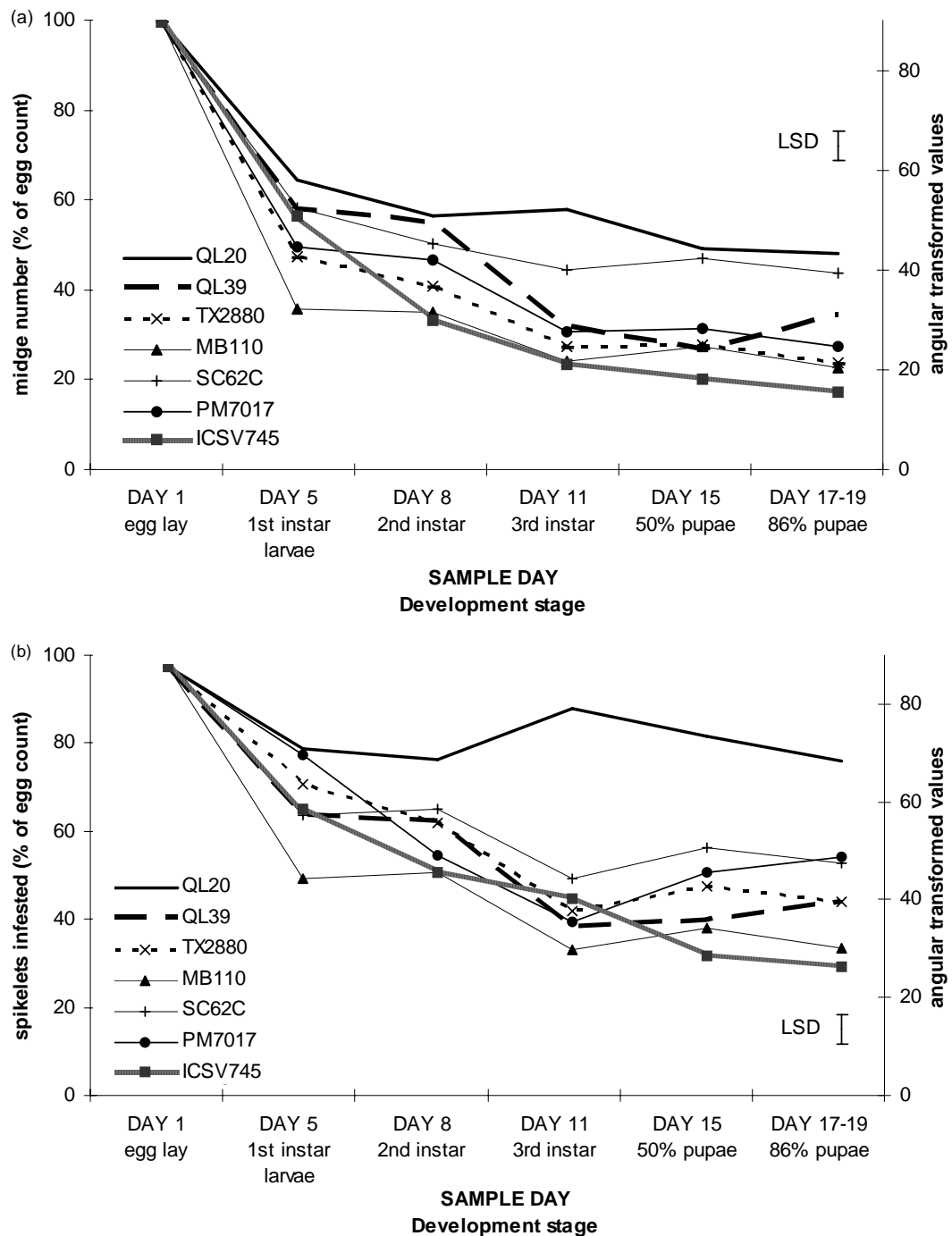
Despite a similar pattern of mortality over time between lines across days, differences in larval mortality between lines occurred over the larval period (Figure 3.3a). Differences in mortality began from day zero to five, between egg lay and the beginning of larval feeding. Mortality over this period was highest in MB110, with numbers dropping to less than 40% of the original egg count, and lowest in QL20 at approximately 65% of the original egg count (Figure 3.3a). Between day five and eight, larval mortality did not occur across all lines except in ICSV745, which recorded a 20% reduction in larval numbers.

Between day eight and 11 during the second half of the larval period, no mortality occurred within QL20, while mortality of approximately 10% occurred in ICSV745, MB110, TX2880 and PM7017, and this increased to 20% in QL39. Between day 11 and the final pupal sample (day 17-19), a majority of mature larvae stopped feeding and pupated, during which time no mortality occurred across all lines.

Over the entire larval period the greatest mortality occurred in ICSV745, MB110, PM7017 and TX2880, while moderate mortality occurred in QL39 (Figure 3.3a). The pattern and level of mortality recorded in SC62C was similar to that of the susceptible line QL20, in contrast to that present in all other resistant lines, which showed higher and more variable patterns of mortality between day 1 and 11.

*Percentage of spikelets infested with eggs*

Midge numbers were also recorded as the percentage spikelets infested to record base line infestation and subsequent damage levels within each spikelet (Table 3.1b). Again the percentage of spikelets infested with eggs was different between lines, highest in QL20 at 86% and lowest in QL39 at 45%.



**Figure 3.3** Midge survival over time in glasshouse cage trials as a percentage of the original egg infestation across seven lines.

(a) Midge number per spikelet as a percentage of egg count (angular equivalent means).

(b) Percentage of spikelets infested as a percentage of egg count (angular equivalent means). Significant differences between lines on each day were recorded in both (a) and (b) above indicated by line LSD ( $p < 0.05$ ).

When these differences were recorded as a percentage of the egg infestation, significant differences in larval mortality were recorded between lines on each day of sampling, across days for all lines, and additionally in the patterns of mortality between lines across days (line, day, and line.day, all  $p < 0.001$ ). The pattern of mortality between lines was significantly different across several sampling periods between the susceptible control QL20 and the six sorghum lines ( $p < 0.001$ ; line.day LSD). In QL20 significant mortality occurred between day zero and five, after which the percentage of spikelets infested remained constant, in contrast to all resistant lines in which the percentage of spikelets infested reduced significantly between day five and 11. Again no significant mortality occurred across all lines from day 11 to pupation when most larvae stopped feeding and pupated ( $p < 0.001$ ; day LSD). Overall the mortality recorded between egg lay and the pupal sample (day 17-19) was highest in the lines ICSV745, and MB110 at over 60% of the original percentage of spikelets infested with eggs, followed by QL39, and TX2880 at approximately 50%, while PM7017 and SC62C produced approximately 40% mortality (Figure 3.3b). In contrast to the resistant lines, QL20 produced a 20% reduction in midge infestation by the end of the larval period.

### **Midge immature feeding position and size**

The position of egg lay within spikelets was different between lines, although in all cases the dominant position was next to the caryopsis (Table 3.2). The overall pattern of midge movement was, as expected, towards the caryopsis, with 99% of all midge larvae across lines feeding against the caryopsis by day 11. Despite this movement of larvae in all spikelets was not always directly towards the caryopsis, and in five of the six resistant sorghum lines a significant percentage of larvae ( $p < 0.05$ ) were found outside the spikelet five days after egg lay. This movement corresponded with a drop in numbers against the caryopsis between egg lay and day five (Table 3.2 and Figure 3.4). In MB110, the combination of significant larval movement outside the spikelet at days 5-8 and initial low percentage of egg lay against the caryopsis resulted in this line producing the lowest percentage of larval feeding against the caryopsis at day eight. By day 11 almost all larvae in all lines were found feeding against the caryopsis.

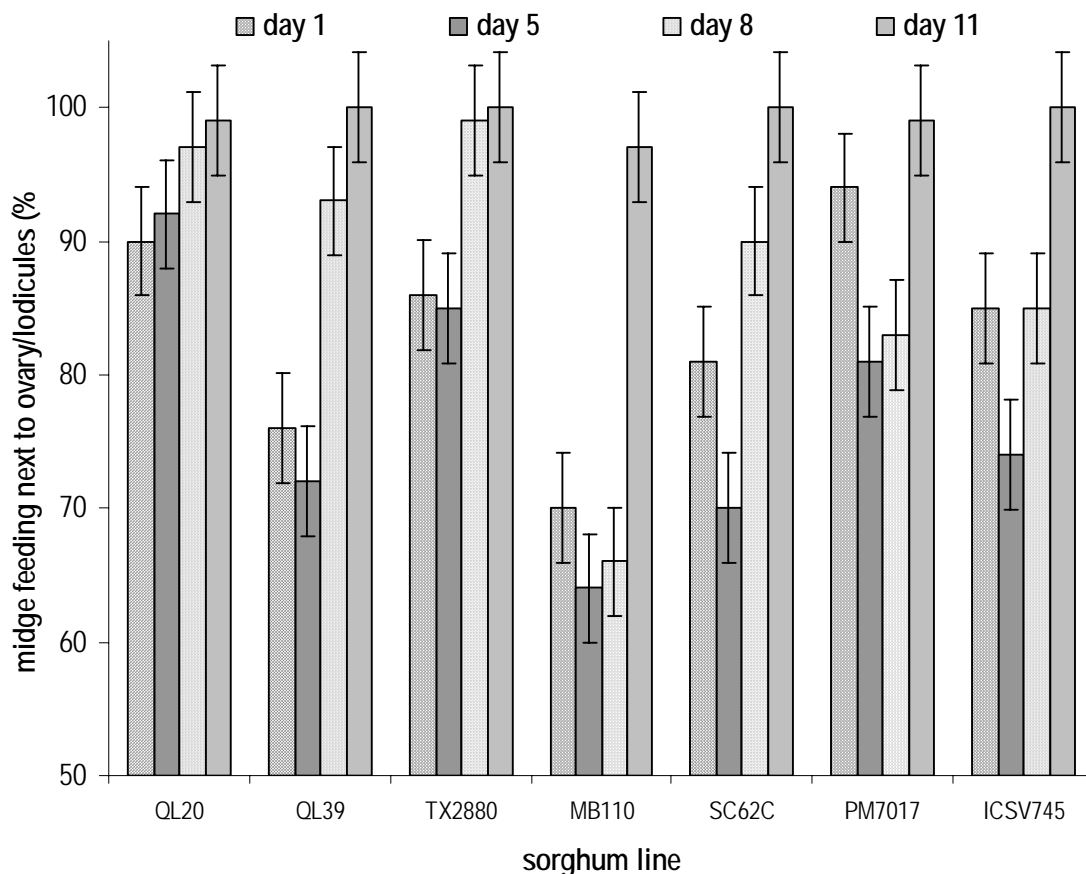


**Table 3.2** Movement of midge larvae over time within the sessile spikelets of seven sorghum lines after natural egg lay in a glasshouse cage trial. Figures for each line are a percentage of the total larvae present at all three positions on that sampling day. Differences between lines on each day at each spikelet position (1-3) are indicated (\*) along with differences across days for all lines (#).

<b>feeding position</b>	<b>line</b>	day 1 (egg)	day 5 (1st instar larvae)	day 8 (2nd instar larvae)	day 11 (3rd instar larvae)	
1. outside spikelet	QL20	-	1 a*	0 a	0 a	
	QL39	-	16 b	0 a	0 a	
	LSD = 7.5*	TX2880	-	2 a	1 a	0 a
	MB110	-	29 c	18 b	0 a	
	SC62C	-	10 b	4 a	0 a	
	PM7017	-	11 b	0 a	0 a	
	ICSV745	-	13 b	6 a	0 a	
LSD = 3.5#	all lines#	-	12 c	4 b	0 a	
2. inside spikelet glume/palea/lemmas	QL20	10 a	7 a	3 ab	0 a	
	QL39	24 bc	12 ab	8 b	0 a	
	TX2880	14 b	13 b	0 a	0 a	
	LSD = 6.9*	MB110	30 c	6 a	16 c	3 a
	SC62C	19 b	21 c	6 ab	0 a	
	PM7017	6 a	9 ab	17 c	1 a	
	ICSV745	15 b	13 b	9 b	0 a	
LSD = 5.5#	all lines	17 c	12 bc	9 b	1 a	
3. inside spikelet caryopsis	QL20	90 cd	92 e	97 c	99 a	
	QL39	76 ab	72 ab	93 c	100 a	
	TX2880	86 bc	85 de	99 c	100 a	
	LSD = 8.2*	MB110	70 a	64 a	66 a	97 a
	SC62C	81 b	70 ab	90 b	100 a	
	PM7017	94 d	81 cd	83 b	99 a	
	ICSV745	85 bc	74 bc	85 bc	100 a	
LSD = 5.7#	all lines	83 b	77 a	87 b	99 c	

Means in columns (\*) or rows (#) at each position (1,2 and 3) followed by the same letter are not significantly different ( $p < 0.05$ )

- No eggs found outside spikelets in rep 1 samples, no other results recorded.



**Figure 3.4** Percentage of midge present against the caryopsis within each line at egg lay, and over the larval period. Bars indicate least significant difference between lines at any day (line.day LSD;  $p=0.05$ ).

Feeding position within the spikelet and line were both found to influence the size of larvae recorded (Table 3.3). The greatest larval growth in all lines occurred between days 5-11 when larvae were estimated to develop from first to third instars. At day five most larvae were estimated to be 2-3 days old at the end of the first instar larval period. At this stage of larval growth no significant differences in larval size were recorded between feeding positions across all lines ( $p<0.05$ ). By day eight however second instar larvae feeding on the caryopsis were on average twice to three times larger than larvae feeding elsewhere within the spikelet, and this trend continued on third instar larvae recorded 11 days after egg lay.

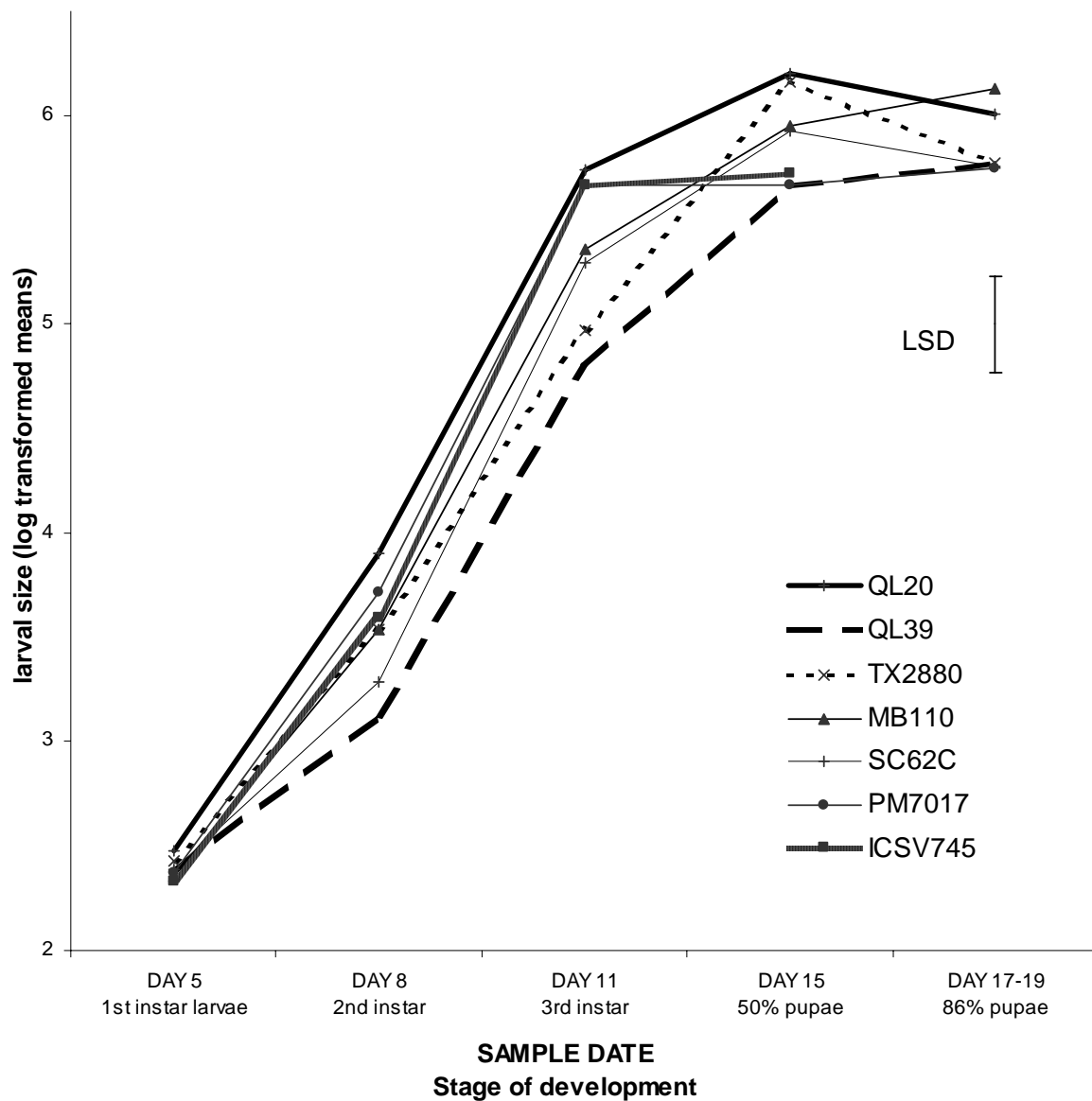
Differences in larval size between lines at each position were also recorded (Table 3.3: Figure 3.5). MB110 recorded significantly ( $p<0.05$ ) larger larvae on the outside

of spikelets and against the inside of glumes from days 5-11. The line QL39 showed a reduced larval growth rate over the larval period, while the final larval size at day 17-19 was not significantly different ( $p < 0.05$ ) to any other line including the susceptible QL20 control. By day 15 when a majority of larvae were fully mature, all other lines showed similar or slightly reduced larval size in relation to QL20. The pupal size in all lines at two sample dates was also similar between lines, while earlier (15 day) pupae were larger than the later sampled pupae at days 17-19 (Table 3.4).

**Table 3.3** Larval size of midge (ocular units; 500 units = 1 mm) at three positions within the spikelet of seven sorghum lines over 12-14 days of larval growth. Log (lg) and back transformed equivalent means (eq. m) are shown after differences between lines on each day (\*) and differences across days for all lines (#) were determined under REML analysis at  $p < 0.05$ .

feeding position	line	day 5 (1st instar larvae)		day 8 (2nd instar larvae)		day 11 (3rd instar larvae)		day 15 (50% pupae)		day 17-19 (86% pupae)	
		eq.m	lg	eq.m	lg	eq.m	lg	eq.m	lg	eq.m	lg
1. outside spikelet	QL20	4	1.7 a*								
	QL39	5	1.8 ab								
LSD (log) = 0.63*	TX2880	4	1.7 a	15	2.8 a						
	MB110	9	2.3 b	9	2.3 a						
	SC62C	8	2.2 ab	11	2.4 a						
	PM7017	7	2.1 ab								
	ICSV745	6	2.0 ab	15	2.8 a						
LSD (log) = 0.69#	all lines	7	2.0 a	12	2.6 a						
2. inside spikelet glume/palea/lemmas	QL20	6	2.0 a	12	2.6 b	64	4.2 a				
	QL39	6	2.0 a	11	2.5 b						
LSD (log) = 0.42*	TX2880	7	2.1 a								
	MB110	6	2.0 a	28	3.4 d	127	4.8 b				
	SC62C	8	2.2 a	6	2.0 a						
	PM7017	5	1.8 a	19	3.0 c	51	4.0 a				
	ICSV745	8	2.2 a	11	2.5 b						
LSD (log) = 0.49#	all lines	7	2.1 a	15	2.8 b	88	4.5 c				
3. inside spikelet caryopsis	QL20	12	2.5 a	54	3.9 c	308	5.7 c	485	6.2 c	402	6.0 a
	QL39	11	2.4 a	26	3.1 a	130	4.8 a	285	5.7 a	326	5.8 a
LSD (log) = 0.46*	TX2880	11	2.4 a	41	3.5 abc	142	5.0 ab	462	6.2 bc	312	5.8 a
	MB110	10	2.4 a	40	3.5 abc	211	5.4 bc	376	6.0 abc	461	6.1 a
	SC62C	11	2.4 a	37	3.3 ab	197	5.3 bc	365	5.9 abc	290	5.8 a
	PM7017	11	2.4 a	44	3.7 b	328	5.7 c	287	5.7 a	303	5.8 a
	ICSV745	10	2.3 a	43	3.6 bc	290	5.7 c	302	5.7 ab		
LSD (log) = 0.44#	all lines	11	2.5 a	35	3.6 b	213	5.4 c	365	5.9 c	385	5.9 c
all positions	QL20	11	2.5 a	48	3.9 c	308	5.7 c				
	QL39	9	2.3 a	22	3.1 a	130	4.8 a				
LSD (log) = 0.43*	TX2880	11	2.5 a	35	3.6 bc	142	5.0 ab				
	MB110	10	2.4 a	26	3.3 ab	207	5.3 bc				
	SC62C	10	2.4 a	25	3.2 ab	200	5.3 b				
	PM7017	10	2.4 a	38	3.7 bc	277	5.6 bc				
	ICSV745	10	2.4 a	32	3.5 abc	290	5.7 bc				
LSD (log) = 0.41#	all lines	10	2.4 a	31	3.5 b	210	5.4 c				

Means in columns (\*) or rows (#) at each position (1,2,3 and 4) followed by the same letter are not significantly different ( $p < 0.05$ ).



**Figure 3.5** Larval size (log transformed ocular units) of midge feeding against the caryopsis/lopicules within seven sorghum lines over the larval period. Differences between lines at any day (line.day LSD) indicated by line LSD bar after REML analysis at  $p < 0.05$ .

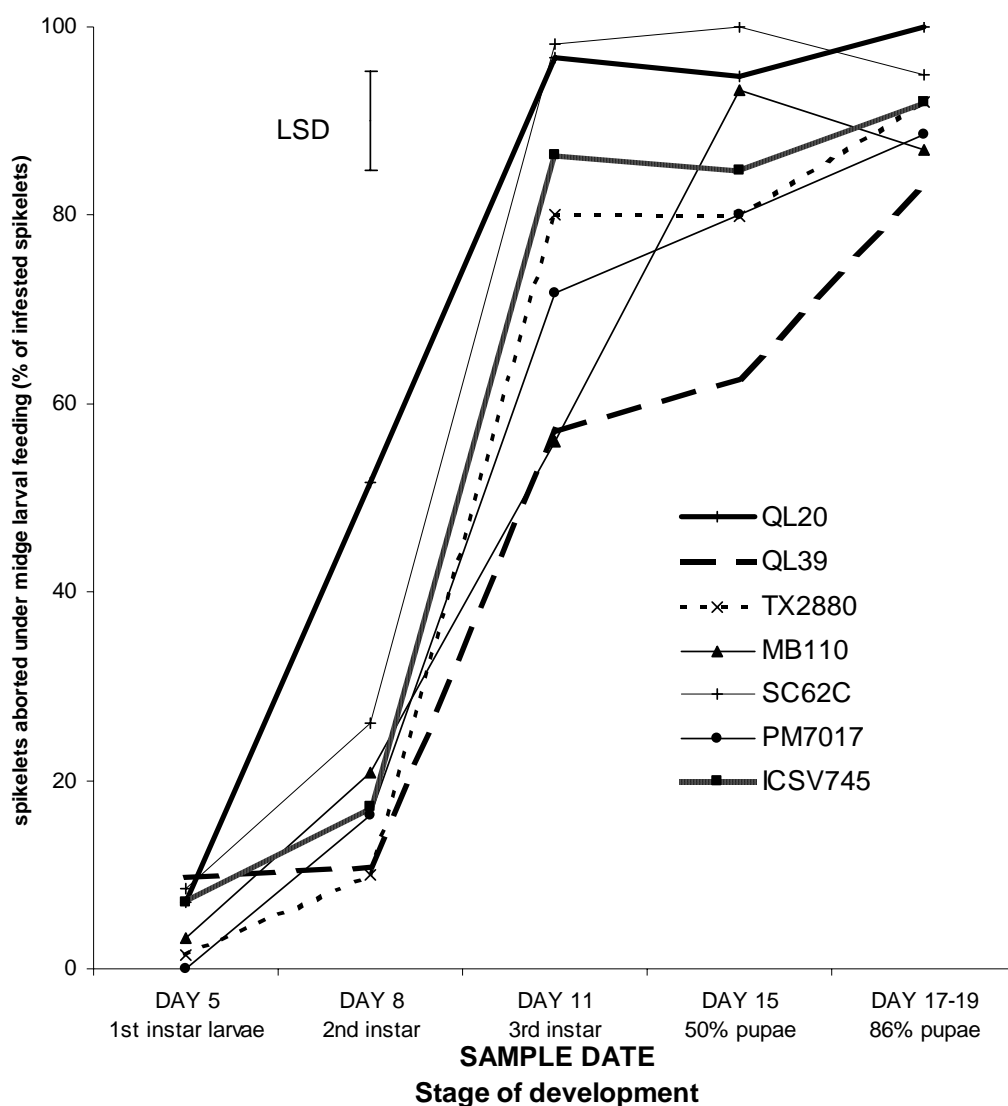
**Table 3.4** Pupal size (ocular units) of midge within seven sorghum lines at two sample dates (fifteen days after egg lay, and again at 17-19 days upon the emergence of the first adult from each panicle of each line). Significant differences in pupal size between lines at each sampling period(\*), and between sampling periods for all lines (#) were recorded on log transformed data (lg) and back transformed equivalent means (eq. m) after REML analysis at  $p < 0.05$ .

LSD=0.16*	day 15 (50% pupae)		day 17-19 (86% pupae)		total	
	eq. mean	lg	eq. mean	lg	eq. mean	lg
QL20	634	6.5 b*	519	6.3 ab	573	6.4 a
QL39	600	6.4 ab	512	6.2 ab	554	6.3 a
TX2880	643	6.5 b	439	6.1 a	531	6.3 a
MB110	571	6.3 ab	497	6.2 ab	533	6.3 a
SC62C	644	6.5 b	543	6.3 b	591	6.4 a
PM7017	544	6.3 ab	475	6.2 ab	509	6.2 a
ICSV745	536	6.3 a	478	6.2 ab	506	6.2 a
all lines (LSD=0.17)#	591	6.4 b	494	6.2 a		

Means in columns (\*) or row (#) followed by the same letter are not significantly different ( $p < 0.05$ ).

### Midge damage, antibiosis and final seed set

A similar pattern of caryopsis abortion (measured as a 50% or greater decrease in caryopsis size under larval feeding) within infested spikelets was observed in all lines, while differences were recorded between lines at various sample dates over the larval period (Figure 3.6). Very few infested spikelets were aborted in any line at day five under first larval instar feeding, however by day eight at the end of the second instar, 50% of the caryopses in QL20 were aborted from larval feeding, while this approached 100% by day 11. In contrast all other resistant lines recorded less than 30% of infested spikelets as aborted by day eight, while mixed results were observed in resistant lines by day 11. At this sample date when a majority of larvae were third instar, the lowest percentage of spikelets aborted was recorded in both QL39 and MB110 at less than 60%, followed by PM7017, TX2880 and ICSV745 at 70-85%. By day 15 when larvae within each line were mature third instar larvae, the percentage of infested spikelets aborted increased to 80% or more in all lines except QL39. Several days later the rate of abortion in QL39 increased to 80%, while the rate of abortion across all other lines were similar, the rate highest in QL20 approaching 100%.



**Figure 3.6** Percentage of infested spikelets aborted due to midge feeding over the larval/pupal period. Differences between lines ( $p < 0.05$ ) across any day indicated (line.day LSD).

Final seed set figures for each line were recorded from pupal sample dissections, 17-19 days after egg lay (Table 3.5). Significant differences ( $p < 0.05$ ) in seed set were recorded between lines, influenced by variations in larval mortality (Table 3.5) and by a reduced rate of abortion of caryopses within infested spikelets over the larval period (Figure 3.6). Very few spikelets in any line were recorded as un-infested and aborted by previous midge feeding. All resistant lines recorded significantly higher

( $p < 0.05$ ) seed set compared to the susceptible control (QL20). MB110 and ICSV745 produced the highest seed set figures largely due to the high percentages of larval mortality in both lines (Table 3.1; Figure 3.3). QL39 also produced high seed set figures due to a combination of low initial egg lay, low larval mortality, and a lower caryopsis abortion percentage under midge feeding (Table 3.1; Figure 3.6). TX2880, SC62C, and PM7017 also showed significant ( $p < 0.05$ ) increased seed set after moderate levels of larval mortality.

**Table 3.5** Summary of midge infestation, antibiosis and seed set within seven sorghum lines after natural cage infestation.

line	% egg	% pupae	antibiosis	% antibiosis+	% seed set
QL20	86 c*	78 d	7 a	9 a	14 a
QL39	45 a	21 ab	24 bc	53 bc	80 cd
TX2880	69 bc	33 bc	36 bc	53 bc	69 bc
MB110	57 ab	18 a	39 cd	69 cd	85 d
SC62C	46 a	26 ab	20 ab	40 b	75 c
PM7017	78 c	41 c	37 cd	43 b	65 b
ICSV745	72 bc	19 ab	53 d	74 d	85 d

Means in columns (\*) followed by the same letter are not significantly different ( $p < 0.05$ ).

+ Antibiosis as a percentage of egg count (egg - pupal / egg \* 100).

## Discussion

### Midge survival and development

While the sampling times chosen in this trial accurately encompassed the entire larval period, the variable egg densities between lines at trial commencement may have confounded results, particularly those of larval size and larval mortality. Despite these limitations some clear contrasting patterns of larval development emerged between several lines in this study that may be caused by the presence of different antibiosis components.

Across all lines most significant mortality occurred before day 11 encompassing the first two larval instars. Larvae underwent the greatest size increase of 4-10 times between days 8-11, coinciding with a majority of third instar larvae feeding against the caryopsis (Table 3.3 and Figure 3.5). As such it was the third instar larvae feeding against the caryopsis that caused irreparable damage to the caryopsis. Those

lines that recorded the greatest larval mortality prior to day 11 also recorded the highest levels of antibiosis that resulted in increased seed set.

Generally larvae feeding against the caryopsis of all lines were larger than larvae feeding elsewhere in the spikelet, except in several resistant lines that recorded larvae outside the spikelet at days five and 11 (Table 3.3). The size difference may be explained as the movement of larvae away from the caryopsis outside the spikelet early in the larval period, particularly in the line MB110 which recorded a drop in the number of larvae against the caryopsis between egg lay and days 5-8. On day five larvae on this line outside the spikelet were of similar size to larvae positioned against the ovary, indicating that such larvae had already fed on the ovary and then moved outside the spikelet. Similarly on day eight and 11, larvae reared on this line positioned on the inside of the glume/palea/lemma were a similar size to larvae feeding against the ovary on the same day. Such larvae were larger than similar larvae feeding against the glume/palea/lemma in all other lines, again indicating that larvae in MB110 were moving away from the caryopsis to feed. This larval movement away from the caryopsis to feed also corresponds with a high level of early larval mortality.

The line QL39 also showed evidence of generating a retarded growth rate, producing smaller larvae than any other line over much of the larval period until the pupal sample, when larvae in this line were similar in size to larvae reared on other lines. While this size difference may be due to antibiotic effects, the final larval and pupal sample of QL39 and other resistant lines was the same size as QL20.

### **Antibiosis components in each line**

The lines MB110 and ICSV745 recorded the highest rates of antibiosis of all lines tested caused by high rates of larval mortality prior to day 11. However both lines appear to contain differing patterns of larval mortality during the early larval period and may contain diverse antibiosis components. The line MB110 in particular appears to contain a unique source of antibiosis that causes larvae to move away from the developing caryopsis within the first five days of larval growth. In contrast



the line ICSV745 contains antibiosis components that cause larval mortality over the entire larval period.

While the line QL39 recorded a unique retarded rate of larval growth that may be due to a diverse mechanism of antibiosis, the final caryopsis abortion in this line was similar to all other lines tested at the end of the larval period. In effect this resistance mechanism is relatively minor as it does not cause significant rates of increased larval mortality prior to caryopsis abortion and subsequently does not result in increased seed set.

## **Conclusions**

While there is evidence to suggest that several lines tested in this trial may contain diverse antibiosis components, any distinct differences between lines were difficult to interpret because of the potential effects of different egg infestations at trial commencement. Even so, the results show that two lines (ICSV745 and MB110) may contain useful and diverse mechanisms of resistance that cause different patterns of larval antibiosis. In order to confirm these results more precise studies need to be repeated under high and consistent rates of initial egg infestation across all lines. This work was made possible by first developing a new screening method and is reported in two parts over the following chapters.

## CHAPTER 4

### The Water Injection Technique

#### Introduction

Studies of larval biology are hindered if high and even initial egg densities cannot be obtained across all genotypes to be studied. This was evident in Chapter 2 and 3 results where several highly antixenotic lines MB110, QL39 and TX2880 recorded highly variable egg infestations even when midge populations were adjusted to attempt to increase egg lay. Past researchers attempting similar studies of midge immatures have recognised this problem, and used artificial egg infestation techniques in an attempt to produce consistent egg infestation across all lines for antibiosis related studies of midge biology. Rossetto *et al.* (1984) and Sharma *et al.* (1993) have described methods of manual egg transfer into sorghum spikelets using a flattened pin or needle to facilitate antibiosis resistance studies of midge biology.

However in my hands, the use of these methods resulted in high levels of mechanical damage and inconsistent egg densities. To overcome this problem I developed a new and more precise method of artificially implanting midge eggs into sorghum spikelets which is based on injecting an aqueous suspension of midge eggs with a micro-pipette. The new water injection method is described in this chapter along with a bioassay to determine the effects of storing midge eggs in water solution under refrigeration for several days and weeks for later use.

## **Materials and Methods**

### **Water injection technique**

#### **Collection of midge eggs**

Eggs were collected after high-density midge egg lay was initiated in flowering spikelets in the laboratory using the method of Franzmann (1996). On each trial day newly-emerged mated female midge were collected mid-morning from cut panicles of sorghum and placed in high densities of 100-300 midge to 20-60 sessile sorghum spikelets. Spikelets were dissected after oviposition under a stereomicroscope, eggs removed with fine forceps and suspended in 5 ml of distilled water within excavated glass blocks, resulting in 200-1000 eggs per block. Immediately prior to injection a small drop (<0.2 ml) of Decon 90® detergent was added to the water in each glass block to prevent eggs sticking together in clumps or sticking to the inside of pipette tips.

#### **Water injection procedure**

Plants to be tested were grown in pots within the glasshouse under normal summer temperatures and provided with adequate water and fertilizer. On each day of water injection an adjustable 0.1-10 µl micropipette (fitted with a standard 2.5 µl plastic tip- distal aperture of 0.5 mm) was used to draw up aqueous suspensions of eggs with the aid of a stereomicroscope. Eggs suspended in water were dispelled at volumes of 0.1-1 µl between the glumes of individual flowering sorghum spikelets pried open by fine forceps.

#### **Water injection technique versus natural cage method**

The above water injection procedure was used at two different densities of egg infestation in two trials on three replications of the same five glasshouse-grown sorghum lines to determine the efficiency and accuracy of the water injection

procedure. The two water injection trial results were further tested against the natural egg lay of a glasshouse cage trial on the same five lines.

### **Water injection trial**

Three plants of each line were selected at flowering and 1-2 rachis of flowering spikelets on each panicle were trimmed to 30 spikelets for injection of exactly two eggs per spikelet. Spikelets from each panicle were sampled one day after injection and stored frozen for later dissection under a stereomicroscope to obtain data on the number and position of eggs. This procedure was repeated on the same five lines at an injection density of 4-6 eggs per spikelet within 20 flowering spikelets of each panicle.

### **Standard cage trial: natural egg lay**

The glasshouse cage trial method described in Chapter 2 was used to obtain natural oviposition of eggs across the same five sorghum lines mentioned above. Glasshouse grown plants were selected for trial over 11 dates in which individual panicles were trimmed to contain 180-200 flowering spikelets, enclosed with wire cages covered with white nylon gauze bags. Based on Chapter 2 ovipositional-antixenosis results, each cage of each line was infested with different numbers of mated females at 9-10 am as follows: QL20 – 20; PM7017 – 30; QL39 – 40; SC62C – 60, and MB110 – 60. Each line was replicated five times. Twenty sessile spikelets were randomly sampled from each panicle one day after trial set up. Samples were stored, dissected, and results recorded as above.

### **Water storage bioassay of midge eggs**

To assess the effect of water storing eggs on egg hatch and subsequent neonate fitness, a laboratory assay was conducted. Eggs were stored in water (in the absence of any detergent) over a range of a few hours at 25°C, or for longer periods of refrigerated storage at 4°C. Approximately 200 four-hour-old eggs were placed in water in excavated glass blocks as previously described, and refrigerated 21, 14, 7, 6, 5, 4, 3, 2 or 1 day(s) before trial commencement. A final number of 8-10 eggs were

pipetted onto the centre of a 9cm moistened filter paper within each petri dish, after obviously mechanically damaged eggs were removed. Ten replications of each treatment were held at  $25\pm 1^{\circ}\text{C}$  under a 12h fluorescent light:12h dark regime in a constant temperature room. Hourly observations of number of eggs hatched along with a record of the position of each neonate took place until all neonates were recorded as moribund or dead. Neonates were deemed to be moribund or dead after not moving following gentle probing with forceps. Neonate position was assessed using a template with concentric circles 1 cm in diameter. All water injection trial and bioassay data were firstly screened for normality, and homogeneity of variances using Bartlett's test, and then analysed using GENSTAT 4.1 software and two-way ANOVA with least significant differences at a 5% probability level.

## **Results**

Preliminary work on the water injection technique, indicated water volumes of 0.1-1  $\mu\text{l}$  were optimum as they avoided egg loss due to overfilling of spikelets, but remained adequate for simple egg retrieval and injection using the micro-pipette. Early trial work also revealed that the water within spikelets rapidly evaporated once plants were placed in the glasshouse, and that the physical disturbance of glumes due to this technique during flowering did not reduce ovary fertilisation and seed set.

### **Water injection technique versus natural cage method**

Three trials were conducted on five sorghum lines. The two water injection trials deposited either two eggs per spikelet, or 4-6 eggs per spikelet, while in the third natural cage trial adult midge densities were adjusted in an attempt to obtain even egg lay across spikelets of each line. Three measures of egg infestation (eggs per spikelet, percentage spikelets infested, and relative egg position) were recorded.

### **Eggs per spikelet**

Both water injection methods produced consistent levels of eggs per spikelet across all lines (Figure 3.3a) regardless of their midge resistance status. Water injection of 4-6 eggs per spikelet produced the same number of eggs per spikelet ( $p\leq 0.05$ ) across

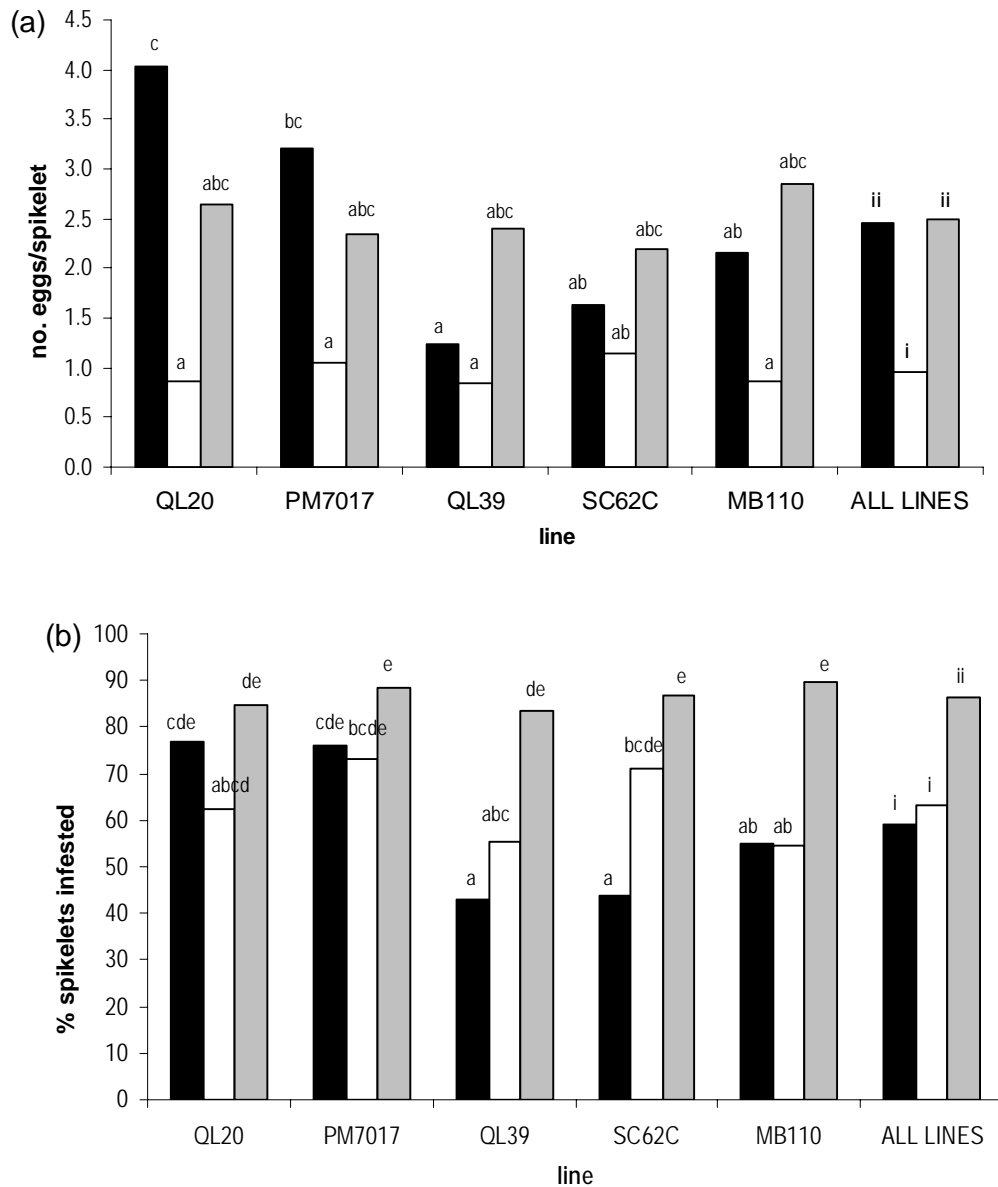
all lines as the natural cage method (2.5 eggs per spikelet). In contrast, under natural egg lay there were consistent differences between host lines.

Both midge susceptible lines QL20 and PM7017 produced higher egg numbers ( $>3.2$  eggs/spikelet) than the antixenotic line QL39 (1.2 eggs/spikelet), while QL20 produced higher egg lay under natural infestation (4.0 eggs/spikelet) than all five lines ( $p \leq 0.05$ ) under water injection of two eggs per spikelet (0.8-1.2 eggs/spikelet)

### **Percentage of spikelets infested**

Inconsistent percentage infestation occurred across lines under the cage technique (Figure 3.3b). The two lines most susceptible to midge egg lay (QL20 and PM7017) contained higher infestations ( $p \leq 0.05$ ) of approximately 75% than the three ovipositional-antixenotic lines (QL39, SC62C and MB110 at less than 60%). Within each density of water injection, there were no differences in percentage egg infestation across lines.

Overall, the water injection of 4-6 eggs per spikelet produced a higher infestation ( $p \leq 0.05$ ) of 88% than either the two eggs per spikelet water injection density (63%), or the natural cage method (60%).



**Figure 4.1** Densities of midge eggs in five sorghum lines one day after egg insertion using three methods: natural caging of mated female midge adults (black); water injection, two eggs per spikelet (white); water injection, 4-6 eggs per spikelet (grey). Columns that have the same letter do not differ significantly from one another ( $p \geq 0.05$ ).

### Relative egg position within the spikelet

Egg lay at three positions within the spikelets of each line (Figure 4.2) illustrated that across lines under natural egg lay there were few eggs oviposited on the inside of

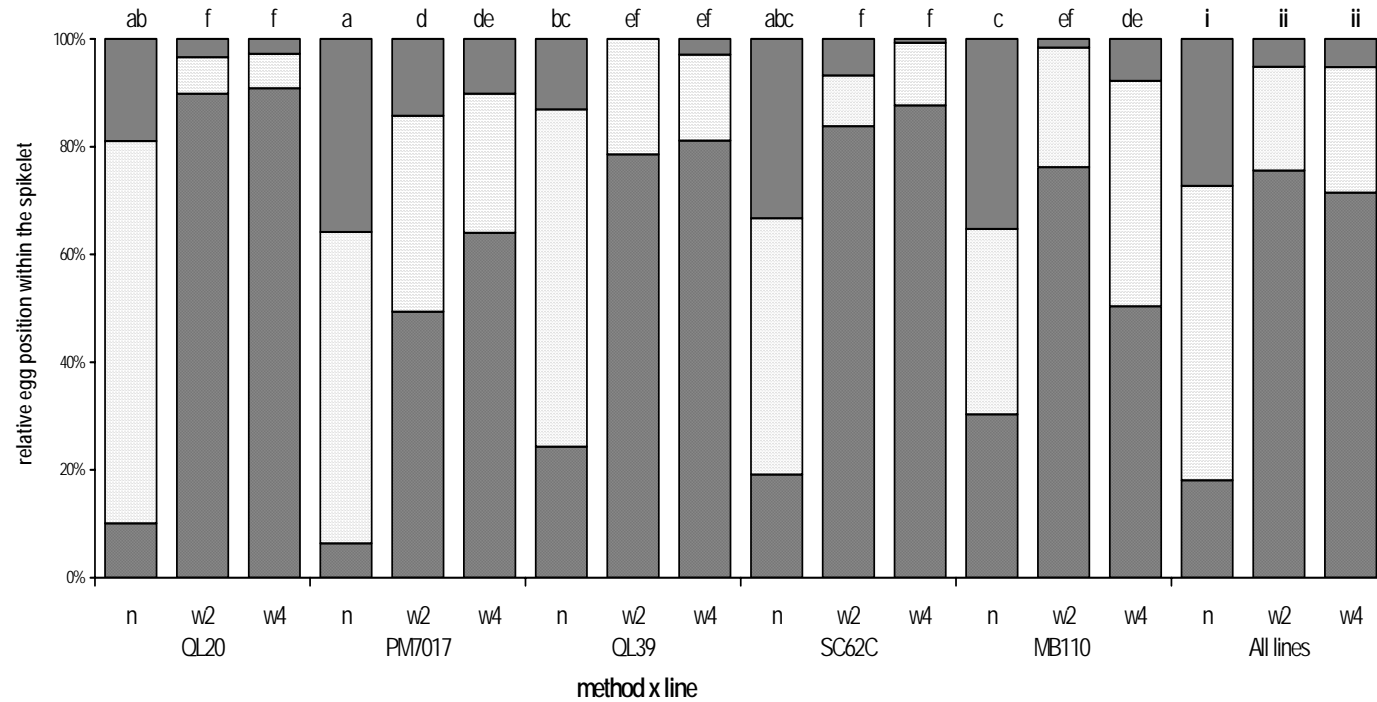
glumes, while most eggs were oviposited on the palea/lemma (50%) or against the ovary (30%). In contrast the dominant egg position across all lines within the spikelet under both water injection methods was against the inside of glumes (70%), significantly higher ( $p < 0.05$ ) than that present under natural egg lay (20%).

Some interactions with genotype occurred within water injection methods at both egg densities. At two eggs per spikelet PM7017 showed fewer eggs (50%) on the inside of glumes than QL20 and SC62C (>80%), and at 4-6 eggs per spikelet PM7017 and MB110 (<70%) also showed fewer eggs on the inside of glumes than QL20 and SC62C (>85%).

### **Water storage bioassay of midge eggs**

A laboratory bioassay was conducted to determine the viability of eggs stored in water. Freshly laid eggs stored in water at room temperature (25°C) for four hours were compared to similar eggs stored in water at 4°C for periods of 1-7 days, 14 days, and 21 days. Measures of egg hatch, neonate survival and movement were used to determine the viability of each storage period.





**Figure 4.2** Relative egg position within the spikelet [glumes (black); palea/lemma (light); ovary (grey)] of five lines using three methods of egg insertion [n – natural caging of mated female midge adults ; w2 – water injection, two eggs per spikelet; w4 – water injection, 4-6 eggs per spikelet]. Statistical analysis was performed on one position of egg lay only (glumes). Columns that have the same letter do not differ significantly from one another on percentage of eggs at the glumes ( $p > 0.05$ ).

## Neonate movement

Larvae were found to move many times their body lengths (<1 mm) from the centre of each petri dish after egg hatch (Table 4.1) across all treatments. The mean distance movements of 0.4-1.2 cm per neonate assume straight-line travel by neonates from the centre of each petri dish directly outward and inward between each 1 cm circle radius. In contrast greatest distance travelled data were recorded in each petri dish as the furthest circle reached by any one neonate, with the maximum of 4.5 cm being the edge of the petri dish. This measure assumes only one way outward travel and as a result the maximum range recorded in days five and seven of Table 4.1 was higher under mean distance than the equivalent greatest distance travelled results.

Consequently all movement data makes estimates of straight-line movements only within defined boundaries. Even so there were significant differences in the maximum distance travelled between treatments ( $p < 0.05$ ), highest at 4.5 cm (the edge of the petri dish) in the day zero treatment, decreasing to 2.5 cm between day 1-4, and further decreasing to a low of 1.5 cm at days 5-7. Mean distance results were not different between treatments, with average distances of 0.4-1.2 cm travelled by each neonate.

**Table 4.1** Survival, development and fitness parameters of midge neonates after eggs were stored for different periods in water at 4°C (Conditions of 25°C, 75% humidity were maintained after storage).

parameter	storage time (days)							
	0	1	2	3	4	5	6	7
<b>survival and development</b>								
% egg hatch	79 a*	57 bc	44 cd	56 bc	52 bcd	64 b	41 d	54 bcd
(range)	(70-100)	(38-75)	(28-67)	(30-78)	(20-70)	(44-100)	(13-75)	(38-67)
time to hatch (hr)	24 a	24 a	23 a	23 a	20 b	19 b	17 c	16 c
(range)	(23-27)	(22-29)	(20-25)	(20-25)	(18-21)	(15-21)	(16-20)	(15-21)
longevity (hr) <sup>^</sup>	29 a	27 ab	26bc	24 c	27 ab	26 bc	27 ab	26 bc
(range)	(25-32)	(22-30)	(22-28)	(22-27)	(23-31)	(22-31)	(25-28)	(21-29)
maximum longevity (hr) <sup>^^</sup>	36 a	30 bc	29 c	32 b	30 bc	32 b	29 bc	31 bc
(range)	(29-42)	(28-32)	(25-32)	(25-35)	(23-35)	(29-35)	(27-35)	(26-34)
<b>movement</b>								
maximum distance travelled (cm) <sup>†</sup>	2.7 a	1.9 b	1.6 bc	1.6 bc	2.0 b	1.4 cd	1.1 d	1.3 cd
(range)	(1.5-4.5)	(1.5-2.5)	(0.5-2.5)	(1.5-2.5)	(1.5-2.5)	(0.5-1.5)	(0.5-1.5)	(0.5-1.5)

\* Means in the same row followed by the same letter are not different at  $p < 0.05$  from a total of 10 replications (petri dishes).

<sup>^</sup> Average lifespan of each neonate from egg hatch to neonate classified as moribund.

<sup>^^</sup> Lifespan of individual neonate to survive the longest in each petri dish.

<sup>†</sup> Maximum distance travelled by any neonate from the centre of each petri dish.

## Discussion

In contrast to natural egg lay, water injection of eggs produced consistent levels of egg deposition across lines with varying levels of ovipositional-antixenosis. Consistent averages of one and 2.5 eggs per spikelet, and 63% and 88% infestation were achieved under injections of two eggs per spikelet, and 4-6 eggs per spikelet respectively (Figure 4.1). This was in spite of egg losses of 30-60% at both densities of water injection, principally due to eggs overflowing outside the spikelets. In contrast, under natural egg lay the two midge-susceptible lines produced 3-4 eggs per spikelet, and approximately 75% infestation in comparison to less than 2.5 eggs per spikelet and 60% infestation of the three lines with higher levels of ovipositional-antixenosis. These differences occurred even when midge densities were adjusted in an attempt to overcome this variation.

The water injection technique does have one potential shortcoming, since the eggs in water-injected spikelets are distributed differently within the spikelet compared to eggs from natural egg lay. The only water injection results of concern are those of egg lay position, where water injected spikelets results contrast with those under natural egg lay. Under natural egg lay the dominant egg lay position within the spikelet was either side of the lemmas and against the ovary. This result confirms that recorded in Chapter 3; Table 3.2 and is similar to that recorded by Waquil *et al.* (1986c) in other sorghum lines, who observed that the majority of eggs were positioned either side of the two lemmas (palea/lemma), and against the ovary. There were subtle differences in relative egg position across lines under water injection, possibly arising from differences in spikelet size and shape. QL20 contains longer, larger glumes than those of the four midge resistant lines, which may explain the highest rate of egg deposition against the glumes in this line. However, despite these subtle differences across lines, water injection deposits most eggs against the inside of the outer glume in contrast to natural egg lay. This is a direct result of the technique, since eggs were injected towards the inside concave surface of the large outer glume. While larvae feeding on the ovary are larger (Waquil *et al.* 1986c) and develop faster (Franzmann, 1993) than those that feed at other positions, most neonates move rapidly towards the ovary to feed irrespective of

egg position, well before the end of the larval period, subsequently causing the seed to abort (Dean 1911; Walter, 1941). Due to this mobility, the altered distribution of eggs resulting from water injection in comparison to natural egg lay is likely to have only a minor effect on larval size and development. Furthermore in antibiosis related studies of midge biology there may be advantage in the consistent egg location produced across lines under water injection where the aim is to determine differences in biology under similar feeding positions within the spikelet.

Bioassay results support the use of water injected eggs stored in water for short periods of four hours or less. When rates of 79% egg hatch (Table 4.1) are coupled with the use of water injection at a density of 4-6 eggs per spikelet, high and consistent infestation levels of neonates result, ideal for antibiosis studies of midge biology. While no published reports of midge egg hatch rates are known, the range of egg hatch in our freshly laid eggs was 70-100%, indicating that very high hatch rates were possible. At a density of 4-6 eggs per spikelet and maximum rates of 30% egg mortality (from above), neonate emergence within each spikelet would reduce from an average of 2.5 to 1.8 (Figure 4.1a). However this reduction is unlikely to have a significant effect on the percentage of spikelets infested. The resulting larval density is ideal for conducting antibiosis studies on post-embryonic development as it eliminates significant larval mortality induced by larval competition, reported by Franzmann *et al.* (1989) at a competition threshold of 2.3 larvae per spikelet. This is good reason for not extending water injection egg densities above 4-6 eggs per spikelet when using the technique to conduct antibiosis larval studies, unlike Rossetto *et al.* (1984) and Sharma *et al.* (1993) who manually transferred 15-25 eggs between the glumes of individual spikelets.

In contrast to the above bioassay results on freshly laid eggs, the egg hatch and fitness of neonates are increasingly compromised on eggs stored at 4°C for 1-7 days, while longer periods of storage result in total loss of viability. At the same time, the eggs underwent significant development during storage at 4°C. Time to hatch decreased from 24 hours to 16 hours, following storage for seven days, representing a 33% decrease in time to hatch under favourable conditions. While the average lifespan (24-29 hours) and movement of larvae (0.4-1.2 cm) were the same on 1-7

day refrigerated treatments, the rate of hatch was lower (44-64%) compared with eggs stored four hours in water at room temperature (79%). This, together with the reductions in both maximum longevity (29-32 hours versus 36 hours) and maximum movement (1.1-2 cm versus 2.7 cm) indicates that the use of un-refrigerated, freshly laid eggs under water injection will produce the highest yield of healthy neonates.

Midge neonates were capable of moving large distances in relation to the size of the sorghum spikelets which they naturally infest. Average distances of 1.2 cm and maximum distances of 4.5 cm were recorded, while neonates were capable of surviving in a moist environment without food for 29 hours. These results suggest the theoretical possibility of extensive midge larval movement not only within spikelets, but from one spikelet to another. Whether this actually occurs requires further investigation since sorghum spikelets provide a protected, sheltered feeding site, conditions widely different from those on the surface of a petri dish.

## **Summary and Conclusions**

The new water injection technique is an ideal method for establishing precise even egg densities within all sorghum lines for further studies of immature development. This method overcomes previously recorded problems with inconsistent egg lay that occur in antixenotic midge resistant genotypes that may subsequently lead to differential larval competition and growth between lines. The technique will enable precise studies of midge antibiosis and tolerance in all midge resistant sorghum genotypes that were previously not possible under natural methods of midge infestation. The use of this new method to further study larval antibiosis and tolerance is described in the following two chapters of this thesis.

## CHAPTER 5

# Using the Water Injection Technique to Study Larval Antibiosis

### Introduction

The water injection technique described in Chapter 4 is a new method that artificially delivers precise egg densities within spikelets of all sorghum genotypes, enabling accurate studies of midge biology. When using this technique, a majority of eggs are delivered to the same location within the spikelet, allowing an equivalent study of neonate movement and feeding over time.

I chose to take advantage of this new technique to conduct an additional study of larval antibiosis on a similar range of lines studied in Chapter 3. In order to fine tune results, an earlier sampling period was chosen to more accurately determine differences between lines at the critical stage of larval establishment and feeding over the first half of the larval period. This chapter describes this study.

### Methods

A similar glasshouse trial to that recorded in Chapter 3 was established on six midge resistant lines, (ICSV745, IS10759, MB110, PM7017, QL39, SC62C) and two susceptible control lines (QL12, QL20). The artificial water injection method of infesting sorghum spikelets with midge eggs (described in Chapter 4) was applied successfully across all midge resistant genotypes. While the procedure again proved to be accurate, it had one disadvantage over the glasshouse cage technique described in Chapter 2 and 3. Water injection takes increased time and effort to produce a similar number of infested spikelets. As a result only eighty flowering spikelets on each panicle were infested. An aqueous solution of 4-6 zero-day-old eggs was injected into individual spikelets on a total of three panicles (replications) of each line. Twenty spikelet samples were taken over four sample dates, designed to

coincide with the periods of egg eclosion and early larval movement and growth (aligned from Chapter 3 results).

day one	day three	day six	day nine
egg	first instar neonates	second instar larvae	third instar larvae
	(≤one day old)	(four days old)	(seven days old)

Exactly the same measures of midge infestation, larval size, larval position within the spikelet and caryopsis abortion were made as described in Chapter 3. In addition differences across all measures were made using the same set of statistical analyses. As such all data from this study may be directly compared to that in Chapter 3 results for differences across trials using different methods of midge infestation.

## **Results**

### **Midge infestation and mortality over the larval period**

At each sample date two measures of midge density were recorded, the number of midge per spikelet, and the percentage of spikelets infested.

#### **Eggs per spikelet**

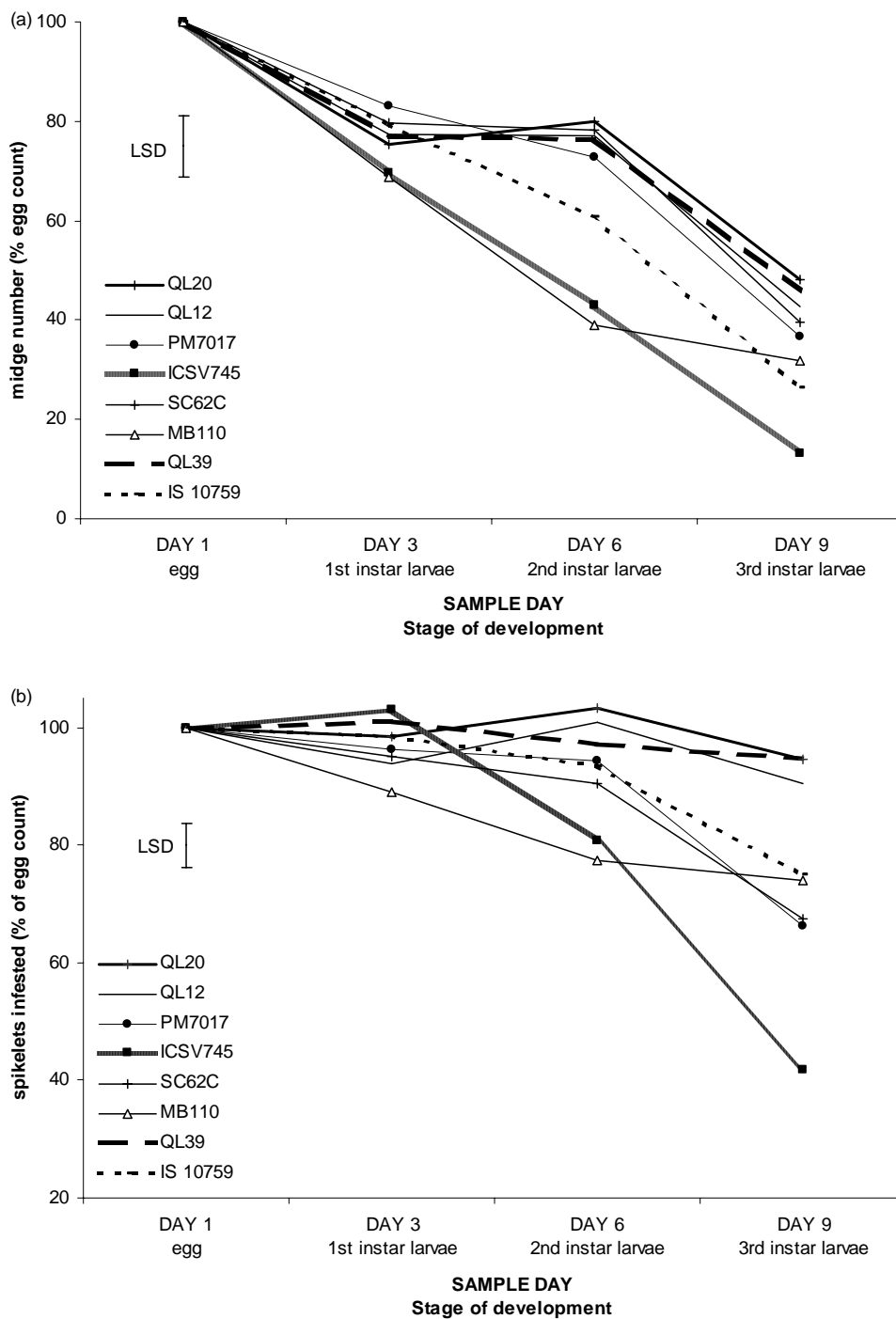
After all spikelets in each line were water-injected with 4-6 eggs, an average of 2.7 eggs per spikelet was recovered across the eight lines, the highest recorded in ICSV745 (3.4 eggs/spikelet), and lowest in SC62C (2.2 eggs/spikelet) (Table 5.1).



**Table 5.1** Midge egg and larval density on two measures of midge infestation in the sessile spikelets of eight sorghum lines after water injection of 4-6 eggs per spikelet. Differences between lines on each day (\*) and between all lines across days (#) were recorded by ANOVA at  $p < 0.05$ .

<b>midge density</b>	<b>line</b>	day 1	day 3	day 6	day 9
		(egg)	(1st instar larvae)	(2nd instar larvae)	(3rd instar larvae)
		mean	mean	mean	mean
<b>(a) eggs per spikelet</b>	QL20	2.7 b*	2.0 b	2.1 d	1.2 c
	QL12	2.8 b	2.0 b	2.1 d	1.1 c
	PM7017	2.4 a	2.1 b	1.8 c	0.8 b
	ICSV745	3.4 c	2.4 c	1.5 b	0.5 a
	SC62C	2.2 a	2.2 b	1.7 bc	0.8 b
	MB110	2.8 b	1.8 a	1.0 a	0.9 bc
	QL39	2.4 a	2.2 b	1.9 cd	1 bc
	IS 10759	3.2 c	2.5 c	1.9 cd	0.9 bc
LSD = 0.24*	all lines	2.7 d	2.15 c	1.7 b	0.9 a
<b>(b) % spikelets infested</b>	QL20	85 a	83 a	87 a	80 a
	QL12	90 a	83 a	85 a	80 a
	PM7017	88 a	85 a	83 a	59 a
	ICSV745	92 a	93 a	75 a	38 a
	SC62C	87 a	82 a	78 a	58 a
	MB110	90 a	80 a	70 a	67 a
	QL39	83 a	84 a	82 a	78 a
	IS 10759	92 a	90 a	85 a	68 a
LSD = 8*	all lines	88 c	85 c	80 b	66 a

Means in columns (\*) or rows (#) followed by the same letter are not significantly different ( $p < 0.05$ ).



**Figure 5.1** Midge survival over time as a percentage of the original egg infestation in eight lines after water injection of 4-6 eggs in each spikelet.

(a) Midge number/spikelet

(b) Percentage of spikelets infested

For both (a) and (b) above LSD between lines at each day shown.

When these small but significant ( $p < 0.05$ ) differences in eggs per spikelet were recorded as a percentage of the original egg eggs per spikelet in each line, differences were observed between lines at each day (line  $p < 0.01$ ), between days across all lines (day  $p < 0.01$ ), while the pattern of mortality between lines across all days was significantly different (line.day  $p < 0.01$ ).

A similar level of mortality of approximately 20% was observed between egg injection and day three across all lines (Figure 5.1a). However between days 3-6 significant differences in mortality were recorded between lines. During this period of early larval feeding when larvae developed from first to second instars, the two susceptible controls (QL12 and QL20), and the resistant lines PM7017, QL39, and SC62C recorded no larval mortality, while larval mortality of approximately 20-30% was observed in IS10759, ICSV745 and MB110. Between 6-9 days, during the second half of the larval period (when larvae developed from second to third instars), significant mortality ( $p < 0.05$ ) of approximately 40% was observed across all lines except MB110 which recorded an insignificant drop in larval numbers.

Overall, a similar pattern of mortality was recorded in the two susceptible controls and the three resistant lines SC62C, QL39 and PM7017. In these lines mortality of approximately 20% occurred between egg lay and day three, while no mortality occurred between days 3-6, and final mortality of 20-30% occurred between days 6-9. The resistant lines ICSV745 and IS10759 both showed a continuous pattern of larval mortality between egg lay and day nine, while MB110 produced a unique pattern of mortality, highest between egg lay and day six and lowest between days 6-9.

### **Percentage of spikelets infested with eggs**

There were no significant differences ( $p < 0.05$ ) between lines in the percentage of spikelets infested with eggs after water injection, all lines averaging a high egg infestation of 88% (Table 5.1). In addition no significant differences ( $p = 0.07$ ) were observed between lines across all days. However when data were recorded as a percentage of the original egg infestation (Figure 5.1b), differences were observed

between lines at each day (line  $p < 0.01$ ), and between days (day  $p < 0.01$ ), and the pattern of mortality was different between lines (line.day  $p < 0.01$ ). Between egg lay and day three, only MB110 recorded a significant ( $p < 0.05$ ) 10% decrease in the percentage of spikelets infested. Between days 3-6 during the first half of the larval period, the two susceptible controls (QL12 and QL20) and the resistant lines QL39, SC62C, IS10759, and PM7017 showed no decrease in infestation. However during this period a significant ( $p < 0.05$ ) reduction in the percentage of spikelets infested of between 10%, and 20% was observed in MB110 and ICSV745 respectively. Between days 6-9 during the second half of the larval period, no decrease in infestation was observed in both susceptible controls (QL12 and QL20) or in the resistant lines QL39 and MB110. However mortality was recorded over this period in all other resistant lines, lowest at 20% in the three resistant lines IS10759, PM7017, and SC62C, and highest at 40% in ICSV745. Overall, the pattern of spikelets infested with midge was similar in QL12, QL20 and QL39 which showed no significant mortality from days 1-9, while this differed in the three resistant lines PM7017, IS10759, and SC62C which showed a reduction in larval infestation between days 6-9. Both ICSV745, and MB110 showed unique patterns of midge infestation. In MB110 the percentage of spikelets infested reduced greatly between egg lay and day six during the first half of the larval period, while the percentage of spikelets infected levelled out (in contrast to all other lines tested) between days 6-9. In ICSV745 a constant pattern of reducing percentage of spikelets infested was recorded over much of the larval period was recorded between days 3-9.

### **Midge immature feeding position and size**

With the water injection technique, the dominant egg position recorded within the spikelets of all lines was on the inside of either glume or against the outside of the palea/lemma (position two), accounting for 93% of all eggs recorded at all three positions (Table 5.2). The remaining 7% of eggs were recorded against the caryopsis (position 3) and on the outside of either glume (position one). Under this consistent egg deposition pattern, larval movement towards the caryopsis was similar between lines. From egg insertion to day three when larvae were only one-day old first instars, the net movement of midge towards the ovary in all lines averaged 37% (41%-4%), entirely accounted for by a significant net movement of midge away from

the inside of the glumes or against the outside of the palea/lemma (93%-56%). Movement of larvae towards the caryopsis increased by a further 31% (72%-41%) across all lines between days 3-6 as larvae developed to second instars, and again by 28% between days 6-9 when an average of 98% of third instar larvae were feeding against the caryopsis. No significant differences ( $p=0.01$ ) were recorded between lines on the small number of larvae present outside the spikelet (0-6%) at days 3-6. By day nine no larvae were recorded outside the spikelets of all lines.

**Table 5.2** Movement of midge within the sessile spikelets of eight sorghum lines over the larval period after water injection of eggs. Figures for each line are a percentage of the total midge present at all three positions on that sampling day.

feeding position	line	sample day (midge developmental stage)			
		day 1 (egg)	day 3 (1st instar larvae)	day 6 (2nd instar larvae)	day 9 (3rd instar larvae)
1. outside spikelet	QL20	1	3	1	
	QL12	0 *	1	0	
	PM7017	5	2	1	
	ICSV745	5	4	1	
	SC62C	2	1	3	
	MB110	6	6	3	
	QL39	2	6	0	
	IS 10759	5	2	0	
LSD = 1.5#	all lines#	3 a	3 a	1 b	
2. inside spikelet glume/palea/lemmas	QL20	96	59	25	0
	QL12	98	62	17	0
	PM7017	85	59	33	0
	ICSV745	91	43	27	0
	SC62C	97	59	31	0
	MB110	89	52	22	3
	QL39	95	58	36	3
	IS 10759	91	57	28	6
LSD = 5.5#	all lines	93 a	56 b	27 c	2 d
3. inside spikelet caryopsis	QL20	3	39	75	100
	QL12	1	37	83	100
	PM7017	10	39	67	100
	ICSV745	3	53	73	100
	SC62C	1	40	66	100
	MB110	5	42	75	97
	QL39	3	36	64	97
	IS 10759	4	41	72	94
LSD = 5.4#	all lines	4 d	41 c	72 b	98 a

Means in columns (\*) or rows (#) at each position (1,2 and 3) followed by the same letter are not sign. different ( $p<0.05$ ).

Significant differences ( $p < 0.05$ ) in larval size were recorded by feeding position and between lines at each feeding position (Table 5.3).

At day three, when larvae were approximately one day old, the average larval size across all lines at all positions was similar at all three positions within the spikelet with few exceptions between lines at each position. However by day six differences in larval size across feeding position became evident. Generally, larvae feeding away from the caryopsis (positions one and two) were similar in size regardless of line, but significantly ( $p < 0.05$ ) smaller than larvae feeding against the caryopsis. Even so, some significant differences in larval size were recorded between lines at each feeding position ( $p < 0.05$ ). Larvae against the caryopsis on day six were smallest in ICSV745 (22) and largest in MB110 (41), while all other lines produced larvae of similar size (28-30). Also on day six, QL39 both recorded the largest larvae of all lines outside the spikelet and the smallest larvae of all lines inside the spikelet at position two away from the caryopsis.

Between days 6-9 larvae underwent their greatest increase in size (approximately three to six fold increase in most cases and up to nine times in some lines). This coincided with larval development between second and third instars and the movement of 98% of all surviving larvae to feed against the caryopsis. At this position by day nine, the size of the larvae in the two resistant lines QL39 (113) and MB110 (195) were smaller ( $p < 0.05$ ) than those reared on QL20 and QL12 (274 and 279). All other lines were similar in size to the two susceptible controls. Larvae feeding inside the spikelet away from the caryopsis were recorded in a very small percentage of spikelets in three lines.

When differences in larval size are viewed as averages at all feeding positions significant differences ( $p < 0.05$ ) between lines were recorded at days six and nine. At day six, ICSV745 recorded the smallest larvae (21), and MB110 the largest (38). However by day nine, ICSV745 recorded similar size larvae to the susceptible lines QL12 and QL20, while MB110 and QL39 recorded larvae approximately two-thirds to half the size respectively of QL12 and QL20.

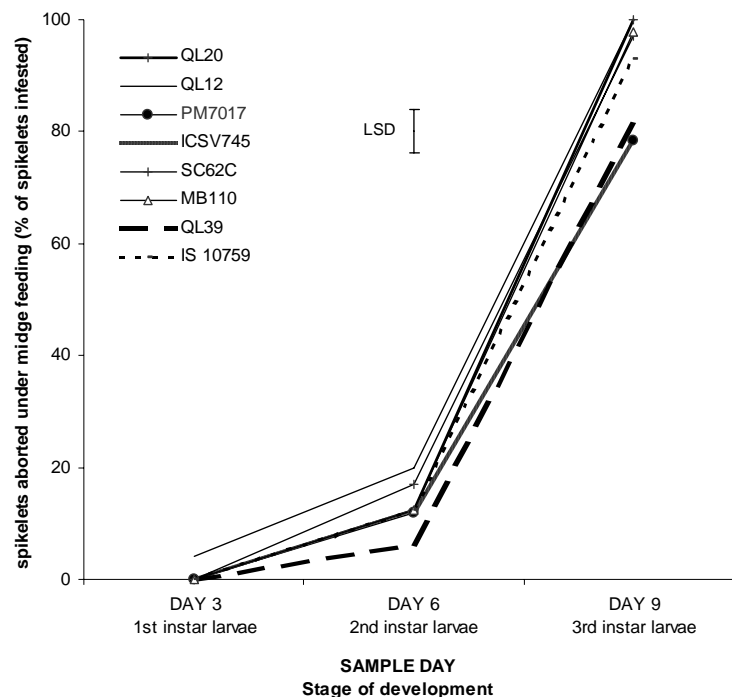
**Table 5.3** Larval size (ocular units; 500 units = 1 mm) of midge at three positions within the spikelet of seven sorghum lines over seven days of larval growth. Log (lg) and back transformed equivalent (eq. m) means are shown after differences between lines on each day (\*) and between days for all lines (#) are recorded under REML analysis at  $p < 0.05$ .

feeding position	line	day 3 (1st instar larvae)		day 6 (2nd instar larvae)		day 9 (3rd instar larvae)		
		eq. mean	lg	eq. mean	lg	eq. mean	lg	
1. outside spikelet	QL20	12	2.6 b	15	2.8 a			
	QL12	6	1.9 a*	15	2.8 a			
	LSD (log) = 0.57*	PM7017	13	2.7 b	13	2.6 a		
	ICSV745	11	2.5 b	13	2.6 a			
	SC62C	15	2.8 b	13	2.6 a			
	MB110	9	2.3 ab	12	2.6 a			
	QL39	13	2.6 b	61	4.1 b			
	IS 10759	13	2.7 b					
LSD (log) = 0.58#	all lines#	13	2.6 a	14	2.7 a			
2. inside spikelet glume/palea/lemmas	QL20	13	2.6 ab	19	3.0 b			
	QL12	13	2.6 ab	15	2.8 ab			
	PM7017	15	2.8 b	16	2.9 ab			
	LSD (log) = 0.21*	ICSV745	14	2.7 ab	16	2.8 ab		
	SC62C	13	2.7 ab	17	2.9 ab			
	MB110	11	2.5 a	18	2.9 ab	63	4.1 b	
	QL39	14	2.7 ab	15	2.7 a	37	3.6 a	
	IS 10759	13	2.7 ab	17	2.9 ab	36	3.6 a	
LSD (log) = 0.13#	all lines	13	2.7 a	17	2.9 b	45	3.8 c	
3. inside spikelet caryopsis	QL20	15	2.8 a	28	3.4 b	274	5.6 c	
	QL12	14	2.7 a	30	3.4 b	279	5.6 c	
	PM7017	15	2.8 a	30	3.4 b	235	5.5 bc	
	LSD (log) = 0.28*	ICSV745	14	2.7 a	22	3.1 a	262	5.6 c
	MB110	16	2.8 a	41	3.7 c	195	5.3 b	
	SC62C	15	2.8 a	30	3.4 b	264	5.6 c	
	QL39	15	2.8 a	28	3.4 b	113	4.7 a	
	IS 10759	16	2.8 a	29	3.4 b	262	5.6 c	
LSD (log) = 0.28#	all lines	15	2.8 a	29	3.4 b	228	5.4 c	
all positions	QL20	14	2.7 a	27	3.3 ab	260	5.6 c	
	QL12	13	2.7 a	30	3.4 b	265	5.6 c	
	LSD (log) = 0.26*	PM7017	15	2.8 a	28	3.4 b	221	5.4 bc
	ICSV745	13	2.7 a	21	3.1 a	247	5.5 c	
	MB110	14	2.7 a	38	3.7 c	182	5.2 b	
	SC62C	14	2.7 a	27	3.3 ab	250	5.5 c	
	QL39	15	2.7 a	26	3.3 ab	104	4.7 a	
	IS 10759	15	2.8 a	28	3.4 b	241	5.5 c	
LSD (log) = 0.25#	all lines	14	2.7 a	28	3.4 b	213	5.4 c	

Means in columns (\*) or rows (#) at each position (1,2,3 and 4) followed by the same letter are not significantly different ( $p < 0.05$ ).

## Midge damage and larval antibiosis

Across all lines, a similar pattern of spikelet abortion was observed over time (Figure 5.2). Spikelets infested with midge did not produce significant ( $p < 0.05$ ) abortion of the caryopsis until day six, when 5-20% of caryopses were recorded aborted. This increased significantly ( $p < 0.05$ ) by day nine across all lines to 80-100%. Even under this similar pattern of caryopsis abortion, significant differences ( $p = 0.05$ ) were recorded between lines at days six and nine. At day six, the two susceptible controls QL12 and QL20 recorded the highest rate of 10-20% abortion of caryopses within infested spikelets, while a low of 5% was recorded in QL39. By day nine, both susceptible controls recorded 100% caryopsis abortion within infested spikelets, while this rate was similar in the resistant lines MB110, SC62C, and IS10759 (90-95%), and significantly ( $p = 0.05$ ) lower in the lines QL39, ICSV745, and PM7017 (approximately 80%).



**Figure 5.2** Percentage of infested spikelets aborted due to midge feeding over the first three-quarters of the larval period (seven out of an estimated average of ten days larval development). Differences between lines at each day indicated by LSD bar ( $p = 0.05$ ).



There were significant ( $p < 0.01$ ) differences in the amount of antibiosis recorded in each line by day nine, when most larvae were close to full size third instar larvae (Table 5.4). The line QL39 produced the lowest level of antibiosis of all resistant lines, similar to the 5-10% present in the two susceptible controls. The resistant lines SC62C, MB110, PM7017, and IS10759 produced moderate levels of larval mortality between 25-34%, while ICSV745 produced the highest level of larval mortality (58%).

**Table 5.4** Summary of midge infestation, antibiosis and seed set within eight sorghum lines after spikelets were water injected with eggs at flowering and sampled nine days later.

<b>line</b>	<b>% egg</b>	<b>% larval</b>	<b>% antibiosis+</b>	<b>% seed set</b>
QL20	85 a	80 d	5 a	20 a
QL12	90 a	80 d	10 a	20 a
PM7017	88 a	59 b	34 b	48 c
ICSV745	92 a	38 a	58 c	65 d
SC62C	87 a	58 b	33 b	38 bc
MB110	90 a	67 b	26 b	32 ab
QL39	83 a	78 cd	5 a	33 bc
IS 10759	92 a	68 bc	25 b	34 bc

Means in columns (\*) followed by the same letter are not significantly different ( $p < 0.05$ ).

+ Percent difference between egg and pupal egg infestation as a percentage of egg count ( $(\text{egg} - \text{larval}) / \text{egg} * 100$ ).

## Discussion

The water injection method overcame the problems of uneven egg density and different neonate hatch positions that confounded results of a similar study of larval biology in Chapter 3. A more accurate view of the effects of antibiosis within genotype over the critical period of larval feeding and caryopsis abortion were recorded and may be compared and contrasted to those recorded in Chapter 3.

## **Midge survival and development**

### **Egg eclosion rates**

In Chapter 4, bioassay results showed that 21% of eggs were infertile. This figure aligns with the results obtained in this study (Figure 5.1a). Consequently, the consistent minimum early mortality observed across all lines of approximately 20% is likely the result of infertile or unhatched eggs.

### **Larval size and movement**

Even though 20% of initial egg infestation was lost due to infertility, an ideal starting density of approximately two larvae per spikelet was established across all lines. Under this ideal initial starting population some evidence for antibiosis-related reduced larval size was recorded in the highly antibiotic line ICSV745 commencing at the onset of larval feeding against the caryopsis (Table 5.3). At day three, ICSV745 contained the highest percentage of midge present against the caryopsis, and then recorded both the greatest larval mortality of all lines between days 3-6 coinciding with the smallest larvae of all lines against the caryopsis at day six. Clearly this line contains antibiosis components related directly to the developing caryopsis. In contrast the line MB110 recorded the opposite affect early on in the larval period. Larvae were larger than average at day six coinciding with the highest larval mortality in any line up to this period, while larval size reduced relative to all other lines tested by day nine. As such these two lines have differing antibiosis components.

QL39 again recorded some evidence for a reduced rate of larval growth by day nine, consistent with the results from Chapter 3. However under a higher midge egg infestation at trial commencement this antibiotic affect did not cause any significant increase in larval mortality.

In contrast to Chapter 3 results, the pattern of larval movement away from the ovary in MB110 and other resistant lines in Chapter 3 was not repeated in this trial. One difference between the study conducted using water injection versus the natural egg lay is the differing initial egg lay position within the spikelets between lines. As a result I suggest that the larval movement outside the spikelet in MB110 and other resistant lines in Chapter 3 under natural egg lay may be a response to a difference in hatch position. It may be that neonates in MB110 and other resistant lines positioned between the ovary and palea/lemma did not establish well at this position, in contrast to larvae feeding elsewhere in the spikelet.

### **Larval mortality**

Several lines recorded unique patterns of larval antibiosis during the first half of the larval period indicating possible differences in antibiosis components.

MB110 showed a different pattern of larval mortality compared to all other lines tested. Early larval mortality of neonates and young larvae was highest in this line, while the mortality decreased between days 6-9 in contrast to all other resistant lines (Figure 5.1) As such the antibiosis in this line is likely to be directly related to some physical barrier that affects initial larvae establishment. The most obvious difference between MB110 and the other lines tested in this trial is that of physical structure. MB110 contains the toughest, most tightly closed glumes of all lines tested in this trial. While this physical structure may contribute to the high level of antixenosis, the same spikelet structure may also make it difficult for larvae to establish feeding. This may also explain the movement of larvae outside this spikelet in this line and several other antixenotic lines in Chapter 3.

The lines SC62C, PM7017, TX2880, and IS10759 appear to contain moderate levels of a potentially similar mechanism of antibiosis. In this study these lines produced a similar pattern of larval mortality when larval growth was greatest between second and third instars.

The level of antibiosis present in ICSV745 is superior to any other line tested even under high levels of egg infestation (Tables 5.1 and 5.4). Larval mortality in this line commenced as soon as larvae began to feed on the caryopsis in each spikelet, and the larval mortality increased in both trials as larval feeding and growth against the caryopsis increased. It is unclear whether the mechanism of antibiosis present in this line is similar to that in the four lines mentioned above. If this is the case, the level of antibiosis present in ICSV745 is superior to any of the other lines tested. This may be in part due to the increased caryopsis and subsequent seed size in this line. The relative amounts of antibiosis present in DJ6514, ICSV197, and ICSV745 were recorded in trial Q (Chapter 2). An increase in seed size corresponds with an increase in the level of larval antibiosis present in each line. ICSV197 is a converted line of DJ6514, both of which contain a relatively small seed size in comparison to ICSV745. Both of these lines recorded significant larval antibiosis, however this was highest in ICSV745 (Chapter 2), a conversion of ICSV197 with a very large seed size. The large caryopsis may be more readily accessible to midge larval feeding in ICSV745, resulting in higher larval mortality under a high percentage of caryopsis feeding early in the midge life cycle. Evidence for this was discussed previously, where at day three in this study (after a majority of eggs were deposited away from the caryopses in all lines), ICSV745 recorded the highest percentage of midge present against the caryopsis of all lines tested and then recorded the greatest larval mortality between days 3-6.

## **Summary and Conclusions**

The resistant lines ICSV745, PM7017, TX2880, IS10759, and SC62C contained significant levels of antibiosis to larvae resulting in larval mortality and an increased seed set of between 20-60% relative to susceptible lines. The mechanism of resistance appears to be linked with the developing caryopsis, as larval mortality increased in all lines as larval feeding against the caryopsis increased.

In contrast the line MB110 may contain a unique antibiosis mechanism, that causes significant displacement of neonates and young larvae. The antibiotic affect does not continue into the second half of the larval period in contrast to all other antibiotic lines tested.

A minor antibiotic mechanism of resistance may be present in QL39. While the growth rate of larvae reared on QL39 was affected, the final larval size and subsequent seed loss within this line was similar to other susceptible lines under moderate or high midge infestations. As such this additional diverse source of antibiosis type resistance is ineffective under higher midge infestation.

Overall the highest level of larval antibiosis was again recorded in the Indian line ICSV745. The level of antibiosis remained constant even under high midge infestation levels and holds great promise as a stand alone mechanism of resistance causing a three fold increase in seed set if incorporated into susceptible lines. In chapters 6 and 7 of this thesis I further examine this source of resistance in more detail.

## CHAPTER 6

### Is There a Link Between Antibiosis and Tolerance?

#### Introduction

Chapter 2 reported evidence for a possible new tolerance mechanism of sorghum midge resistance that appears to be restricted to Indian grain sorghum germplasm derived from the resistant source line DJ6514. Further results in Chapters 3 and 5 showed that these lines directly related to DJ6514 contain a potentially unique and highly effective antibiosis mechanism that affects larvae after they begin feeding on the developing caryopsis. So antibiosis and tolerance may be linked mechanisms of resistance. One possible explanation for the link between antibiosis and tolerance in these lines is that the specific antibiosis components within the developing caryopsis (either physical or chemical) also cause tolerance by changing larval feeding behaviour. Larvae could be deterred from feeding directly on the developing caryopsis feeding elsewhere in the spikelet for some time, after which normal feeding against the caryopsis occurs. This critical period of feeding away from the caryopsis may be long enough for both the larvae and caryopsis to survive in many spikelets, resulting in the observed tolerance.

One way to test the effects of different plant components on larval growth, feeding behaviour, survival and development is to carry out bio-assay trials using an artificial diet with different larval instars being exposed to specific plant components incorporated into the diet itself.

Unfortunately, various attempts at the University of Queensland to develop an artificial diet for midge larvae have proven to be unsuccessful (Diana Liu & Ekhllass Jarjees, pers. comm. 2000).

Based on these results I decided not to attempt to develop an artificial diet but rather to carry out the work *in situ* with whole sorghum caryopses.

While several different feeding situations may be hypothesised within the spikelet of tolerant and non-tolerant lines, the following three scenarios may be used to effectively demonstrate tolerance as follows:

(a) Firstly, deterrent properties within the developing caryopses of tolerant lines are absent or reduced in non-tolerant lines, or else attractant properties of other parts of the spikelets in tolerant lines are absent or reduced in non-tolerant lines. In order to examine this scenario, larvae could be artificially restricted to feed on the caryopsis alone.

(b) Secondly, midge larvae must be able to feed and develop away from the developing caryopsis within the spikelet of tolerant lines. Even more precisely, it may be that some genotypes contain a better environment for larval growth and survival away from the developing caryopsis. In this case higher rates of tolerance may occur. In order to test this, larvae could be artificially restricted to feed away from the caryopsis.

(c) Finally, if tolerance is simply a result of antixenosis to caryopsis feeding in antibiotic genotypes, then genotypes without tolerance may similarly exhibit tolerance under the same circumstances where they escape larval feeding on the caryopsis for a similar period. It may be possible to artificially delay feeding on the developing caryopsis by delaying egg oviposition within susceptible and resistant genotypes.

In order to determine an answer to the above three feeding scenarios [(a), (b) and (c)], I developed three separate sets of trial work to test each. The results of this work are presented in this chapter.

## Methods

The water injection method was employed over three types of trials on a narrow range of glasshouse pot grown midge susceptible, antixenotic and antibiotic/tolerant lines related to DJ6514. The two antibiotic and tolerant lines ICSV745 and PIONEER 3 respectively, were tested in all trial work. In previous testing over four years, ICSV745 consistently recorded high rates of antibiosis and low rates of tolerance, while PIONEER 3 consistently recorded moderate rates of antibiosis with high rates of tolerance (chapter 2; table 2.4). At least one of the susceptible lines (QL12 and QL20), and antixenotic lines (QL39 and 90562) were also included in each trial according to the availability of flowering plants. In one trial the closely related antibiotic/tolerant lines DJ6514, and ICSV197 were also tested.

### Isolating larval feeding to the developing caryopsis

Several attempts were made to trim the glumes and lemmas of summer grown plants in the lines QL12, QL20, ICSV745 and PIONEER 3 immediately after midge oviposition at anthesis to isolate midge larval feeding to the developing caryopsis. However in all cases this method failed, causing caryopsis abortion within all spikelets even when attempted under dissection microscopy. I was unable to insert a suitable barrier between the lemmas and developing caryopsis in any sorghum line without damaging the developing caryopsis. So these attempts to develop a method to isolate larval feeding to the developing caryopsis *in situ* failed.

In a separate experiment, attempts were made to establish larval feeding on the same lines by dissecting out intact developing caryopses and placing them in moistened petri dishes in various sheltered light and dark environments. Despite efforts to shelter midge larvae, at all instars no larval feeding or growth was observed. Consequently I was not able to design any experiments that would isolate larval feeding to the caryopsis in any lines.



## **Larval feeding with the caryopsis absent**

### **Developing the procedure**

A technique to remove the caryopsis from QL12 spikelets at flowering was successfully developed. The technique involved lightly squeezing the base of sessile spikelets at flowering in such a way as to “pop” out the developing caryopsis. The caryopsis was then gently removed using jewelers forceps, leaving the lodicule, palea and lemmas within the spikelet undamaged and intact.

Using this procedure on 20 sessile spikelets of QL12, half the spikelets were immediately sampled and dissected to check that the caryopsis was completely removed without causing damage to any other part of the spikelets. Five days later the other half of the spikelets were dissected. In all cases where this procedure was used all remaining parts of the floret were found to be intact and healthy.

Further work determined that the absence of the caryopsis in spikelets of several lines made the water injection technique highly precise because of the increased cavity within the spikelet. Under this situation, the water injection technique accurately deposited 90-100% of all eggs injected when used at an egg density of two eggs per spikelet.

### **Trial on four lines**

Single pot grown plants of each line, QL12 (susceptible), 90562 (antixenotic), ICSV745 (antibiotic) and PIONEER 3 (tolerant) were selected on the day of trial at mid flower. Three flowering rachis branches on each plant were then trimmed back to contain 9-12 sessile spikelets from which the complete developing caryopsis was removed to leave all other floral parts intact. The water injection technique was then used to inject two eggs per spikelet across all lines. No egg sample was taken as the water injection technique proved to be extremely accurate in initial trial work. All

spikelets were sampled 15 days after trial commencement to coincide with late larval development. All samples were frozen for later dissection.

### *Collation of results*

Larval infestation and size was measured using the methods described in Chapter 3 and 5. However the presence of high numbers of very small neonate sized emaciated larvae greatly complicated results, because the likelihood of survival of such larvae remained very low. Previous studies by this author [Trial (a) this chapter] showed that a high proportion of these larvae were unlikely to feed normally or develop through to pupation and cause kernel abortion. Instead they may survive for a time with no increase in size and eventually die. es. Consequently these larvae have been recorded separately to larger healthy larvae that are likely to develop normally.

Larval survival and infestation was recorded as the percentage of spikelets with larvae present. Individual larval size records (using an ocular micrometre: 500 units = 1 mm) were then used to further classify the infestation of spikelets to incorporate the observed high rates of small larvae within spikelets of all lines as follows:

small larvae – emaciated larvae ( $\leq 100$  units in size)

large larvae (or pupae) – healthy larvae ( $> 100$  units in size)

All infestation and larval size data were then recorded according to the above two categories.

### *Statistical analyses*

Analysis of variance and least significant difference ( $p < 0.05$ ) were used to discern any differences between lines in the percentage of spikelets infested with larvae. However analysis of larval size data between lines was not performed due to the low number of larvae that were above 100 ocular units in size.

### **Artificially Delayed Feeding against the Caryopsis (Egg deposition delayed four days)**

Further trial work was attempted in pot grown glasshouse plants designed to artificially delay for several days the egg deposition and subsequent larval hatch, establishment, feeding and growth on the developing caryopsis in a range of sorghum lines. This experiment was conducted to determine if delaying feeding on the caryopses allowed both the grain and larvae to develop in antibiotic sorghum lines.

In order to create this situation, the water injection technique was used on several QL12, DJ6514, and ICSV197 panicles, delaying egg insertion until 2-6 days post anthesis. Early attempts at this technique led to high rates of mechanical damage. This was particularly difficult in lines infested five or six days post anthesis when it was not possible to pry open the glumes of spikelets and inject eggs accurately without damaging the spikelets, or dislodging the developing caryopses. However after some practice, I found it was possible to successfully inject the above lines with eggs directly from above into the cavity just below the apex of the spikelet with very limited prying open of the glumes. This resulted in high rates of egg insertion (80% of spikelets infested) and very low rates of mechanical damage to the caryopsis (less than 1% of spikelets aborted).

A larger trial was then planned using this technique on a range of susceptible, antixenotic, antibiotic and tolerant lines four days post-anthesis. This time period post-anthesis was the maximum time point at which high rates of water injection could be maintained under very low rates of caryopsis abortion.

#### **Testing procedure**

The water injection technique was again used to screen a total of seven lines. These included the susceptible lines QL12 and QL20, the antixenotic line 90562, and the antibiotic and tolerant lines DJ6514, ICSV745 and ICSV197. Also included was the highly tolerant line PIONEER 3, related to DJ6514.

Individual panicles were simultaneously chosen at mid-flower and placed aside for water injection. On each panicle a total of 3-4 rachis branches were trimmed back to leave a total of 10-15 flowering spikelets. Four days later the water injection procedure at a density of 4-6 eggs per spikelet was used to inject three rachis branches of each plant. In addition one additional rachis branch of the lines QL12, 90562 and QL12 was injected to determine the accuracy of the technique. Time constraints and limited available midge egg supply meant that this was not carried out across all lines.

The extra three rachis branches from three of the seven lines tested were immediately sampled and dissected to determine the success rate of egg injection within the spikelets of each line, and to record any signs of mechanical damage.

All plants were then caged according to the glasshouse cage test procedure described in Chapter 2. All spikelets were left until the first midge imagos emerged into the cages of three lines, 19 days after water injection. This sampling period was chosen to coincide with pupal development.

### **Collation of results**

#### *Egg infestation figures*

Only three of the eight lines were sampled for egg infestation, based on the assumption that all lines would record equal egg infestation levels due to the accuracy of the water injection technique (Chapter 4). Evidence for this assumption was confirmed when all three lines sampled recorded a consistent range of 79-81% of spikelets with eggs at an average egg density of 2-3 eggs per spikelet. Consequently all lines were assumed to contain a similar starting egg density. (80% spikelets infested; 2-3 eggs per spikelet).

#### *Larval infestation, midge damage and record of tolerance*

Measures of infestation, tolerance and larval feeding position within the spikelet were recorded similarly to the methods described in Chapters 2, 3 and 4. However when larval size and tolerance data were collated, the record of high numbers of

spikelets containing small larvae at different feeding positions within spikelets that contained filled caryopses led to a change in the way both larval size data and tolerance data were collated and analysed.

To account for the high numbers of small larvae within filled spikelets of all lines, larvae were again [see section (b)] classified into two distinct larval sizes according to their likelihood of causing abortion to the caryopsis, either less than 100 units in size, or greater than 100 units in size.

The record of high numbers of small larvae within spikelets that contained filled caryopses also affected the record of tolerance. To account for this two modified definitions of tolerance were recorded as follows:

Tolerance (a)

= The number of sessile spikelets containing larva(e)/pupa(e) greater than 100 units in size within spikelets containing filled caryopsis where larval feeding did not reduce caryopsis size below 75% normal size.

Tolerance (b)

= The number of sessile spikelets containing larva(e)/pupae of all sizes feeding anywhere in the spikelet where larval feeding did not reduce caryopsis size below 75% normal size.

#### *Statistical analysis*

Analysis of variance and LSD was used to discern any differences in measures of percentage of spikelets aborted under midge feeding and tolerance [(a) or (b)] between lines. However very small sample sizes of larvae greater than 100 units in size at all feeding positions within aborted and tolerant spikelets made statistical analysis of differences in larval size between lines at all feeding positions inappropriate.

## Results

### Isolating larval feeding to the developing caryopsis

No useful results were obtained because it was not possible to develop a method that effectively isolated the developing caryopsis.

### Larval feeding with the caryopsis absent

Tolerance was observed in all four lines when the caryopses were artificially removed from all sessile spikelets on two measures of larval infestation (Table 6.1). While all lines initially recorded 90-100% of spikelets infested with eggs post water-injection, the percentage of spikelets infested with larvae of all sizes reduced approximately 10-20% across all lines when spikelets were sampled 15 days later. However, when small larvae (unlikely to survive through to pupation) were removed from sampling, the percentage of spikelets infested with larvae ranged between 47-62%, approximately 50% of the original egg density.

**Table 6.1** Percentage of spikelets infested with larvae 15 days post water injection on four lines where the caryopses of all spikelets were artificially removed prior to egg infestation (Initial egg infestation estimates of 90-100%).

line	% spikelets infested	
	with all larvae	small removed <sup>^</sup>
QL12	80 *	55
90562	69	62
ICSV745	79	46
PIONEER 3	73	47

\* No significant differences ( $p < 0.05$ ) were recorded between lines for either measure of spikelet infestation.

<sup>^</sup> Data after small emaciated larvae unlikely to survive through to pupation were removed from results.

Across all lines, 50-70% of total larvae recorded in both aborted and tolerant spikelets were less than 100 units in size, with no significant differences between lines ( $p < 0.05$ ).

### **Artificially Delayed Feeding against the Caryopsis (Egg deposition delayed four days)**

When egg infestation was delayed four days, the record of larval infestation and tolerance 15 days later was similar across all susceptible, antixenotic and antibiotic/tolerant lines (Table 6.2). All lines recorded similar rates of larval infestation (50-74%), and caryopsis abortion (20-43%).

From the 28-34 spikelets of each line that were sampled and dissected, a total of 100 larvae, and three pupae were recorded and of these approximately half (52) were recorded feeding against the caryopses.

When larval size was recorded relative to the position of midge feeding and record of caryopsis abortion, the following patterns of larval size and midge damage was recorded across all lines:

Larval size was greatest within aborted spikelets of all lines, where 100% of larvae were greater than 100 units in size (500 ocular units = 1 mm). Within tolerant spikelets, 70% of larvae feeding against the caryopses of tolerant spikelets were greater than 100 units in size, while 18% of larvae feeding elsewhere in the spikelet were greater than 100 units in size.

**Table 6.2** Midge larval infestation, damage and tolerance within seven lines infested with midge eggs four days post-anthesis.

line	larval feeding		% tolerance within infested spikelets	
	% spikelet infested ^	% spikelets aborted	(a)	(b)
90562	74 *	43	42	57
DJ6514	50	36	55	64
ICSV197	59	27	45	73
ICSV745	58	29	39	71
PIONEER 3	63	20	44	80

^ Initial egg infestation of 80% recorded on three lines. All other lines assumed to contain similar egg infestation.

\* No significant differences recorded between lines on any data in columns at (p<0.05).

(a) only tolerant spikelets with seed <75% normal size & larvae <100 units in size feeding against caryopsis

(b) spikelets with seed <75% normal size & larvae of all sizes at any feeding position

Under a delayed midge egg infestation of four days, all lines recorded low to moderate levels of percentage of spikelets aborted between 20-43% (Table 6.2). The bottom and tops of this range were similarly recorded in the QL12 (24%) and QL20 (56%) susceptible control lines.

High and consistent rates of tolerance within infested spikelets were recorded across all lines (Table 6.2). When tolerance was recorded as the presence of large midge larvae feeding against filled caryopses [Tolerance (a)], all lines recorded a similar range of 31-55% tolerance within infested spikelets. This increased further to 44-80% when small larvae feeding away from the caryopsis in all lines were included in the record of tolerance. No significant ( $p < 0.05$ ) differences were recorded between lines for either measure of tolerance.

## **Discussion**

Failure to obtain any data on the effects of midge larval feeding on the caryopsis alone [section (a)] prevented direct proof of any unique anti-feeding properties of caryopses of DJ6514, and the related genotypes ICSV745 and ICSV197.

In many ways the results obtained from section (b) and (c) also create more questions than answers. While there is some evidence for similar and significant levels of larval survival away from the caryopsis within the spikelets of both susceptible and resistant genotypes in the remaining trial data [section (b) and (c)], the unusually retarded larval growth rates across all lines in both trials made interpretation of results uncertain, due to the unknown fate of both larvae and developing caryopses within these spikelets. One way to overcome this problem would have been to have multiple sampling times to track the fate of emaciated or small larvae.

Despite these problems, the results from both these trials highlight several issues to do with midge larval survival and development across all lines regardless of midge resistance status. In both trials we see evidence for the critical timeframe under which midge larval development occurs within the sorghum spikelet. If the caryopsis is either not present, or larval feeding against the caryopsis is delayed by



up to four days, then both survival and growth rates are reduced in a majority of larvae (Table 6.2). The results emphasize the importance of the developing caryopsis in providing the necessary nutrition for larval development. Positive results in section (a) would have showed this more directly. The results from both Chapter 3 (Tables 3.3 and 3.7) and the results obtained in sections (b) and (c) in this chapter highlight the clear correlation between feeding position and larval size across both susceptible and resistant lines. Larvae still feeding on the glumes/palea/lemmas at day six, eight, or nine in these trials were many times smaller than those feeding on the caryopsis during the same period. When the caryopsis was removed altogether in the trial in section (b) of this chapter, this effect is even more pronounced. Six to nine days after egg lay, 50-70% of larvae were similar in size to first instar larvae.

These facts also highlight that previously classified ‘tolerant’ genotypes (Chapter 2) do not record higher rates of tolerance than other genotypes when midge larval feeding against the caryopsis is delayed several days. So their caryopses are no different in coping with midge larval feeding.

## **Summary and Conclusions**

These experiments provide indirect evidence that the unique tolerance mechanism of resistance within the line DJ6514 and its derivatives is the result of antixenosis to caryopsis feeding due to the antibiotic anti-feeding properties of developing caryopses within these lines.

While the role of the caryopsis in deterring larval feeding could not be tested directly (section (a)), the tolerance could be artificially created in all lines when larval feeding against the caryopsis was delayed [section (c)]. The observation of tolerance in antibiotic genotypes (Chapter 2) was not due to these lines having caryopses that are any more ‘tolerant’ to abortion under larval feeding, but due to some other factor. A change in larval feeding behaviour is a likely reason for this observation given that larvae survive and grow at similar reduced rates away from the caryopses of all lines when they are forced to feed away from the caryopsis [section (b)].

Therefore, I conclude that tolerance observed in Chapter 2 as the presence of midge larvae against filled caryopses is directly related to a change in larval feeding behaviour best described as antixenosis to caryopsis feeding. I hypothesize that the tolerance observed in genotypes related to DJ6514 is due to the unique anti-feedant properties of the caryopses in the spikelets of these lines creating a change in larval feeding behaviour within the spikelets of these lines resulting in an escape mechanism for caryopsis survival as larvae feed elsewhere in the spikelet.

## CHAPTER 7

### The Inheritance of Antibiosis

#### Introduction

The antibiosis identified in the Indian lines DJ6514, ICSV745 and ICSV197 is being used in the Australian public sorghum breeding program as an ideal new source of midge resistance. Understanding the inheritance of antibiosis may not only assist breeders in their efforts to rapidly incorporate this new source of resistance into Australian grain sorghum, but it may also aid our understanding of the underlying cause behind such resistance. A recent study of the inheritance of antibiosis was made in a recombinant inbred population, coupled with a genetic mapping study using PCR-based markers (Tao *et al.* 2003). In this chapter the phenotypic data from this study are presented along with the screening results from several breeding populations to determine a more detailed understanding of the inheritance of antibiosis.

#### Methods

##### Population development and selection

Several breeding populations developed by the QDPI&F breeding team were used for this study:

One F5/6 RIL population of the cross 90562 x ICSV745 (122 plants)

One F2 population of the cross B35 x 62191 (81 plants)

Three F1 populations (6-10 plants of each cross)

a) 31945 x ICSV745

b) A35 x ICSV745

c) A35 x 31945

Two antibiotic genotypes (ICSV745 and 62191) were chosen for testing. The line 62191 was selected as an F6 recombinant inbred line (RIL) from the cross of 90562

x ICSV745. In repeated testing on eight panicles, over two years, this line recorded consistently high levels of antibiosis (65%) similar to that present in ICSV745.

Several diverse origin non-antibiotic lines were utilised in different crosses. The lines B35 and its genetically identical male sterile counterpart A35 were used repeatedly. These lines are highly susceptible to midge egg lay, and contain no antibiosis. The lines 90562 and 31945 contain high and low-moderate levels of antixenosis respectively, while both contain no antibiotic lines in their pedigree (Henzell pers. comm. 2003).

Two different methods of screening were deployed over four years on the three populations. In the RIL population a detailed glasshouse screening method was used, while a field trial was used to screen both the F1 and F2 populations.

### **F5/6 Recombinant Inbred Lines (ICSV745 x 90562)**

This population was phenotyped (by this author) and then linked to genetic mapping data, to determine the genetic nature of antixenosis (from 90562) and antibiosis (from ICSV745) (Tao *et al.* 2003). This study identified a single QTL for antibiosis, explaining 34.5% of the variance in the population. However for the purposes of this study only antibiosis phenotype results have been presented.

### **Screening Method**

Glasshouse trials were conducted over three summers using the cage screening method described in Chapter 2. In all cases a range of 3-5 replicates (panicles) of each recombinant inbred line (RIL) were screened each year, while several RILs were screened over more than one year testing to confirm the accuracy of results across days and years.

On each trial day, flowering panicles of all (RILs) chosen and at least one panicle of ICSV745 and 90562 were selected at mid-flower and trimmed to contain 120-150 flowering spikelets. Plants were then placed in wire cages enclosed with white nylon gauze bags. Forty mated female sorghum midge were introduced into each cage at 9-

11 am and left to oviposit in each enclosed panicle. Two samples (50-80 sessile spikelets) were then taken from each panicle; an egg sample (one day after trail commencement); and a pupal sample (taken upon the emergence of first adult from the remaining spikelets on each panicle).

The percentage of i) spikelets infested with eggs and ii) pupae, were obtained by microscope dissections of each sample as per Chapter 2 results.

### **Data collection and analysis**

Antibiotic phenotypic data were calculated as described in Chapter 2 results using the following formula;

Antibiosis = difference between % eggs and % pupae as a percentage of the original egg count.

Means were generated from the 3-6 panicles tested on each RIL and compared directly to two distinctly different ranges of scores recorded in the two parent lines. This was possible due to a similarly accurate and diverse range of antibiosis scores recorded across all days testing in both parent lines and RI lines. This was confirmed under statistical analysis where no significant day x day, or year by year interactions were recorded. As such ANOVA was used to predict RI mean scores using common effects estimated across blocks, and years. Predicted means for antibiosis were then directly compared to the range of scores recorded in the resistant to determine the ratio of segregation of antibiosis within the population. Despite the accuracy of the procedure in some cases sampling error led to the record of higher pupal infestation than egg counts. As this result was not biologically possible, all negative differences were converted to zero for the purposes of presentation.

Chi squared analysis of the segregation of resistance was used to determine the goodness of fit of the single gene model.

### **F1 and F2 populations**

### **Screening method**

In both the F1 and F2 populations, field grown plants were screened using a modified cage method (Chapter 2). Each trial (F1 or F2) was conducted separately using the same methodology. Plants were grown through to booting and then covered in muslin cloth bags until the first day of flowering at which time bags were removed to expose panicles to midge oviposition for 4-8 days (dependent on panicle size and midge density). All plants flowered over a two week period in all populations (82 F2 lines, 6-10 plants of each F1 cross), however panicles of the parent lines only flowered over a narrow 5-10 day period. This coincided with the beginning (B35) and middle (62191) of flowering in one F2 population, and the beginning (B35, 31945) and end (ICSV745) of the flowering period in the other F2 populations. Consequently any differences in testing conditions across days could not be analysed in reference to all parent control lines in both populations. However in order to counter this possible effect, two samples (replications) of data were taken on each panicle 3-4 days apart in both populations to spread sampling over a longer period of testing covering a wider range of environmental conditions. Panicles of each plant were enclosed in muslin cloth bags until the beginning of flowering, at which time heads were exposed to natural midge infestation for 4-6 days. During this period of exposure two egg samples were taken on each panicle, one 2-3 days after midge exposure (on the top half of the panicle) and another 2-4 days after the first sample (on the bottom half of the panicle). Sample sizes and sampling method were exactly the same as those described in the RIL population (this Chapter). Upon taking the last sample, all adult midge were removed from the panicle and muslin cloth bags were tied onto panicles to prevent damage. No pupal sample was taken, instead two measures of seed set were made on the top and bottom half of the panicle of each line 2 weeks later.

### **Data collection and analysis**

Antibiotic phenotype data on all lines were gathered using the same method described above, with one minor change to the antibiosis formula because visual seed set scores on individual panicles were used in place of pupal samples to measure final midge infestation.

Antibiosis = difference between % eggs and % midge damage at seed set as a percentage of the original egg count.

Individual antibiosis scores for each line were calculated from the average of two scores on each panicle. Because parent lines were not tested on the same days as all F2 plants, statistical analysis of differences in means between parent lines and F1 or F2 progeny scores was not appropriate. Despite this limitation all resistant and susceptible parent lines recorded almost identical patterns of antibiosis scores to those recorded in the RIL glasshouse study (this Chapter). Consequently day x day or line x day differences across all trials were assumed again to be non-significant.

The pattern of inheritance of antibiosis in the F1 and F2 populations was then determined by viewing the range of antibiosis scores in the progeny relative to those recorded in the parent lines in each trial.

In the F1 data, reduced panicle scores (6-10 per genotype) did not allow Chi squared analysis of data, and simple visual interpretation was used to determine the goodness of fit of inheritance patterns based on a single gene model. In the F2 population (81 lines) a Chi squared analysis was used to test the goodness of fit of segregation ratios based on a single gene model.

## **Results**

### **F5/6 Recombinant Inbred Lines (ICSV745 x 90562)**

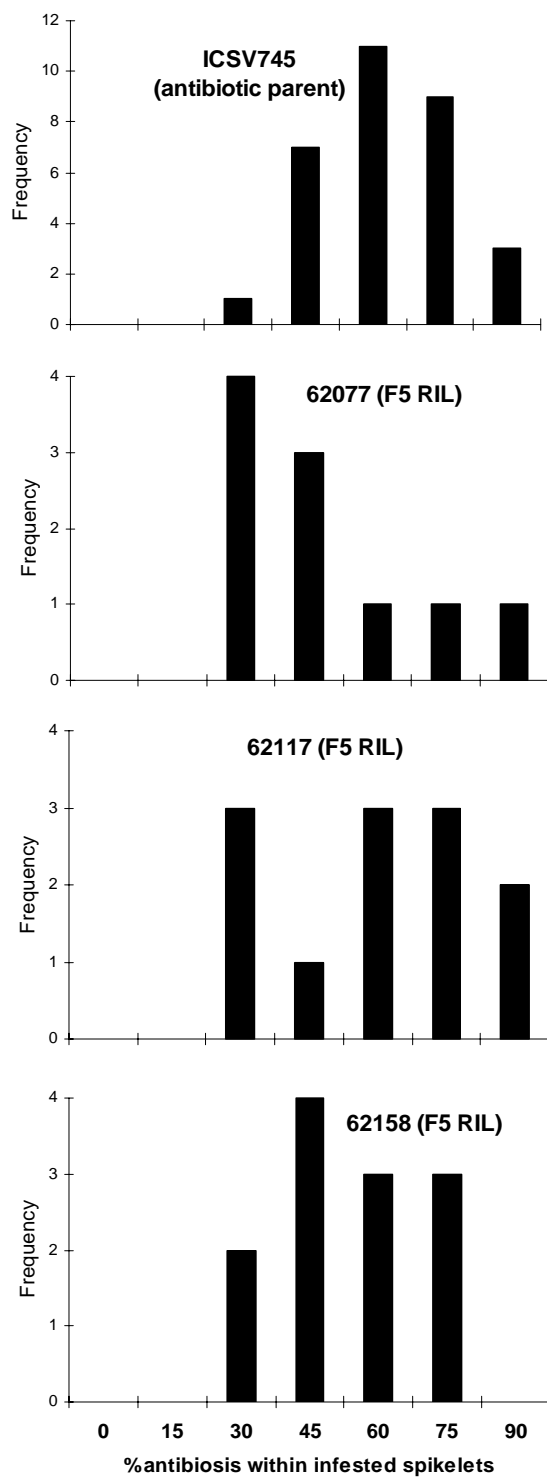
A total of seven RILs were repeatedly tested across all years, along with the parents (90562 and ICSV745). From these data a similar range of antibiosis scores was recorded in the antibiotic parent (ICSV745) and subsequently identified resistant RILs, distinct from that present in the susceptible parent (90562) and similarly identified susceptible RILs in most days testing. The only exceptions to this occurred across three days testing data in the first year's screening, where both parents and two repeatedly tested RILs recorded inconsistent results. As such these

three days testing results were removed from analysis, effectively reducing two of the scores of repeatedly tested RILs from analysis

### **Antibiosis scores in parent lines and repeatedly tested RILs**

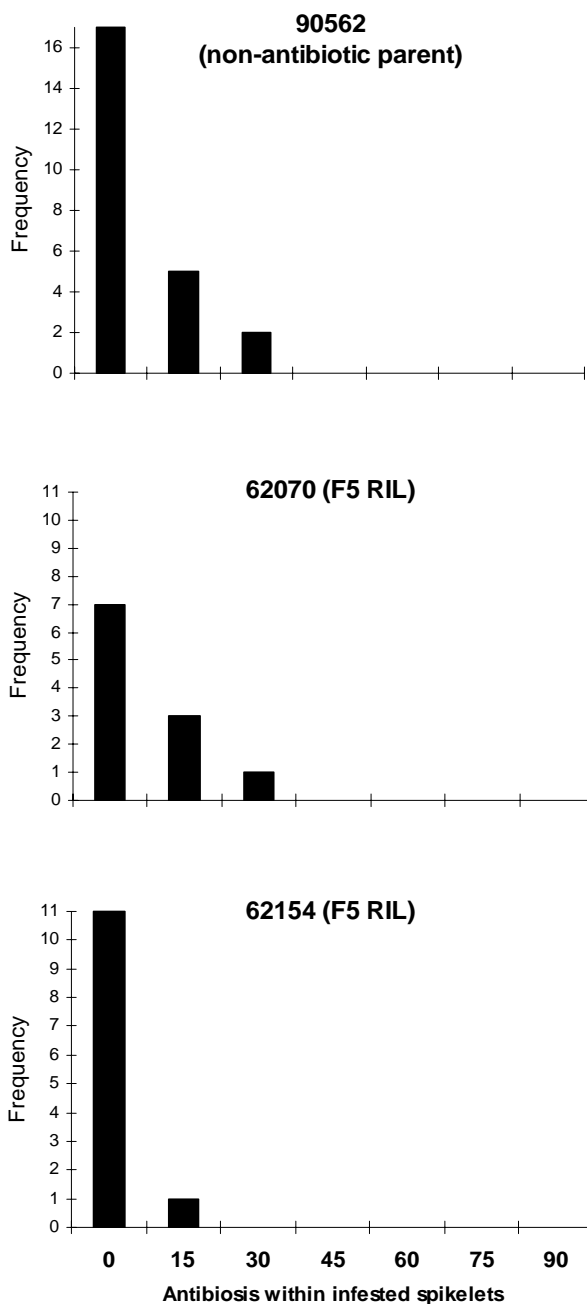
A similar broad range of antibiosis scores was recorded in the resistant parent and three antibiotic RILs (Figure 7.2). While one resistant RIL (62117) recorded 61% antibiosis identical to that recorded in ICSV745, two other repeatedly tested RILs (62007 and 62158) recorded slightly lower antibiosis scores of 49-55%. In all cases a broad range of antibiosis scores were recorded, indicating that either the method or testing conditions used to measure antibiosis were prone to sampling errors, or that the expression of the antibiosis trait in antibiotic resistant lines was variable.





**Figure 7.1** Distribution of individual antibiosis scores in three antibiotic F5 recombinant inbred lines repeatedly tested over three years compared to those recorded in the antibiotic parent line ICSV745.

A significantly different ( $p < 0.01$ ) distribution of scores was recorded in the susceptible (antixenotic) parent 90562 and two susceptible RILs (Figure 7.2). In these lines a consistent narrow distribution of antibiosis scores was recorded between 0-30%, highly skewed towards zero, resulting in mean scores of 0-5% across 90562 and both non-antibiotic RILs (62070 and 62154).



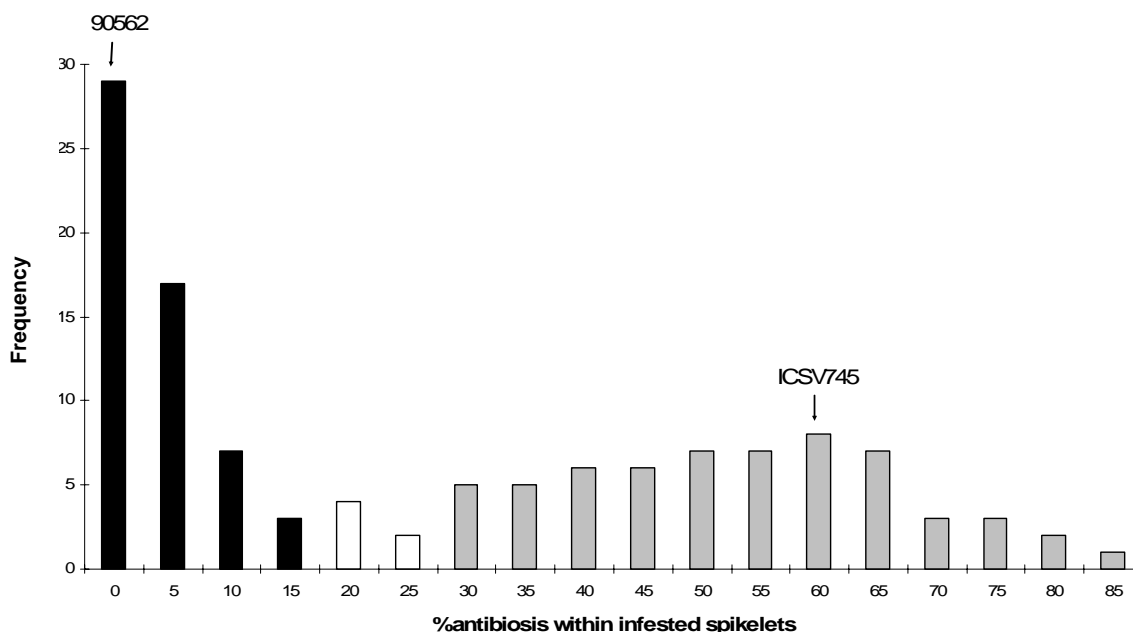
**Figure 7.2** Distribution of individual antibiosis scores in two antibiosis susceptible F5 recombinant inbred lines repeatedly tested over three years compared to those recorded in the susceptible (antixenotic) parent line 90562.

### **Segregation of antibiosis scores in RIL population**

The frequency and mean antibiosis scores in the antibiotic (ICSV745) and antixenotic (90562) parents and repeatedly tested RILs (Figures 6.2 and 6.3) were used to predict an expected grouping of non-antibiotic means between zero to 30 percentage antibiosis, and a separate distribution of means between 30 to 90 percentage antibiosis respectively. The actual distribution of mean antibiosis scores from 3-5 replicates of all 122 RILs (Figure 7.3) was similar to that predicted. Two clear distributions of scores were recorded in line with that recorded in both parents between 0-15% antibiosis (susceptible) and 30-90% antibiosis (resistant). Despite the accuracy of phenotyping, a small number of RILs recorded scores between 21-29% antibiosis outside the expected range of the two parents. When these scores were removed from analysis a total of 53 (49%) of RILs recorded no antibiosis compared to 55 (51%) with antibiosis. This clear 1:1 distribution of scores is highly significant under Chi-square analysis ( $p < 0.01$ ) for a single gene segregation ratio.

A simple test for epistasis in the parent line 90562 was also conducted by comparing the mid-point antibiosis score of the two parents relative to the mean score in the RIL population. A midpoint score of 22% antibiosis was recorded between the two parents (90562 and ICSV745), not significantly different from the mean score of 20% recorded across all 122 RILs. This result provides evidence for a lack of antibiosis gene interactions between the resistant and susceptible parents, and adds evidence for the presence of a simply inherited single gene for antibiosis.

A further simple analysis of the top range of antibiosis scores present in the RIL population also shows that there is no evidence for transgressive segregation (ie lines outside parental scores) in this population. While a small number of RILs recorded antibiosis scores of over 85% antibiosis, ICSV745 similarly recorded a similar percent of higher antibiosis scores over three years of testing, consequently these scores were never significantly ( $p < 0.05$ ) higher than that recorded in ICSV745.



**Figure 7.3** Frequency distribution of mean antibiosis scores on a total of 122 F5 RILs from the cross ICSV745 x 90562. Scores are averages from 3-6 replicated testings of each RIL over one to three years testing. Grey bars indicate the range of scores recorded in the resistant antibiotic parent ICSV745 over 3-5 replications in all trials, black bars likewise for the susceptible (non-antibiotic parent) 90562, and white bars indicate scores not recorded in either parent.

## F1 and F2 populations

### Midge oviposition across populations

Field testing conditions in both the F1 and F2 populations varied markedly. In both trials highly variable weather conditions were experienced over the two weeks of flowering (initial midge oviposition), affecting the subsequent midge infestation within individual panicles.

Conditions for midge oviposition and larval development were best in the F1 trial, where all panicles tested recorded consistently high midge densities of 30-70 ovipositing per panicle (total per panicle over 4-8 days) over the two weeks of flowering. These ideal midge pressures led to high and consistent egg infestation

figures in all hybrid and parent lines between 85-100% (percentage of sessile spikelets infested with eggs).

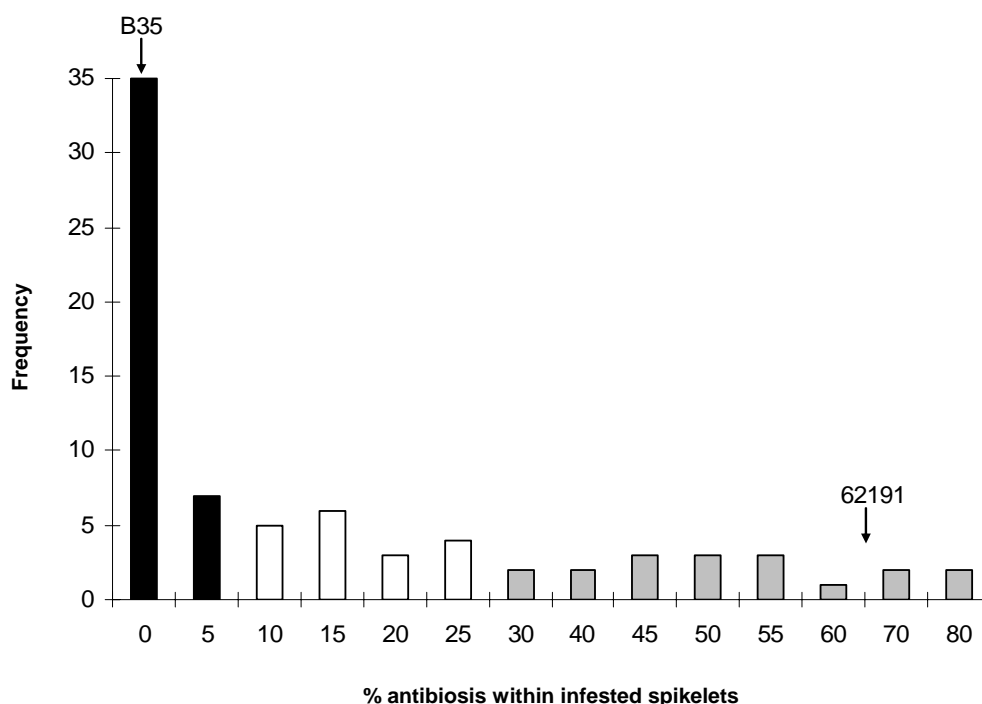
In the F2 population, a greater range of midge pressures were recorded across the 82 F2 lines (and 6-10 parent lines) exposed to midge oviposition during the two week flowering period. Midge pressures of 3-70 midge per panicle were recorded over the two weeks of flowering, resulting in highly variable levels of midge infestation between 5-100% in the 82 F2 lines. This effect was similarly recorded in the parent line 62191 (5-45% egg infestation across 8 panicles) but not in the susceptible parent line B35 where a high and consistent egg lay of 90-100% was recorded in 6 panicles.

### **Inheritance of antibiosis in the F2 population**

Despite variable testing conditions and variable levels of egg infestation in the resistant parent (62191) relative to the susceptible parent (B35, two distinct antibiosis scores (within infested spikelets) were recorded in each. B35 recorded a narrow range of antibiosis within infested spikelets of between 0-5% (average of zero) across six panicles. In contrast the antibiotic parent line 62191 recorded a wider range of antibiosis scores between 25-83% (average of 65%) across eight panicles. This broad and variable range of antibiosis scores is similar to that recorded in the F5 population, where the resistant line ICSV745 and the repeatedly tested resistant RILs similarly recorded a wide range of antibiosis scores between 30-90% (Figure 7.1).

When antibiosis was recorded in the 81 individuals within the F2 population, most lines recorded consistent antibiosis scores across the two samples made on each panicle (top or bottom half). However in three of the 82 lines, antibiosis scores on each panicle were recorded greater than one standard deviation (24%) of the mean (two scores), and consequently these data were removed from results. In the remaining 78 lines the antibiosis scores averaged between 0-81%, a similar range to that recorded across both parents.

The range of antibiosis scores of the two parent lines were used to determine the expected groupings of susceptible (0-5%) and antibiotic (25-80%) F2 lines. Using this criterion, two or three clear distribution frequencies were observed (Figure 7.4).



**Figure 7.4** Frequency distribution of mean antibiosis scores on a total of 78 F2 lines from the cross B35 x 62191. Scores are averages from 2 replicated testings on one panicle of each line (top and bottom of the panicle). Grey bars indicate the range of scores recorded in the resistant antibiotic parent 62191 over 8 panicles, black bars likewise for the susceptible parent line B35 over 6 panicles, and white bars indicate scores not recorded in either parent.

While 42 lines (54%) recorded a similar narrow range of antibiosis scores to the susceptible parent line B35 and 18 lines (23%) recorded a similar range of scores to that present in the antibiotic line 62191, a group of 18 lines (23%) recorded scores between 10-25%, outside the range of scores present in either parent.

If antibiosis is inherited as a single gene (interpreting data from the RIL population this chapter), then based on a simple interpretation of the previous result, the inheritance of antibiosis is likely to fall under one of the three following patterns of inheritance:

Antibiosis is recessive (frequency distribution - 3 susceptible: 1 resistant)

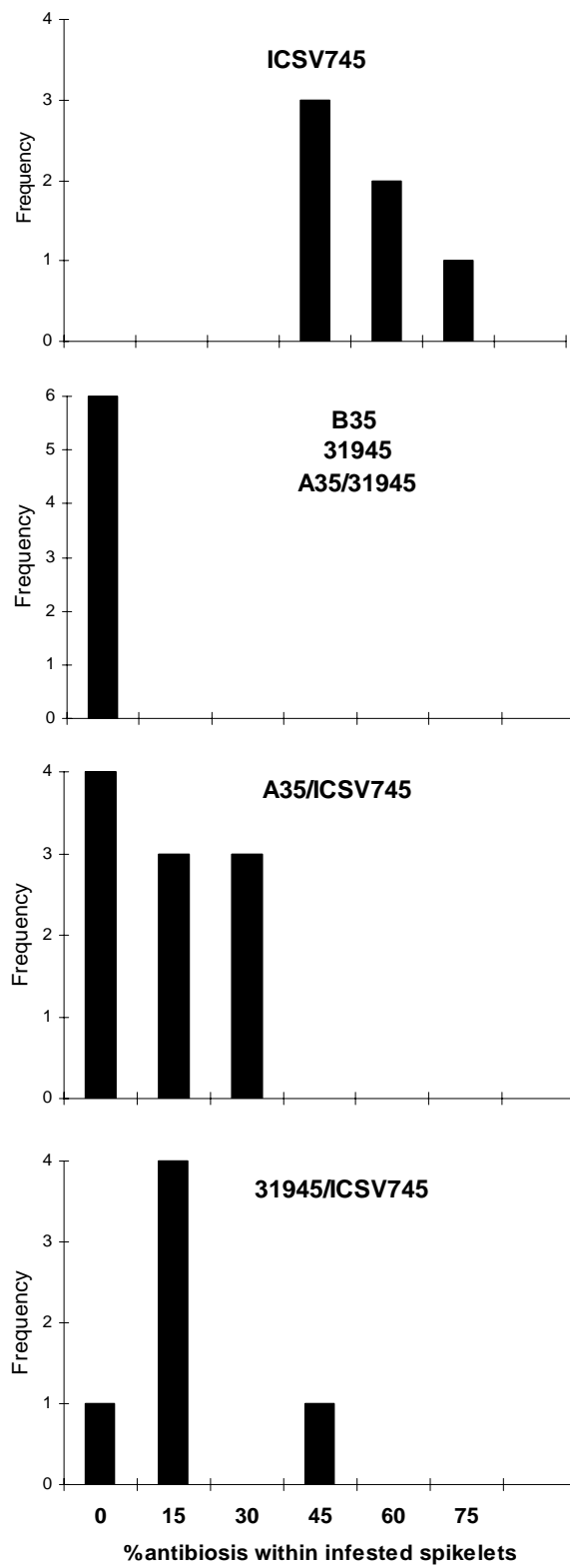
Antibiosis is partially dominant (1 susceptible: 2 partially resistant: 1 resistant)

Antibiosis is dominant (1 susceptible: 3 resistant)

Looking at the frequency distribution (Figure 7.4), only one pattern of inheritance fits these data, that of a 3:1 recessive inheritance of antibiosis. No matter which way the data are considered (as either two or three frequency distribution categories), the dominant class of antibiosis scores recorded align with those present in B35 between 0-5% antibiosis). These susceptible data makes up more than 50% of the lines tested (41 out of 78 scores). As such whether the intermediate data (partially resistant scores) are removed from analysis (42:18), or included in results and assigned to either a susceptible or resistant category [at a cut off of 17%; (53 susceptible: 25 resistant)], both fit a clear 3:1 distribution ( $p > 0.05$ ).

### **Inheritance of antibiosis in the F1 hybrids**

Under ideal scoring conditions, the lines B35, 31945 and the hybrid A35/31945 all recorded almost identical antibiosis scores between 0-3% (average of 0) across each of the six panicles tested for each genotype (Figure 7.5). In contrast the antibiotic line ICSV745 recorded a range of antibiosis scores between 44-70% (average of 55%). The two hybrids developed from crosses of susceptible and resistant parent lines recorded a range of antibiosis scores distinctly different to either of the resistant or susceptible parents. In the F1 hybrid A35/ICSV745 scores between 0-32% (average of 5%) antibiosis were recorded across 10 panicles. In the second F1 hybrid 31945/ICSV745 a range of scores between 7-43% (average of 31%) were recorded across six panicles. The results in both these hybrids indicate a partial dominance pattern of inheritance, with individual and subsequent mean antibiosis scores falling between the range of either the susceptible and antibiotic parent lines.



**Figure 7.5** Distribution of individual antibiotic scores (calculated from the average of two scores at the top and bottom of each panicle) in the antibiotic parent line



62191, the susceptible (non-antibiotic) parent lines (B35, 31945) and three F1 hybrids developed from these lines.

## Discussion

The clear 1:1 segregation ratio recorded in the RIL population strongly suggests that the antibiosis present in DJ6514 and its derivatives ICSV197 and ICSV745 is simply inherited as a single gene. These results confer with the molecular mapping results derived from this population, where only one quantitative trait loci (QTL) on chromosome 8 (J) was found to be associated with antibiosis (Tao *et al.* 2003).

It is interesting to note the similar broad spread of antibiosis scores recorded between 30-90% in all of the repeatedly tested resistant lines (ICSV745, 62077, 62117, 62158 and 62191) over multiple years of testing. (Figures 7.1 and 7.3-7.5). In contrast to this broad range of scores, the range of scores is much narrower in the susceptible genotypes (90562, 62070, 62154, B35/A35 and 31945) tested in the same trials where over 90% of antibiosis scores were recorded in a narrow range between 0-10% (Figures 7.2-7.5). Sampling errors or other experimental errors are unlikely to account for this difference in the spread of scores between resistant and susceptible lines under the same trial conditions, because the same affect was measured under different trial methodologies and different glasshouse and field environmental conditions. The broader range of antibiosis scores in resistant lines versus susceptible lines may thus be a real biological affect. There are several possible explanations for this pattern of antibiosis expression. Modifier gene(s) may mask or enhance the effects of antibiosis in different genetic backgrounds. Alternatively the physical or chemical components that effect antibiosis may be expressed variably under slightly different environmental conditions, further compounded by genetic background. Finally, regardless of growing conditions it may be that the antibiotic components present in the developing caryopsis are expressed in resistant lines at only slightly higher average amounts (10-20%) than that present in susceptible lines, leading to variable rates of midge larval survival when this baseline expression varies by relatively low amounts across plants, or even across individual spikelets.. This affect may be further exacerbated in heterozygous plants, where the baseline

expression falls mid way between susceptible and resistant levels. The expected affect is most consistent with the data recorded in the F1 and F2 populations where low to intermediate expression of antibiosis was recorded in heterozygous progeny, closer on average to the average susceptible parent score. Under this senario, in heterozygous progeny slightly below average expression of resistance may no longer affect midge larvae. While the F1 scores indicate a resistance pattern for partial dominance of susceptibility, the F2 scores show a more complete dominance pattern for susceptibility with some unexplained intermediate scores that align to the F1 pattern of inheritance.

## **Summary and Conclusions**

The antibiosis mechanism of resistance derived from DJ6514 is clearly inherited by a single gene. The expression of antibiosis is variable from plant to plant even in homozygous resistant form, and greatly diluted and even more variably expressed in heterozygous form. Consequently antibiosis is not a dominantly inherited trait and in practical breeding terms the single antibiosis gene identified in resistant lines will need to be deployed in both sides of the breeding program to obtain homozygous resistant progeny to ensure a full expression of antibiosis in commercial hybrids.

## Chapter 8

### General Discussion and Future Directions

#### Introduction

In this thesis several new sources of antibiosis type midge resistance have been isolated and characterised in some detail using newly developed methods. In the two best lines identified in these studies, antibiosis was found to occur either during the initial period of larval establishment or later on in the larval period, indicating two potentially diverse sources of antibiosis type resistance. In this discussion I outline the value of each of these resistance mechanisms along with a discussion of the potential long term durability of each resistance mechanism if they are incorporated into commercial hybrids. In the final part of this chapter the future directions of this research are discussed.

#### Different Mechanisms of Antibiosis

While antibiosis to larvae has been well documented in midge resistance studies, the cause of antibiosis remains unclear. Various researchers have linked antibiosis to tannins (Santos & Carmo 1974; Sharma 1985, 1993), spikelet morphology and increased grain development rate (Sharma 1993; Sharma *et. al.* 1990), however in all cases correlations were made from a diverse range of midge resistant lines, some of which contained little or no antibiosis. Similarly Sharma in all three studies failed to record any significant tannins or reduced larval size or weights within the highly antibiotic line DJ6514 and its derivatives ICSV745 and ICSV197.

In all the above cases no in depth analysis was made of midge larval development over time to determine the biological likelihood of these correlations between lines. The studies conducted in this thesis however do shed some light on the likelihood of the chemical, or structural cause of antibiosis within the spikelet in several lines.

### **Antibiosis during larval establishment**

A diverse pattern of early larval mortality was recorded in the line MB110, which in two studies repeatedly recorded over 40% mortality of first and second instar larvae, coinciding with larval movement away from the caryopsis (Chapters 3 and 5). In contrast to all other lines tested this mortality decreased during the last half of the larval period when all larvae were present feeding against the developing caryopsis. Consequently it is unlikely that the mechanism of antibiosis in this line is related to chemical properties present in the caryopsis that are toxic to larval growth.

Instead it may be that larvae reared on this line have initial difficulty feeding against the developing caryopsis, caused by some component of internal spikelet morphology. A cramped spikelet environment caused by small, tough and tightly closed glumes may be one likely explanation. Indeed MB110 contained the shortest, toughest and most tightly wrapped glumes, of all lines tested (unpublished data). While these characteristics are also likely to be the cause of the extremely high rates of ovipositional-antixenosis in this line, they may also create a cramped internal spikelet environment that makes initial larval feeding and establishment against the caryopsis difficult.

### **Antibiosis caused by the developing caryopsis**

A second pattern of larval antibiosis was observed in several antibiotic lines, which recorded increased larval mortality over time as larval feeding against the developing caryopsis increased over time. The clearest examples for this were observed under the water injection studies in Chapters 4 and 5. In contrast to natural egg lay, the water injection technique artificially deposited over 85% of eggs between the lemmas and glumes of all lines, away from the developing caryopsis (Chapter 5). As movement to the caryopsis to feed increased over time, so a proportional increase in larval mortality was similarly observed in the antibiotic lines SC62C, TX2880, PM7017, IS 10759 and ICSV745. The pattern was most evident in ICSV745 which only recorded larval mortality from day three corresponding to an increase of larval feeding against the caryopsis from 3-53% (Table 5.3 and Figure 5.1). In fact larvae forced to feed away from the developing caryopsis in this line (when the caryopsis

was artificially removed) survived at similar rates to those feeding at the same locations in other antixenotic and susceptible lines [Chapter 6; Trial (b)]. Consequently, it is clearly the developing caryopsis that causes larval mortality in this line.

## **Value of Antibiosis Mechanisms**

### **Antibiosis during larval establishment**

While the antibiosis mechanism present in MB110 is unique and effective, it may be caused by spikelet morphology characters that also effect high levels of ovipositional-antixenosis. The purpose of this study was to identify new source of resistance with different mechanisms that would broaden the genetic base of resistance in the breeding program. As such selection of this source of antibiosis may not achieve this outcome.

In addition QDPI&F breeders have found it difficult to produce agronomically acceptable germplasm from this line (Henzell pers com.). In particular seed threshing at harvest in this line and lines developed from this material is very poor, directly related to the tough tightly closed glumes that cause high levels of midge resistance. Consequently the resistance present in MB110 is unlikely to be selected for further use in the Australian sorghum breeding program.

### **Antibiosis caused by developing caryopsis**

The highest levels of larval antibiosis were caused by larval feeding against the caryopsis in lines developed from the Indian land race line DJ6514.

This material under repeated testing in over 20 separate replicated combined glasshouse and field trials has shown the following positive characteristics:

- Antibiosis is effective over the entire larval period at all levels of midge infestation and will directly increase seed set three-fold.
- This antibiotic caryopsis has an anti-feedant effect that causes a change in larval feeding behaviour which contributes an additional form of resistance – ‘antixenosis to caryopsis feeding’

- Mechanism is directly related to properties within the developing caryopsis alone, is inherited equally in all seed types (red, brown, testa present absent etc.) and is independent of glume morphology
- Is inherited by a single gene from a diverse Indian background, specifically from the Indian land race line DJ6514

Despite these positives, the above source of antibiosis may be of little value to the breeding program if the resistance is not durable. For this reason, I believe that the issue of resistance durability needs to be addressed to ensure that the resistance is deployed in the most sustainable way.

### **The issue of resistance durability**

History shows that single gene biochemically-based sources of insect resistance are often rapidly overcome by avirulent insect biotypes. A classic example of this occurred in wheat with respect to an insect very closely related to the sorghum midge, the Hessian fly, *Mayetiola destructor* Say (Painter, 1951).

In the above case the build up of insect biotypes resulted from selection in the insect population in response to exposure to resistant varieties and in most cases has been reported to occur on a gene for gene basis where both the plant and insect contain respective avirulence and virulence genes. The intensity and duration of this selection and the initial frequency and recessive or dominant nature of both insect and plant resistance genes were the major factors that governed the rate of biotype development (Bouhssini *et al.* 2001). At present in Hessian fly and most other economic CCecidomyiidae, the exact chemical induced plant response and subsequent insect-plant interaction is still very poorly understood even though many virulence and avirulence genes have been identified through genetic mapping (Harris *et al.* 2003).

In the case of sorghum midge there is little current evidence for avirulent biotype development of sorghum midge populations within antibiotic or antixenotic cultivars.

While one report of potential resistance breakdown was reported in Kenya within DJ6514 and its derivatives (Sharma *et al.* 1999), this author later associated this situation to an environment by genotype effect caused by low temperatures during flowering and grain fill (Sharma *et al.* 2003). Consequently, while there is some evidence for resistance breakdown of antibiosis by resistance breaking biotypes, a more likely explanation for the susceptibility of these lines is a variable expression of the physio-chemical causes of resistance within the developing caryopsis under adverse environmental conditions. This interpretation of data may be consistent with what was observed in my results in Chapter 7, where variable expression of antibiosis was recorded in both homozygous resistant lines, and even more so in heterozygous lines under only slightly different environmental conditions.

Apart from this one example no other reports of resistance breakdown exist and this may be due to some unique factors associated with the biology and population dynamics of sorghum midge in sorghum.

Franzmann (2004) made a specific examination of several factors in Australia that may limit the build up of avirulent biotypes in sorghum that are common across all resistant cultivars. Firstly, several alternative hosts, forage sorghum and Johnston grass are constantly available in Australia throughout much of the year, particularly over summer when grain sorghum is grown. Johnston Grass is known to be the dominant host of the first two sorghum midge generations, confirmed in survey results by Franzmann (2004). Both forage sorghum and Johnston grass as sorghum midge hosts are completely susceptible to sorghum midge development and produce inter-mixing sorghum populations that are presumed conspecific (Congdon *et al.* 2002).

Secondly, in what is probably a unique situation in the case of the sorghum midge and sorghum, a large number of pedicellate spikelets (that contain no caryopsis) occur in all sorghum plants that act as suitable hosts (Franzmann 1993b). In my studies these pedicellate spikelets were infested in significant numbers on the lines DJ6514, ICSV197 and ICSV745 and produced similar numbers of midge larvae to those that survived in the sessile spikelets of these antibiotic lines (Table 2.1).

Importantly these larvae in the same study developed at similar rates across susceptible and antibiotic genotypes. As such there is no evidence for the expression of antibiosis in pedicellate spikelets, while significant levels of midge infestation and development are known to occur equally in both susceptible and resistant lines.

This study also showed an additional factor that reduces selection pressure for resistance within antibiotic cultivars within the sessile spikelet. Antixenosis to caryopsis feeding (tolerance) was only observed in antibiotic lines related to DJ6514 (Chapters 2 and 6). This escape mechanism (tolerance) further reduces the selection pressure on the build up of possible midge biotypes within the midge population in these cultivars.

Within antibiotic cultivars there may also be further potential to select lines that contain higher rates of tolerance and lower rates of direct larval mortality (antibiosis). In Chapter 2 results a commercial elite breeding line (Pioneer 3) recorded similar levels of total seed set to that in ICSV745 from the combined effect of direct larval mortality (16-41%) and higher rates of tolerance (24-62%). Under this situation the midge population within the sessile spikelets was reduced by only 10-33% compared to those reared on ICSV745 where the midge population was reduced by 42-80%.

## **Future Directions**

### **Breeding for antibiosis using marker-assisted selection**

Within the QDPI & F breeding program advances in several areas are being made by using molecular markers to tag valuable breeding traits such as midge resistance (Tao *et al.* 2003). Breeding for antibiosis within the Australian breeding program is expected to be made simple and highly efficient due to the more recent development of marker-assisted selection using PCR-based molecular markers that are closely associated with the antibiosis mechanism of resistance (Hardy *et al.* 2001). This approach is underway within elite breeding populations and will allow the precise selection of antibiosis in select segregating populations without expensive large scale



phenotype field trials that are often affected by variable midge populations. Analysis of these trials is made further difficult by the presence of the antixenosis mechanism of resistance.

Current trial results indicate that the introgression of the antibiosis QTL into midge susceptible genetic backgrounds results in the expression of a level of resistance equivalent to a 4-5 rated hybrid under the Australian midge rating scheme. In addition the introgression of the antibiosis QTL into lines with a with a low but useable level of antixenosis (2-4 rating) generates lines that record a very high level of resistance equivalent to that of a 7 or 8+ hybrid (Hardy & Jordan 2006). Commercial hybrids with this level of resistance are rarely likely to require insecticide control (Chapter 1: Table 1.1).

### **Cloning the antibiosis gene**

The ability to isolate and clone cereal plant genes for insect resistance genes is now possible and has been completed in several cereal crops (Pan *et al.* 2000). In wheat, several Hessian fly insect resistance genes have been cloned, while in all other CCecidomyiidae, no published reports exist as yet. Consequently, while latest plant mapping data reveal a high level of synteny between cereal genomes, as yet there is no clear evidence for orthologous gene action across midge species across cereal crops (Harris *et al.* 2003).

In the case of sorghum, researchers at the Texas A&M University in partnership with Australian QDPI&F researchers are attempting to obtain the antibiosis gene of interest using a map based cloning approach (Hardy & Jordan 2006). Current mapping results confirm that a single gene is present on a part of the sorghum chromosome seven that has a high level of synteny with a part of rice chromosome four (unpublished data). As such there is a possibility that the resistance mechanism may be common or similar to a gene that is present in rice. This work remains highly promising and is likely to reveal the exact cause of antibiosis and produce near perfect in-gene molecular markers for antibiosis. Under this situation a greater understanding of the durability of the antibiosis mechanism may be possible, while at

the same time antibiosis may be rapidly deployed into commercial hybrids as the markers are likely to be effective in all sorghum breeding programs.

## **Summary and Conclusions**

The source of antibiosis derived from DJ6514 characterised in this study is genetically diverse from other resistance sources currently present in Australian commercial hybrids and highly effective, capable of increasing the level of resistance many fold. When combined with low levels of antixenosis in most elite breeding lines, the combined expression of both resistance mechanisms in this material results in a level of resistance that approaches practical field immunity under Australian conditions. At present the exact cause of antibiosis remains unclear, as does our understanding of the long term durability of this resistance mechanism. A greater understanding of the causes of resistance and the potential for resistance breakdown may be gained if new gene cloning technologies enable researchers to uncover the physiological process within the caryopsis that causes this resistance.

Despite these unknowns the results recorded in this study combined with a more detailed view of the dynamics of sorghum midge in Australia show that the antibiosis mechanism of resistance is not likely to breakdown (Franzmann, 2004). Consequently QDPI&F sorghum breeders continue to advance this new source of midge resistance in elite breeding populations. This process is expected to be made highly efficient by the recent development of molecular markers for the antibiosis gene. In the near future Australian grain sorghum producers will have access to sorghum hybrids that are highly midge resistant to the point of practical field immunity, a situation ideal for sustainable sorghum production in Australia.

## List of References

- Adamson, D., Thomas, G., & Davis, E. 1997, 'An Economic Estimate of Helicoverpa's Effect on Australian Agricultural Production,' Cooperative Research Centre for Tropical Pest Management, Brisbane.
- Baxendale, F.P., & Teetes, G.L. 1981, 'Production of unisexual progenies by the sorghum midge, *Contarinia sorghicola*,' *Annals of the Entomological Society of America*, Vol. 74, pp. 412-413.
- Baxendale, F.P., Teetes, G.L., & Sharpe, P.J.J. 1984, 'Temperature-dependent model for sorghum midge (Diptera: Cecidomyiidae) spring emergence,' *Environmental Entomology*, Vol. 13, pp.1566-1571.
- Berquist, R.R., Rotar, P., & Mitchell, W.C. 1974, 'Midge and anthracnose head blight resistance in sorghum,' *Tropical Agriculture (Trinidad)*, Vol. 51, pp. 431-435.
- Binns, M.R. & Nyrop, J. P., 1992, 'Sampling insect populations for the purpose of IPM decision making,' *Annual Review of Entomology*, Vol., 37, pp. 427-453.
- Bowden, J. & Neve, R.A. 1953, 'Sorghum midge and resistant varieties in the Gold Coast,' *Nature*, Vol. 172, pp. 551.
- Coquillett, D.W. 1898, 'A cecidomyid injurious to seeds of sorghums,' *USDA Agricultural Division Entomological Bulletin*, Vol 18, pp. 81-82.
- Cronholm, G., Knutson, A., Merchant, M., & Teetes, G.L. 1993, 'Managing insect and mite pests of Texas sorghum,' *Texas Agricultural Extension Service B-1220*, College Station.
- Dean, W.J. 1911, 'The sorghum midge' *USDA Entomological Bulletin*, Vol. 85, pp. 39-58.
- Diarisso, N.Y. 1997 'Spikelet flowering time and morphology as causes of sorghum resistance to sorghum midge (Diptera: Cecidomyiidae),' *Ph.D. thesis*, Texas A&M University, College Station.
- Ding, H., Lamb, R. & Ames, N. 2000, 'Inducible production of phenolic acids in wheat and antibiotic resistance to *Sitodiplosis mosellana*,' *Journal of Chemical Ecology*, Vol. 26, pp. 969-985.
- Doggett, H. 1988, *Sorghum*, Longman Scientific and Technical, New York, 2<sup>nd</sup> ed.  
 Ervin, R.T., Khalema, T.M., Peterson, G.C., & Teetes, G.L. 1996, 'Cost/Benefit analysis of a sorghum hybrid resistant to sorghum midge(Diptera:Cecidomyiidae),' *Southwestern Entomologist*, Vol. 21, pp. 105-115.

Doering, G.W., & Randolph, N.M. 1963, 'Habits and control of the sorghum midge, *Contarinia sorghicola*, on grain sorghum,' *Journal of Economic Entomology*, Vol. 56, pp. 454-459.

FAO. 1995, *Production Yearbook, FAO Statistics Series*, Food and Agriculture Organization of the United Nations, Rome.

Faris, M.A., Lira, Mde A., & Veiga, A.F.S.L. 1979, 'Stability of sorghum midge resistance,' *Crop Science*. Vol. 19, pp. 577-580.

Farrell, J.A.K. 1977, 'Plant resistance to insects and the selection of resistant lines,' *New Zealand Entomologist*, Vol. 6, Issue 3, pp. 244-261.

Fisher, R.W. 1981, 'Adult emergence and oviposition of the sorghum midge, *Contarinia sorghicola* (Coquillett),' in relation to abiotic environmental influences' M.S. thesis, Texas A&M University, College Station.

Fisher, R.W., & Teetes, G.L. 1982, 'Effects of moisture on sorghum midge (Diptera; Cecidomyiidae) emergence,' *Environmental Entomology*, Vol 11, pp. 946-948.

Fisher, R.W., Teetes, G.L., & Baxendale, F.P. 1982, 'Effects of time of day and temperature on sorghum midge emergence and oviposition,' *Texas Agricultural Experimental Station*, PR-4029.

Fisher, K.S. & Wilson, F.L. 1975, 'Studies of grain production in *Sorghum bicolor* (L. Moench). III. The relative importance of assimilate supply, grain growth capacity and transport system,' *Australian Journal of Agricultural Research*, Vol. 26, pp. 11-23.

Forrester, N.W. 1987, 'Diapause development in the sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera; Cecidomyiidae),' *Bulletin of Entomological Research*, Vol. 77, 581-587.

Forrester, N. W., Cahill, M., Bird, L. J. & Layland J. K. 1993, 'Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera:Noctuidae) in Australia. *Bulletin of Entomological Research*, Supplement Series. No. 1.

Franzmann, B.A. 1988, 'Components of resistance to sorghum midge in grain sorghum,' *Proceedings of Ninth Australian Plant Breeding Conference*, pp. 277-278.

Franzmann, B.A. 1990, 'Midge oviposition on post-flowering panicles,' *Sorghum Newsletter*, Vol. 31, pp. 60.

Franzmann, B. A. 1993 a, 'Ovipositional antixenosis to *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in Grain Sorghum,' *Journal of the Australian Entomology Society*, Vol. 32, pp. 59-64.

- Franzmann, B.A. 1993 b, 'Development of *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in pedicellate sorghum spikelets,' *Journal of the Australian Entomological Society*, Vol. 32, pp. 65-66.
- Franzmann, B.A. 2004, 'Resistance to sorghum midge and management of panicle pests in sorghum,' *PhD dissertation*, School of Life Sciences, University of Queensland.
- Franzmann, B.A. 1996, 'Evaluation of a Laboratory Bioassay for Determining Resistance Levels to Sorghum Midge *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in Grain Sorghum,' *Journal of the Australian Entomological Society*, Vol. 32, pp. 65-66.
- Franzmann, B.A. & Butler, D.G. 1993, 'Compensation for reduction in seed set due to sorghum midge (*Contarinia sorghicola*) in midge-susceptible and midge-resistant sorghum (*Sorghum bicolor*),' *Australian Journal of Experimental Agriculture*, Vol. 33, pp. 193-196.
- Franzmann, B.A., Butler, D.G., Henzell, R.G., Fletcher, D. & Cutler, J.H. 1996, 'A sorghum industry scheme assigning midge resistance levels to commercial hybrids,' *Proceedings Third Australian Sorghum Conference, Tamworth, 20 to 22 February, 1996* (eds M.A. Foale, B.W. Hare & R.G. Henzell), pp. 359-363. Institute of Agricultural Science, Melbourne.
- Franzmann, B.A. & Hardy, A.T. 1996, 'Testing the host status of Australian indigenous sorghums for the sorghum midge,' *Proceedings Third Australian Sorghum Conference, Tamworth, Occasional Publication No. 93*. pp. 365-367.
- Franzmann, B.A. & Lloyd, R.J. 1995, 'Sorghum midge *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae) in Australia produces unisexual progeny,' *Australian Entomologist*, Vol. 22, pp. 69-70.
- Franzmann, B.A., Lloyd R. J., & Zalucki, M. P. 2006 'Effect of soil burial depth and wetting on mortality of diapausing larvae and patterns of post-diapause adult emergence of sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Diptera: Cecidomyiidae),' *Australian Journal of Entomology*, Vol. 45, pp. 192-197
- Franzmann, B.A., Page, F.D. & Modini, M.P., 1986, 'Evaluating midge resistance in grain sorghum' *Proceedings of the 1<sup>st</sup> Australian Sorghum Conference*, pp. 3.20-3.26.
- Franzmann, B.A. & Sharma, H. C. 2002, 'Orientation of Sorghum Midge, *Stenodiplosis sorghicola*, Females (Diptera: Cecidomyiidae) to Color and Host-Odor Stimuli,' *Journal of Agricultural Urban Entomology*, Vol. 18, pp. 237-248.
- Franzmann, B.A., Titmarsh, I.J., Greig, J.C., & McAlpine, J.D. 1989, 'Life table studies of sorghum midge in midge-susceptible and resistant grain sorghum,' *Proceedings Australian Sorghum Workshop, Toowoomba*, pp.122-129.

- Franzmann, B.A. & Vaschina, B.K. 1989, 'Sorghum midge oviposition in preflowering and flowering panicles,' *Proceedings Australian Sorghum Workshop*, Toowoomba, pp. 119-121.
- Franzmann, B.A. & Zalucki, M. P. 1993, 'Effect of resistant sorghum on sorghum midge, *Contarinia sorghicola* (Coquillett) populations – a simulation study,' In: *Pest Control and Sustainable Agriculture* (eds SA Corey, DJ Dall & WM Milne), pp. 323-325. CSIRO Publications, Melbourne, Australia.
- Gagne, R.J. 1989, '*The plant-feeding gall midges of North America.*' Comstock Publishing Associates, Cornell University Press, USA.
- Hallman, G.J., Teetes, G.L., & Johnson, J.W. 1984, 'Relationship of sorghum midge (Diptera: Cecidomyiidae) density to damage to resistant and susceptible sorghum hybrids,' *Journal of Economic Entomology*, Vol. 77, pp. 83-87.
- Hamilton, R.I., Subramanian, B., Reddy, M.N., & Rao, C.H. 1982, 'Compensation in grain yield components in a panicle of rainfed sorghum,' *Annals of Applied Biology*, Vol. 101, pp. 119-125.
- Harding, J. 1965, 'Ecological and biological factors concerning the sorghum midge in 1964,' *Texas Agriculture Experimental Station*, MP-773, College Station.
- Harris, K.M. 1961, 'The sorghum midge,' *Annals of Applied Biology*, Vol. 84, pp. 114-118.
- Hardy, A. T., & Franzmann, B.A.. 2001, 'The Relationship between Spikelet Flowering Time and Ovipositional Antixenosis Resistance to the Sorghum Midge', *Proceedings of the Fourth Australian Sorghum Conference*, Kooralbyn – Gold Coast Hinterland, CD Rom.
- Hardy, A. & Jordan D. 2006 'Making Australian grain sorghum immune to the mighty midge! (The current status of sorghum midge resistance in Australia)', *Proceedings of the Fifth Australian Sorghum Conference*, Gold Coast, CD Rom.
- Hardy, A., Tao, Y., & Franzmann, B. 2001, 'New Host-Plant Resistance to the Sorghum Midge and the Use of Molecular Marker Mediated Selection', *Proceedings of the Fourth Australian Sorghum Conference*, Kooralbyn – Gold Coast Hinterland, CD Rom.
- Harris, K.M. 1979, 'Descriptions and host ranges of the sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae), and of eleven new species of *Contarinia* reared from Gramineae and Cyperaceae in Australia,' *Bulletin of Entomological Research*, Vol. 69, pp.161-182.
- Harris, M.O., Stuart, J.J., Modan, M., Nair, S., Lamb, R.J. and Rohfritsch, O. 2003, 'Grasses and gall midges: plant defense and insect adaptation,' *Annual Review of Entomology*, Vol. 48, pp. 549-577.
- Henzell, R.G., Franzmann, B.A., & Brengman, R.L. 1994, 'Sorghum midge research in Australia,' *International Sorghum and Millets Newsletter*, No. 35, pp. 41-47.

Henzell, R.G., & Gillieron, W. 1973, 'Effect of partial and complete panicle removal on the rate of death of some Sorghum bicolor genotypes under moisture stress,' *Queensland Journal of Agriculture and Animal Science*, Vol. 30, pp. 291-299.

Henzell, R.G. & Hare, B.W. 1996, 'Sorghum Breeding in Australia – Public and Private Endeavours', *Proceedings of the Third Australian Sorghum Conference*, Occasional Publication No. 93. pp.159-171.

Henzell, R.G., Peterson, G.C., Teetes, G.L., Franzmann, B.A., Sharma, H.C., Youm, O., Ratnadass, A., Toure, A., Raab, J. & Ajayi, O. 1996, 'Breeding for resistance to panicle pests of sorghum and pearl millet,' *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*, Lubbock, Texas, September, 1996, pp. 255-280. INTSORMIL & ICRISAT.

Harris, K. M. 1961, 'The sorghum midge, *Contarinia sorghicola* (Coq.) in Nigeria,' *Bulletin of Entomological Research*, Vol. 52, pp. 129-146.

ICRISAT, 1992, 'The medium-term plan,' International Crops Research Institute for the Semi-Arid Tropics, Patancheru, AP. India.

Jadhav, R. & Jadhav, L.D. 1978, 'Studies on preliminary screening of some sorghum hybrids and varieties against earhead midge (*Contarinia sorghicola* Coq.),' *Journal of the Maharashtra Agricultural Universities*, Vol. 3, pp. 187-188.

Johnson, J.W., Rosenow, D.T., & Teetes, G.L. 1973, 'Resistance to the sorghum midge in converted exotic sorghum cultivars,' *Crop Science*, Vol 13, pp. 754-755.

Jordan, D.R., Tao, Y.Z., Godwin, I.D., Henzell, R.G., Copper, M., & McIntyre C.L., 1998, 'Loss of genetic diversity associated with selection for resistance to sorghum midge in Australian sorghum,' *Euphytica*, Vol. 102 pp. 1-7.

Jotwani, M.G. 1978, 'Investigations on insect pests of sorghum and millets with special reference to host plant resistance. Final Technical Report (1972-77),' *Research Bulletin of the Division of Entomology, New Delhi, India*: Indian Agricultural Research Institute, 114 pp.

Kennedy, G.C., Gould, F., Deponti, M.B., & Stinner, R.E. 1987, 'Ecological agricultural genetic and commercial considerations in the deployment of insect-resistant germplasm,' *Environmental Entomology*, Vol. 16, pp. 327-338.

Kogan, M., & Ortman, E.E., 1978, 'Antixenosis – a new term proposed to replace Painter's 'non-preference' modality of resistance,' *Bulletin of the Entomological Society of America*, Vol. 24, pp. 175-176.

Kulkarni, K.A. 1985, 'Screening sorghums for midge resistance,' *Proceedings of the International Sorghum Entomology Workshop*, 15-21 July, 1984, pp. 265-274. Texas A&M University and ICRISAT.

- Lamb, R.J., McKenzie, I.L., Wise, R.S., Barker, P.S., & Smith, M.A.H. 2000, 'Resistance to *Sitodiplosis Mosellana* (Diptera: Cecidomyiidae) in spring wheat (Gramineae),' *The Canadian Entomologist*, Vol. 132, pp. 591-605.
- Henzell, R.G., Bregman, R.L. & Page, F.D. 1980, 'Transference of sorghum midge resistance into agronomically acceptable lines,' In; *Proceedings of the Australian Agronomy Conference*, Lawes, April 1980, p. 233.
- Martins, A.J. 1977 'Influencia do teor de tanino em genótipos de sorgo, *Sorghum bicolor* (L.) Moench, sobre o ataque da *Contarinia sorghicola* (Coquillett, 1898) (Dip. Cecidomyiidae). (InPT.),' *M.S. thesis, Universidade Federal do Ceara*, Fortaleza, Brazil, 44 pp.
- Maxwell, G.G. & Jennings, P.R. 1980, 'Strategies of race-specific phytoparasitism and its control by plant breeding,' *Genetika*, Vol. 9, pp. 237-255.
- Melton, K.D. & Teetes, G.L. 1984, 'Effects of resistant sorghum hybrids on sorghum midge (Diptera: Cecidomyiidae) biology,' *Journal of Economic Entomology*, Vol. 77, 626-631.
- Modini, M.P., Page, F.D., & Franzmann, B.A. 1987, 'Diurnal oviposition activity in grain sorghum by *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae),' *Journal of the Australian Entomology Society*, Vol. 26, pp.293-294.
- Murray, D., Ferguson, J., Lloyd, R., Hopkinson, J., Maclean, S. & Powell, R. 2001, 'Advances in heliothis management on grain sorghum in Australia,' *Proceedings of the Fourth Australian Sorghum Conference*, Kooralbyn – Gold Coast Hinterland, CD Rom.
- Murray, D., Miles, M. & Ferguson, J. 2000, 'Area-wide management of heliothis – results of current studies,' *Proceedings of the Tenth Australian Cotton Conference*, Brisbane, pp. 11-17.
- Murray, D.A.H, Monsour, C.J., Teakle, R.E., Rynne, K.P. & Bean, J.A. 1995, 'Interactions between nuclear polyhedrosis virus and three larval parasitoids of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae),' *Journal of the Australian Entomological Society*, Vol. 34, pp. 319-322.
- Murray, D.A.H. & Zalucki, M.P. 1994, 'Spatial distribution and mortality of *Helicoverpa* spp. pupae on the Darling Downs, Queensland,' *Journal of the Australian Entomological Society*, Vol. 33, pp. 193-198.
- Natarajan, K. & Chelliah, S. 1985, 'Studies on the sorghum grain midge, *Contarinia sorghicola* Coquillett, in relation to environmental influence,' *Tropical Pest Management*, Vol. 31, pp. 276-285.
- Page, F.D. 1979, 'Resistance to sorghum midge (*Contarinia sorghicola* Coquillett) in grain sorghum,' *Australian Journal of Experimental Agriculture and Animal Husbandry*, Vol. 19, pp. 97-101.



- Painter, R.H. 1951, *Insect resistance in crop plants*, University Press of Kansas, Lawrence.
- Pan, Q., Wendel, J., Fluhr, R. 2000, 'Divergent evolution of plant MBS-LRR resistance gene homologues in dicot and cereal genomes,' *Journal of Molecular Evolution*, Vol. 50, pp. 203-213.
- Passlow, T. 1958, 'Parasites of sorghum midge, *Contarinia sorghicola* (Coq.) in Queensland' *Queensland Journal of Agriculture and Animal Science*, Vol. 15, pp. 35-36.
- Passlow, T. 1965, 'Bionomics of sorghum midge (*Contarinia sorghicola* (Coq.)), in Queensland, with particular reference to diapause,' *Queensland Journal of Agriculture and Animal Science*, Vol 22, pp. 149-167.
- Passlow, T., Franzmann, B.A., & Allsopp, P.G. 1985, 'Sorghum insect problems in Australia,' In: *Proceedings of the International Sorghum Entomology Workshop*, 15-21 July 1984, pp. 65-72. Texas A&M University and ICRISAT.
- Pendleton, B. B., Teetes, G.L., & Peterson, G.C. 1994, 'Phenology of sorghum flowering' *Crop Science*, Vol 34, pp. 1263-1266.
- Peterson, G. C., Johnson, J. W., Teetes, G. L., & Rosenow, D. T. 1985, 'Registration of midge resistant sorghum germplasm,' *Crop Science*, Vol. 25, pp. 372.
- Peterson, G. C., Teetes G.L., & Pendleton, B. B. 1994, 'Resistance to the sorghum midge in the United States,' *International Sorghum and Millets Newsletter*, Vol. 35, pp. 48-63.
- Rossetto, C.J. 1977, 'Tipos de resistencia de sorgo *Sorghum bicolor* (L.) Moench, a *Contarinia sorghicola* (Coquillet, 1898). (In Pt.)', *Thesis, Universidade Estadual Paulista, Jaboticabal*, Sao Paulo, Brazil, 34 pp.
- Rossetto, C. J., Goncalves, W., & Diniz, J.L.M. 1975, 'Resistencia de variedade AF-28 a mosca do sorgo, *Contarinia sorghicola*, no ausencia de outras variedades,' *Anais Da Sociedade Entomologica Do Brazil*, Vol 4, pp. 16-20.
- Rossetto, C.J., Nagai, V., & Overman, J. 1984, 'Mechanisms of resistance in sorghum variety AF-28 to *Contarinia sorghicola* (Diptera: Cecidomyiidae),' *Journal Economic Entomology*, Vol. 77, pp.1439-1440.
- Russell, G.E. 1978, 'Plant Breeding for Pest and Disease Resistance,' *Butterworth Technical Publication, London*, pp. 313.
- Santos, J.H.R., & Carmo, C.M. 1974, 'Evaluation of resistance to *Contarinia sorghicola* by sorghum lines from Cameroon, Africa collection in Ceara, Brazil,' *Sorghum Newsletter*, Vol. 17, pp. 12-13.

Schalk, J.M., & Ratcliffe, R. H. 1976, 'Evaluation of ARS program on alternative methods of insect control: host plant resistance to insects,' *Bulletin of Entomological Research*, Vol. 22, pp. 7-10.

Sharma, H.C. 1985, 'Screening for sorghum midge resistance and resistance mechanisms,' *Proceeding of the International Sorghum Entomology Workshop*, Texas A&M University and ICRISAT, pp. 89-95.

Sharma, H.C. 1993, 'Host plant resistance to insects in sorghum and its role in integrated pest management,' *Crop Protection*, Vol. 12, pp.11-34.

Sharma, H.C., Agrawal, B.L., Abraham, C.V., Vidyasagar, P., Nwanze, K.F., & Stenhouse, J. W. 1994, 'Registration of nine sorghum lines with resistance to sorghum midge: ICSV 692, ICSV 729, ICSV 730, ICSV 731, ICSV 736, ICSV 739, ICSV 744, ICSV 745, and ICSV 748,' *Crop Science*, Vol. 34, pp. 1425-1426.

Sharma H.C., Franzmann B.A. & Henzell R.G. 2002 'Mechanisms and diversity of resistance to sorghum midge, *Stenodiplosis sorghicola*' *Euphytica*, Vol. 124, pp. 1-12.

Sharma H.C & Hariprasad K. V. 2002 'Flowering events in sorghum in relation to expression of resistance to sorghum midge, *Stenodiplosis sorghicola*' *Euphytica*, Vol. 127, pp. 411-419.

Sharma, H.C., Leuschner, K. & Vidyasagar, P. 1990a, 'Factors influencing oviposition behaviour of the sorghum midge, *Contarinia sorghicola* Coq.,' *Annals of Applied Biology*, Vol 116, 431-439.

Sharma, H.C., Leuschner, K. & Vidyasagar, P. 1990b, 'Componental analysis of the factors influencing resistance to sorghum midge, *Contarinia sorghicola* Coq.,' *Insect Science and its Application*, Vol 11, No. 6, pp. 889-898.

Sharma, H. C. & Teetes G. L. 1995, 'Yield loss assessment and economic injury levels for panicle-feeding insect pests of sorghum,' *Proceedings of the International Consultative Workshop on Panicle Insect Pests of Sorghum and Pearl Millet*, Niamey, Niger, pp. 125-134.

Sharma, H.C. & Vidyasagar, P. 1994, 'Antixenosis component of resistance to sorghum midge, *Contarinia sorghicola* Coq. in *Sorghum bicolor* (L.) Moench.' *Annals of Applied Biology*, Vol. 124, pp. 495-507.

Sharma, H.C., Vidyasagar, P., & Leuschner, K. 1990, 'Componental analysis of the factors influencing resistance to sorghum midge, *Contarinia sorghicola* Coq.,' *Insect Science Applications*, Vol. 11, pp. 889-898.

Sharma, H.C., Vidyasagar, P., & Subramanian, V. 1993, 'Antibiosis component of resistance in sorghum to sorghum midge, *Contarinia sorghicola*,' *Annals of Applied Biology*, Vol. 123, pp. 469-483.

- Shyamsunder, J., Parameswarappa, R., Nagaraja, H.K., & Kajjari, N.B. 1975 'A new genotype in sorghum resistant to midge (*Contarinia sorghicola* Coq.),' *Sorghum Newsletter*, Vol. 20, pp. 68.
- Sosa, O. & Foster, J.E. 1976, 'Temperature and the expression of resistance in wheat to the Hessian fly,' *Environmental Entomology*, Vol. 5, No. 2, pp. 333-336.
- Stephens, J.C., & Quinby, J.R. 1934, 'Anthesis, pollination, and fertilization in sorghum,' *Journal of Agricultural Research*, Vol. 49, pp. 123-136.
- Stern, V.M., Smith, R.F., Bosch, van den R & Hagen, K.S., 1959 'The integrated control concept', *Hilgardia, A Journal Published by the California Agricultural Experiment Station*. Vol. 29, pp. 81-101.
- Summers, C.G. 1975, 'Daily adult emergence in the sorghum midge, *Contarinia sorghicola*,' *Environmental Entomology*, Vol. 4, pp.495-498.
- Taley, Y.M., Deore, B.P., & Thakare, D.R. 1971, 'Bionomics of *Contarinia sorghicola* Coquillet (Diptera:Cecidomyiidae) nonpreference for a resistant hybrid sorghum,' *Journal of Economic Entomology*, Vol. 33, pp. 202-208.
- Tao, Y.Z., Hardy, A., Drenth, J., Henzell, R.G., Franzmann, B.A., Jordan, D.R., Butler, D.G. & McIntyre, C.L. 2003, 'Identifications of two different mechanisms for sorghum midge resistance through QTL mapping,' *Theoretical and Applied Genetics*, Vol. 107, pp. 116-122.
- Tao, Y.Z., Jordan, D.R., Henzell, R.G., & McIntyre, C.L. 1998, 'Construction of a genetic map in a sorghum RIL population using probes from different sources and its alignment with other sorghum maps,' *Australian Journal of Agricultural Research*, 49, pp. 729-736
- Teakle, R.E., Page, F.D., Sabine, B.N.E. & Giles, J.E. 1983, 'Evaluation of heliothis nuclear polyhedrosis virus for control of *Heliothis armigera* on sorghum in Queensland, Australia,' *General and Applied Entomology*, Vol. 15, pp. 11-18.
- Teetes, G.L., & Johnson, J.W. 1978, 'Insect resistance in sorghum,' *Proceedings of the 33<sup>rd</sup> Annual Corn and Sorghum Research Conference*, Chicago, IL. pp.167-189.
- Teetes, G.L. 1985, 'Insect resistant sorghum in pest management,' *Insect Science and its Applications*, Vol. 6, pp. 443-451.
- Teetes, G.L., Becerra, M.L., & Peterson, G.C.1986, 'Sorghum midge (Diptera: Cecidomyiidae) management with resistant sorghum and insecticide,' *Journal Economic Entomology*, Vol. 79, pp. 1091-1095.
- Tryon, H. 1894, 'The insect enemies of cereals belonging to the genus *Cecidomyia*,' *Transactions of the Natural History Society of Queensland*, Vol. 1, pp. 81-83.
- Vanderplank J. E.1968, 'Disease Resistance in Plants' *New York Academic Press*, USA.

- Walter, E.V. 1941, 'The biology and control of the sorghum midge,' *USDA Technical Bulletin*, pp.778.
- Waquil, J.M. 1985, 'Resistance modality of hybrid sorghum to sorghum midge (Diptera: Cecidomyiidae),' Ph.D. dissertation, Texas A&M University, College Station.
- Waquil, J.M., Teetes, G.L., & Peterson, G.C., 1986a, 'Sorghum midge (Diptera: Cecidomyiidae) adult ovipositional behaviour on resistant and susceptible sorghums,' *Journal of Economic Entomology*, Vol. 79, pp. 530-532.
- Waquil, J.M., Teetes, G.L., & Peterson, G.C., 1986b, 'Adult sorghum midge (Diptera: Cecidomyiidae) nonpreference for resistant hybrid sorghum,' *Journal of Economic Entomology*, Vol. 79, pp. 455-458.
- Waquil, J.M., Teetes, G.L., & Peterson, G.C., 1986c, 'Comparison of immature sorghum midge (Diptera: Cecidomyiidae) development on resistant and susceptible sorghum,' *Journal of Economic Entomology*, Vol. 79, pp. 833-837.
- Wise, I., Lamb R. & Smith M. 2001 'Domestication of wheats (graminaceae) and their susceptibility to herbivory by *Sitodiplosis mosellana* (Diptera:Cecidomyiidae),' *The Canadian Entomologist*, Vol. 133, pp. 255-267.
- Wiseman, B.R., Widstrom, N.W., & McMillian, W.W. 1972, 'Flight movements and color preference of the sorghum midge,' *Journal of Economic Entomology*, Vol. 65, pp. 767-770.
- Wiseman, B.R., Duncan, R.R., & Widstrom, N.W. 1988, 'Registration of SGIRL-MR-3 and SGIRL-MR-4 midge resistant sorghum germplasms,' *Crop Science*, Vol. 28, pp. 202-203.
- Wuensche, A.L. 1980, 'An assessment of plant resistance to the sorghum midge, *Contarinia sorghicola*, in selected lines of *Sorghum bicolor*,' *Ph.D. thesis*, Texas A&M University, College Station, TX, USA, 193 pp.
- Young, W. R., & Teetes, G.L. 1977, 'Sorghum entomology,' *Annual Review of Entomology*, Vol. 22, pp. 193-218.