Chapter 6

Evaluation of Resistant Restorer Lines in Hybrid Combinations

Summary

Ten selected restorer lines (R-lines) were test-crossed with A89 (A-line) to produce F_1 hybrids that were evaluated in the field for resistance to *A. helianthi*. Two of the ten R-lines were selected for further evaluation based on the performance of these hybrids. Five commercial A-lines and the public line A89 were crossed with two restorer lines (R_1 and R_2), to produce twelve F_1 hybrids. These hybrids, their parental lines and three commercial hybrids were exposed to generated epidemics of *A. helianthi* at two field sites. Area under the disease progress curves (audpcs) were calculated for each line, from disease severity ratings (DSRs) taken from budding to late flowering. The commercial A-lines were as susceptible to *A. helianthi* as the susceptible line A89. R_1 and R_2 had high levels of resistance. Generally, resistance of the F_1 hybrids was greater than their midparent estimates, indicating a high degree of dominance for genes controlling resistance in the resistant parents R_1 and R_2.

6.1 Introduction

In sunflower, hybrids are generally produced by crossing two inbred parents (Figure 6.1). For ease of production, a cytoplasmic male sterile (*cms*) or A-line is crossed with a line which has fertility restoration genes, commonly known as the restorer or R-line (Fick, 1978). Seed stocks of the sterile A-line are maintained by crossing sterile A-line plants with a line which is genetically identical (isoline) except that it produces pollen. This line is often called the 'maintainer' or B-line and since it does not have fertility restoration genes, all progeny arising from the cross are sterile. The R-line is usually branched and produces numerous flowers over a period of several weeks, while the A-line is unbranched and produces a single flower head. During flowering, several rows of florets open each day and become receptive for fertilisation. This process continues for a period of 4-7 days. The extended flowering period of the branched R-line ensures the availability of pollen for fertilisation while the sterile A-line is flowering. Hence the

availability of pollen throughout this period is important for maximum seed production. Genes that control branching are recessive, hence the F_1 hybrids derived from crossing branched R-lines and single-headed A-lines have only a single flower head.

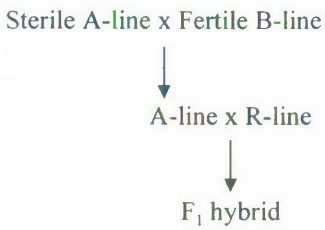

Figure 6.1. *The lines and process used in the production of single-cross sunflower hybrids.*

Disease resistance has commonly been incorporated in commercial hybrids via the restorer parent. Part of the reason for this is that the breeding and maintenance of *cms* parents is tedious and time consuming compared to the breeding of restorer lines. Dominant, single gene resistance is easily utilised by this method and as such has been exploited by sunflower breeders, particularly in relation to sunflower rust (*Puccinia helianthi* Schw.) and downy mildew (*Plasmopara halstedii* [Earl.] Berl & de Toni). Another factor that may have contributed to the reliance on restorer lines for resistance, was that the line HA89 provided sunflower breeders with an A-line of such a high standard, that it was not quickly or easily surpassed, and was therefore widely used for many years. This allowed breeders to concentrate their efforts on the development of restorer lines. Consequently, a vast number of restorer lines have been generated by sunflower breeders throughout the world. Together, these factors perhaps delayed the widespread development of superior female lines. Although many R-lines are available for public use, very few A-lines have been made available. This is because most A-line development is done by private seed companies who do not release inbred lines for public use.

When disease resistance is inherited in a simple Mendelian fashion, as with independent dominant genes, the resistance phenotype expected from a given cross is highly

predictable. Hence, genotypes can be determined from an examination of phenotypes, not only because the rules of inheritance governing dominance follow specific patterns but also because environment may have little effect on the expression of the phenotype. In contrast, where resistance is conditioned by many genes of minor effect, as is often the case with quantitative types of resistance, phenotypes do not reflect genotypes. This is due to a) the collective effect of genes which may exhibit little or no dominance and each gene either adds to or subtracts from the expression of the trait (Mather and Jinks, 1977) and b) the influence of environment on the expression of the trait (Falconer, 1989). The simplest description of this model is:

the phenotypic value = a genetic effect + an environmental effect + interaction

This implies that in the absence of dominance effects, the resistance of F_1 progeny arising from a cross between two lines of known phenotype is very difficult to predict. This has important implications in breeding for resistance to *A. helianthi* in sunflower, because all commercial hybrids are the F_1 progeny of crosses between specific inbred lines. Therefore, where QR is concerned, the final evaluation of any inbred line must be made by testing it in hybrid combinations. The degree of dominance and the additivity of genetic effects can then be determined, and appropriate breeding strategies formulated. But also, in order to obtain a more reliable estimate of the resistance phenotypic, hybrids must be tested across environments so that the environmental effects on the phenotype can be separated from genetic effects.

When resistance is of a quantitative nature, the 'midparental' pattern of inheritance can be used to predict the mean resistance of progeny from the mean resistance of their parents (Gilbert, 1989). This pattern implies that the average genetic values of resistance for all offspring is half-way between the genetic values of the two parents. This empirical model is based on the premise that males and females contribute equally to the genotypes of their offspring.

Since we can only measure phenotypic values, we can expect deviations from the midparent pattern due to the interaction between genetic and environmental effects. The magnitude of these effects can be determined by testing parental lines and their offspring in different environments. Theoretically, the magnitude of environmental effects could be determined by the degree of phenotypic variation among clones or alternatively, inbred lines. Deviations of the F_1 from the midparent give an indication of the level of dominance (Warner,1952).

The slope of the regression of progeny on midparent is recognised as a measure of heritability and is true providing the midparent pattern and the above equation are both correct. If a particular phenotype does reflect pure genetic effects, then the slope of the regression would equal one. Deviations from one occur because of the effect of environment on the expression of the phenotype and because of the collective effect of genes that increase or decrease the character. Heritability is usually determined from the regressions of F_2 parent on F_3 progeny data obtained from experiments that follow some specific mating design (Gardner, 1963).

The simultaneous segregation of the many genes that are thought to control QR cause populations to be characterised by continuous variation. Hence, in any one family, there is so much variation that predictions of individuals cannot be made. At best, family means can be predicted in accordance with the the midparent pattern (Gilbert, 1989).

Because of the way in which sunflower hybrids are produced, it is necessary for parental lines exhibiting quantitative traits to be tested in hybrid combinations. In this study, R-lines with resistance to *A. helianthi* were crossed with a range of A-lines and tested in the field using a generated epidemic of *A. helianthi.* The area under the disease progress curve (audpc) was used to evaluate and compare resistance among hybrid combinations.

6.2 Materials and Methods

6.2.1 Preliminary selection of R-lines

The testing of hybrids and their parents in replicated field trials is extremely demanding of resources. In order to accomodate this constraint, a decision needed to be made whether to test a large number of lines in small field plots, or alternatively, test a few lines in larger field plots. Due consideration of available resources led to acceptance of the latter choice. Thus, the few lines that would be tested in hybrid combination would need to be carefully selected, so a preliminary evaluation of R- lines was carried out.

Table 6.1. *Sunflower lines used in hybrid combination with B89 and oil contents of the hybrids grown in the field in 1992.*

Experiment Codes	Pedigree	Pedigree Codes	% oil content of hybrid
H_1	Arpop//Rpop/Charata	10020.11.2.28	40.4
H_2	Arpop//Rpop/Charata	10020.11.3.13	39.0
H_3	Arpop//Rpop/Charata	10020.11.4.20	39.0
H_4	Arpop//Rpop/Charata	10011.4.3.18	37.0
H_5	Arpop//Rpop/Charata	10011.4.3.16	36.5
H_6	ARpop//QSR1	10014.6.1.20	37.7
H_7	ARpop//QSR1	10014.6.1.11	37.4
H_8	ARpop//QSR1	10008.3.3.5	43.7
H_9	Arpop//Rx677	10019.1.2.10	35.3
H_{10}	Rpop//Charata	Rpop//Charata 3.2.1	36.3
B89	–	–	40.2
[a]Hysun 45CQ	–	–	40.9
[b]Suncross 40R	–	–	40.9
[c]Advance	–	–	41.6

[a] = commercial hybrid of Pacific Seeds Pty Ltd
[b] = commercial hybrid of Agseed research Pty Ltd
[c] = commercial hybrid of Pioneer Hi-Bred Australia Pty Ltd
– = unknown

Ten R-lines (Table 6.1) whose resistance to *A. helianthi* was evaluated in 1991 (Chapter 4), were selected for testcrossing. These lines were crossed with emasculated B89 plants to produce ten F_1 testcross hybrids. These testcross hybrids, as well as three commercial hybrids (Table 6.1), were planted in the field in 1992, but lack of disease development prevented their assessment for resistance to *A. helianthi*. Instead of waiting until the following season to repeat the field trial, the sunflower breeders from the three private

seed companies operating in Australia were invited to inspect the hybrids and evaluate them according to their agronomic attributes.

Seed of each hybrid was sampled for oil content using a Nuclear Magnetic Resonance (NMR) oil analyser. Consideration of oil content and agronomic attributes led to the selection of two R-lines lines, 10020.11.4.20 and 10008.3.3.5. These will be referred to as R_1 and R_2 respectively. These lines were used for further testing in hybrid combination with a range of commercial A-lines.

6.2.2. Hybrid production and field trial design

The two restorer lines R_1 and R_2 were crossed with the public line B89 as well as 5 proprietry A-lines, to produce twelve F_1 hybrids. The A-lines, hybrids and their experiment codes are shown in Table 6.2.

Table 6.2. *Hybrids that were produced from crosses between six A-lines and two selected R-lines*

Codes for A-lines	Source of A-lines	Codes for hybrids made with [a]R_1	Codes for hybrids made with [b]R_2
A_1	HA89 is a public line	[c]A_1R_1	A_1R_2
A_2	Pacific Seeds Pty Ltd	A_2R_1	A_2R_2
A_3	Pacific Seeds Pty Ltd	A_3R_1	A_3R_2
A_4	Agseed-Research Pty Ltd	A_4R_1	A_4R_2
A_5	Agseed-Research Pty Ltd	A_5R_1	A_5R_2
A_6	Pioneer Hi-Bred Pty Ltd	A_6R_1	A_6R_2

[a] = Refers to the restorer line 10020.11.4.20

[b] = Refers to the restorer line 10008.3.3.5

[c] = Refers to the A- and R-line combinations used to produce each F_1 hybrid. .

A large quantity of seed of each hybrid was required so that they could be tested at multiple field sites by each of the private-company sunflower breeders. Hybrid seed was therefore produced in the field in 1993. Each restorer line was grown with each of the six A-lines inside an open-weave fabric tent that was designed to exclude insects. A beehive

containing a nucleaus colony of bees was placed inside each tent at flowering to facilitate cross pollination. Seed was harvested from each A-line in January, 1994.

The two R-lines, six A-lines, twelve hybrids and the three commercial hybrids, Hysun 45CQ (Pacific Seeds), Suncross 41 (Agseeds Research) and Advantage (Pioneer Hi-Bred), were planted at two field sites on 10th February, 1994. Site1 was at the Queensland Department of Primary Idustries (QDPI) Research Station located at Gatton and Site2 was at QDPI research station at Kingsthorpe. These sites were the same as those used for the experiments described in Chapter 5. Due to the short period of time between harvest and planting, all hybrid seed was treated with Ethrel® (100ppm ethylene) to break germination dormancy. Seed was planted in 12.5m rows, with an inter-plant spacing of 0.2m and an inter-row spacing of 0.75m. Lines were randomised in three replications, however the A-lines and R-lines were kept together as a randomised group within each replicate (block) to reduce the adverse effects of competetion exerted by the more vigorous hybrids. Two rows of the susceptible line B89 were planted perpendicular to the ends of each treatment row and extended the full width of each replicate. These rows were spray inoculated with spores of *A. helianthi* when plants were at the V4 growth stage, then again 1 and 2 weeks later. Overhead misting was applied for 8h following each inoculation and then daily for 3–4 days after the inoculation, for a period of 4–6h. Disease assessment began at Site1, 61 days after planting when plants were at growth stage R2–R4 and at Site2, 67 days after planting when plants were at growth stage R1–R3. Plants at Site1 were assessed again at 70, 78, 85 and 92 days after planting. Plants at Site2 were assessed again at 78, 84 and 91 days after planting. Ten plants at intevals of about 1m were assessed in each row, allowing a space of about 1.75m from the ends of the rows to the first and last plants assessed. The 3rd or 4th pair of leaves of each assessed plant was marked with red paint, so that the same leaves were assessed at each assessment time. The proportion of diseased leaf tissue was determined for each marked leaf pair using a modified pictorial key (Appendix III).

6.2.3 Analysis of data

Area under the disease progress curves (audpcs) were calculated for all plants assessed at both sites. Data were log-transformed before comparing audpc means with Scheffe's test for significant differences (SSD). The difference between the midparent audpc and the F_1 hybrid audpcs were calculated for each sunflower line. Linear regression and Spearman's rank correlation were used to determine the degree of correlation between the resistance of sunflower lines grown at each field site. A combined analysis of variance was conducted and variance components partitioned to provide estimates of genotype x environment interactions. The g x e interaction was estimated as σ^2_{ge} = (interaction mean square - error mean square).

6.3 Results

6.3.1 Audpcs of parental lines and F_1 hybrids.

Disease intensity (audpc) at Site2 was on average, almost twice that observed at Site1. The restorer lines R_1 and R_2 were the most resistant parental lines at both sites (Table 6.3). Overall, there was no difference in resistance between the A-lines, however at Site1, A_4 had a significantly lower level of infection than all other A-lines.

At Site2, A_3R_2 was the most resistant hybrid, but there was no significant difference in resistance between any of the other hybrids (Table 6.4). At Site1, A_2R_1 was the most susceptible hybrid and A_5R_2 the most resistant. All other hybrids had the same level of resistance.

Audpcs for the sunflower lines grown at sites 1 and 2 were well correlated. Spearman's ranking of the lines gave a correlation coefficient of 0.750, while a coefficient of

determination (R) of 0.895 was obtained from the regression of audpcs at Site1 with Site2.

Table 6.3. *Area under the disease progress curves (audpcs) for the R-and A-lines grown at field sites 1 and 2. Values followed by the same letter are not significantly different.*

	Audpc means			
	Site 1		Site 2	
Parental lines	[a] transformed	untransformed	transformed	untransformed
R_1	4.148 b	68.38	4.143 c	74.53
R_2	4.265 b	82.90	4.180 c	87.87
A_1	4.915 a	165.23	4.954 a	186.63
A_2	4.851 a	137.57	5.228 a	233.77
A_3	4.762 a	134.83	4.953 ab	180.95
A_4	4.328 b	83.69	4.675 b	130.68
A_5	4.977 a	155.49	4.934 ab	173.65
A_6	4.760 a	137.48	4.656 b	147.68

[a] *Both transformed and untransformed data are presented. Untransformed data were used to generate the midparent values for the F_1 hybrids shown in Table 6.5. Audpc means were compared using log-transformed data.*

Table 6.4. *Area under the disease progress curves (audpcs) for F_1 hybrids grown at field sites 1 and 2. Values followed by the same letter are not significantly different.*

	[a] Audpc means	
Hybrid lines	Site 1	Site 2
A_1R_1	3.765 ab	4.074 a
A_2R_1	4.087 a	4.169 a
A_3R_1	3.830 ab	4.255 a
A_4R_1	3.902 ab	4.212 a
A_5R_1	4.019 ab	4.417 a
A_6R_1	4.009 ab	4.281 a
A_1R_2	3.458 b	3.880 a
A_2R_2	3.763 ab	4.258 a
A_3R_2	3.572 ab	3.481 b
A_4R_2	3.727 ab	4.088 a
A_5R_2	2.781 c	3.883 a
A_6R_2	3.791 ab	4.220 a
Hysun 45CQ	3.489 b	3.786 a
Suncross 41	4.002 ab	4.111 a
Advantage	3.977 ab	4.444 a

[a] *Data were log-transformed for analysis. Transformed data are presented.*

6.3.2. Differences between F_1 hybrids and midparent audpcs

Midparent audpcs for each hybrid (Table 6.5) were calculated by averaging the untransformed audpcs for the A- and R-line parents shown in Table 6.3. Overall, the hybrids were more resistant than was expected from their midparent values. This is reflected in the negative values that were obtained when the midparent audpcs were subtracted from the F_1 hybrid audpcs (F_1-MP; Table 6.5). At both field sites, all hybrids based on R_2 except for A_4R_2, were significantly more resistant than expected from the calculated midparent values. At Site1, five of the six hybrids based on R_1, were significantly more resistant than expected from the calculated midparent values, but at Site2, only three of these had audpcs smaller than their midparent. The hybrid A_4R_1 had the same resistance as the midparent at both field sites. At both sites, hybrids made with line R_2 were on average slightly more resistant than hybrids made with R_1.

Table 6.5. *Differences between F1 hybrid and midparent audpcs calculated from untransformed area under the disease progress curves (audpcs) for plants grown at sites 1 and 2.*

	Audpc means					
	Site 1			Site 2		
Hybrid code	F_1 hybrid	Midparent	F_1 - MP	F_1 hybrid	Midparent	F_1 - MP
A_1R_1	46.87	116.76	-69.89 *	80.74	130.06	-49.26 *
A_2R_1	65.64	102.98	-37.34 *	81.34	154.15	-73.80 *
A_3R_1	50.08	112.46	-62.38 *	106.95	127.74	-20.79
A_4R_1	53.48	76.00	-22.52	91.13	102.61	-11.48
A_5R_1	63.28	111.90	-48.62 *	107.16	124.10	-16.94
A_6R_1	57.52	102.93	-45.41 *	104.50	111.11	-66.10 *
Mean	**56.15**	**102.03**	**-47.69** *	**95.57**	**124.96**	**-39.72** *
A_1R_2	40.89	123.60	-82.71 *	68.81	137.25	-60.29 *
A_2R_2	63.53	109.80	-46.27 *	100.51	160.80	-60.30 *
A_3R_2	46.44	108.47	-62.03 *	60.83	134.40	-75.70 *
A_4R_2	52.97	82.90	-29.93	89.64	109.28	-19.64
A_5R_2	21.80	118.80	-97.00 *	74.86	130.76	-55.84 *
A_6R_2	54.29	110.28	-55.99 *	86.85	117.78	-30.93 *
Mean	**46.65**	**109.50**	**-62.32** *	**80.25**	**131.71**	**-50.45** *

* = *F1 mean and midparent are significantly different.*

6.3.3. Combined analysis of Variance for Sites 1 and 2.

Table 6.6 shows the combined analysis of variance for the two field sites. As expected, there were significant differences between sunflower lines. Significant differences between replicates within sites indicated that disease was not uniform across the trial sites. The lines x sites interaction was not significant, which suggests that the g x e interaction was small. The estimate of g x e from Table 6.6 was -0.105, which must be assumed to be zero.

Table 6.6. *Combined analysis of variance for audpcs of sunflower lines grown at the two trial sites.*

Source	df	Mean Square	F
Sites	1	2.241	3.0 *
Reps within sites	4	0.618	3.1 *
Lines	22	1.160	5.8 **
Lines x sites	22	0.095	<1
Error	88	0.199	

* = *significant at 0.05 level*
** = *significant at .01 level*

6.3.4. Thousand grain weights and oil contents of hybrids

An early frost at Site2 during late anthesis caused damage to flowers and so prevented sampling to determine the 1000 grain weights and oil content of each hybrid line. Table 6.7 shows the 1000 grain weights and oil contents of hybrids grown at Site1.

Hybrids A_2R_1, and Advantage had heavier seed than all other hybrids except for A_2R_2, and A_3R_1. There was little difference in seed weight between the remaining hybrids. Suncross 41 had the lowest oil content and all other hybrids fell into overlapping groups. Although hybrids A_1R_2, A_2R_2 and A_3R_2 had the highest oil contents, they were not significantly higher than the majority of hybrids.

Table 6.7. *Thousand grain weights and percentage oil yields for each F_1 hybrid grown at Site1. Values followed by the same letter are not significantly different.*

F_1 Hybrid Code	1000 grain wt. (g)	Oil content (%)
A_1R_1	50.24 bc	43.37 ab
A_2R_1	66.80 a	40.63 b
A_3R_1	65.45 ab	42.77 ab
A_4R_1	46.42 c	39.90 b
A_5R_1	49.34 bc	42.57 ab
A_6R_1	54.68 bc	42.8 ab
A_1R_2	48.32 bc	45.97 a
A_2R_2	62.10 ab	44.50 a
A_3R_2	56.46 b	44.83 a
A_4R_2	47.77 bc	44.00 ab
A_5R_2	49.23 bc	42.80 ab
A_6R_2	53.20 bc	44.23 ab
Hysun 45CQ	55.69 bc	41.53 b
Suncross 41	51.60 bc	38.13 c
Advantage	67.90 a	42.40 ab

6.4 Discussion

Under the epidemic conditions experienced at both field sites, hybrids made with the selected restorer lines R_1 and R_2 had the same levels of resistance as the commercial hybrids Hysun 45 CQ, Suncross 41 and Advantage. However, disease intensity was low at Site1 and moderate at Site2, so it is not known how these hybrids would perform under conditions of greater disease intensity. Previous studies have shown that the expression of resistance can be affected by inoculum load and environmental conditions (Carson and Medhi, 1983; Kong *et al.*, 1996). Indeed, the ten lines that were tested at two field sites (Chapter 5) under different disease intensities (audpcs) were better separated at high disease levels (Site1) than at low disease levels (Site2). Although relatively high levels of disease may be required to differentiate resistance in certain lines, there may be a fine line between 'just enough disease' and 'too much'. Obtaining the right balance under field conditions is extremely difficult. Hence, lines need to be tested at many locations and over a number of years so that performance can be determined in a wide range of

environments. The lines used in this study were planted at eight different locations in the Central Highlands of Queensland over two years by the plant breeders from the three commercial seed companies, but disease development was prevented by severe drought conditions. In this study, both R-lines showed commercial promise in terms of the seed weights and oil contents produced by their hybrids, but rigorous testing would be required to confirm this.

When this study began, there were no A-lines publicly available that had resistance to *A. helianthi*. In general, the proprietry A-lines used in this study were susceptible to *A. helianthi* and had levels of resistance similar to the susceptible standard line, B89. Because of the way that sunflower hybrids are produced and the nature of resistance to *A. helianthi,* it is likely that levels of resistance in hybrids would be greatly improved if resistance could be incorporated into both parents. From a breeding point of view, this is a difficult and time consuming task, as resistance would need to be maintained throughout the process of converting a superior inbred line to an A-line. In Chapter 4, crosses between moderately resistant lines were shown to result in lines with improved resistance. If gains in resistance following a single cross were sustainable with increasingly resistant parents, then theoretically, highly resistant hybrids could be produced. In reality however, gains in resistance probably plateau out, and may in some cases decline. In fact, there is no guarantee that resistance will be improved in crosses between resistant parents, because we do not know how the genes conferring resistance interact. For example, hybrids made with A_4 were only as resistant as the predicted midparent value, indicating no gain in resistance even though all other hybrids had increased resistance when crossed with the same R-lines.

Deviations of the F_1 from the midparent value indicate a level of genetic dominance. At both field sites, hybrids made with R_1 and R_2 had audpcs approximately equal to or lower than the audpcs of R_1 and R_2, indicating a high degree of dominance or even over-dominance among the gene(s) contributing to resistance. Crosses involving line A_4 are anomalous, because there appears to have been little or no dominance. According to the additive-dominance model (Mather and Jinks, 1977), it could be inferred that on balance, genes in A_4 subtract from the expression of resistance conferred by R_1 and R_2, whereas

genes in the other A-lines add to it. In order to determine the degree of dominance (non-additive) relative to additive effects, a structured mating design such as the diallel, would be required. This would take several years to complete and may lead to an understanding of the genetics and inheritance of a limited number of lines. Commercial sunflower breeders would not undertake this kind of study, preferring to seek resistance by testing as many hybrid combinations as is possible and understand none of the inheritance. From their point of view, methods that locate resistance quickly and easily are a commercial reality.

Using large populations, gains in resistance can be made relatively quickly, but a point is reached where improving resistance becomes increasingly difficult. Moreover, this 'end-point' level of resistance might be of little commercial value. Further progress might then only be possible through an understanding of the genetics and inheritance of resistance. For example, it would be interesting to look at the F_2 of the crosses used in this study to see if the high level of dominance found among the F_1 indicated resistance conferred by one or a few dominant genes.

While it is tempting to view the high level of dominance effects and the lack of g x e effects as support for partial resistance conferred by single dominant genes, it is possible that differences in environment between the two sites were too small to cause any g x e effect. Testing over a wider range of environments would be required to resolve this.

Although the R-lines used in this study were developed during a relatively short period of selection, they were highly resistant to *A. helianthi* compared to the susceptible line B89 and the A-lines used in commercial hybrid production. The high levels of resistance in F_1 hybrids using these lines is encouraging, but further testing is required before their commercial usefulness can be determined.