Chapter 4

Initial Selection of Resistant Germplasm

Summary

Sources of resistance to *A. helianthi* were not available at the time this research began. Therefore, locally developed inbred restorer lines (F_4) were accessed from a public breeding program and screened for resistance to *A. helianthi* in the field using a generated epidemic. Of the 37 lines that were evaluated, 32 had less disease than the susceptible line B89. Twelve lines had approximately half the amount of disease of B89 and 7 lines had less than one third of the disease found on B89. Variation for disease reaction within lines was high. Although no lines were immune, plants with very low levels of disease were observed in many of the lines.

4.1 Introduction

At the time that this research began, sources of resistance to *A. helianthi* had not been identified in Australia and only a few sources of resistance had been identified elsewhere. Consequently, commercial hybrids with resistance to *A. helianthi* were not available or being developed in Australia.

In order to further develop a greenhouse assay and to verify its usefulness as an aid to selection, it was necessary to identify a number of lines whose field reactions to *A. helianthi* were known. Germplasm that expressed a range of reaction types would be the most useful in evaluating the greenhouse assay. It was also decided that by targeting sources of resistance which could be rapidly utilised for the production of commercial hybrids, germplasm development time could be reduced. Therefore, 37 developmental inbred restorer lines (F_4) were accessed from the seedstore of the discontinued public breeding program located at the Department of Primary Industries Hermitage Research Station, Hermitage, Queensland. If individuals with high levels of resistance could be identified among these restorer lines, they could be tested almost immediately for

performance in hybrid combinations. Alternatively, lines with moderate levels of resistance would provide a good basis for the further development of resistant germplasm in a recurrent selection program.

Visual estimation of disease severity using pictorial keys are subject to observer bias and are often imprecise (Parker, Shaw and Royle, 1995). Rating sunflower plants for reaction to *A. helianthi* is not only time consuming, but is also difficult because of the density of the leaf canopy as the plants approach maturity. The selection process would be greatly improved if a simple, accurate and rapid method of estimating disease levels were available. Allen (1981) suggested that the number of senesced leaves per plant could be used to predict levels of *A. helianthi* infection on different sunflower lines. In this initial selection trial, this suggestion was tested, as a good correlation between senescence and disease severity would simplify further selection.

4.2 Materials and Methods

The 37 restorer lines used were F_4 selections obtained from outcrossing in mixed sunflower populations. The basic pedigrees of each population and the codes used to identify the selected lines are described in Table 4.1.

The 37 restorer lines plus the susceptible line B89 were grown in the field in 1991 and were tested for *A. helianthi* resistance using a generated epidemic. Seed stocks of many of the lines were low and viability of some stocks was poor, so with the exception of B89, all lines were planted in 10 m rows without replication. Four replicates of B89 were included. The standard row spacing of 75 cm was used, but plant spacing was reduced from the standard 30 cm to 20 cm. It was expected that a greater plant density would facilitate epidemic development.

Lines were planted in 4 blocks. In each block, the susceptible line B89 was planted as the first row and every third row thereafter, so that each restorer line was flanked by a row of B89 on one side and by another restorer on the other. The rows of B89 plants were spray-

Table 4.1. *Pedigrees and identification codes for the thirty-seven restorer lines that were selected for field evaluation of their resistance to* A. helianthi.

Pedigree	Codes used to identify selections
[a]ARpop // [b]Rpop/ [c]Charata	10020-5-2; 10020-5-3; 10020-5-4; 10020-11-1; 10020-11-2; 10020-11-3; 10020-11-4 10011-3-1; 10011-3-2; 10011-3-3; 10011-4-2; 10011-4-3; 10011-4-4;
Rpop//Charata	Rpop//Charata-3-2-1
ARpop // [d]SFM-3	10005-2-2; 10005-2-3 10016-3-1; 10016-3-2 10016-4-1
ARpop // [e]QSR1	10008-3-2; 10008-3-3 10014-5-1; 10014-5-2; 10014-5-3 10014-6-1; 10014-6-2; 10014-6-3
ARpop // [f]Rx677	10007-13-2; 10007-13-3 10015-2-1 10019-1-1; 10019-1-2
QSR1	QSR1
Charata	Charata 3-2-1
SFM-3	SFM-3
Rx677	Rx677
Rpop	Rpop

[a] *ARpop was a population derived from wild H.annuus plants that were selected for A. helianthi resistance by Dr. J K Kochman*

[b] *Rpop was a population selected by Dr D. George and was based on restorer lines released by the United States Department of Agriculture (USDA).*

[c] *Charata 3-2-1 was from a diverse Argentinian sunflower population known as Charata Inta.*

[d] *SFM-3 is an S_4 selection from the backcross of H petiolaris with cms HA 89 and is known for its resistance to sunflower moth (Roger, Thompson and Seiler,1982).*

[e] *QSR1 is a single headed restorer line developed by Dr D. George and was based on selections from Charata Inta. It was released by the Queensland Department of Primary Industries as a public line.*

[f] *Rx677 was selected by Dr D. George from a population derived from crosses between USDA restorer lines and bulk restorer pollen obtained from a commercial breeding program.*

inoculated with conidia of *A. helianthi* when plants were at the V6-V10 growth stage. Overhead misters were located in the trial plot and provided fine mist for 6-8 h periods, 3-4 times per week depending on the frequency of rainfall. This facilitated epidemic development in the rows of inoculated B89 plants that flanked the restorer lines. At anthesis, the proportion of leaf area infected was estimated for the lower leaves of all uninoculated plants, using a modified version of the pictorial key (Appendix II) devised by Allen, Brown and Kochman, (1983c). The number of senesced leaves per plant was recorded for 24 of the lines. The growth stages at the time of assessment varied from R6.0-R8.0 due to different maturities of the lines. Growth stages were designated according to the stage represented by at least 80 percent of individual plants in a line.

Prior to flowering, paper bags were placed over the main flower buds of all plants and secured to prevent cross pollination. Seed was harvested from selected plants, catalogued and stored in a freezer (-8°C)for further greenhouse and field studies.

An analysis of variance was performed on data for percentage leaf area infected and the standard deviation calculated to give an indication of the level of variability within each line. The number of senesced leaves per plant was regressed with leaf area infection data, to determine whether the number of senesced leaves could be used to predict the level of leaf infection.

4.3 Results

There was a large amount of variation within each line for reaction to *A. helianthi* when measured as the percentage of leaf area infected (see standard deviations in Table 4.2). However, individual plants with less than 5% leaf area infected could be selected from most lines. In general, lines could be grouped as having either low (0-5.9%), low to moderate (6-10.9%), moderate to high (11-15.9%) or a high (>16%) level of leaf tissue

infected. If these are designated as disease severity classes (DSC) 1 to 4, with Class 1

Table4.2. *Mean ratings for percentage leaf area diseased and number of senesced leaves for 37 sunflower restorer lines grown in the field and exposed to a generated epidemic of* A. helianthi.

Line	No.of plants	Mean % leaf area diseased. Standard deviation in brackets	[a] DSC	Mean number of senesced leaves
B89(susceptible)	100	19.0 (4.3)	4	10
ARpop//Rpop/Charata				
10011-3-1	29	9.8 (6.3)	2	11
10011-3-2	35	11.4 (5.2)	3	11
10011-3-3	33	8.7 (4.5)	2	11
10011-4-2	18	5.8 (2.3)	1	11
10011-4-3	30	5.3 (3.4)	1	13
10011-4-4	19	7.5 (3.4)	2	12
10020-5-2	35	10.5 (4.5)	3	-
10020-5-3	30	14.1 (5.8)	3	-
10020-5-4	33	12.3 (5.0)	3	-
10020-11-1	23	4.1 (2.8)	1	10
10020-11-2	29	4.3 (2.6)	1	9
10020-11-3	22	3.5 (1.3)	1	10
10020-11-4	25	4.0 (3.3)	1	10
Rpop//Charata	26	6.3 (1.9)	2	12
ARpop//QSR1				
10008-3-2	25	10.6 (6.5)	3	11
10008-3-3	15	5.7 (3.4)	1	11
10014-5-1	31	9.9 (4.3)	2	14
10014-5-2	21	10.1 (2.9)	2	13
10014-5-3	29	11.5 (5.2)	3	14
10014-6-1	27	7.2 (3.1)	2	13
10014-6-2	9	9.0 (5.4)	2	13
10014-6-3	10	12.2 (4.5)	2	12
ARpop//SFM3				
10005-2-2	15	16.6 (5.7)	4	-
10005-2-3	21	12.9 (6.2)	3	-
10016-3-1	18	22.7 (9.5)	4	-
10016-3-2	27	20.0 (7.6)	4	-
10016-4-1	14	18.8 (9.7)	4	-
ARpop//Rx677				
10007-13-2	36	8.8 (4.5)	2	11
10007-13-3	27	14.3 (7.4)	3	10
10015-2-1	22	13.5 (6.0)	3	-
10019-1-1	27	15.9 (5.0)	3	-
10019-1-2	19	7.0 (2.6)	2	-
QSR1	18	9.7 (3.7)	2	13
Charata	16	14.4 (4.3)	3	10
SFM3	31	17.6 (7.0)	4	-
Rx677	33	12.7 (6.3)	3	-
Rpop	36	16.7 (9.3)	4	-

[a] *DSC = Disease Severity Class 1, 2, 3 and 4 = 0-5, 6-10.9, 11-15.9 and >16% of leaf area infected infection on the lowest pair of unsenesced leaves respectively.*

being the lowest and Class 4 the highest proportion of leaf area infected, then the majority of lines fell into Classes 2 (12 lines) and 3 (12 lines). Six lines were in Class 4 with infection levels similar to the susceptible line B89. Six of the seven lines in Class 1 were originally derived from the population composed of Arpop, Rpop and Charata (Table 4.2).

The number of senesced leaves per plant was poorly correlated (r =0.03) with the percentage of leaf area infected.

Maturity within lines was quite uniform. Growth stage differences between lines were not large. The difference in maturity between the least and most mature lines (R6.0 to R8.0) was probably about 7 days.

4.4 Discussion

This initial field evaluation of lines was based on infection data obtained from single rows of plants bordered by the suscept (B89) on one side and a line of unknown resistance on the other. Under these conditions, the resistance of some lines may have been biased due to non-uniform disease pressure caused by differences in susceptability of adjacent lines. For example, high inoculum loads arising from highly susceptible neighbours might cause a resistant line to appear susceptible. Conversely, a susceptible line might appear resistant if its neighbours were either resistant or had a low level of disease due to micro-environmental influences. Thus, the effects of interference between neighbours can be extended, such that the level of infection on any one plant is dependent, more or less, on the infection levels of every other plant. These effects are difficult to measure and control (Last, 1978; Jenkyn, 1981) in trials containing multiple lines with various levels of resistance. The field plan used in the initial selection was convenient in terms of space and seed supplies, but it is acknowledged that some 'mis-selection' of plants may have occurred as a result of the effects of interference.

About two-thirds of the lines that were screened in this field trial, had intermediate levels of resistance compared to the suscept B89. About one-fifth of the lines had less than one-third of the level of infection of the susceptible line B89. The high level of variability within lines for percentage leaf area infected, indicated that individuals with levels of infection much lower than the population mean, could be selected. Whether these represented true resistance or had simply "escaped" by being exposed to low inoculum levels and/or less favourable environmental conditions, could not be determined until selfed selections could be tested. Nevertheless, a large number of selections were made from the 37 restorer lines. Some of these could be tested in hybrid combinations to gauge their suitability for continued development. In addition, all of the selections could be used as the basis of a new population suitable for phenotypic recurrent selection.

Because susceptibility of sunflower tissue increases at and during anthesis, (Allen, Brown and Kochman, 1983b) differences in disease levels between lines can be simply due to age-mediated differences in susceptibility. It is not possible to overcome this problem in a single field trial. Delaying the rating of lines until a specific growth stage does not eliminate the problem because lines are then exposed to different environmental conditions and inoculum loads over time. The expression of quantitative resistance can be greatly influenced by these factors. Careful notes on maturity dates and epidemic development from multiple field sites can help to reduce this problem. Nonetheless, it was thought that the small differences in growth stage between the lines tested, may have contributed little to the differences in infection between lines. The maximum observed difference in phenology (R6.0 to R8.0) would have amounted to a period of about seven days. For most lines, the difference would have been much less than this.

One of the main objectives of this screening trial was to identify lines ranging in their susceptability to *A. helianthi.* These lines could then be used in further field trials to develop better techniques for identifying and characterising resistance and in turn, data obtained from field trials could be used to evaluate the accuracy of the greenhouse assay

described in Chapter 3. The sensitivity and robustness of the greenhouse assay would be better determined by using lines that exhibited a broad range of susceptability. Hence, a core set of lines ranging in their susceptability to *A. helianthi* was selected from the lines evaluated in this study (Table 4.3). The commercial hybrid, Hysun 45 CQ (Pacific Seeds Pty Ltd) was thought to have some resistance to *A. helianthi* and was therefore included for comparison. These lines were used in the field and greenhouse experiments described in Chapters 5, 6 and 7. The experiment codes given in Table 4.3 will be used hereafter when referring to specific sunflower lines.

Table 4.3. *Lines selected for further experimental work, their experiment and selection codes, pedigrees and their disease severity classes (DSC) as determined from a single unreplicated field trial.*

Experiment Code	Pedigree	Single plant selections	[a]DSC
P1	ARpop//Rpop/Charata	10020-11-1-18	1
P2		10020-11-2-28	1
P3		10020-11-3-13	1
P4		10020-11-4-20	1
P5		10011-4-3-18	1
P6		10011-4-4-16	2
P7	ARpop//QSR1	10014-6-1-20	2
P8		10014-6-1-11	2
P9		10008-3-3-5	1
P10	ARpop//Rx677	10007.13.2.7	2
P11	ARpop//Rx677	10019-1-2-10	2
P12	Rpop//Charata	—	2
P13	HA-B89	—	4
P14	[b]Hysun 45 CQ	—	?
P15	QSR1	—	2
P16	Charata-3-2-1	—	3
P17	RX677	—	3
P18	Rpop	—	4

[a] DSC = *Disease Severity Classes 1, 2, 3 and 4 = 0-5, 6-10.9, 11-15.9 and >16% of leaf area infected on the lowest pair of unsenesced leaves respectively.*

[b] *Commercial hybrid of Pacific Seeds Australia Pty Ltd.*

? = *Reaction type unknown at time of assessment.*