CHAPTER 5

USE OF RECURRENT PHENOTYPIC SELECTION TO IMPROVE QUANTITATIVE RESISTANCE OF A SUNFLOWER POPULATION TO *SCLEROTINIA MINOR*.

5.1 Summary

Breeding sunflower for resistance to sclerotinia wilt is difficult because of the destructive nature of the disease. Immunity to the disease is not known and plants rarely become partially infected. Once infection is established then plant death follows. Resistance to sclerotinia wilt caused by *Sclerotinia sclerotiorum* is considered to be under polygenic control with additive effects. Recurrent phenotypic selection should help to accumulate various favourable loci and alleles if individuals with the desirable phenotype can be accurately identified. The 'Pot Base Inoculation' method was tested as a technique to select for increased resistance to *Sclerotinia minor* in recurrent phenotypic selection of a sunflower population.

Fifteen inbred sunflower lines identified in earlier experiments as possessing some resistance to *Sclerotinia minor* were intercrossed twice in the absence of the disease and then subjected to three cycles of recurrent phenotypic selection. Over 3 cycles of screening the percentage mortality of the population compared to the mean of the four check lines decreased from 100.4% to 27.4% while the rate of lesion extension and mean time from inoculation to expression of basal stem lesions showed little change. The screening method of Castaño *et al.* (1992) was applied to the Cycle 3 population in an attempt to select for increased resistance of shoot tissue. Field grown plants of Cycle 3 were screened using the detached petiole test of Martinson (1992). The nett lesion lesion length of the population was less than that of RHA 801.

The Cycle 4 population developed from selected Cycle 3 individuals could be regarded as being as resistant to *S. minor* as RHA 801 if the the parameter 'rate of lesion extension' is best correlated with field resistance. Higher mortality and shorter delay from inoculation to appearance of basal stem lesions indicate that, for these parameters, the population was more susceptible than RHA 801. The screening cycles did not result in the selection of plants with decreased root density.

Screening of 20 S_0 families derived from the Cycle 3 plants inoculated by the Martinson (1992) method or survivors of the Cycle 4 population inoculated by the pot base method revealed that a high level of resistance was present in some families as expressed by lower mortality, longer mean delay from inoculation to appearance of basal stem lesions and slower mean rates of lesion extension than RHA801.

Over ninety partially inbred (S_2 and S_3) families were screened at two field sites as single row replicates in a modified augmented design. The second site consisted of head-to-row plots selected from the first site. There was a distinct increase in the number of test plots with adjusted area under the disease progress curve less than RHA801 in the second trial indicating that selection for increased resistance was still necessary and possible during inbreeding.

A number of selections were provided to and screened by Dr Maria Bazzalo in Argentina for resistance to head rot caused by *Sclerotinia sclerotiorum*. In many cases there were variations in the reactions within a line to inoculation on different days. This may have been due to heterogeneity within the selections such that differences in time to anthesis represented differences in susceptibility to the head rot. However, several lines (eg Cycle3-81-1-3-1, Cycle-91-1-3-2, Cycle-91-1-3-3, Cycle3-100-1-1) gave consistently resistant reactions compared to the controls on two inoculation dates.

5. 2 Introduction

The approaches available for breeding for resistance to diseases in crops depend on the heritability of that resistance. Where it can be demonstrated that a single or few genes condition a suitable level of resistance then simple pedrigree or backcross breeding strategies can be applied to transfer the resistance into superior genotypes. These methods have been successfully utilised for many years in breeding various crops for resistance to the obligately parasitic smut, rust and downy mildew fungi, various bacteria and some viruses. For many diseases however the inheritance of resistance is rather more complex and is often described as being under polygenic control (Hooker and Saxena, 1971). In pathosystems involving polygenic resistance there is often a continuous scale of phenotypic expression as a result of interactions between genotype and external factors such as inoculum pressure and environmental conditions. Hence, this resistance is often described as being quantitative or partial since it is not absolute (Geiger and Heun, 1989; Parlevliet, 1992).

Polygenic resistance is conditioned by a number genes each of which contribute a small effect and has been recognized against a large number of pathogens in a variety of hosts

(Simons, 1972). Individual genes are usually undetectable (Kim and Brewbaker, 1977; Wilcoxson, 1981; Leonard and Mundt 1984) and the number of genes involved can at best be only estimated. These estimates can be biased by the action of single genes of higher action or by non-genetic factors (Baker, 1984; Geiger and Heun, 1989). In the future, molecular biological techniques such as Quantitative Trait Loci (QTL) analyses using correlations between genetic markers and phenotypic expression of a trait may provide useful tools in the estimation of the relative importance of different loci in the control of quantitatively inherited traits (Paterson, Lander, Hewitt, Peterson, Lincoln and Tanksley, 1988; van Ooijen, 1992; Stuber, 1992).

Traditional plant breeding methods used to manipulate polygenic resistance are similar to those used for other complex traits such as yield. The method chosen is based on the inheritance of the trait (Allard, 1960). Where resistance is under the control of additive gene action and is quantitatively inherited then recurrent phenotypic selection can be used to increase the frequency of desirable alleles. Recurrent phenotypic selection involves the following steps (based on Fehr, 1987);

1. Intermate a number of parents which possess different alleles from diverse sources controlling the trait of interest,
2. Continue to intermate the progeny for as many generations as possible (within constraints of time and resources) o allow for maximum recombination of genes,
3. Expose this base or Cycle 0 population to selection for the desirable phenotype,
4. Intermate superior individuals to constitute the Cycle 1 population,
5. Continue selection and intermating to produce Cycle x populations with improved mean performance and individuals with superior performance,
6. Futher development is dependent on whether the improved population is to be used as a cultivar *per se* or as a source of superior individuals for use as pure-line cultivars or inbreds.

In summary, recurrent phenotypic selection is a means of increasing the frequency of desirable genotypes in a heterogeneous source population thereby increasing the likelihood of isolating superior individuals.

Sunflower is a predominantly hybrid crop based on a very efficient cytoplasmic male sterility (*cms*) system (Fick, 1978). Two separate breeding strategies are therefore used (Figure 5.1); one designed to produce fertile inbred lines that maintain the sterility of isogenic *cms* lines and, the second, to produce inbred lines that restore fertility in hybrids with cytoplasmic sterile lines. The lines that restore fertility are termed restorer or R lines while those that maintain sterility are described as maintainer or B lines. The cytoplasmic male sterile line is the A line (Skoric, 1988). Crosses between the sterile A line and the

fertility restoring R line produces the hybrid F_1 seed grown by farmers.

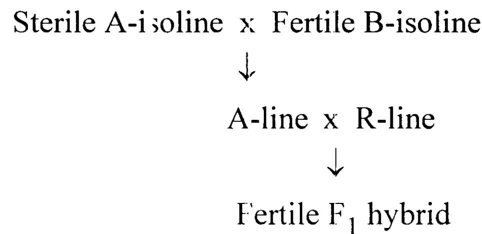

Figure 5.1. Simple representation of the process involved in producing single-cross hybrid seed.

Resistance to sclerotinia diseases in sunflower would seem to be a character that could be improved through the use of recurrent phenotypic selection. Differences in the susceptibility of sunflower to sclerotinia wilt have been recognised for many years but there are no accurate analyses available on the number of genes responsible for these differences. Fick and Gulya (1980) evaluated the incidence of wilt in 20 hybrids derived from 11 female parents with 6 male parents. The regression coefficient (b=0.31) calculated from the female parent-offspring regression suggested that resistance was heritable but only at a low level. Large Specific Combining Ability (SCA) effects occurred as the performance (disease incidence) of some hybrid combinations was better than expected from the performance of the parental lines. Significant SCA effects for resistance to sclerotinia wilt were also detected in the breeding lines and hybrids evaluated in other studies (Fick, Gulya and Auwater, 1983; Vranceanu, Pirvu, Stoenescu and Iliescu, 1984; Pirvu, Vranceanu and Stoenescu, 1985). In contrast, Tourvieille de Labrouhe and Vear(1990) examined 36 hybrids derived from six female and six male parents and concluded that GCA (General Combing Ability) effects were greater than SCA effects. Furthermore, the regression coefficient (R=0.58) for the regression between parental lines and hybrids indicated a relatively high level of heretibility for the germplasm examined. In general, inheritance of resistance is considered complex and under the control of several genes. Additive gene effects were considered to be more important than dominance and epistatic gene effects (Fick *et al.*, 1983; Pirvu *et al.*, 1985).

The sunflower inbred line RHA 801 (Figure 5.2) has had a consistently superior performance with regard to low susceptibility to sclerotinia wilt caused by *S. sclerotiorum* in the United States (Gulya, 1985; Miller, 1992) and wilt caused by *S. minor* in Victoria (Porter, Clarke and Woodroofe, 1994) as well as in controlled glasshouse and field inoculations against both fungi (Sedun and Brown, 1989). The following experiments were conducted to test whether the pot base inoculation method could be used as a selection technique in a phenotypic selection breeding program to improve the resistance of sunflower to wilt caused by *Sclerotinia minor* and, more specifically, to produce a

diverse source of germplasm with resistance comparable or superior to that of RHA 801.

5.3 Experimental

5.3.1. General materials and methods

i Production of sunflower plants

The fifteen sunflower lines used to develop the breeding population are described in Table 5.1. These lines were chosen because they demonstrated superiority in one of the selection parameters used (mortality, delay or rate of lesion extension) and also represented diverse germplasm sources. Plants were raised in standard potting mix in 10cm diameter plastic pots. Small pots were used so that more plants could be grown in the limited space available in the glasshouse. Glasshouse culture was chosen because the University of New England at an altitude of 1042m provides only a short growing season for reliable field work.

The scheme of Schneiter and Miller (1981) was used to describe Growth Stages of both glasshouse and field grown plants. Symbolism used to describe breeding status of lines is consistent with System II as recommended by Fehr(1987).

Figure 5.2 A plot of RHA801 growing at Numurkah, Victoria.

Table 5.1. The fifteen sunflower lines used to establish the population used for recurrent phenotypic selection.

UNE #	Line	Source
2	RHA 801	United States, S_3 selection from a population formulated from the inbred lines RHA 271, RHA 273, RHA 274, R 344, and R 494 (Roath *et al.*, 1981)
10	CM526	Canada, interpollination of 50 undescribed Russian lines (Huang, 1982)
14	PacR1	Australia, Pacific Seeds proprietry inbred selected from a R-line rust resistant population
20	*	United States, Bushland #9109-1, a selection from *cms* HA89 /*Helianthus exilis* produced by Dr.G. Seiler (United States Department of Agriculture)
28	*	Romania, synthetic population of unknown formulation
29	*	Soviet Union, DM-3 Composite prepared in United States by Miller and Gulya(1985) from selections of the open pollinated cultivar 'Progress' which included *Helianthus tuberosus* in its pedigree (Pustovoit 1978, as cited by Skoric, 1992).
32	*	As #29, different selection
38	*	United States, Bushland #7605-2, a selection from *cms* HA89 / *H. paradoxus* produced by Dr. G. Seiler (United States Department of Agriculture)
39	*	Romania, selfed selection made in Australia by Pacific Seeds from Romanian hybrid 'Romsun 22'
40	*	Romania, selfed selection made in Australia by Pacific Seeds from Romanian hybrid 'Select'
42	*	Romania, synthetic population of unknown formulation
43	*	Romania, synthetic population of unknown formulation
60	77-5-67-8A	Canada, selection derived from HA61 (Sedun, 1986)
71	*	Argentina, selection of Saenz Peña 74-2-1 (composite produced by intermating various Russian cultivars with the cultivars 'Klein', 'Record', 'Horizon', 'OS2', and a synthetic line (produced from VNIIMK 1646, VNIIMK 6540, VNIIMK 8883, wild *H. annuus* and *H. petiolaris*) (A. Luciano, *pers. comm.*)
77	953-88-3	United States, selection from the natural cross between the cultivar 'Sunrise' and wild annual sunflowers in Texas, U.S.A. (Putt and Sackston, 1957).

1. * Introduced and reselected by Pacific Seeds Pty. Ltd in the absence of pressure from *Sclerotinia* spp.

ii. Fungal isolate and inoculation of plants.

The culture of *Sclerotinia minor* used throughout these studies was accession UNE#3 that was originally isolated from an infected sunflower plant from the Liverpool Plains area of New South Wales. Unless otherwise stated the pot base inoculation method described in earlier chapters was used. Approximately 25 colonized millet grains (≅ 0.5g) were placed in the base of the pot and the root ball replaced. The parameters used to assess resistance were mortality, linear rate of lesion extension and time (delay) from inoculation to appearance of a basal stem lesion.

5.3.2 Re-evaluation of the 15 parental sunflower lines for reaction to inoculation with Sclerotinia minor.

The fifteeen sunflower lines used to establish the population were inoculated under controlled conditions to examine their reaction compared to the check line *cms* HA89.

Materials and method

Ten plants of each sunflower line were raised in a controlled environment cabinet under a regime of 24/18°C (day/night), 12h photoperiods at 350µE.m^{2}.sec^{-1} and relative humidity 60/80% (day/night). Plants were watered twice daily. All plants were inoculated at 35d after sowing when plants were beginning to bud.

Results

The 15 parental lines varied considerably for each of the parameters assessed (Table 5.2). Root indices ranged from 1.3 for #2 (RHA 801) to 3.7 for #10 (CM526). Only 2 plants of #42 had been killed by 21d after inoculation while 9 plants of #29 and #38 were killed. Eight plants of the suscpetible check *cms* HA89 were killed. The mean delay from inoculation to expression of basal stem lesions was longest for #29 (15.9d) and shortest for #42 (9.0d). The mean delay for the susceptible check was 13.4d. The mean rate of lesion extension was slowest for #2 (RHA 801) and most rapid for #60 (77-5-67-8A). This degree of variability was considered desirable if genetic recombination for low mortality, long delay from inoculation to lesion expression and slow rate of lesion extension was to be obtained. At the same time it must be recognized that, depending on the assessment parameter, some lines were more susceptible than the susceptible check *cms*HA89.

Table 5.2. Reaction of fifteen sunflower lines and a check line to inoculation with *S. minor*[1].

UNE Accession #	Root Index	Mortality (/10)	Mean Delay (d)	Rate of Lesion Extension ($mm.d^{-1}$)
2	1.3	6	12.3 *abcde*	5.17 *e*
10	3.7	7	12.6 *abcde*	7.67 *cde*
14	2.9	7	14.7 *ab*	9.78 *abc*
20	3.3	8	10.3 *bcde*	8.39 *bcde*
28	2.3	7	13.0 *abcde*	9.08 *abcd*
29	3.3	9	15.9 *a*	7.77 *cde*
32	3.4	7	10.7 *bcde*	9.42 *abc*
38	3.3	9	9.5 *de*	8.06 *cde*
39	1.8	7	13.2*abcde*	10.3 *abc*
40	2.6	5	12.8 *abcde*	6.49 *cde*
42	2.2	2	9.0 *e*	11.17 *a*
43	2.4	5	10.0 *cde*	6.03 *de*
60	1.7	3	14.0 *abc*	11.40 *ab*
71	3.4	8	13.7 *abcd*	6.96 *cde*
77	2.6	6	12.2 *abcde*	8.83 *abcd*
Check(*cms* HA89)	3.1	8	13.4 *abcde*	8.87*abcd*
L.S.D.(P=0.05)	0.81	4		

1. Means in each column with at least one lower case letter in common are not significantly different as determined with Duncan's Multiple Range Test ($P \leq 0.05$).

5.3.3 Development of the Cycle 0 sunflower population

The base or Cycle 0 population was developed from the 15 sunflower lines described in Table 5.1. Recombination was initiated by intermating the lines according to a partial diallel design. The crosses made are shown in Table 5.3. Further crosses were made but only those crosses where sufficient seed was obtained and those that allowed best representation of each line were used to create the population. The initial decision to use 15 lines in at least 5 crosses each prevented equal representation. For example, 15 lines involved in crosses at least 5 times results in 75 possible haploid combinations or 37.5 diploid combinations. One line must be represented in 6 crosses to achieve 38 diploid combinations. The line #71 was used in 6 crosses because Saenz Peña 74 of which it is a derivative is a potential source of several other characters such as rust resistance (Gulya, 1985*b*) which would be useful in the development of superior hybrid cultivars.

Table 5.3. Crosses made to produce F_1's used to establish the partial diallel sunflower population.

UNE #	2	10	14	20	28	29	32	38	39	40	42	43	60	71	77
2	-														
10	X	-													
14	X	X	-												
20	X	X	-	-											
28	-	-	-	-	-										
29	-	-	-	X	-	-									
32	X	-	-	X	X	-	-								
38	-	-	X	-	X	X	X	-							
39	-	-	-	-	X	-	-	X	-						
40	-	-	-	-	X	X	-	-	-	-					
42	-	-	-	-	X	X	-	-	X	-	-				
43	-	-	X	-	-	-	-	-	X	X	-	-			
60	X	X	-	-	-	X	-	-	X	-	X	-	-		
71	-	-	-	X	-	-	X	-	-	X	X	X	-	-	
77	-	X	X	-	-	-	-	-	-	X	-	X	-	X	-
# crosses involved	5	5	5	5	5	5	5	5	5	5	5	5	5	6	5

Crossing was performed by hand emasculating glasshouse grown plants and pollinating with pollen from other plants (Putt, 1941) . When the plants matured and dried-down the F_1 seed was harvested, cleaned and stored at 5°C for 2 months to break any germination dormancy that might be present. Fifty seed taken from each of the 38 crosses was then bulked. This seed was divided into equal parts which were split-planted into multi-compartment seedling flats in the glasshouse with three weeks between sowings. Seedlings were transplanted by hand into a field plot at the University of New England's Laureldale Research Station on 13 December, 1990 and 4 January, 1991 when the majority of seedlings were at V2 Growth Stage. These plantings provided temporal isolation at anthesis from other sunflowers that were planted on the Research Station.

The seedlings were spaced 20cm apart in rows 20m long with an inter-row spacing of 75cm. Rows from each planting were planted in alternate pairs. A syringe and needle was used to apply 0.2 - 1 ml of a solution containing 120 ppm of the chemical emasculant gibberellic acid A_3 (GA_3) and 0.01% Triton-X100 (Miller, 1987) to the floral face of plants at the early reproductive stage R1 in alternate rows. The treatment was repeated 5

days later. Treated plants were examined as anthesis commenced and those with apparently healthy anthers or pollen were discarded. This design provided alternate rows of male sterile and fertile plants of two different maturities (Figure 5.3). This split-planting was designed to facilitate maximum chance of crossing between plants of different maturities, that is, late flowering plants of the first planting could cross with early flowering plants of the second planting.

Function[1]	M	F	M	F	M	F..................	M	F	M	F	
Planting	1	1	2	2	1	1..................	2	2	1	1	
Plants	x	x	x	x	x	x	x	x	x	x	
	x	x	x	x	x	x	x	x	x	x	
	.	.	.	.	.	.	.	.	.	.	
	.	.	.	.	.	.	.	.	.	.	
	x	x	x	x	x	x	x	x	x	x	x
	x	x	x	x	x.................x		x	x	x		

1. M- male fertile F- chemical induced male sterility

Figure 5.3. Design used to obtain alternate rows of fertile and sterile plants from a split-planting.

Intermating (cross-pollination) was allowed to proceed via natural populations of pollinating insects. Ten seeds were collected from each chemically sterilized plant after it had matured. This seed was bulked to form the Cycle 0 population.

5.3.4 Phenotypic Recurrent Selection of the sunflower population for resistance to Sclerotinia minor *by using the Pot Base Inoculation method.*

Phenotypic recurrent selection commenced once the Cycle 0 population was created.

Materials and method

The Pot Base Inoculation method was used to screen the Cycle 0 and subsequent populations for resistance to *Sclerotinia minor*. Four hundred plants of each cycle were planted in 10cm diameter pots and raised in the glasshouse. As well, 20 plants of the sunflower inbred RHA801 and hybrid Pacific Seeds Pac3435 were also grown to serve as resistant checks. The inbred line *cm*.HA89 and hybrid Dekalb Dk3903 were included as susceptible checks. All plants were inoculated when 50% of plants were assessed to have achieved or passed the growth stage R1. A few plants failed to reach the stage for

inoculation due to abnormal growth or cultural accidents but over 390 plants of the population were inoculated at each screening. The Cycle 0 was screened during autumn, Cycle 1 during spring, Cycle 2 during summer and Cycle 4 during winter/spring.

The parameters recorded included flowering time, root rating at inoculation, time until lesion expression (delay), rate of lesion extension and number of plants killed. Further disease development in plants without lesion expression was inhibited 28 days after inoculation by applying 50ml of a 0.12% (w/v) suspension of the fungicide Benlate DF (50% benomyl) to each pot to suppress or kill the pathogen. Each plant was examined before treatment with the fungicide to ensure that inoculation had been conducted by removing the pot and examining the root ball. Benomyl is toxic to *Sclerotinia* species (Herd and Phillips, 1988) and the rate was as recommended by Engelhard and Waltz (1986) for the control of *Fusarium* species in pots of ornamental plants and was therefore not thought to be at phytotoxic levels.

Intermating of surviving (Cycles 0-2) or selected (Cycle 3) plants was achieved by emasculating by hand and pollinating with pollen that had been stored. The diverse germplasm used resulted in flowering being spread over a period of 20-30 days. Plants that flowered in discrete 5 day periods were emasculated in the morning and the anthers and pollen collected and stored at $5^{o}C$ in Petri dishes enclosed in plastic bags that also contained the desiccant silica gel. The stored pollen was then used to pollinate plants emasculated in the following 5 day period. In this way all plants (except those that flowered in the first 5 day period which were used only as pollen sources) acted as pollen sources and pollen recipients, self pollination was avoided and intermating between plants differing in maturity was achieved.

Results

The benomyl drench did not have any apparent phytotoxic side-effects on the treated plants. The effectiveness of the treatment in controlling further disease development was not certain because in other experiments very few new cases of basal stem lesions occurred in plants after 21d post- inoculation.

The percentage mortality at 21 days after inoculation of the sunflower population differed dramatically in each of the screening experiments (Figure 5.4). Mortality of 77.8% when the Cycle 0 population was screened had dropped to 17.1% when the Cycle 2 population was screened. Mortality of the Cycle 4 population developed from the Cycle 3 population which was screened by the method of Castaño *et al.* (1992) (Experiment 5.3.5) rose to 83.6%.

Four check lines were included in each screening to provide a basis for comparing improvement in population performance and reduce the impact of seasonal variation. Compared to the the mean of the two resistant checks the mortality of the population decreased from 111.1% to 48.9% between Cycle 0 to Cycle 2 and increased to 133.8% for the Cycle 4 population. Mortality of the population was consistently less than the mean of the two susceptible checks.

The genetic gain as measured from the changes in mortality over the different cycles can be interpreted from the regression of mortality for each cycle on cycle number. The decrease in mortality between the Cycle 0 poulation and Cycle 2 population was linear (r = -0.990, P = 0.089) with an average loss of 36.5 mortality units per cycle. Inclusion of the Cycle 4 population in the regression removed all linear relationships.

The mean rate of lesion extension for the population was equal to or greater than the mean of the two resistant checks and less than the mean of the two susceptible checks (Figure 5.5). Active selection for reduced rate of lesion extension along the stem is not possible because even slow growing lesions result in plant death precluding the use of superior individuals in breeding.

The selection pressure applied with he pot base inoculation method was for increased delay between inoculation and appearance of basal stem lesions. The delay for the population increased from 88.1% to 06.3% relative to the mean of the resistant checks between Cycle 0 and Cycle 2 but decreased to 78.2% in Cycle 4 after no selection pressure was placed on this character by the screening applied to the Cycle 3 generation (Figure 5.6). Compared to the mean of the susceptible checks the delay of the population plants expressing lesions reached 116.2% or 2d by Cycle 2 but decreased to 103.4% or 0.4d at Cycle 4.

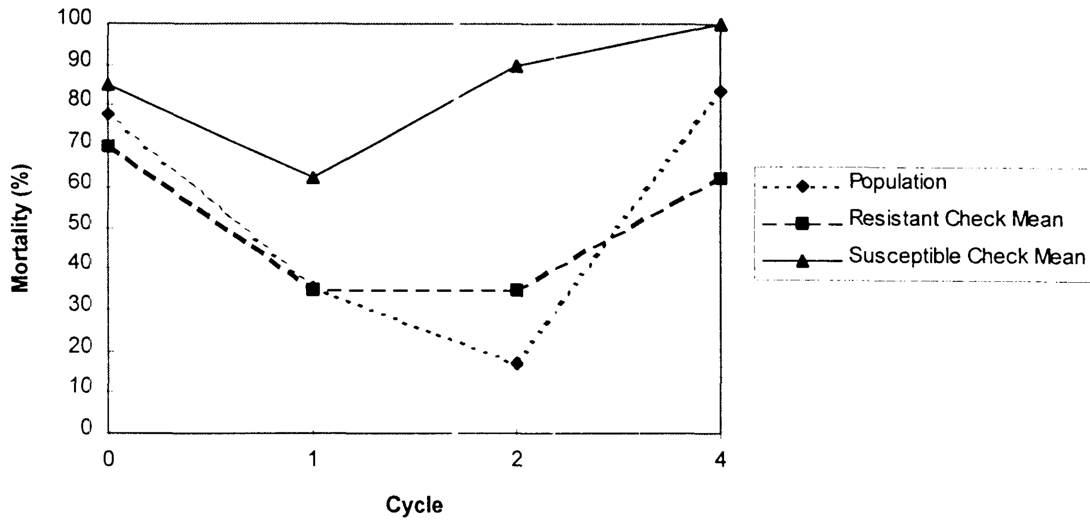

Figure 5.4 Mortality in a recurren. sunflower population screened for resistance to *Sclerotinia minor* compared to the mι ans of two susceptible (*cms*HA89 and Dk3903) and two resistant (RHA801 and Pac3435) :heck lines.

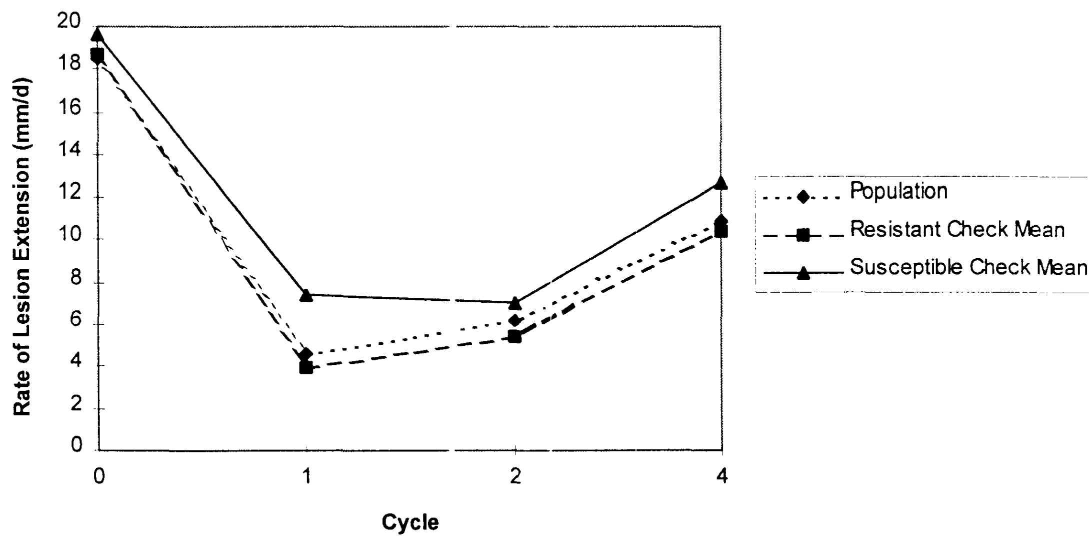

Figure 5.5 Mean rate of lesion exten;ion in a recurrent sunflower population screened for resistance to *Sclerotinia minor* comp ιred to the means of two susceptible (*cms*HA89 and Dk3903) and two resistant (RHA801 ınd Pac3435) check lines.

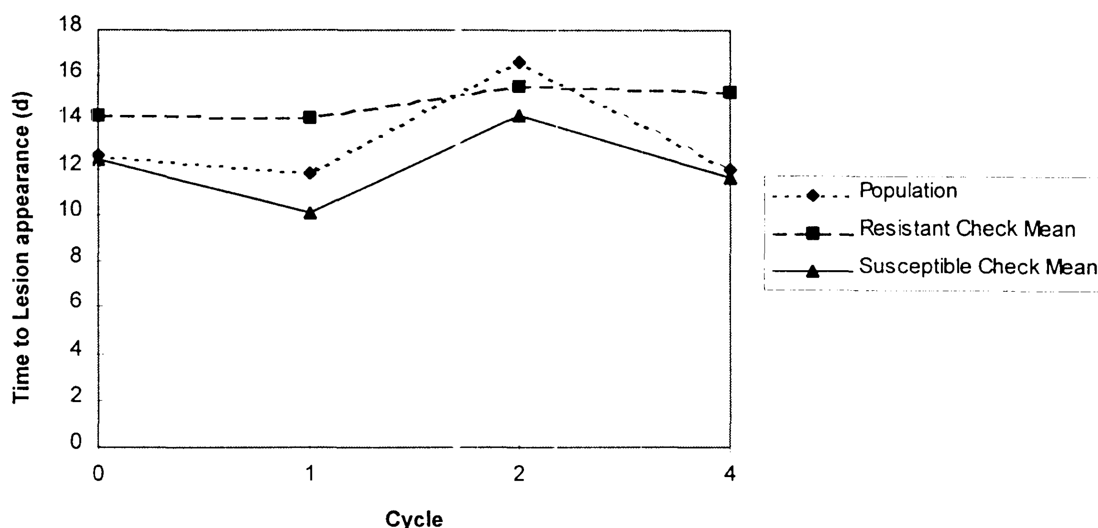

Figure 5.6 Delay in time to lesion appearance in a recurrent sunflower population screened for resistance to *Sclerotinia minor* compared to the means of two susceptible (*cms*HA89 and Dk3903) and two resistant (RHA801 and Pac3435) check lines.

Root indices for the population were closest to those of *cms* HA89 and showed no tendency towards the reduced root growth observed for RHA801 (Table 5.4). Lower mortality levels in the screening experiments could not be explained through escape, that is, lack of contact between roots and inoculum.

Table 5.4. Root indices determined for the sunflower population compared to four check genotypes.

Germplasm Screened	Root Index[1]			
	Cycle 0	Cycle 1	Cycle 2	Cycle 4
Population	2.34	2.28	2.05	2.77
RHA 801	1.43	1.33	1.60	1.37
cms HA89	2.33	2.20	2.00	2.18
Pac 3435	2.86	2.33	2.84	2.25
Dk3903	2.13	2.87	2.87	2.90

1. Root index assessed from the base of the exposed root ball at inoculation. A rating of 0 = no roots visible, 4 = >75% of surface occupied by roots.

5.3.5 Alternative assessment of the Cycle 3 Population using the method of Castaño et al. (1992).

The Cycle 3 population was screened by an alternative method in an attempt to increase pressure for selection of genotypes with higher levels of shoot tissue resistance. The leaf inoculation method of Castaño *et al.* (1992) was used because it permitted plants to be screened without killing the shoot and, according to the authors, the results were 'frequently correlated with levels of resistance to root attack'.

Materials and Methods

One hundred seedlings of the Cycle 3 population were grown in the glasshouse in 10cm diameter plastic pots. Ten plants of each of the check sunflower varieties RHA801, *cms*HA89, Pac 3435 and Dk 3903 were grown for comparison. All plants were inoculated when 50% of plants were assessed to have achieved the growth stage R1.

Plants were inoculated by placing a 1cm diameter agar disc taken from the margin of a vigoursly growing culture of *S. minor* grown on Potato Dextrose Agar against the end of the main vein on the upper surface of a leaf with the mycelium in contact with the leaf. The end of the leaf and inoculum were then covered with aluminium foil. Two fully expanded leaves at mid-plant height of each plant were inoculated. Plant reaction was assessed by measuring the rate of lesion extension along the main vein and the rates for the two leaves per plant were averaged to give a mean reaction of the plant.

Infected leaves were removed from Cycle 3 plants with mean rates of lesion extension less than $20mm.d^{-1}$. These plants were intercrossed to produce the Cycle 4 population.

Results

All plants inoculated with this method developed leaf lesions. The mean rate of lesion extension of the Cycle 3 population was lower than any of the four checks but this difference was not statistically significant ($P>0.05$) (Table 5.5). The rate at which lesions grew along the leaf varied from $14.4mm.d^{-1}$ to $37.6mm.d^{-1}$ for individual Cycle 3 plants. This was a greater range than observed on the inbred and hybrid checks and indicated that selection for increased resistance for this character might be possible. The most susceptible (greatest rate of lesion extension) Cycle 3 plants were more susceptible than any of the check plants. Susceptibility for lesion extension along leaves had therefore been carried through the previous selections where this character was not assessed. The usefulness of this method must be questioned when the rates of the check lines were not significantly

different while their field susceptibilities differ markedly (Experiments 4.3.1 and 4.3.3).

Table 5.5 Mean rate of lesion extension on sunflower leaves of Cycle 3 population plants and four checks.

	Population	RHA 801	*cms*HA89	Pac 3435	Dk 3903
Rate of Lesion Extension[1] (mm.d^{-1})	24.88 *a*	26.93 *ab*	28.08 *b*	25.47 *a*	25.17 *a*
Range (mm.d^{-1})	14.4-37.6	23.5-29.0	25.7-30.8	22.8-29.5	21.9-28.9
Population as % of Check		92.4	88.6	97.7	98.8

1. Means in row followed by at least one lower case letter in common are not significantly different ($P<0.05$) as determined by Duncans Multiple Range Test.

Nineteen Cycle 3 plants were selected on the basis of having leaf lesions that progressed at less than 20mm.d^{-1}. These plants were intercrossed randomly to constitute the Cycle 4 population screened in the previous section.

5.3.6 Screening of field grown Cycle 3 plants with the detached petiole method of Martinson (1992).

An attempt was made at generating field data from a field screening of the Cycle 3 population. Previous efforts at field screening Cycle 0-2 populations in the disease nurseries on the University of New England's Laureldale Research Farm had failed due to extremely poor establishment of the seed produced from plants grown in small pots in the glasshouse.

Materials and methods

A small field screening nursery on the research farm was prepared by incorporating sunflower stems infected with *S. minor* and containing numerous sclerotia. The stems were collected from an earlier screening trial and stored over-winter at room temperature in hessian sacks. The stems were cut into 5cm lengths, dispersed onto the site at a rate of 15.m^{-2} and incorporated into the soil with a rotary-hoe.

The screening trial consisted of 5m long two row plots of the Cycle 3 population, each plot bordered on one side by a plot of RHA 801 and on the other side by a plot of *cms*HA89. These inbreds were to be used as resistant and susceptible checks, respectively. Each treatment was replicated four times. Seed was hand-sown at an inter-row spacing of

75cm and seedlings were thinned to a spacing of 20cm between plants.

The plants were examined regularly for occurrence of sclerotinia wilt. Wilt was not observed in any of the plots as the plants approached anthesis. The opportunity was taken therefore to assess relative susceptibi ty by the method of Martinson (1992) as described in Experiment 4.3.7. Single petioles were excised from the tenth youngest leaves of 100 Cycle 3 population plants (ie 25 per plot) and 20 plants of each of the checks (ie 5 per plot). Petioles were returned to the laboratory, surface sterilised, placed in test tubes containing agar cultures of *S. minor* and incubated at 24°C for 24h, then 31°C for 24h before returning to 24°C for 24h. The change in lesion length before and after the incubation at 31°C was measured. Plants of the Cycle 3 population were also examined to check whether the morphological phenotype of RHA 801 was being unintentionaly selected for in the small pot screening for resistance to *Sclerotinia minor*.

Results

Only 4 plants (2%) of *cms*HA89 had developed sclerotinia wilt by maturity. None of the RHA 801 or Cycle 3 population plants developed wilt. Examination of plant morphology confirmed that none of the Cycle 3 population plants closely resembled RHA 801. Most of the plants (75%) were monocephalic and those with branches tended to be taller, have different branching patterns and leaf and capitulum shapes than RHA801. The use of the Martinson (1992) method for non-destructive assessment of sunflower for resistance to *Sclerotinia* revealed more variable nett lesion lengths among the Cycle 3 population plants than the check inbred lines (Table 5.6). The average lesion length for the population was, however, less than for both of the checks.

Table 5.6 Comparison of Nett lesion lengths of Cycle 3 sunflower plants and two check inbred lines.

	Population	RHA 801	*cms*HA89
Growth Stage	R4-R5.2	R5.1	R4
Nett Lesion Length (mm)[1]	3.75 *a*	4.1 *a*	4.8 *a*
Range (mm)	1.0-12.0	1.0-9.0	2.0-10.0
Population Mean % of Checks		91.5	78.1

1. No significant differences (P>0.05) between nett lesion lengths were detected as determined by Duncan's Multiple Range test.

5.3.6 Glasshouse Screening of a number of S_0 selections of the Cycle 3 and Cycle 4 populations.

Commercial sunflower production is based on the culture of hybrids produced by crossing at least two inbred lines. Before any germplasm created by recurrent phenotypic selection can be used in the production of uniform hybrids the germplasm must be made homozygous or at least homogeneous for the phenotypes of interest. The reaction of the selfed progeny of a number of plants of the Cycle 3 population that had been screened by the method of Martinson (1992) and some survivors of the Cycle 4 population that had been screened by the pot base inoculation method were examined.

Materials and method

One hundred Cycle 3 plants were screened for resistance to *Sclerotinia minor* using the method of Martinson (1992)(Experiment 5.3.6). All these plants were self-fertilised by enclosing the capitula with paper bags before anthesis had commenced. This prevented the introduction of pollen from neighbouring plants by wind or insects. Similarly, plants that survived the glasshouse screening of the Cycle 4 population (Experiment 5.3.4.) were self-fertilised by enclosing the capitula in paper packets. The seed from these plants was designated S_0, the first self-fertilised generation.

Ten plants of ten S_0 selections from each Cycle were grown in the glasshouse and screened by the pot base inoculation method Fifteen plants of RHA801 and *cms*HA89 were included as resistant and susceptible checks, respectively.

Results

The autocompatibility (~self-fertility) either through autogamy (self-pollination of a single flowers) or geitonogamy (pollination among flowers on a single capitulum) of the plants as determined from seed production following bagging varied considerably from less than 10 % to over an estimated 60%. Emphasis was therefore put on screening families with higher autocompatibility because a) more seed was available and b) the ability to produce seed in the absence of pollinating insects is an important selection criterion in commercial sunflower breeding since hybrids will be grown in a wide range of environments including some where the presence of pollinators is low.

At least two of the S_0 families had a level of resistance to *Sclerotinia minor* superior to the resistant check RHA801. The selections Cycle4-115 and Cycle4-131 demonstrated lower mortality, longer mean delay from inoculation to appearance of basal stem lesions and slower mean rates of lesion extension than RHA801 (Table 5.7). Cycle4-135 had the longest mean delay of all families (16.5 days), a low rate of lesion extension and 80%

mortality. Three families (Cycle4-177, Cycle4-53 and Cycle3-36) were considered to be more susceptible than *cms*HA89 based on more rapid rates of lesion extension. Cycle4-53 also had high mortality and a shorter mean delay than *cms* HA89. This indicated that genes for susceptibility were being maintained in the population.

Table 5.7 The reaction of S_0 families from the Cycle 3 and Cycle 4 populations to inoculation with *Sclerotinia minor* (Ranked in descending order of rate of lesion extension).

Line	Pot Base Root Indices	Mortality (%)	Delay (days)	Rate of Lesion Extension (mm.d^{-1})
Cycle4-177[1]	2.83	80	12.60	14.05
Cycle4-53	3.40	90	10.56	11.02
Cycle3-36	2.63	50	12.50	9.61
cms HA89[2]	2.94	100	11.22	9.43
Cycle3-7	3.50	60	15.00	9.20
Cycle3-5	2.55	60	14.00	8.26
Cycle3-60	2.44	80	14.75	8.04
Cycle3-56	3.00	90	12.29	7.83
RHA801[2]	2.16	66.7	15.30	7.54
Cycle3-96	3.40	30	13.25	7.39
Cycle3-37	2.85	90	11.44	7.36
Cycle3-31	2.41	60	12.25	6.98
Cycle4-129	3.06	80	11.71	6.79
Cycle3-77	3.40	60	13.75	6.57
Cycle4-127	3.05	30	12.33	6.31
Cycle4-131	3.56	40	15.50	6.26
Cycle4-135	2.90	80	16.50	5.61
Cycle4-62	3.35	90	12.50	5.58
Cycle4-106	3.33	80	12.00	5.08
Cycle4-115	3.15	30	15.80	4.84
Cycle3-22	3.20	50	14.57	4.52
Cycle4-27	2.60	80	12.38	3.99
L.S.D.(P=0.05)	0.56	50	2.981	2.687

1. Designation: CycX-Y; X- Cycle from selection was made, Y- plant number 2. Check lines

5.3.8 Field screening of early inbred selections from Cycle 3 and Cycle 4 for resistance to **Sclerotinia minor.**

The production and testing of a large number of inbred selections from population requires special strategies. Field testing offers the best oppurtunity for testing large numbers of lines but, even so, resources (space and seed) may be extended if replicated trials are used. A number of unreplicated designs have been proposed which allow selection of the top n% of genotypes by adjusting genotypic values to compensate for field heterogeneity while providing an estimate of the precision of selection (Lin and Poushinsky, 1983; Lin and Poushinsky, 1985; May, Kozub and Schaalje, 1989). These Modified Augmented Designs (MAD) use various grid designs where plots of a control variety or varieties are placed systematically and randomly throughout the trial area to provide information on field heterogeneity. The MAD (type 1) design uses a Latin Square layout while the MAD (type 2) design is meant for long, rectangular plots. Analysis of individual test plot results involves transformation or adjustment of the data with respect to the result achieved for the relevant check plots. In this way the adjusted result for a test plot with high disease incidence may be lower than a test plot in another part of the field with a lower disease incidence because the check plots for the first test plot were more severely affected than those for the second test plot.

Materials and method

Over 100 plants survived the glasshouse screening described in the previous experiment. All these plants were allowed to self-fertilise by enclosing the capitula with paper bags before anthesis had commenced.

Two field sites were established near Toowoomba, Queensland, and infested by incorporating sunflower stems infected with *S. minor* collected from the University of New Englands' Laureldale Research Station and artificially inoculated sunflower capitula. A modified augmented design (type 2) (Lin and Poushinsky, 1985) was used. Whole plots were 9m long and contained 9 single row plots 75cm apart. The central row in each whole plot was RHA 801 as the main check variety while additional plots of CM526 and *cms*HA89 were randomly scattered throughout the trial site to provide check sub-plot data. Seed was oversown and seedlings thinned to a spacing of 20cm. This provided between 40 and 50 plants per plot. The capitula of two or more plants per test plot were enclosed in paper packets before anthesis commenced to force self-fertilisation. Seed from those selfed plants which did not develop sclerotinia wilt was collected for sowing in subsequent screening experiments or nurseries.

Disease incidence in each plot was assessed every 7 days until maturity. Wilted plants were examined for the presence of a basal stem lesion and were marked with red aerosol paint if a lesion was evident to avoid recounting in subsequent assessments. Area under the disease progress curves were determined for cumulative disease development (as a proportion of total plot plant number) over time. The experiment was designed and analysed using Agrobase/4™ (Agromix Software Inc., Manitoba, Canada).

Results

In the first screening nursery, 49 selections (or 54%) produced adjusted AUDPC's less than the RHA801 test entry, 87 were less than *cms*HA89 and 7 were greater than *cms* HA89. The distribution of adjusted AUDPC's as percentages of the adjusted AUDPC for RHA801 as shown in Figure 5.7 reveal that some lines were highly susceptible with adjusted AUDPC's of over 400% of that of RHA801. In plots with high disease incidence the likelihood of bagged plants developing wilt was much greater with the consequence that these lines were discarded and not progressed. The effect of this can be seen in Figure 5.8 where the number of selections with adjusted AUDPC's less than RHA801 was 73 (or 78% of total). The number of highly susceptible selections also decreased.

The eight sunflower inbred lines used throughout the studies reported in this thesis were also included as test plots in the second nursery. The relative susceptibilty of these lines was similar to those determined in the replicated screening trials described in the previous chapter of this thesis (Table 5.8 ; Experiment 4.3.1.).

Table 5.8. Area under the disease progress curves (AUDPC) as assessed and following statistical adjustment for eight sunflower inbred lines (ranked by increasing adjusted AUDPC).

Line	Assessed AUDPC	Adjusted AUDPC[1]
HA124	1.1	5.62
PacA1	6.7	6.29
CM526	13.3	8.25
RHA801	42.4	9.86
*cms*HA89	50.3	10.65
PacA3	82.3	13.85
PacR2	120.3	17.65
CM497	112.1	18.73

1. Adjusted using Method 3 (Agrobase/4, agronomix Pty Ltd.)

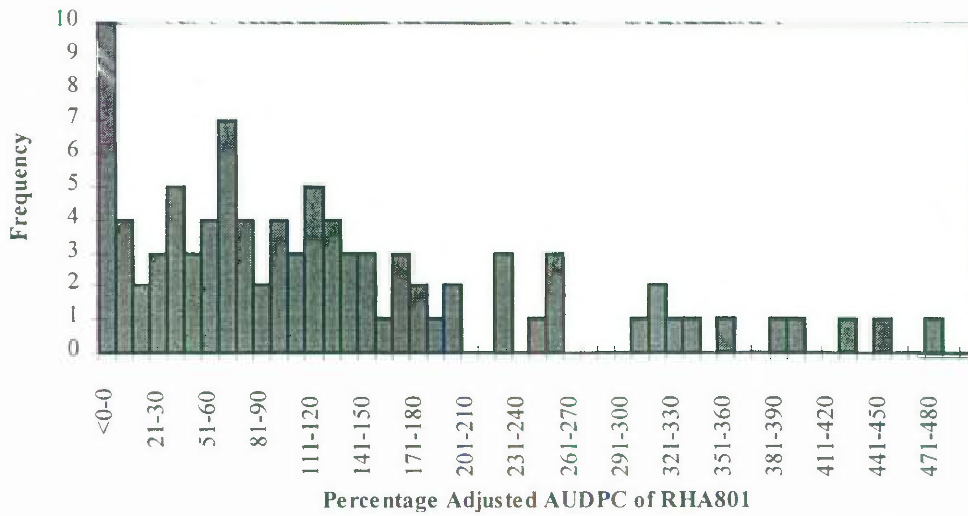

Figure 5.7. Frequency distribution histogram of adjusted AUDPC's for 91 partially inbred sunflower lines relative to the resistant check RHA801 (set at 100%)

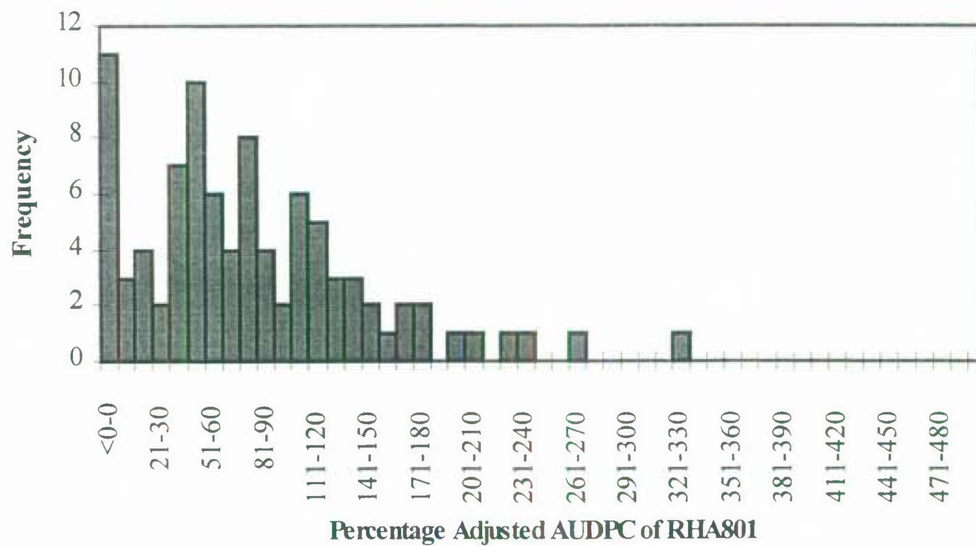

Figure 5.8. Frequency distribution histogram of adjusted AUDPC's for 93 partially inbred sunflower lines selected from the lines represented in Figure 5.7 relative to the resistant check RHA801 (set at 100%)

***5.3.9 Co-operative screening of selected lines for resistance to sclerotinia head rot caused by* Sclerotinia sclerotiorum.**

An opportunity to have a number of lines screened for reaction to head rot was accepted. Seed of thirty lines varying from S_2 to S_3 were supplied to Dr Maria Bazzalo (Zeneca Agricola), Balcarce Research Station, Argentina. Results of screenings are as provided by Dr. Bazzalo.

Material and methods

The head rot screenings conducted by Zeneca Agricola were conducted in the field at the Balcarce Research Station, Argentina during autumn. Test plots were planted along with serial plantings of two inbred sunflower check lines which provided susceptible and resistant lines for comparison. Plants were inoculated at anthesis (G.S. 5.1 - G.S. 5.2) by spraying the floral face of the primary capitulum with 1ml of an aqueous suspension of ascospores ($10^5.ml^{-1}$) and immediately covering the capitulum with a paper bag to maintain humidity. The bag was removed after 72h. Plants were inspected at maturity for symptoms which were rated on a 1-9 scale where 1 was totally destroyed and 9 was without visible symptoms. The ratings obtained from the test lines were then compared to those of the check plants inoculated on the same date.

Results

For many of the lines screened the reaction produced by inoculating individuals on different days were inconsistent (Table 5.9). Individuals inoculated on a particular day might develop little head rot while those of the same line inoculated two days earlier or later were badly rotted. Failure of the inoculation procedure is discounted due to consistent results obtained with the check lines. These inconsitencies may have been due to heterogeinity in the lines being tested. Some lines however did produce consistent results. Lines such as Cycle3-74-1-1-1, Cycle3-74-1-1-2, Cycle3-77-1-1 and Cycle3-94-3-1 were moderately susceptible compared to the checks while Cycle3-81-1-3-1, Cycle3-91-1-3-2, Cycle3-91-1-3-3 and Cycle3-100-1-1 were highly resistant.

Table 5.9 Results of screening 29 sur flower lines developed through recurrent phenotypic selection for resistance to *Sclerotinia minor* wilt for resistance to head rot caused by *Sclerotinia sclerotiorum*[1].

			Inoculation	Date		
Line	13.2	15.2	17.2	20.2	22.2	27.2
Resistant Check	8.6	8.8	8.1	7.3	-	-
Susceptible Check	2.9	3.7	2.8	2.6	-	-
Cycle3-19-1-3-2			8.4	3.4		
Cycle3-22-2-1	8.3					
Cycle3-22-7-1	6.6					
Cycle3-27-8-1	5.8					
Cycle3-27-8-2	3.6					
Cycle3-27-8-3	2.7					
Cycle3-36-1-1	6.9					
Cycle3-36-1-3	4.5					
Cycle3-40-1-1					2	9
Cycle3-40-1-4	9	3				
Cycle3-40-1-7	6.6					
Cycle3-40-2-2	7.2					
Cycle3-56-2-1	3.2					
Cycle3-66-2-2-1	5.6					
Cycle3-74-1-1-1			4		2.7	
Cycle3-74-1-1-2			5		3	
Cycle3-77-1-1			4.9	6.3		
Cycle3-77-1-2				4.0	5.0	
Cycle3-77-1-3			8.3	4		
Cycle3-81-1-3-1		8.7	7.3			
Cycle3-83-1-2	6.3					
Cycle3-85-2-2	7.8					
Cycle3-91-1-3-2	9	9				
Cycle3-91-1-3-3	7.8	8.7				
Cycle3-94-3-1	3.3		5.5			
Cycle3-100-1-1				7.8	8	
Cycle3-100-1-2				6.6	7.5	
Cycle4-115-9-1	4.8	6				
Cycle4-177-2-1				3		

1. Disease severity was scored on a 1-9 scale where 1 = totally destroyed and 9 = no visible symptoms.

5. 4 Discussion

Breeding for resistance to plant diseases follows the strategies used for any other trait. Genetic recombination is first established by intermating individuals with differing genotypes and then selecting for desirable phenotype recombinations. For phenotypes

controlled by many genes several generations of intermating and selection may be necessary before individuals with the optimal combination of genes occur at a high enough frequency to be found in small populations. Pedigree selection within F_2 populations derived from intermating elite lines has been used extensively in the improvement of crops such as maize and sunflower (Hallauer, 1992) but this approach limits genetic variability to the parental lines included in the cross. Recurrent phenotypic selection is an alternative breeding strategy designed to increase the frequency of optimal genetic recombinations through the selection of desirable phenotypes among a genetically diverse population. It is especially suitable in the breeding for traits with low heritability (Hanson, Probst and Caldwell, 1967).

An accurate means of detecting desirable phenotypes is essential in recurrent phenotypic selection. A pot base inoculation method was used in the screening experiments reported in this chapter. The use of small pots allowed for a reasonable population size that could be grown and screened throughout the year in the glasshouse. The selection of plants that did not develop a basal stem lesion using this inoculation procedure after a predetermined time is non-destructive. The destructive nature of sclerotinia diseases in killing infected plants has continually been a problem in breeding for resistance (Tourvieille de Labrouhe and Vear, 1990). In the period following inoculation with the pot base method all sunflower plants show some wilting but only those plants were the fungus grows through the roots and colonises the stem base were killed. The remainder where root colonisation failed to reach the stem continued to grow to maturity albeit with various degrees of debilitation such as stunting and leaf necrosis.

In the experiments reported in this chapter, hybrid checks were used along with the inbred checks since in the initial cycles of recombination it could be expected that significant heterosis would occur in the population which would decrease in later generations as more individuals shared common genotypes. The check lines were used to indicate the success of the inoculation or, alternatively, the likelihood of escapes occurring. The mean mortalities of the two susceptible checks over the four inoculations were 85%, 62.5%, 90% and 100% while those for the resistant checks were 70%, 35%, 35% and 62.5%. The low mean mortality of the susceptible checks of 62.5% was due to only 30% of *cms* HA89 plants being killed while 95% of Dk 3903 plants were killed. This failure to kill more plants of *cms*HA89 cannot be explained although many of the plants developed severe wilt but did not have basal stem lesions by the twenty-first day after inoculation. Nelson and Christianson (1993) also noted that, in a comparison of three sunflower lines following root inoculation, HA89 was the first to wilt but took longer to develop stem lesions than one of their 'resistant' lines.

The killing of 85-100% of plants of susceptible checks equates to 0-15% survivors or 'escapes'. In a population of 400 plants as many as 60 plants surviving the screening could have been as susceptible as *cms*HA89 or Dk 3903. However, over three cycles of screening and intermating the mean mortality of the population decreased from 91.5% to 19.0% of the mean of the two susceptible checks. This gain indicates the rapid accumulation of genes conditioning resistance selectable by the pot base inoculation method or very rapid loss of genes conditioning susceptibility. Vear and Tourvieille de Labrouhe (1984) found when selecting a recurrent sunflower population for resistance to head rot caused by *Sclerotinia sclerotiorum* that very rapid progress was made in the early generations as highly susceptible genotypes were eliminated.

The use of the pot base inoculation method to screen immature plants allows selected plants to be used as both female and male gene contributors. In this way genetic gain for the desired phenotype is much greater (twice) than would be the case if the selected plants were only pollen recipients or were selfed and the progeny tested for resistance before further intercrossing (Fehr, 1987). Jenkins *et al.* (1954) demonstrated that quantitative resistance to *Exserohilum turcicum* (Pass.) Leonard & Suggs in maize (*Zea mays* L.) could be rapidly increased by screening young plants and intercrossing selected individuals. Similarly, Graham *et al.* (1965) were able to screen young lucerne (*Medicago sativa* L.) for resistance to common leaf spot (*Pseudopeziza medicaginis* (Lib.) Sacc.) before intercrossing only the resistant selections.

In contrast, Vear and Tourvieille de Labrouhe (1984) screened sunflower for resistance to sclerotinia head rot and because plants with greater resistance could only be selected after flowering the plants were allowed to self and the progeny from selected individuals was intercrossed before the population could again be screened. This procedure took two growing seasons to complete a cycle. Analysis of selfed progeny was also performed in the recurrent selection for resistance to *Sclerotinia sclerotiorum* in *Phaseolus vulgaris* L. (Lyons, Dickson and Hunter, 1987). Good progress was made in increasing the level of resistance in bean as assessed by a subjective rating of lesion type and a quantitative measure of lesion length.

If this breeding method can achieve rapid accumulation of genes for resistance it can also lead to rapid loss of these genes if they are not fixed in the population. That is, the genes may be lost if they are not in a homozygous state in all individuals since a proportion (depending on the number of loci involved) of the progeny from intercrosses between heterozygous individuals would revert to susceptibility. The concentration of favourable genes were apparently eliminated in the Cycle4 population created from the Cycle 3 population which was screened by the method of Castaño *et al.* (1992). Hypotheses to

explain the loss of performance in the Cycle 4 population include; i) resistance genes present in the heterozygous condition after a small number of recombinations were lost when the screening emphasis changed during the Cycle 3 population, ii) there were a large number of escapes from the Cycle 2 population that were not detected by the leaf inoculation method used to screen the Cycle 3 population and consequently increased the level of susceptibility in the Cycle 4 population, iii) the number of individuals screened in the Cycle 3 population and the number recombined to give the Cycle 4 population were too low to allow satisfactory recombination of resistance genes, iv) escapes occurred in the Cycle 2 population due to relative differences in the effects of environment on the susceptibility of the germplasm, and v) inoculum pressure was too high and this masked any quantitative resistance that was present. The last proposal is not supported by earlier findings that susceptibility to this inoculation approach is not greatly affected by inoculum quantity (Experiment 3.3.4.).

The idea of a large number of escapes from the screening of the Cycle 2 population is not supported by the high mortalities of the susceptible checks *cms*HA89 and Dk3903. Screening by the method of Castaño *et al.* (1992) selected on the basis of resistance of leaf tissue to lesion development and was reported by the authors to be 'frequently correlated with levels of resistance to root attack'. This correlation may not however be strong. Independence of resistance to *Sclerotinia* on the different organs of sunflower has been reported (Tourvieille de Labrouhe and Vear, 1990). The pot base inoculation method does require that the fungus infects and successfully colonises the roots from the pot base to the stem base. If resistance in roots is independent to that in leaves then it might be expected that changing the emphasis of the screening to resistance from one organ to another may lead to a loss of resistance in the first organ. It was shown in the present work that resistance to head rot was also present in some elite lines chosen for their wilt resistance while in others it was absent. This supports the hypothesis of independence of resistance to sclerotinia diseases in the different organs of sunflower and shows that resistance in different organs is not exclusive.

The effect of environmental conditions on the susceptibility of sunflower to sclerotinia wilt must be also be considered. It is known that daylength and temperature can influence the development of disease (Orellana, 1975; Experiment 3.3.3). The Cycle 0 population was screened in the glasshouse during autumn, Cycle 1 during spring, Cycle 2 during summer and Cycle 4 during winter/spring. The warmer temperatures and higher light intensities in the glasshouse during spring and summer may have modified the relative susceptibility of the sunflower plants. However, the susceptibility of the check lines at each screening does not support the contention that glasshouse conditions were unfavourable for disease development. Since there is also no information in the literature

as to whether there are specific genotype x environmental interactions in the sunflower:*Sclerotinia* pathosystem any speculation that the susceptibility of the population, as a whole, showed greater sensitivity to environmental influences than the uniform checks would be unfounded, at this stage.

The screening of the $S_{0:1}$ and later families suggested that with further inbreeding and selection a number of new genotypes with resistance equal to or superior to the inbred line RHA801 might be readily developed. This line has consistently shown high levels of resistance to *Sclerotinia sclerotiorum* wilt in the United States (Gulya, 1985; Miller, 1992) and to *Sclerotinia minor* wilt in Victoria (Porter, Clarke and Woodroofe, 1994), and in the experiments described in this thesis. Unfortunately, RHA801 is not a vigorous inbred (Figure 5.2) and has limited root growth (Experiment 6.3.1). The population and inbred families described in this chapter had much greater root growth, different plant morphologies and variability in flowering times under pot culture than RHA801. If these characteristics are maintained in the field then the lines derived from this population will provide valuable new sources of genetic diversity for resistance to sclerotinia wilt.

CHAPTER 6

RELATIONSHIP BETWEEN ROOT GROWTH AND SUSCEPTIBILITY OF SUNFLOWER LINES TO SCLEROTINIA WILT IN GLASSHOUSE AND FIELD EXPERIMENTS.

6.1 Summary

The sunflower:*Sclerotinia minor* pathosystem is unusual among sclerotinia diseases in that, along with sunflower wilt caused by *S. sclerotiorum*, infection is initially established on host roots. It is possible therefore that root characteristics might influence the frequency with which plants become infected. In this chapter the hypothesis that total root growth is positively correlated with disease incidence was tested for eight sunflower inbred lines in two field trials and pot experiments. In addition, the effect of shoot on root susceptibility was tested in a reciprocal grafting experiment.

In the pot experiments the root index, as measured by estimating the amount of the base of the root ball covered with roots, was found highly correlated to root dry weight ($P<0.01$) but was not correlated with seedling susceptibility to *S. minor*. In plants grown in the two field there were various correlations between physical characters of plants (eg height, leaf area, days to flower, root length density and estimated average lateral root diameter) but the only measure with a significant correlation with disease incidence at anthesis was days to flower at both sites ($r=0.78$, $P<0.05$; $r=0.67$, $P<0.10$). The physical quantity of roots in the soil did not seem therefore to be a primary determinant of the final incidence of sclerotinia wilt, at least for the eight sunflower inbred lines used in this study.

The role of the shoot in modifying the susceptibility of plants to sclerotinia wilt was demonstrated by grafting different combinations of four sunflower inbred lines. In two experiments using RHA801, PacR2 and PacA1 as scions grafted onto rootstock of CM497 the time taken for lesions to appear at the stem base were significantly longer than in plants of CM497 grafted onto CM497. In these combinations the number of plants killed was also reduced. The effect of RHA801 on resistance was expressed by shoots and roots while in PacA1 the effect was mostly due to roots. The use of PacR2 as root stock increased susceptibility but its use as a scion had little effect on altering the susceptibility of root stocks.

6.2 Introduction

The incidence of soil-borne diseases has long been recognised as being influenced by the soil environment. Soil reaction (pH), nutrient levels, temperature, water potential, organic matter and gaseous composition affect the growth of plant pathogens and other soil microflora that may interact with the pathogens (Colhoun, 1973; Shipton, 1984). The influence of the host on the soil environment is mediated by the growth of roots and the presence of exudates. The nature and extent of plant roots influences the incidence of pathogen infection by soil-borne pathogens. The density and position of roots in the soil in relation to pathogen inoculum will determine the potential for contact and subsequent infection. Such a relationship has been found between root density of cotton and infection by *Verticillium dahliae* (Huisman, 1982).

Growth of roots of annual plants usually follow a sigmoidal growth curve where exponential growth precedes an asymptote that coincides with the reproductive phase. Sobrado and Turner (1986) found that the dynamics of root growth of a cultivated sunflower followed this scheme. The dry weight of the total root systems increased rapidly to a maximum at anthesis and then levelled out. Study of the root systems of cultivated sunflowers has shown, in a range of soil types, that the greatest root length density (linear length of roots per unit volume of soil) is in the upper 15-20cm of soil (Mason, Meyer, Smith and Barrs, 1983; Leach and Feale, 1987). Water deficits in the upper layers of soil may lead to increased growth by deeper roots (Sobrado and Turner, 1986). Sampling to determine differences in maximum root growth between sunflower cultivars grown without water stress can therefore be made at anthesis.

Grafting has also been used as a means of investigating various physiological processes in plants such as the relative importance of root and shoot genotypes on yield, drought tolerance and other phenotypic traits (Sanders and Markhart, 1990; White and Castillo, 1992). Roots and shoots exchange carbohydrates, water, mineral ions, and growth substances. Roots produce cytokinins and abscisic acid which can have dramatic effects on the growth and senescence of shoots (Sitton, Itai and Kende, 1967; Hubick and Reid, 1988; Zhang and Davies, 1990). The mineral composition of shoots may be influenced by differences in the ability of rootstocks to take-up Ca^{2+}, Mg^{2+} and K^{+} (Carles, Calmes, Alquier-Bouffard and Magny, 1966) Shoots supply roots with carbohydrates as energy sources for metabolic activity and growth. Understanding the relative importance of the morphological and physiological importance of roots and shoots to yield performance of sunflower has been considered necessary in the identification of ideotypes to be manipulated by breeding (Arnoux, 1978). In sunflower, the reciprocal grafting experiments of Robinson (1968) and Dyakov (1992) demonstrated that the shoot genotype

controlled traits such as maturity, stem length, grain yield and oil yield.

In this chapter, the hypothesis under test was whether root growth ie. root length density is correlated with incidence of sclerotinia wilt in sunflower varieties growing in small pots and artificially inoculated as well as in naturally infected plants in field plots. The role of the shoots on disease susceptibility was also tested in small pot experiments by establishing grafts.

6.3 Experimental

6.3.1. Relationship between root length density and the incidence of Sclerotinia minor *wilt in eight sunflower inbreds growing in the field.*

The root length densities of the sunflower inbred lines, RHA801, PacR2, CM497, CM526, PacA1, PacA3, *cms*HA89 and HA124 were determined in two field trials. The sites were in uninfested soil adjacent to the sclerotinia wilt screening trials LD91 and LD92 (refer Chapter 4). The soil at these sites was a chocolate silty clay loam of basaltic origin (Stace, Hubble, Brewer, Northcote, Sleeman, Mulcahy and Hallsworth, 1972). Agronomic preparation of the site included chisel ploughing to a depth of 25cm, followed by discing and finally rotary-hoeing just before sowing. Sunflowers were hand sown in 4 row plots with 50 cm between the 2.5m long rows and 25cm between plants within the row. A randomised complete block design was used with 4 replicate blocks. The sites were irrigated to the same schedule as the screening sites ie. approximately 12.5mm irrigation every ten days unless this amount or more was provided by rainfall. Irrigation was applied 10 days after rain. Weeds were controlled by manually removing seedlings as they appeared.

Root length density of lateral roots was determined for two plants sampled at random in the middle two rows when plants reached 50% anthesis. The sampling scheme used was based on that described in 'Figure 6b' of van Noordwijk, Floris and de Jager (1985). Six 8cm diameter, 15cm deep soil cores were taken in a regular pattern in relation to the chosen plants. Cores were cut into segments corresponding to depths 0 -5 cm, 5 - 10 cm and 10 - 15 cm. They were stored at $4^{o}C$ before soaking for 72 hours at $4^{o}C$ in a 0.5% (w/v) solution of Calgon™ to disperse clay colloids. Root material was extracted using a combination of sieving and floatation then gently blotted dry with paper towel and weighed for fresh weight determination.

Root length was estimated by the root intercept method of Tennant (1975). Roots were

dispersed on a glass tray placed over a 1cm x 1cm grid. The frequency at which the grid lines were intersected by roots was determined. Root length was estimated as the number of intercepts multiplied by 0.7857 (the length conversion factor)(Tennant, 1975). The quadratic mean root radius (r) was calculated from the relationship $(F_{wr}/L_r\pi)^{1/2}$ where F_{wr} is the fresh weight of roots and L_r is the length of roots (Mackay and Barber, 1985). The accuracy of this calculation is dependent on the density of root tissue being $1g.cm^3$. The density of sunflower roots was not determined in the present experiments but was assumed to be approximately one.

Other data collected in the trial were plant height, leaf number, leaf area and time from sowing to 50% anthesis. Leaf area of extant leaves was estimated by measuring the length and breadth of leaves and applying the formula; Leaf area= length x breadth x correction factor (0.7) (English, 1976). Anthesis reached 50% when at least 50% of florets on 50% of plants in a plot had opened.

Results

The eight inbred sunflower lines varied in the morphological characters assessed at both trial sites (Tables 6.1 and 6.3). The multi-headed lines RHA801 and PacR2 were generally shorter (height of primary capitulum), supported a smaller leaf canopy and had the lowest root length density of lateral roots. Among the monocephalic lines HA124 and PacA1 were also shorter, with smaller canopies and root length densities. Plants in the autumn trial (LD92) were larger on the whole than those in the summer trial (LD91). Roots systems excavated from LD92 as described in Experiment 7.4.7 and shown in Figure 6.1 demonstrate the variability in the extent of root systems between the inbred lines.

Regression analysis for relatedness between these morphological characters and cumulative mortality revealed few significant correlations. At the LD91 site (Table 6.2) flowering time was not significantly correlated to any of the morphological characters but there was a significant positive correlation with cumulative mortality ($r=0.78$, $P<0.05$). Similarly, at the LD92 site time to flowering was significantly correlated with cumulative mortality ($r=0.67$, $P<0.1$). Disease incidence at anthesis was higher in lines that took longer to reach anthesis. Root length density was positively correlated with mean diameter of roots at both sites and indicated that in bigger root systems the average diameter of roots increased. Neither of these variables were highly correlated with cumulative mortality at either site although both were positive and could be used to explain up to 56% of the variation in mortality.

Table 6.1 Relationship between plant characteristics at flowering of eight inbred sunflower lines grown in a disease free site adjacent to sclerotinia screening site LD91 and the cumulative incidence of *Sclerotinia minor* wilt recorded in the disease trial at the comparable time.

Line	Shoot			Root		Cumulative Mortality
	D.F.50[1] (days)	Height (cm)	Leaf Area (cm^2)	R.L.D.[2] ($cm\ cm^{-3}$)	Mean Ø[3] (mm)	(%)
RHA 801	84	93.6	1432.3	0.352	0.107	7.3
Pac R2	84	74.4	1609.1	0.338	0.111	15.1
CM526	88	108.6	2028.9	0.924	0.142	9.0
CM497	96	85.3	1980.2	0.951	0.118	36.2
Pac A1	84	79.1	1807.2	0.593	0.122	1.6
Pac A3	84	114.1	4586.4	1.086	0.132	21.9
*cms*HA89	92	102.0	3509.4	1.166	0.149	21.2
HA124	84	95.1	1895.7	0.979	0.106	5.0
L.S.D. (P=0.05)		9.152	851.0	0.614	0.0058	15.4

1. D.F. 50 - days from sowing to 50% flowering. 2. R.L.D. - root length density. 3. Ø- diameter.

Table 6.2 Correlation matrix of variables in Table 6.1[1]

Variable	DF_{50}	Height	Leaf Area	R.L.D.	Root Ø	Mortality
DF_{50}	1.0	0.038ns	0.095ns	0.496ns	0.407ns	0.775**
Height		1.0	0.700*	0.681*	0.599ns	0.095ns
Leaf Area			1.0	0.698*	0.625*	0.405ns
R.L.D.				1.0	0.639*	0.426ns
Root Ø					1.0	0.240ns
Mortality						1.0

1. Level of significance of correlation: ns (P> 0.10), * (0.10>P<0.10), ** (0.05>P<0.01), *** (p<0.01).

Table 6.3 Relationship between plant characteristics at flowering of eight inbred sunflower lines grown in a disease free site adjacent to sclerotinia screening site LD92 and the cumulative incidence of *Sclerotinia minor* wilt recorded in the disease trial at the comparable time.

Line	Shoot			Root		Cumulative
	D.F.$_{50}$[1]	Height (cm)	Leaf Area (cm^2)	R.L.D.[2] ($cm\ cm^{-3}$)	Mean Ø[3] (mm)	Mortality (%)
RHA 801	80	109.6	2216.2	0.488	0.116	47.8
Pac R2	75	76.7	1921.6	0.425	0.120	50.1
CM526	90	121.0	2267.8	0.886	0.141	36.3
CM497	100	110.4	3018.1	0.762	0.135	90.1
Pac A1	82	104.3	4236.2	0.511	0.127	51.43
Pac A3	92	128.7	4386.2	0.905	0.136	74.8
cms HA89	92	102.5	4229.3	1.003	0.143	86.7
HA124	88	109.7	2.55.9	0.461	0.102	47.2
L.S.D.	(P=0.05)	7.448	953.9	0.145	0.0017	26.62

1. D.F. 50 - days from sowing to 50% flowering. 2. R.L.D. - root length density. 3. Ø- diameter.

Table 6.4 Correlation matrix of variables in Table 6.3[1]

Variable	DF$_{50}$	Height	Leaf Area	R.L.D.	Root Ø	Mortality
DF$_{50}$	1.0	0.623*	0.370ns	0.724**	0.515ns	0.671*
Height		1.0	0.337ns	0.548ns	0.305ns	0.104ns
Leaf Area			1.0	0.557ns	0.579ns	0.605ns
R.L.D.				1.0	0.872**	0.562ns
Root Ø					1.0	0.495ns
Mortality						1.0

1. Level of significance of correlation: ns (P>0.10), * (0.10>P<0.10), ** (0.05>P<0.01), *** (p<0.01).

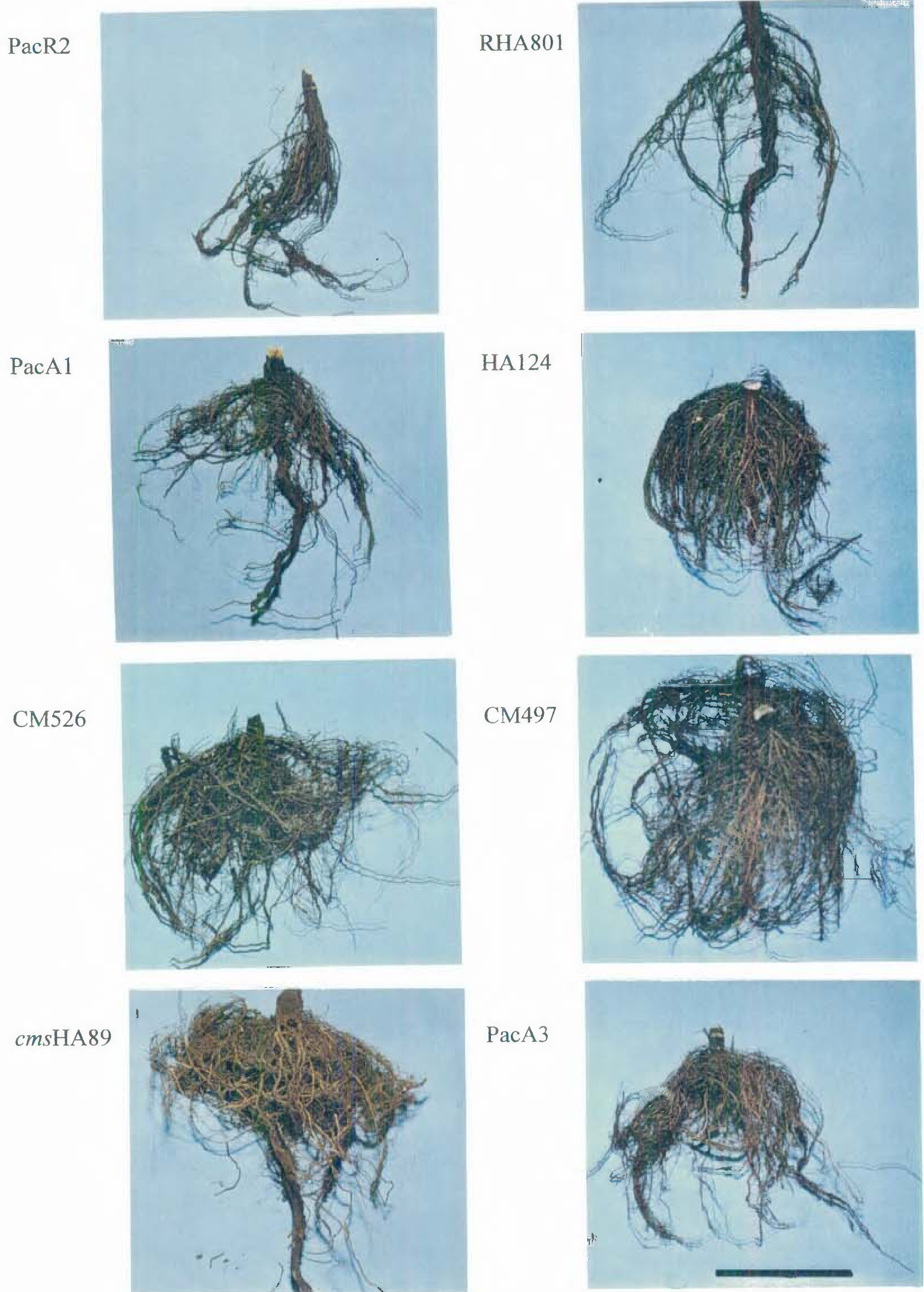

Figure 6.1 Dried root systems of eight inbred sunflower lines excavated from LD92 as described in Experiment 7.4.6. (Bar= 10cm)

6.3.2. Relationship between pot base root index and root density

While developing the pot base inoculation method differences in the amount of root growth between the lines were observed. These differences seemed to be consistent between different trials. This experiment was designed to examine the relationship between the area of roots exposed on the base of the root ball and the total density of roots in the pot.

Materials and method

Seedlings of the eight inbred sunflower lines; RHA801, PacR2, CM497, CM526, PacA1, PacA3, *cms* HA89 and HA124 were grown in the standard potting mix in 10cm diameter plastic pots with flat bottoms. Fifteen replicates were arranged in a completely randomised design. Two or three seeds were sown 2cm deep as close as practicable to the centre of the pot. These were thinned to a single seedling before the first true leaves developed. Pots were thoroughly watered with tap water each morning and allowed to drain freely. Five pots of each line were chosen at random thirty-five days after sowing, inverted and the pot dislodged from the root ball by gently tapping the rim on the edge of the bench. Area of the bottom surface of the root ball occupied with exposed roots was estimated using the key as described in the General material and methods section (Chapter 2). Root dry weights were taken after the roots were removed from the stems, washed free of potting medium and dried at 80°C for 36h. The remaining ten replicates were inoculated with *S. minor* UNE#3.

The experiment was conducted twice and the combined data was analysed using factorial analysis of variance (MSTAT, Michigan State University)

Results

Root Index for the eight inbred sunflower lines was highly correlated ($r=0.85$, $P=0.007$) with total root dry weight, and can therefore be used as an easy way to estimate total root growth in the pot. There were no significant correlations ($P>0.1$) between root index or root dry weight and any of the parameters of disease susceptibility. This suggests that it is not the physical quantity of root in the pot that matters but characteristics of the roots themselves.

The results of the small pot experiment and the field experiment reported in the previous section were subjected to regression analysis (Table 6.7). It is acknowledged that this may not be a valid comparison since the pot grown plants were assessed at early bud stage (G.S. R1) while the field grown plants were at mid-flowering and were expected to have attained maximum root biomass. Nevertheless, there were significant correlations between root length densities determined from the field trials and root index as determined in small pots.

Table 6.6 Relationship between root growth visible at the bottom of root ball, total root growth for sunflower seedlings raised in small pots and susceptibility to *S. minor* (ranked by ascending root index).

Inbred	Root Index	Root Dry Weight (g)	Delay (days)	Rate (mm.d^{-1})	Mortality (/10)
RHA801	1.89	0.5393	12.8	7.7	8
PacR2	2.00	0.5930	7.6	13.9	7
CM526	2.23	0.7075	10.9	8.2	8
HA124	2.41	0.8692	14.6	8.4	5
PacA3	2.48	0.8440	11.9	12.3	8
PacA1	2.50	0.9779	10.9	9.6	7
CM497	3.07	0.9044	11.9	14.1	9
cms HA89	3.30	1.0470	13.0	11.8	6
L.S.D.(P=0.05)	0.56	0.2747	3.1	2.53	4

Table 6.7 Correlation matrix of comparison between root growth in small pots and field grown plants.

	Field		Small Pot	
Variable	R.L.D.(LD91)	R.L.D. (LD92)	Root Index	Root Dry Weight
R.L.D.(LD91)	1.0	0.788**	0.744**	0.716**
R.L.D.(LD92)		1.0	0.638*	0.462ns
Root Index			1.0	0.851***
Root Dry Weight				1.0

1. Level of significance of correlation: ns (P>0.10), * (0.10>P<0.10), ** (0.05>P<0.01), *** (p<0.01).

6.3.3. Susceptibility of grafted sunflower plants to **Sclerotinia minor.**

Grafting offers the opportunity to test the relative effect of different shoot genotypes on the susceptibility of sunflower roots to *Sclerotinia minor*.

Materials and method

The sunflower genotypes RHA801, PacR2, PacA1 and CM497 were used in reciprocal grafting experiments to examine the effect of different root and shoot genotype combinations on the susceptibility of sunflower to *S. minor*. RHA801 and PacR2 are both

multi-headed fertility restorer inbred lines that differ in their susceptibility to *S. minor* in both field and glasshouse tests (Experiment 4.3.2) while PacA1 and CM497 are both single-headed inbred lines that also differ in their susceptibility (Experiment 4.3.2). The experiment was repeated with all four lines (Table 6.8).

Seedlings were grown in multi-compartmental polystyrene flats with a single seedling per 2.5x2.5 cm compartment. Grafts were performed when the primary leaves began to expand (5-7 days after sowing). Cleft grafts were made by cutting the hypocotyl 1.5cm below the cotyledonary node into a 'V-shape' with either a razor blade or scalpel. The scion was immediately placed in the cleft of a root stock, and the graft held in place and protected from desiccation by wrapping with a small piece of adhesive masking tape. The use of fungicides to protect the seedlings while graft union established was avoided in case of residual problems with later inoculations. Grafted plants were placed under a plastic canopy and exposed to reduced light levels for 2-3 days. The canopy was removed for one day before the seedlings were returned to the glasshouse bench. Seven days later the survivors were transplanted to 10cm pots and transferred to glasshouse benches. The graft union was well formed by this stage (Figure 6.1).

Plants were inoculated with *S. minor* JNE#3 by the pot base method at 30-40 days after sowing. Plants were either at the late vegetative or early reproductive growth stages. Data collected included root index as observed when the plants were inoculated, mortality after 21 days and the period between inoculation and expression of a lesion at the stem base. The rate of lesion extension was not analysed because of the uneven effect of the graft callus on lesion progression. Many lesions did not progress beyond the graft while in other cases the callus did not inhibit lesion progression. Plant height at inoculation was also measured in the second experiment.

Table 6.8. Reciprocal grafts used in Experiments.

	Scion			
Stock	RHA801	PacR2	PacA1	CM497
RHA801	*	*	*	*
PacR2	*	*	*	*
PacA1	*	*	*	*
CM497	*	*	*	*

The data from the two experiments has been presented separately due to heterogeneity of the data.

Grafted seedlings in excess of the ten replicates of each treatment used in the glasshouse experiments were transplanted to a field site infected with *S. minor* (LD91) in a preliminary study to test the feasibility of establishing a field trial of grafted plants. One hundred plants in all were transplanted. Transplants were provided with 5min of over-head irrigation at hourly intervals during daylight hours for the first 48h after transplanting.

Figure 6.1 A successful sunflower graft.

Results

Grafting resulted in over 90% survival except in cases where the restorer lines RHA801 and PacR2 were used as root stocks for PacA1 and CM497. The restorer lines produced smaller seed which resulted in seedlings having thinner hypocotyls. Grafting success between these lines and the seedlings of PacA1 and CM497 which produced seedlings with thicker hypocotyls ranged between 70 and 85%. At least 20 seedlings were grafted to be certain of obtaining the 10 plants of each combination to be inoculated with *S. minor*.

The field transplanted seedlings suffered high rates of mortality. As early as one week after transplanting several seedlings were found with stem lesions and sclerotial primordia. Isolations confirmed the presence of *S. minor*. In the following month more plants were killed so that by the time the plants reached early reproductive phase (G.S. R1) over 40% had been killed. This compared to observations in the adjacent screening trial where the same inbreds used in the grafting experiment were planted where less than 1% of plants established from seed were killed by G.S. R1 (Experiment 4.3.1).

In glasshouse experiments grafts with CM497 as scion showed the greatest alteration in root parameters. Root indices were higher for stocks of the other three lines with CM497 as scion in both experiments. In grafted plants with CM497 as scion the time taken from inoculation to appearance of basal stem lesions was reduced as a general trend and the mortality of plants was also higher compared to the CM497/CM497 graft. The reverse situation was also evident. The use of CM497 root stock in combination with the other lines as scions reduced susceptibility to *S. minor*.

Table 6.9 Effects of the interaction between shoot or root genotypes on growth and susceptibility of sunflower to *Sclerotinia minor*[1] . Experiment 1.

	Scion Genotype			
Stock Genotype	RHA801	PacR2	PacA1	CM497
		Root Index		
RHA801	1.8 -	1.8ns	2.0ns	2.8 *
PacR2	1.8ns	1.3 -	1.2ns	2.5**
PacA1	1.8 *	1.6 *	2.2 -	2.4ns
CM497	2.1**	2.7 *	3.4ns	3.8 -
		Delay (days)		
RHA801	15.0 -	17.0ns	16.5ns	13.5ns
PacR2	17.0 *	12.5 -	13.5ns	10.3ns
PacA1	18.0**	12.0ns	13.0 -	12.2ns
CM497	13.5**	1[illegible].8ns	13.3**	10.8 -
		Mortality (/10)		
RHA801	4 -	2ns	4ns	8 *
PacR2	6ns	5 -	4ns	8ns
PacA1	2ns	5ns	5 -	5ns
CM497	2 *	4 *	3 *	10 -

1. Means of heterologous grafts tested against control homologous grafts in pair wise comparisons tested for significance by Students t-test. Effects of shoots on root susceptibility tested (eg RHA801/CM497 versus RHA801/RHA801). Statistical significance, ns- not significant, *- significant at 5% level, **- significant at 1% level.

The summary of the effects of all combinations, although not statistically analysed, are shown in Table 6.11. Resistance in RHA801 may be mediated by both shoot and root since grafts with RHA801 as scion or root-stock resulted in extended times from inoculation to appearance of a stem base lesion (delay) and lower mortality. In contrast the effect in PacA1 is stronger when this line was used as a root stock.

Table 6.10 Effects of the interaction between shoot or root genotypes on growth and susceptibility of sunflower to *Sclerotinia minor*[1] . Experiment 2.

	Scion Genotype			
Stock Genotype	RHA801	PacR2	PacA1	CM497
	Height (cm)			
RHA801	68.4 -	68.9ns	68.8ns	72.4ns
PacR2	72.9ns	71.4 -	72.8**	70.9ns
PacA1	71.7ns	60.3 *	60.0 -	65.5ns
CM497	74.6ns	66.1ns	72.3**	66.0 -
	Root Index			
RHA801	2.2 -	2.2 ns	2.6 *	2.8 *
PacR2	2.7 *	2.4 -	3.3**	3.3**
PacA1	2.3 ns	2.2 ns	2.1 -	2.9**
CM497	3.3 ns	2.8 ns	3.0 ns	3.0 -
	Delay (days)			
RHA801	17.3 -	15.7 ns	15.0 ns	14.0 ns
PacR2	13.3 ns	11.6 -	10.3 ns	11.4 ns
PacA1	15.0 *	15.7 *	17.0 -	14.8 *
CM497	11.7**	10.6**	10.8 *	8.0 -
	Mortality (/10)			
RHA801	3 -	5ns	4ns	6 *
PacR2	9ns	8 -	10ns	6ns
PacA1	3ns	3ns	3 -	4ns
CM497	5 *	6ns	7ns	10 -

1. Means of heterologous grafts tested against control homologous grafts in pair wise comparisons tested for significance by Students t-test. Effect of roots on shoot height analysed (eg. CM497/RHA801 versus RHA801/RHA801) while effects of shoots on root susceptibility tested (eg RHA801/CM497 versus RHA801/RHA801). Statistical significance, ns- not significant, *- significant at 5% level, **- significant at 1% level.

Table 6.11 Summary Table of Combined Means from Tables 6.9 and 6.10

Stock Genotype	Scion Genotype	Root Index	Height[1] (cm)	Delay (days)	Mortality (/10)
RHA801	all	2.28	69.6	15.5	4.5
PacR2	all	2.32	72.0	12.5	7.1
PacA1	all	2.19	64.4	14.4	3.8
CM497	all	3.02	69.8	11.4	5.9
all	RHA801	2.27	71.9	15.1	4.3
all	PacR2	2.15	66.7	13.4	4.8
all	PacA1	2.48	68.5	13.7	5.0
all	CM497	2.95	68.7	11.9	7.2

1. Plant height was only recorded in one experiment.

6.4 Discussion

The importance of roots on the incidence of sclerotinia wilt and stem base rot may be influenced by an interaction between root volume in the soil and exudates from the roots which affect sclerotial germination and growth of rhizosphere inhabitants that might influence development of the pathogen. The propagules of many fungal pathogens do not germinate spontaneously in soil. They are stimulated to germinate by exudates from the host or even non host species. Positive effects of root exudates on germination of fungal propagules have been reported for *Sclerotium cepivorum* Berk. (Coley-Smith, 1960), *Rhizoctonia fragariae* (Husain and McKeen, 1963), *Fusarium solani* (Mart.)App. & Wr. f sp *phaseoli* (Stroth and Snyder, 196 .); *Fusarium solani* f sp *pisi* (Short and Lacy, 1974) and *Plasmodiophora brassicae* Wor. (MacFarlane, 1952). Exudation of carbohydrates was thought responsible for the germination of *F. solani* while the exudates of onion may include volatile antibiotic substances that inhibit the growth of rhizosphere microflora but stimulate germination of *S. cepivorum*(Coley-Smith and Holt, 1966; King and Coley-Smith, 1968). In many other cases the nature of the exudates has not been characterised.

Frenzel (1957) reported that the amino acids leucine, valine, glutamine, glutamic acid, alanine, asparagine, serine, phenylalanine, threonine and aspartic acid were present in the exudates of the roots of *Helianthus annuus*. Juo and Stotzky (1970) showed that the exudation of proteins from sunflower roots varied dramatically with time after sowing. Protein yields decreased from 130μg (20d post-sowing) to only 20μg (55d post-sowing) before rapidly increasing to over 160μg (61-90d post-sowing). However, they did not

describe the cultivar used or the growth stages corresponding to the sampling times. It would have been interesting to correlate their results with growth stage and periods of maximum attack of roots by *Sclerotinia minor* or *S. sclerotiorum*. It is possible that of the mixture of compounds exuded by sunflower roots some may be toxic to *Sclerotinia* and the lack of these may lead to increased infection. Alternatively, some of the compounds are probably stimulatory to fungal growth and lowered production of these compounds may decrease infection.

Attempts to establish transplanted grafted sunflower seedlings in soil infested with *S. minor* were not successful with many plants being killed in the first weeks after transplanting. These plants were killed during vegetative growth where little disease develops in plants established from seed. It is possible that root damage suffered during transplanting released compounds into the soil that stimulated germination of sclerotia. Furthermore the damage might have resulted in the death of roots which as a consequence became more susceptible to attack by a necrotrophic pathogen such as *S. minor*. Stress experienced by the plant during acclimatisation after transplanting might have reduced the capacity of the remaining roots to resist colonisation. In plants established from seed vegetative growth is characterised by vigorous growth of root systems with little necrosis so the same opportunities presented to the fungus by transplanted plants will not be present. The propagules of other soilborne plant pathogenic fungi such as *Thielaviopsis basicola* (Berk. and Br.) Ferr. and *Sclerotium cepivorum* Berk. are stimulated to germinate by wounds to plant roots (Coley-Smith and Hickman, 1957; Linderman and Toussoun, 1968).

The relationship between shoot and root growth of plants is tightly regulated with a number of genetically programmed alternative developmental patterns exhibited in response to external stimuli such as photoperiod and light interception, moisture stress, shoot and root temperatures, soil acidity, and nutrient availability (Kasperbauer, 1990). Studies of genetically identical lines under different growth conditions has led to conclusions that plants will only invest sufficient carbon resources to roots to ensure that the shoot can be supported and subsequent seed production can be achieved (Kasperbauer, 1990). The grafting experiments reported in this chapter also demonstrated that shoot genotype can influence root growth and susceptibility to *Sclerotinia minor*. Use of RHA801 (a line which produces low shoot biomass in the field) as scion onto root stocks of CM497 (which produces larger shoots) resulted in a significant decrease in root growth compared to CM497 grafted onto CM497. Fewer of the RHA801/CM497 grafted plants were killed and the time taken for stem lesions to appear was greater on these plants than on CM497/CM497 grafted plants. The mechanism behind the reduced susceptibility was not determined. However, one possible explanation was that there were reduced

movement of assimilates from the shoot of RHA801 to the roots of CM497 and this lowered the carbohydrate status of the roots and subsequently the amounts of root exudates.

Davet and Serieys (1987) found that the concentration of reducing sugars in sunflower roots and stem bases increased up to the commencement of flowering and then decreased rapidly. The lower concentration of reducing sugars was correlated with increased infection of the tissue by *Macrophomina phaseolina*. The levels of reducing sugars were higher in stem bases of resistant than susceptible hybrids. *M. phaseolina* may be described as a 'low sugar disease' (Lewis, 1973). It is possible that higher concentrations in roots before anthesis would result in increased exudation of these compounds. However, the effect of different levels of reducing sugars on growth of *Sclerotinia* species has not been reported.

Compounds released from roots may favour sclerotial germination, fungal growth and infection by *Sclerotinia* species. Rimmer and Menzies (1983) compared the effects that root exudates from seedlings of corn (*Zea mays* L.), rapeseed (*Brassica napus* L.) and sunflower had on the germination of sclerotia of *Sclerotinia sclerotiorum*. Exudates collected from sunflower roots, compared to the other two species, increased the myceliogenic and carpogenic germination of several isolates of *S. sclerotiorum* but the effect was not consistent for all isolates of the fungus. The chemical compounds involved were not identified. Myceliogenic germination in three isolates was inhibited by exudates from corn and rapeseed. It is possible that non-host species such as corn and host species such as rapeseed where roots are not normally attacked exude into the soil chemicals inhibitory to *Sclerotinia* which partly explains their resistance.

Plant roots exudates may also contain toxic substances. Sunflower species including *H. annuus* liberate a number of compounds some of which are allelopathic to a range of crop and weed species (Wilson and Rice, 1968; Irons and Burnside, 1982; Schon and Einhellig, 1982; Leather, 1983). In *Helianthus rigidus* Desf. autotoxicity may also exist (Curtis and Cottam, 1950). Allelopathy is an ecological means of reducing competition. In its natural environment *H. annuus* is regarded as an early coloniser of disturbed sites. Environmental stresses caused by over-population and unavailability of nutrients can increase the concentration of potentially allelopathic phenolic compounds within *H. annuus* tissue (Hall, Blum and Fites, 1982). Under those conditions allelopathy would provide a distinct advantage for survival. Exudation of these compounds from roots or release after decomposition provide a means of reducing germination of competitive plant species.

The application of plant breeding methods to sunflower might be expected to have lead to

a reduction in the allelopathic capacity of genotypes. Crops are selected to yield at high plant populations without any negative inter-plant effects. However, Leather(1983) demonstrated that leachates from a number of commercial sunflower hybrids were more inhibitory to the germination of *Brassica kaber* (DC.)L.C.Wheeler seeds than those of a wild *H. annuus* line. At present there is no experimental evidence as to whether there are large differences in the rate and type of root exudation of allelopathic compounds with fungistatic or fungicidal effects that might explain the differences in incidence of sclerotinia wilt on various sunflower genotypes.

Recent research has shown that plants can produce a number of peptides and proteins with toxicity to fungi. Some of these are preformed in organs such as seeds and are released on germination or damage to the seed (Terras *et al.*, 1995). This may provide a protective buffer around the seed. Pathogenesis-related proteins may also possess anti-fungal activity but are expressed following challenge by the pathogen or other stresses. Sunflower leaf tissue treated with acetylsalicylic acid, oxalic acid, heavy metal ions or ultraviolet radiation was shown to produce proteins of the chitinase, β-1,3-glucanase, and osmotin classes of pathogenesis-related proteins (Jung, Maurel, Fritig and Hahne, 1995). Further research may examine whether any of the proteins exuded by sunflower roots possess anti-fungal activity since the host has been shown capable of producing anti-fungal proteins.

The relationship between the root system of sunflower and the incidence of root infection by *Sclerotinia minor* is complex. Root mass may affect the likelihood of a root and fungal propagule being in a suitable position for infection to occur (English and Mitchell, 1988). It has been proposed that the greater incidence of sclerotinia wilt caused by *S. sclerotiorum* after anthesis is due to the presence of a greater root mass and the loss of the ability as seen in the developing root system to slough-off damaged or infected roots (Nelson, 1984; Nelson and Christianson, 1993). Burgess *et al.* (1992) observed that in pots and in the field in the sunflower growing area of Victoria where *Sclerotinia minor* wilt of sunflower is most prevalent that root growth of a sunflower hybrid and an inbred increased dramatically between floral bud initiation and mid-bud (Growth Stage 3.3) and this coincided with the first occurrence of wilt. However, the results presented in this chapter suggest that root density is not the primary determinant of susceptibility to sclerotinia wilt caused by *S. minor*. This reinforces the decision to study a diverse range of genotypes rather than a very small number. It would be possible within the present group to select combination to support opposing hypotheses. For example, a comparison between RHA 801 and CM497 would support the hypothesis that disease incidence was directly related to root length density. However, a comparison between CM526 and PacR2 suggests that greater root length density enhances resistance. It can be concluded that root length density is not a suitable ideotype for use in selecting sunflower for resistance to *Sclerotinia minor* wilt.