CHAPTER 10

GENERAL DISCUSSION

The work reported in this thesis confirmed that sunflower is highly susceptible to *Sclerotinia minor* with the main symptom being a root and stem base rot leading to wilting and death of the plant. This disease has been a major constraint to sunflower production in the highly productive irrigation areas of northern Victoria (Porter and Clarke, 1992) and an increasing problem on the Liverpool Plains area of New South Wales. At present however, *S. minor* has not been reported to be economically important overseas. There have been no publicly funded programs to breed sunflower for enhanced resistance to *S. minor* in Australia although there have been projects looking at screening commercial and experimental hybrids in fields infested with the fungus (Clarke, 1982; Porter *et al.*, 1989). Consequently, little is known about the nature of resistance to *S. minor* in sunflower. Sunflower breeding in Australia is conducted by private companies. The priorities of those companies has been most often dictated by the requirements of the larger northern seed markets where sunflower rust (*Puccinia helianthi*) is the major disease problem. The studies reported in this thesis were designed to obtain information on the efficacy of selecting for resistance to *S. minor* and the nature of resistance in sunflower.

Another species of *Sclerotinia, S. sclerotiorum,* is a major disease of sunflower internationally causing various diseases including root and basal stem rots, middle stem rots, bud rot and head or capitulum rot (Masirevic and Gulya, 1992). There is a large amount of literature describing selection of sunflower for resistance to wilt caused by *S. sclerotiorum*. This literature was reviewed in Chapter 3 with the intention of identifying methods that would also be suitable for screening sunflower for resistance to *S. minor* as well as identifying host germplasm that might be tested further. The breeding of crops for resistance to any disease requires an appropriate method of screening that is efficient and effective. That is, efficient in that a large number plants must, if necessary, be screened with minimal possibility of escapes and effective in that the method must accurately distinguish between host lines exhibiting different levels of reaction to the pathogen. A wide range of approaches were reported but very few reported relationship between the results of direct (using the pathogen) or indirect (without the fungus) artificial screenings with the relative susceptibility of the same germplasm under natural conditions.

Sedun and Brown (1989) published the only report on the use of artificial inoculation to test sunflower for resistance to *S. minor*. In essence, the method involved growing plants in small pots, inserting inoculum into a hole at the edge of the pot and assessing the subsequent linear rate of lesion extension on the stem. These authors did not consider that the time or delay taken from inoculation to appearance was a consistent measure for use in assessing different sunflower lines. A modification of their method was developed and tested in Chapter 3. This pot base inoculation method was performed on sunflower seedlings grown in small pots (10cm) and involved removal of the root ball, placing inoculum in the base of the pot and replacing the root ball. It was hoped that uniform placement of inoculum relative to the stem base would reduce variation in the time taken from inoculation to appearance of a basal stem lesion therefore making this measurement a useful parameter to assess the ability of the fungus to colonise the roots. Root colonisation is important in the field epidemiology of sclerotinia wilt but is avoided by many inoculation methods where the stem only is inoculated.

Optimisation of the pot base inoculation method showed that millet grain colonised by *S. minor* was suitable provided colonies were young and large numbers of sclerotial initials and sclerotia had not formed. The number of millet grains placed in the bottom of the pot did not consistently affect the proportion of plants killed. An ambient temperature around 20°C was most conducive to disease development. The pot base inoculation method was then used to screen a number of inbred sunflower lines, sunflower hybrids and wild accessions of sunflower. In every screening experiment variation among sunflower accessions for resistance to *S. minor* was apparent. These included genotypes that could be considered more susceptible to *S. minor* than the susceptible check *cms*HA89 and more resistant than the resistant check RHA801 which had originally been chosen as checks on the published reaction of these lines to sclerotinia wilt caused by *S. sclerotiorum* (Miller, 1992; Mancl and Gulya, 1993).

From the lines screened eight were chosen for generation of field data against which further glasshouse and laboratory results could be compared. These lines were chosen because sufficient seed was available for comprehensive experimentation and they represented the range of susceptibility observed. Two disease screening sites were established on the heavy clay soils at the University of New Englands' Laureldale Research Farm (Chapter 4). Apart from occasional plants that wilted before budding most disease became evident in plots after budding and rapidly increased between budding and anthesis so that in several of the sunflower lines most plants had been killed by anthesis. This contrasts with the observations made by several authors on the progress of wilt caused by *S. sclerotiorum* where over 70% of disease incidence occurred after flowering (Dorrell and Huang, 1978; Hoes and Huang, 1985; Nelson *et al.*, 1989). Huang and Kozub (1990) considered that infection of sunflower at early stages of growth was due to germination of injured and partly melanised sclerotia of *S. sclerotiorum* and that later infection required root exudates to stimulate germination of intact sclerotia. It is uncertain whether a similar situation exists with the incorporation of sclerotia of *S. minor* into the soil that many are wounded and primed to germinate precociously. It seems unlikely that precocious germination was occurring since very few young plants (<Growth Stage V8) were seen with wilt. Nevertheless the trials revealed differences in susceptibility between the inbred sunflower lines as measured by disease incidence at anthesis and area-under-the-disease-progress-curve.

Analysis of disease progress curves suggested that the epidemics progressed with some attributes of polycyclic diseases such as an initial lag phase followed by a period of rapid disease increase followed by an assypmtote. Maximum rates of disease progress in the screening trials occurred between budding and flowering. This period coincides with rapid expansion of the root system of sunflower (Sobrado and Turner, 1986). It is likely that the sigmoidal disease progress curves are the result of the rapidly increasing root mass increasing the number of infection courts as well as increasing the likelihood of plant-to-plant spread through root contacts rather than free dispersal of inoculum. It is intuitive that sclerotia of *S. minor* do not move far within a season unless significant soil disturbances occur. Artificial inoculation of plants in the field (Experiment 4.3.5) showed that the incidence of disease increased with plant age while the susceptibility of tissue as assessed by the rate of lesion extension decreased. This suggests that as the plant ages the tissue becomes less susceptible to progress by the fungus while the higher incidence may be a result of more roots being available to be infected. Changes in the susceptibility of sunflower stems with plant age have also been reported with *S. sclerotiorum* by Auger and Nome (1970) for plants inoculated by inserting colonised toothpicks into the stems and by Bazzalo (1982*a*) for plants inoculated by placing colonised grain at the stem base. Sunflower stems were found to be most susceptible at the early bud stage. This is the stage at which at the pot base inoculations were performed.

The data generated in the field trials were compared with results of inoculation experiments. Best comparative rankings were obtained between the rate of lesion extension following inoculation using the pot base method and disease incidence at anthesis and AUDPC for both field trials. Rate of lesion extension using the inoculation method of Sedun and Brown (1989) was not as effective at ranking the reaction of the eight inbred sunflower lines used. The delay from inoculation to the appearance of stem lesions following inoculation by the pot base method, although less variable than that produced by the inoculation method of Sedun and Brown (1989), had no relationship with the reaction of the lines in the field. Correlation experiments using twelve commercial and experimental sunflower hybrids however provided evidence that delay might be useful as a measure for predicting field performance. The artificial inoculation methods were applied at late vegetative stages/early reproductive stages when the plants at the same growth stage in the field are most susceptible. However, many field grown plants may escape infection at this stage due to low root volumes leading to low frequency of interception of soil-borne sclerotia and only become infected at later stages when the tissue is intrinsically more resistant. This may be one explanation for the inability to produce strong relative rankings between inoculation and field data. The resistance of several host genotypes at early budding and later growth stages needs to be examined to find whether the relative resistance between the genotypes at the early growth stage is consistent with relative resistance at the later growth stages.

Effective glasshouse screening methods should be useful during the breeding process rather than only being applicable to the evaluation of finished inbred lines or hybrids. Inoculation methods such as that used by Sedun and Brown (1989) where the measure of susceptibility is the rate of lesion extension may be unsuitable for screening segregating populations since low rates of lesion extension indicative of greater resistance result in plant death but just at a slower rate than in lines where lesion extension is fast. In general, inheritance of resistance to sclerotinia wilt caused by *S. sclerotiorum* is considered complex and under the control of several genes. Additive gene effects were considered more important than dominance and epistatic gene effects (Fick *et al.*, 1983; Pirvu *et al.*, 1985). Studies into the inheritance of resistance have shown that, depending on the germplasm intermated, resistance is heritable at a low level with large Specific Combining Ability (SCA) effects relative to General Combining Ability (GCA) effects (Fick *et al.*, 1983; Vranceanu *et al.*, 1984; Pirvu *et al.*, 1985). Such quantitative resistance is amenable to mass selection approaches such as recurrent phenotypic selection (Fehr, 1987).

Chapter 5 described the use of the pot base inoculation method in a recurrent phenotypic selection program. The utility of the inoculation procedure was demonstrated by the creation of a large number of partially inbred sunflower lines which were shown in subsequent field screening trials to be more resistant than the line RHA801. Warnings were also provided with the ease with which resistance could be lost within the germplasm if the selection pressure was changed. Continual selection is necessary until all the loci required for resistance are fixed. A fortuitous outcome of the breeding for resistance to *Sclerotinia minor* wilt was the identification of several lines with useful levels of resistance to head rot caused by *Sclerotinia sclerotiorum*.

Further isolates of *Sclerotinia minor* and *S. sclerotiorum* should be tested for comparative capacity to produce root and basal stem rot of sunflower. Sedun (1986) suggested that *S. minor* is better adapted as a root pathogen of sunflower than *S. sclerotiorum* which he suggested was better adapted for carpogenic germination and infection of aerial plant parts. The higher selection pressure applied by screening sunflower populations for resistance to *S. minor* should provide higher levels of resistance in sunflower to *S. sclerotiorum* but this needs to further tested.

The breeding procedure used above is of the 'black box' or 'empirical' type (Marshall, 1987) where nothing is known of the traits or characteristics that actually contribute to reducing the incidence or severity of the disease. Polygenic resistance such as is predicted for resistance to *S. minor* in sunflower is conditioned a number genes each of which contribute a small effect and has been recognised 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 and 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 *et al.*, 1988; van Ooijen, 1992; Stuber, 1992). These molecular techniques may not however give an understanding of the underlying biology of resistance. It was considered worthwhile therefore to assess a number of sunflower lines for ideotypes or plant characteristics that are correlated with the degree of susceptibility among host genotypes. If a particular ideotype can be found that determines to a large extent the susceptibility of sunflowers to *Sclerotinia minor* then molecular biological techniques may be used to find markers for that trait.

Morphological characteristics assessed among eight inbred sunflower lines grown in the field included time taken to anthesis, height, leaf area, root length density and mean lateral root diameter (Chapter 6). Of these traits only time from sowing to anthesis strongly correlated with disease incidence at anthesis. Less disease was evident at anthesis in sunflower lines that were earliest to flower. In contrast, Fick and Gulya (1980) found that days to 50% anthesis was not correlated (r=0.05) with the relative susceptibility of sunflower hybrids to wilt caused by *Sclerotinia sclerotiorum*. The difference between the result reported in this thesis and those of Fick and Gulya (1980) possibly reflect differences in the epidemiology of the two wilt diseases. Up to 90% of plants killed by *S. minor* in the field plots described in this thesis were killed at anthesis whereas up to 70% of sunflower plants killed by *S. sclerotiorum* were killed after anthesis (Nelson *et al.*, 1989). The failure to find a significant relationship between the density of roots and disease incidence means that more factors beside interception of inoculum are involved in the reaction of roots. Sedun (1986) observed in root viewing chambers that lesion progression on the roots of one sunflower line (RHA801) was slower than on the roots of the sunflower hybrid Pacific Seeds Hysun 32.

Root systems can be modified through plant breeding and selection (Clarke and McCraig, 1993). It is possible therefore that if a particular root ideotype(s) related to reduced susceptibility to sclerotinia wilt could be identified then that trait(s) could be introgressed into other host genotypes. An advantage of the pot base inoculation method is that the extent of root growth in the pot can be assessed. A simple root index calculated from the amount of root exposed on the base of the root was shown to be correlated with the total mass of roots in the pot. The root index calculated for the eight inbred sunflower lines ranked the lines in a similar order as found in the field. As well the root index of the recurrent phenotypic population did not show bias towards reduced root mass and remained greater than that of both the resistant (RHA801) and susceptible (*cms*HA89) check lines.

The ideal way to test the importance of roots on incidence of sclerotinia wilt would be to generate isolines of host genotypes differing in root characteristics, for example, root volume or lateral root thickness. Such a study was impractical given the time constraints of the present work so another less desirable approach was taken, that of grafting. Reciprocal grafting of four sunflower inbred lines was performed to demonstrate the contribution of the whole plant on the susceptibility of sunflower to *Sclerotinia minor* (Experiment 6.3.3). Replacing the shoot of CM497 with that of RHA801 reduced root growth, and mortality and time from inoculation to lesion appearance at the stem base. A similar situation occurred when PacA1 was the scion. The use of CM497 as the scion increased the susceptibility of the roo stocks. Further examination is necessary to elucidate the mechanisms behind these observations.

During pathogenesis *Sclerotinia* spp. release a number of plant tissue macerating enzymes and the toxin oxalic acid that acts synergistically with some of these enzymes. Several of these enzymes have acidic optima for activity which can be provided by the presence of oxalic acid. As well, disruption of pectins in the middle lamella and perturbation of plasma membranes are enhanced when calcium is withdrawn by chelation with the oxalate anion. Given that different sunflower genotypes are known to vary appreciably in content of many plant nutrients (Blamey *et al.*, 1980) it was considered possible that calcium may be one of those nutrients and that variation in calcium content between host genotypes could contribute to variation in disease susceptibility. Several experiments (Chapter 7) were performed to test what effect alteration of gross calcium status through varying the supply of calcium had on disease susceptibility. Increased calcium content did reduce the rate at which stem lesions progressed. Analysis of tissue calcium for the eight inbred sunflower lines however did not show a correlation between calcium content in roots and stem bases and disease incidence although concentration of the element did vary significantly between lines.

Among the elements that could be readily assayed using inductively coupled plasma (I.C.P.) analysis there was a strong positive correlation between total phosphorus in roots and disease incidence. Variation in phosphorus content among sunflower germplasm has been reported (Blamey *et al.*, 1980; Seiler, 1986) but this is the first indication that there may be a relationship between phosphorus content and susceptibility to sclerotinia wilt. Application of phosphate fertilisers have been associated with increased incidence and severity of a diverse range of diseases including *Septoria nodorum* on wheat (Leath *et al.*, 1993), *Peronospora farinosa* f.sp. *betae* on sugarbeet (Fargasova and Bojnansky, 1993) and *Verticillium dahliae* on lucerne (Isaac, 1957). However in Experiment 7.4.5 additional phosphorus was not applied so variation in the content of this element was a genotypic response to available phosphorus. Phosphorus is such an important element fundamental to almost all, if not all, metabolic processes that it is difficult to suggest why higher tissue levels would render the host more susceptible to *Sclerotinia minor*. During pathogenesis free phosphates released from host cells may act with oxalate produced by the fungus to chelate calcium and cause further cellular disruption. Alternatively, the phosphorus status of the plants may have been an indication of differences in mycorrhizal colonisation. Further studies may validate these results and elucidate the mechanisms behind the observations.

Sunflowers produce a wide of phenolic compounds (Duke, 1992) some of which have been suggested as being toxic to *Sclerotinia sclerotiorum* (Bazzalo *et al.*, 1987). The production of preformed and induced phenolic compounds active against *Sclerotinia* has stimulated the search for phenolic markers involved in resistance of sunflower to these fungi. Twenty-five peaks were found in High Performance Liquid Chromatography (H.P.L.C.) of solvent extracts from healthy sunflower plants and those infected by *Sclerotinia* spp. or injured with liquid nitrogen (Hémery *et al.*, 1987). Susceptible genotypes had fewer peaks in the H.P.L.C. profiles of healthy tissue and accumulated less total phenolics than the relatively-more resistant varieties. The presence of preformed phenolics was considered necessary for plants to show resistance. In a more recent study 3 peaks, numbered 4, 6 and 9 based on order of appearance in the chromatograms, were found to be closely correlated with resistance of sunflower leaves to infection by *S. sclerotiorum* (Castaño *et al.*, 1992). In Chapter 9 it was found that two compounds present in tissue infected with *Sclerotinia minor* but absent in healthy tissue were the coumarins scopoletin (7-hydroxy-6-methoxycoumarin) and ayapin (6,7-methylenedioxycoumarin). These were found to be toxic to mycelial growth of *S. minor* and germling growth from ascospores of *S. sclerotiorum*. These compounds may represent some of the peaks identified by Hémery *et al.* (1987). The expression levels of these two coumarins in sunflower can be altered to higher levels through somaclonal variation (Roseland *et al.*, 1989) but it may be deleterious to express these phytoalexins at tissue concentrations that would be toxic to *Sclerotinia* spp. since they can also have adverse effects on the plant (Einhellig *et al.*, 1970). Sunflower has also been shown to have mechanisms for the metabolism of scopoletin

(Gutierrez *et al.*, 1995). To increase the levels of scopoletin appreciably may require the down-regulation of these metabolic pathways as well.

Future Research

- Use the pot base inoculation method in a commercial sunflower breeding scenario in conjunction with selection for agronomically desirable traits

- Determine whether resistance is stable against other isolates of *Sclerotinia minor* and *S. sclerotiorum*.

- Determine how the relative susceptibility at early budding compares with later growth stages for a larger number of genotypes.

- Further examine the relationship of nutrient levels especially phosphorus and disease susceptibility and whether mycorrhiza are involved.

- Further examine whether the ability of sunflower tissue to neutralise oxalic acid as found by bathing leaf discs in a solution of oxalic acid can be developed into an efficient means of indirect screening for resistance.

- Demonstrate whether expression of heterologous oxalate degrading enzymes in sunflower tissue will provide resistance.

- Better understand the metabolic pathways that lead to accumulation of coumarin phytoalexins and how these pathways may be manipulated.

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APPENDIX 1.

Description of sunflower germplasm used.

- **Inbred sunflower lines**

The following is a list of the inbred sunflower lines referred to in the body of this thesis. Pedigree and characteristics of these lines are given.

UNE#	Line	Source/Description[1]
Public Inbred Lines		
1	RHA801	United States, S_3 selection from a population formulated from the inbred lines RHA271, RHA273, RHA274, R 344 and R 494 (Roath *et al.* 1981).
2	HA61-1	United States. selection from cross 953-88-3/'Armavirsky 2497' (Fick and Zimmer, 1975).
3	*cms* HA89	United States. reselected from CM303 itself selected from the Russian cultivar 'VNIIMK 8931' by the Canadian Department of Agriculture (United States Department of Agriculture Release Notice)
4	HA124	United States, selected from the Russian cultivar 'VNIIMK 8883'
5	HA292	United States, BC_3F_6 selection of the cross between the cultivar 'Commander' as the recurrent parent and 'Mennonite RR' (Fick and Zimmer, 1976).
6	HA304	United States, BC_3F_2 selection of the cross between the cultivar 'Commander' as the recurrent parent and 'Mennonite RR' (Fick and Zimmer, 1976).
7	HA305	United States, sister BC_3F_2 selection to HA304 (Fick and Zimmer, 1976).
8	HA335	United States, BC_2F_4 single plant selection of HA89*3/*H. annuus* 423 (Miller and Gulya, 1988).
9	HA340	United States, BC_2F_4 single plant selection of HA89*3/*H. argophyllus* 415 (Miller and Gulya, 1988).
10	HA-R1	United States, composite of 25-45 S_3 plants that trace to a single S_2 plant of the Argentinian cultivar 'Pergamino 71/538' (Gulya, 1985*b*).
11	HA-R2	United States, composite of 25-45 S_3 plants that trace to a single S_2 plant of the Argentinian cultivar 'Impira INTA' (Gulya, 1985*b*).
12	HA-R4	United States, composite of 25-45 S_3 plants that trace to a single S_2 plant of the Argentinian cultivar 'Saenz Peña 74-1-2' (Gulya, 1985*b*).

Continued		
13	HA-R5	United States, composite of 25-45 S_3 plants that trace to a single S_2 plant of the Argentinian cultivar 'Guayacan INTA' (Gulya, 1985*b*).
14	CM361	Canada, released as a S_3 selection from the Russian cultivar 'VNI MK 8931' and considered to have a measure of resistance to Sclerotinia wilt in the field (Dedio, Hoes and Huang, 1983).
15	CM497	Canada, released as a S_3 selection following interpollination of 50 early flowering, high oil Russian lines (Huang, 1982).
16	CM526	Canada, interpollination of 50 early flowering, high oil Russian lines (Huang, 1982).
17	77-5-67-8A	Canada, selection of HA61 (Sedun, 1986).
Unadapted lines involving crosses with wild species		
18	953-88-3	United States, selection from a natural cross between the cultivar 'Sunrise' and wild annual sunflowers in Texas, U.S.A. (Putt and Sackston, 1957).
19	SFM#1	United States, S_4 selection from a backcross involving *cms*HA89 and *Helianthus petiolaris* Nuttall (Rogers *et al.*, 1983).
20	SFM#2	United States, S_4 selection from a backcross involving *cms*HA89 and *Helianthus tuberosus* L (Rogers *et al.*, 1983).
21	SFM#3	United States, S_4 selection from a backcross involving *cms*HA89 and *Helianthus petiolaris* Nuttall (Rogers *et al.*, 1983).
22	Bushland #7605-2	United States, selection from cross *cms*HA89/*Helianthus paradoxus* Heiser made by Dr. G. Seiler (United States Department of Agriculture).
23	Bushland #9109-1	United States, selection from cross *cms* HA89/ *Helianthus exilis*-774 Gray made by Dr. G. Seiler (United States Department of Agriculture).
24	Bushland #7608-2	United States, selection from cross *Helianthus paradoxus*/HA89/ /*H. argophyllus* made by Dr. G. Seiler (United States Department of Agriculture).
25	Bushland #8401-3-2-1	United States, selection from cross *cms* HA89/ *Helianthus annuus*-21 made by Dr. G. Seiler (United States Department of Agriculture).
26	Bushland #535-1-1	United States, selection from cross *cms* HA89/ *Helianthus argophyllus*-1-22-1 made by Dr. G. Seiler (United States Department of Agriculture).

Continued

27	Bushland #9401-1-1	United States, selection from cross *cms* HA89/ *Helianthus debilis sylvestris*-367 Heiser made by Dr. G. Seiler (United States Department of Agriculture).
28	Bushland #8401-3-5-1	United States, sister selection of UNE#25
29	Bushland #9109-1	United States, selection from cross *cms* HA89/ *Helianthus exilis*-774 made by Dr. G. Seiler (United States Department of Agriculture).
30	Bushland #9401-1-2	United States, selection from cross *cms* HA89/ *Helianthus debilis sylvestris*-367 made by Dr. G. Seiler (United States Department of Agriculture).
31	Bushland #7309-2	United States, selection from cross *cms* HA89/ *Helianthus deserticola* Heiser made by Dr. G. Seiler (United States Department of Agriculture).
32	Bushland #568-1-1	United States, selection from cross *cms* HA89/ *Helianthus petiolaris*-3-161 made by Dr. G. Seiler (United States Department of Agriculture).
33	Bushland #8401-3	United States, sister selection to UNE#25
34	Bushland #9605-1	United States, selection from cross cms HA89//*cms* HA89/ *Helianthus paradoxus* made by Dr. G. Seiler (United States Department of Agriculture).
35	Bushland #9101-1	United States, selection from cross *cms* HA89/ *Helianthus exilis*-774 made by Dr. G. Seiler (United States Department of Agriculture).
36	Bushland #8401-3-4-1	United States, sister selection to UNE#25
37	Bushland #9104-1	United States, selection from cross *cms* HA89/ *Helianthus argophyllus*-420-2 made by Dr. G. Seiler (United States Department of Agriculture).
38	Bushland #8809-1	United States, selection from cross *Helianthus paradoxus*/HA89/ /*H. argophyllus* made by Dr. G. Seiler (United States Department of Agriculture).
39	Bushland #8401-3	United States, selection from cross *cms* HA89/*H. annuus*-21 made by Dr. G. Seiler (United States Department of Agriculture).
40	Bushland #7605-2	United States, selection from cross *cms* HA89//*cms* HA89/*H. petiolaris fallax* Heiser made by Dr. G. Seiler (United States Department of Agriculture).
41	Bushland #7607-4-1	United States, selection from cross *cms* HA89/*H. paradoxus*-9-1 made by Dr. G. Seiler (United States Department of Agriculture).

Continued		
42	Bushland #7505-2	United States, selection from cross *cms*HA89/*H. paradoxus*-2 made by Dr. G. Seiler (United States Department of Agriculture).
43	Proprietary	Australia, Pacific Seeds F_4 selection from the cross *cms*HA89//CM5 / *H. argophyllus* designed to give early rust resistant germplasm.
44	Proprietary	Australia, Pacific Seeds F_5 selection from the cross *cms* HA89 // CM5 / *H. argophyllus* derived from a different F_2 plant than #43.
45	Proprietary	Australia, Pacific Seeds sister F_5 selection to #44 derived from a common F_2 plant.
46	953-88-3(-2-3)	Australia, Pacific Seeds reselection from 953-88-3
Proprietary Inbreds		
47	Pac R1	Australia, Pacific Seeds S_4 selection from a restorer composite (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
48	Pac R2	Australia, Pacific Seeds F_4 selection from the cross Pehuen INTA//RHA271/a selection from the USDA 1970R composite (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
49	Pac A1	Australia, Pacific Seeds S_5 selection from a sterility maintainer population, converted to cytoplasmic male sterility (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
50	Pac A2	Australia, Pacific Seeds S_5 selection from a different sterility maintainer population to #49, converted to cytoplasmic male sterility (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
51	Pac A3	Australia, Pacific Seeds F_4 selection from the cross HA89//HA89/'Pergamino 71/538', converted to cytoplasmic male sterility (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
52	Pac A4	Australia, Pacific Seeds S_5 selection from a different sterility maintainer population to #49, converted to cytoplasmic male sterility (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
53	Proprietary	Australia, Pacific Seeds S_4 selection from the DM-3 composite prepared in the United States by Miller and Gulya (1985) from selections of the Russian cultivar 'Progress' (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
54	Proprietary	Australia, Pacific Seeds S_4 selection from the DM-3 composite originating from a different S_2 plant to #53

Continued

55	Proprietary	Australia, Pacific Seeds S_4 selection from the Argentinian cultivar 'Saenz Peña 74-2-1' which is a composite formulated from various Russian cultivars, 'Klein', 'Record', 'horizon', 'OS2' and a synthetic line produced from VNIIMK 1646, VNIIMK 6540, VNIIMK 8883, wild *H. annuus* and *H. petiolaris* (Aurelio Luciano, *pers. comm.*).
56	Proprietary	Australia, Pacific Seeds S_4 selection from the Argentinian cultivar 'Saenz Peña 74-2-1' originating from a different S_3 plant to UNE#55 (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
57	Proprietary	Australia, Pacific Seeds S_4 selection from the Argentinian cultivar 'Saenz Peña 74-2-1' originating from a different S_1 plant to UNE#55 (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
59	Proprietary	Australia, Pacific Seeds S_4 selection from the Argentinian cultivar 'Saenz Peña 74-2-1' originating from a different S_1 plant to UNE#56 and UNE#57 (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).(Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
59	Proprietary	Australia, Pacific Seeds S_4 selection from the Argentinian cultivar 'Saenz Peña 74-1-2' which is similar in pedigree to 'Saenz Peña 74-2-1' (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
60	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian hybrid 'Felix'. This hybrid is supposedly tolerant to Sclerotinia wilt caused by *Sclerotinia sclerotiorum* (Miller and Gulya, 1990)
61	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian hybrid 'Felix' originating from a different S_1 plant to UNE#60.
62	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian hybrid 'Romsun 22'. This hybrid has been observed to have field resistance to *Sclerotinia sclerotiorum* in Europe (Vranceanu *et al.*, 1987).
63	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian hybrid 'Select'. This hybrid is supposedly tolerant to Sclerotinia wilt caused by *Sclerotinia sclerotiorum* (Miller and Gulya, 1990).
64	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian synthetic population Pop. Sint 1B of unknown formulation but supposedly with resistance to *Sclerotinia sclerotiorum* (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
65	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian synthetic population Pop. Sint. ICRF of unknown formulation but supposedly with resistance to *Sclerotinia sclerotiorum* and fertility restoration genes (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).

continued

66	Proprietary	Australia, Pacific Seeds S_3 selection from the Romanian synthetic population Pop. Sint. 105 of unknown formulation but supposedly with resistance to *Sclerotinia sclerotiorum* and *Phomopsis helianthi* (Mr. Alan Scott, Pacific Seeds Pty Ltd, *pers. comm.*).
67	Proprietary	Australia, Pacific Seeds S_5 selection from 'Select' but derived from a different S_2 from #63
68	Proprietary	Australia, Pacific Seeds S_4 selection from the DM-3 composite originating from a different S_2 plant to #53
69	Proprietary	Australia, Pacific Seeds S_4 selection from the DM-3 composite originating from a different S_2 plant to #53

- **Sunflower hybrids used**

The following sunflower hybrids were used during the research reported in the body of this thesis. The companies that provided these hybrids are also listed. Pedigree information on these hybrids was restricted since their composition is proprietary information.

Agseeds Research

Suncross 40+
Suncross 40R
AgX 3740
AgX 8740
AgX 9040
AgX 9391
AgX 7240
AgX 9313
AgX 9340

Dekalb

Dk610
Dk36630
Dk36823
Dk3903

Pacific Seeds Pty Ltd

Hysun 32
Hysun 33
Hysun 35
Hysun 44
Pac 2592
Pac 9454
Pac 8619
Pac 2515
Pac 2574
Pac 2576
Pac3 435
Pac 7622
Pac 9433
Pac 8699
Pac 3679

Pioneer Seeds

F61

Queensland Department of Primary Industries

89/QRS1
89/2-13
89/6-1
89/7-13
89/11-1
89/6-5

Wild *Helianthus* Collections

A number of *Helianthus* species have escaped cultivation and have established wild populations throughout eastern Australia. The following accessions were provided by Pacific Seeds Pty Ltd.

U.N.E. Accession Number	Species	Collection Area[1]
HS09	*Helianthus debilis*	Boonarga, QLD
HS10	*H. debilis*	Chinchilla, QLD
HS13	*H. argophyllus*	Emu Park, QLD
HS25	*H. argophyllus*	Yepoon, QLD
HS28	*H. annuus*	Warren, NSW
HS32	*H. annuus*	Warren, NSW
HS33	*H. annuus*	Kairo, QLD
HS35	*H. annuus*	Gunnedah, NSW
HS39	*H. annuus*	Gunnedah, NSW
HS40	*H. annuus*	Gilgandra, NSW
HS41	*H. annuus*	Gilgandra, NSW
HS46	*H. annuus*	Coolah, NSW
HS47	*H. annuus*	Coolah, NSW
HS48	*H. annuus*	Moree, NSW
HS49	*H. annuus*	Gurley, NSW
HS53	*H. annuus*	Dalby, QLD
HS55	*H. annuus*	Curlawa, NSW
HS57	*H. annuus*	Pomona, QLD
HS58	*H. annuus*	Bogabilla, NSW
HS59	*H. annuus*	Springsure, QLD
HS64	*H. annuus*	Roma, QLD
HS65	*H. annuus*	Roma, QLD
HS90	*H. annuus*	Goondiwindi, QLD

1. QLD Queensland. NSW New South Wales

APPENDIX 2.

Nutritional status of field sites on the University of New England's Laureldale Research Farm.

Soil samples were collected and supplied to Moree Agricultural Laboratories, Moree, New South Wales as instructed by that company.

Test	*Sclerotinia minor* field screening site			
	LD91		LD92	
	Result	Unit	Result	Unit
pH (in water)	5.04		5.20	
pH (in $CaCl_2$)	4.67		4.81	
Nitrogen	50	ppm	27	ppm
Phosphorus	22	ppm	16	ppm
Sulphur	24	ppm	22	ppm
Potassium	343	ppm	2680	ppm
Calcium	7040	ppm	6215	ppm
Magnesium	5055	ppm	5220	ppm
Sodium	360	ppm	222	ppm
Copper	15	ppm	9	ppm
Zinc	5	ppm	2	ppm
Manganese	821	ppm	765	ppm
Iron	886	ppm	877	ppm
Boron	2.87	ppm	2.58	ppm
Electrical Conductivity	0.46	$mS.cm^{-1}$	0.26	$mS.cm^{-1}$
Cation Exchange Capacity	79.76	meq.%	76.23	meq.%
Exchangeable Sodium	1.95	%	1.26	%
Calcium/Magnesium ratio	0.84		0.71	