CHAPTER 4

COMPARISON OF FIELD AND POT-BASED SCREENING OF SUNFLOWER FOR RESISTANCE TO SCLEROTINIA WILT CAUSED BY *SCLEROTINIA MINOR*.

4.1 Summary

Two *Sclerotinia minor* disease screening sites were established on the University of New England's Laureldale Research Farm These sites were used to generate field data on the relative susceptibility to *Sclerotinia minor* wilt of a number of inbred sunflower lines and hybrids. These results provided data against which the results of glasshouse and controlled environment screening experiments were tested.

Disease progress of *Sclerotinia minor* wilt was characterised by very low incidence before budding followed by rapid disease increase after budding so that most plants that were killed in total had been killed by anthesis. Disease progress was best linearised by the Gompertz model which suggested that the epidemics were polycyclic but the shape parameter of the Weibull probability distribution function transformation of the disease progress curves did not consistently indicate that the epidemics were polycyclic. The apparent rate of disease progress (b) calculated from the Gompertz model was lowest for HA124, PacA1, CM526 and RHA801 in both screening trials. The area under the disease progress curve (AUDPC) was also calculated. In the two screening trials the AUDPC was not statistically different between PacA1, RHA801, CM526 and HA124. AUDPC was greatest for CM497, *cms*HA89 and PacA3. PacR2 fell between these two groups.

The AUDPC and disease incidence at anthesis were used as parameters of field reaction against which parameters from pot assays were compared. The distribution of rankings of results for the eight inbred sunflower lines from the two trials were very similar. The eight inbred sunflower lines were also screened using the pot base inoculation or Sedun and Brown (1989) methods. The ranking of linear rate of lesion extension of the eight lines inoculated by the pot base method in the pot assays was very similar to the rankings of disease incidence at anthesis and AUDPC for the same eight lines in the two field screening trials. The linear rate of lesion extension following the inoculation method of Sedun and Brown (1989) showed a weaker correlation to the distribution of field results from the second screening trial only.

Twelve commercial and experimental sunflower hybrids were also screened in the field and pots. AUDPC was lowest for two experimental hybrids, Pac 3435 and Pac 9433, and highest for another two experimental hybrids, Pac 7622 and AgX8740. Pac 3435 is the F_1 cross of two resistant inbreds PacA1 and RHA801 while Pac 7622 is the F_1 of the more susceptible inbreds PacA3 and PacR2. This indicated that, in specific cases at least, hybrid performance could be predicted from the performance of the constitutive inbreds. The ranking of the hybrids in the field and after inoculation with the pot-base method showed that mortality, the time taken from inoculation to lesion appearance and linear rate of lesion extension in the pot experiments were correlated with AUDPC. A disease index calculated from the parameters of disease assessment used in the pot experiment showed a correlation with disease incidence (mortality) in the field and AUDPC ($r=0.787$, $P<0.01$).

Another field experiment was conducted to examine the effect of plant maturity on susceptibility of sunflower to *S. minor*. Plants from vegetative to seed development stages were artificially inoculated by inserting inoculum into the soil beside the stems. The number of plants that developed basal stem lesions increased with plant age, the rate of lesion extension along the stem decreased with plant age and the time taken for lesion to first appear at the stem base tended to decrease with age for one hybrid (Suncross 40R) and increase after an initial decrease for the second sunflower hybrid (Dk3903).

Three other glasshouse screening methods were examined for comparability to field rankings. When sclerotia were incorporated into the potting mix at sowing the final mortality was least and the mean time taken for stem based lesions to appear was greatest in PacA1, HA124, CM526 and RHA801. The same four inbred sunflower lines were most resistant as determined by time to death and rate of lesion extension after inoculation with the method of Bazzalo *et al.* (1991). The more indirect screening of detached petioles as described by Martinson (1992) showed PacA1 and HA124 to be most resistant however average nett lesion length was not correlated with field performance.

4.2 Introduction

The ability to accurately select progeny possessing the phenotypic traits required is fundamental to any plant breeding program. The screening of breeding lines for resistance to plant pathogens also requires methods of selecting better performing individuals. Glasshouse assays are often used to reduce the environmental influences on the host:pathogen interaction but to be of any use they must discriminate between genotypes in a manner comparable to that which would occur in the field under conditions of natural infection. It is also necessary to understand the epidemiology of the disease in the field so

that selection for components of quantitative resistance in the glasshouse can be placed in perspective.

A comparison of the advantages and disadvantages of field experiments and growth chamber experiments in the development of epidemiological models of plant diseases have been reviewed by Aust and Kranz (1988). Briefly, field experiments allow a more holistic examination of epidemics without the generation of artifactual conclusions in a way that is directly relevant to practical applications. The value of field experiments is often reduced by the agronomy of the host if only one experiment can be run in a season or if the disease fails to develop. Experiments conducted under controlled conditions have the advantages of controlled exposure to environmental parameters, as well as exposure to different isolates and inoculum densities of the pathogen. Nevertheless the conclusions generated under controlled conditions must still be verified under field conditions (Rotem, 1988).

Measurement of disease intensity can be expressed as disease incidence and/or disease severity. Incidence is the percentage or proportion of diseased plants or plant parts in the sample or population while severity is the percentage or proportion of host tissue covered by symptoms of the disease. Incidence is commonly used for diseases such as systemic virus diseases and wilts that affect entire plants. Disease incidence is readily assessed by counting the number of plants in a population that show disease symptoms. Shoot symptoms are most often used to identify diseased plants although for many wilt diseases it is the roots that are infected. Root sampling would however provide better information on the timing of infection and actual number of plants infected but this form of sampling is usually destructive (the plant is removed from the experiment) and can be tedious and time-consuming. Shoot wilting is the means most commonly used to assess the incidence of sclerotinia wilt diseases in sunflower (Hoes and Huang, 1985; Holley and Nelson, 1986; Clarke, Porter and Woodroofe, 1992)

Sufficient data must be collected to allow epidemics developing in the different treatments to be compared. This is essential when assessing differences in the quantitative resistance of host cultivars. Five or more assessments should be made at appropriate intervals (Campbell, 1986; Madden, 1986). Berger (1988) suggested that epidemics could be compared by analysing disease progress to provide an estimate of the rate at which the epidemic is progressing. These rate estimates could then be used to calculate the times when the disease reached some critical level, for example, 50%, or the level at a particular growth stage.

Disease progress curves can be characterised in a number of ways. Models of disease development can be either 'theoretical' if they are based on some prior biological assumptions or 'empirical' if the model is based on the data obtained without any

assumptions about the underlying biological mechanisms acting (Madden, 1986). The models used within plant disease epidemiology have been adopted from other disciplines concerned with the dynamics of population increase (Madden, 1980). Van der Plank (1963) suggested that disease progress during an epidemic could be considered to follow an increase comparable to the accumulation of either simple interest or compound interest. In 'simple interest diseases' the disease increases without concurrent increase and spread of inoculum that initiates further infection during the current season. Such diseases are also called monocyclic and the monomolecular transformation can be used to linearise the data. This transformation is $Y= \ln(1/1-y)$ where y = proportion of diseased plants or diseased tissue in the range $0<y<1$. 'Compound interest diseases' are characterised by having concurrent production of inoculum and re-infection during a season. These diseases are polycyclic as typified by the rust diseases of cereals and to transform the sigmoid disease progress curves to linearity van der Plank(1963) suggested the use of the logistic equation. The logistic transformation equation is: $\text{Logit}(y) = \ln(y/(1-y))$ where y = proportion of diseased plants or diseased tissue in the range $0<y<1$.

Other transformations have since been examined and found to more applicable to different pathosystems. Adoption of each transformation is often based on the ease with which it may be applied. Berger(1981) compared the use of the Gompertz and logistic equations to describe plant disease progress. The Gompertz transformation equation is: $\text{gompit}(y) = -\ln(-\ln(y))$ where y = proportion of diseased plants or diseased tissue in the range $0<y<1$. The Gompertz transformation was found more applicable to pathosystems with asymmetrical disease progress curves than the logistic transformation especially where the point of inflection was less than 0.5. The Weibull probability distribution function (PDF) and cumulative distribution has been described as a flexible model to describe plant disease progress (Pennypacker, Knoble, Antle and Madden, 1980). This model includes shape and location parameters which can be manipulated so that a wide range of disease progress curves can be described.

Often it is assumed that the transformation that best fits the data gives an indication of the underlying biology of the epidemic. Such assumptions can be profoundly wrong (Huisman, 1982; Pfender, 1982). Problems with the accuracy of interpretation arises because the model is fitted to the phenotypic expression of the epidemic and may not account for variations in plant and pathogen growth and changing environmental conditions. For example, rapid increases in the incidence of infected plants may be the result of flushes of root growth providing more infection courts rather than multiplication of the pathogen. That is, increased root growth increases the effective inoculum density. Differences in inoculum density have been reported to affect when an epidemic commences (location parameter), the shape of the curve (shape parameter), the rate of

disease progression (rate parameter), and the final amount of disease (position of the asymptote)(Campbell, 1986).

The area under the disease progress curve (AUDPC) has been used to 'summarise' the development of epidemics. Fry (1978) considered AUDPC better than the rate of epidemic progress or final level of disease for estimating the effects of host resistance on development of potato late blight. Waggoner (1986) supported the use of AUDPC because it can use all available data on disease intensity, did not obscure variations in rate of epidemic development that may occur in other transformations of the data and it does not generate estimates biased by the asymptotes of the epidemic (Neher and Campbell, 1992). Values for AUDPC can be easily normalised (Fry, 1978) by dividing AUDPC by the total possible area which is product of the duration of assessment (days) and 1.0 (maximum proportion of disease incidence or severity) or 100 (percent disease incidence or severity). Calculated AUDPC's can be compared using standard analysis of variance (ANOVA) techniques (Madden, 1986). An alternative method is to calculate an index of cultivar resistance by calculating the area under the plot of the disease proportion of test hosts against the disease proportion of a check or standard (McRae and Platt, 1987).

Few thorough studies have been conducted into the epidemiology of sclerotinia wilt diseases but a conceptual understanding of how epidemics progress must be developed so that effective artificial screening methods can be applied. Jarvis and Hawthorne (1972) were interested in identifying whether the progression of lettuce drop caused by *S. minor* followed the monocyclic (monomolecular) or polycyclic (logistic) models. They argued that if inter-plant spread of the disease occurred then the logistic model would provide a better fit to the disease progress curve. Furthermore, they attempted to determine whether the occurrence of disease spread along the row could be calculated using the method of Roach (1968). This method uses the χ^2 test to compare the occurrence of chains of infected plants as the disease spreads plant-to-plant with what is expected if infection is random. After comparing a number of epidemics they failed to identify a consistent trend, some epidemics were better described by the monomolecular equation while for others the logistic equation provided the better fit. Evidence of consistent plant-to-plant spread was not found.

Fravel, Adams and Potts (1992) studied the effects of increasing application rates of inoculum of the mycoparasite *Sporidesmium sclerotivorum* on the incidence of lettuce drop by comparing disease progress curves. The logistic equation was fitted to the data and the rate parameter and inflection point were used to compare epidemics. The inflection point is the time when the curve changes concavity and disease increase is at the maximum. If the maximum asymptote corresponds to a disease incidence of 100% then the inflection

point also represents the time when disease incidence is 50%. The inflection point also describes the location of the curve with respect to time.

Soil-borne root-infecting sclerotial fungi such as *Verticillium dahliae* and *Macrophomina phaseolina* have been assumed to cause 'simple interest' diseases while fungi that produce mobile zoospores that spread the disease between plants during a season may produce 'compound interest' diseases (Campbell, 1986). Huang and Hoes (1980) showed that root-to-root spread of *Sclerotinia sclerotiorum* occurred between sunflower plants when plants were separated by less than 30cm. Mean time taken for plant-to-plant (stem base-to-stem base) spread was 1.5 weeks for plants separated by 10cm and 4.3 weeks for plants separated by 30cm. Nelson *et al.* (1989) studied the effect of four sunflower plant densities (37-74 x $10^3.ha^{-1}$) on the progression of sclerotinia wilt caused by *Sclerotinia sclerotiorum* in eight field experiments. Analysis with the Weibull model showed that few of the disease progress curves were satisfactorily explained by either the monomolecular or logistic equations. Failure of plant-to-plant spread to generate a polycyclic disease progression was attributed to the use of 76.2 or 91.4 cm inter-row spaces which prevented row-to-row spread thereby restricting progression of the disease to the one dimension along rows. Inoculum density was found to be positively correlated with the rate of disease progress but it did not seem, from the data presented, that the location in time of the beginning of the epidemic was affected. Campbell (1986) predicted that higher inoculum density should increase the likelihood of host root - pathogen interceptions leading to a greater number of infections per plant and earlier expression of disease symptoms. The interaction of other factors such as plant age or environmental constraints on sclerotial germination and infection might explain the results of Nelson *et al.* (1989).

The objectives of the investigations reported in this chapter were two-fold:
Firstly to investigate epidemic development in a number of sunflower lines under field conditions; and secondly to determine the ability of glasshouse screening methods to produce results that correlate well to field results.

4.3 Experimental

4.3.1. Field Screening of eight inbred lines for resistance to Sclerotinia minor.

Two field screening nurseries were established on the University of New England's Laureldale Research Station (Latitude 30° 31' S, Longitude 151° 39' E, Altitude 1042m). Each of the sites was originally considered to be free of *Sclerotinia minor*. Site 1 (LD91) was used for the artificial inoculation of sunflower lines during the summer of 1990/91 as described in Experiment 4.3.5. At the end of that experiment the site was cultivated and left fallow. Site 2 (LD92) was originally annual rye grass (*Lollium* spp.) pasture in 1990.

The pasture was killed with two applications of glyphosate (1.5L.ha^{-1}a.i.) and cultivation. During the summer of 1990/91 the site was planted with the sunflower hybrid Pacific Seeds Hysun 34. Capitula of plants 6m apart were inoculated by inserting toothpicks colonised with *S. minor* when the plants were approximately 2 weeks-post-anthesis. A mass of sclerotia were produced in each inoculated capitulum. The total biomass was incorporated into the soil by cultivation when the plants had matured.

To determine the density of sclerotia in the soil at each site, soil samples were taken following the final cultivation before sowing. Soil cores 7cm in diameter were taken to a depth of 10cm at 30 random positions determined by using a random number generator to direct the position. The soil cores were air dried, homogenised and 50g sub-samples taken. Sclerotia were extracted using the wet sieve and glycerol centrifugation method of Abd-Elraik and Lorbeer (1980).

The eight sunflower inbred lines screened were RHA801, Pac R2, CM526, CM497, Pac A1, Pac A3, *cms*HA89 and HA124 These were chosen because as inbreds each was expected to be relatively genetically homogeneous, adequate quantities of seed were available and the lines were considered representative of the range of susceptibility in sunflower to sclerotinia wilt available the time of these experiments. RHA801 and Pac R2 have upper stem branching and were fertility restorers for the PET1 *cms*. The other inbred lines were all monocephalic. HA124, CM526 and CM497 have hermaphroditic florets while Pac A1, Pac A3 and *cms*HA89 were all male (pollen) sterile. The lines Pac R2, Pac A1 and Pac A3 were proprietary inbreds supplied by Pacific Seeds Pty. Ltd. (Toowoomba, Australia).

Site 1 (LD91) was hand-sown on 17 October, 1991 and matured during summer. The screening trial consisted of 4 row plots each 10m long with an inter-row spacing of 50cm arranged in a randomised complete block design with 4 replicates. Plots were oversown and then manually thinned to 4 plants.m^{-1} to give an equivalent plant density of 80000.ha^{-1}. Irrigation was applied throughout the season by over-head sprinklers. An equivalent of 25mm of irrigation was applied every 10 days unless an equal or greater quantity of rain fell in which case irrigation was resumed 10 days after the cessation of the rainy period. Meteorological data was obtained from the University's weather station situated on the research station within 200m of the trial site.

Site 2 (LD92) was hand-sown on 25 January, 1992 and matured during autumn. This screening trial was a repeat of the first trial except that 6 replicates were used and plot lengths were reduced to 8m. This site was 800m from the weather station.

Plots in both trials were examined periodically for incidence of wilt. Regular assessments at 4-7 day intervals commenced once wilt became apparent. The number of wilted plants in the data rows with visible lesions at the stem bases were counted and the capitula were marked with spray paint to avoid recounting in subsequent assessments. The growth stage of each treatment was recorded at each assessment using the scheme of Schneiter and Miller (1981).

Areas under the disease progress curves (AUDPC) were calculated using a computer program that estimates AUDPC by trapezoidal integration (Berger, 1988). The 'NLIN' program published by Berger (1988) was used to fit the logistic, Gompertz and Weibull probability distribution functions to the data. The Plot function of MSTAT was used to further study regressions, correlations and significance of goodness-of-fit of the linearisations as calculated using Student's t-test. The slopes (b) of the transformed lines gave estimates of the rate of increase in disease incidence. The Weibull model also contains a location parameter (a) and a shape parameter (c). The location parameter (a) was set to the day of the first assessment minus 1. The shape parameter (c) varies with the disease progress curve from 1.0 for monocyclic diseases to 3.6 for polycyclic diseases were the point of inflection is 0.5 times the duration of assessment.

Relative rates of disease progress at each assessment time were calculated using the formula;

$$dy_t/dt = [h_1^2.y_{t+1} + (h_2^2-h_1^2).y_t - h_2^2.y_{t-1}] / [h_1.h_2.(h_1 + h_2)]$$

where h_1 = time between disease incidence assessments t-1 and t, h_2 = time between t and t+1 and y = disease incidence (Jeger. 1980).

Results

At the time of sowing the mean sclerotial density at Site 1 was 13.kg^{-1} oven-dried soil (range 0.8 - 24.kg^{-1}) while at Site 2 sclerotial density was 51.kg^{-1} oven-dried soil (range 0-116.kg^{-1}).

The weather conditions during the two screening trials are shown in Figure 4.1 (a-d). During the periods that both trials were being conducted there were malfunctions of the weather station that resulted in data not being collected. In LD91 which ran from October through to February the mean daily air temperatures were between 10 and 25^{o}C while in LD92 which was planted in January and matured in May a definite cooling can be seen. Total precipitation recorded at LD91 was 566mm consisting of 416mm of rainfall and 150mm through irrigation. At LD92 total precipitation was 456mm consisting of 281mm of rainfall and 175mm through irrigation. Mean daily soil temperatures at 5 and 10cm depths followed the trends of air temperatures but with smaller fluctuations. It should be

noted that these soil temperatures were not recorded in the trial site but at the established weather station where the probes were buried beneath turf.

The progression of disease epidemics in the eight inbreds for the two screening sites are presented in Figures 4.2 (a-h) and 4.3 (a-h). Using formula of Berger (1980) it was possible to determine the period when the rate of disease epidemic development was greatest. The plots of the relative rate of epidemic development as proportion of plants dying per day against time are shown as bars in Figure 4.2 (a-h) and Figure 4.3 (a-h). It is apparent that in the trial at LD91 disease progress is bimodal with the greatest rates of disease progress before anthesis followed by a late season increase in disease coinciding with a period of high precipitation (Figure 4.1a). Disease levels were much higher in the screening trial at LD92 with the greatest relative rates of disease progress before anthesis.

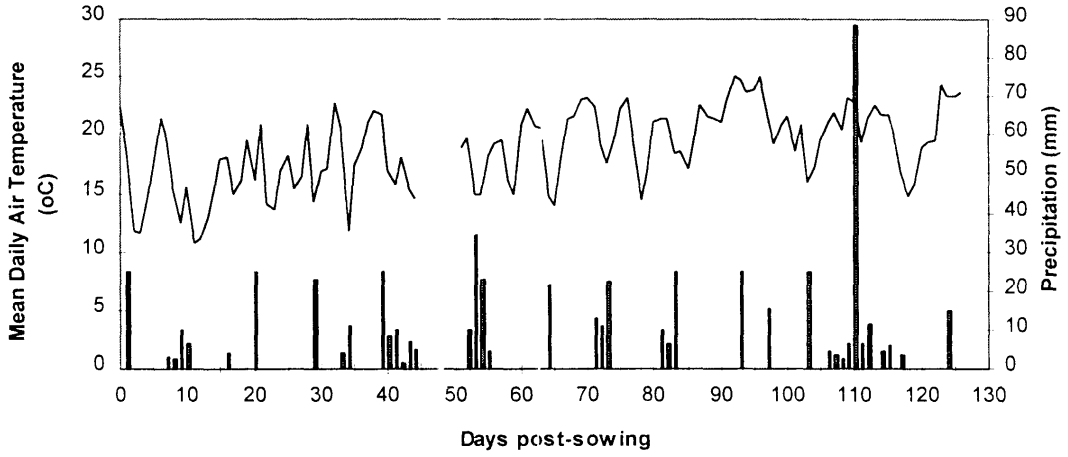

Figure 4.1 *a.* Mean daily air temperatures and precipitation recorded during conduct of the LD91 screening trial.

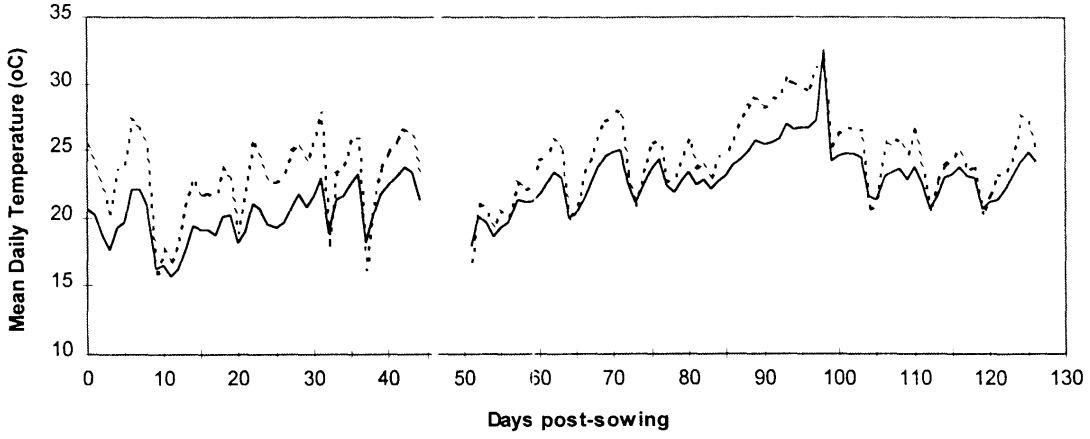

Figure 4.1 *b.* Mean daily soil temperatures recorded at 5cm (···) and 10cm (-) during conduct of the LD91 screening trial.

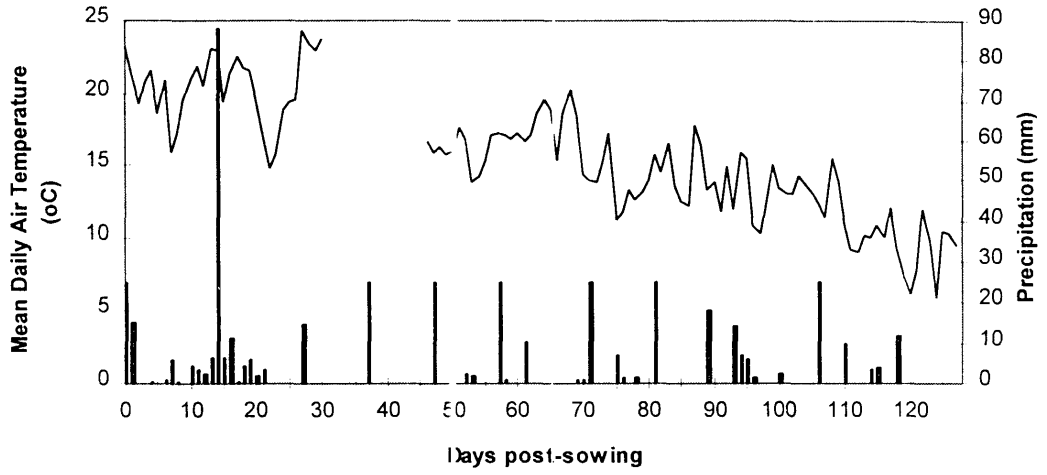

Figure 4.1 *c.* Mean daily air temperatures and precipitation recorded during conduct of the LD92 screening trial.

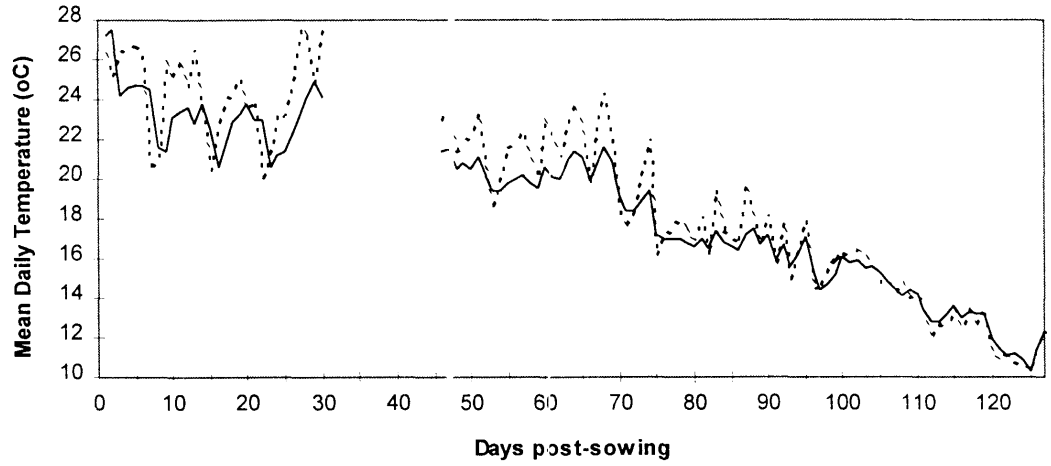

Figure 4.1 *d.* Mean daily soil temperatures recorded at 5cm (···) and 10cm (-) during conduct of the LD92 screening trial.

The cumulative number of plants with wilt increased with time for the 8 inbreds in both trials. Linear regression applied to plots of cumulative wilt incidence transformed with four models showed that of the fixed models the Gompertz transformation was usually superior as determined by the coefficient of determinations (Table 4.1) although all models produced statistically significant fits ($P<0.01$). Compared to the logistic model, the

gompertz transformation provided be ter linearisation of the data for all lines at both sites. This phenomenon is related to the better ability of the Gompertz model to transform asymmetrically sigmoidal curves suc ı as these generated when there was a rapid increase in the rate of disease early in the growth cycle. In some cases (eg Pac A1 and HA89 at LD91 and CM526, CM497, Pac A1, and *cms*HA89 at LD92) the flexible Weibull model provided better fit but with a range of shape parameters. The shape parameter (c) of the Weibull model estimates the point of inflection of the disease progress curve. The disease progress curves at LD91 were all asymmetrical ($1 < c < 3.6$) and closer to the monocyclic model than the disease progress curves for the same sunflower lines at LD92.

Choice of transformation model also affects the estimation of the rate of disease progress. The apparent rate of disease progress (b) as estimated by the logistic transformation was greatest for PacA1 in both trials while the rates derived through transformation with either the Gompertz, monomolecular or Weibull models were the lowest or among the lowest for any line. The highest rates as determined through transformation with either the Gompertz, monomolecular or Weibull models were seen in CM497, Pac A3 and *cms*HA89 at both trials. This corresponded well with the incidence of mortality seen in these lines (Table 4.2)

The area-under-the-disease progress curves (AUDPC) calculated for the eight inbred sunflower lines are shown in Table 4.2. The repeatability of the results for AUDPC were good as indicated by the coefficient of correlation ($r = 0.779$, $P=0.023$) between the two screening trials. The inbred lines can be classed into three groups based on the criteria used to assess disease susceptibility (ie estimated rate of disease progression and AUDPC): lines with a high level of resistance (RHA801, PacA1, CM526 and HA124), lines with a low level of resistance (CM497, *cms*HA89 and PacA3) and lines that fall between these groups (Pac R2). These lines therefore provided a good cross-section of the variability in resistance available in sunflower against sclerotinia wilt caused by *S. minor*.

Comparison of the incidence of mortality at anthesis and at the completion of assessment showed that more than half the plants wilted before anthesis (LD91: mean 56.5%, range 29.1-75.3%; LD92: mean 81.4%, range 64.9-95.5%)(Table 4.2).

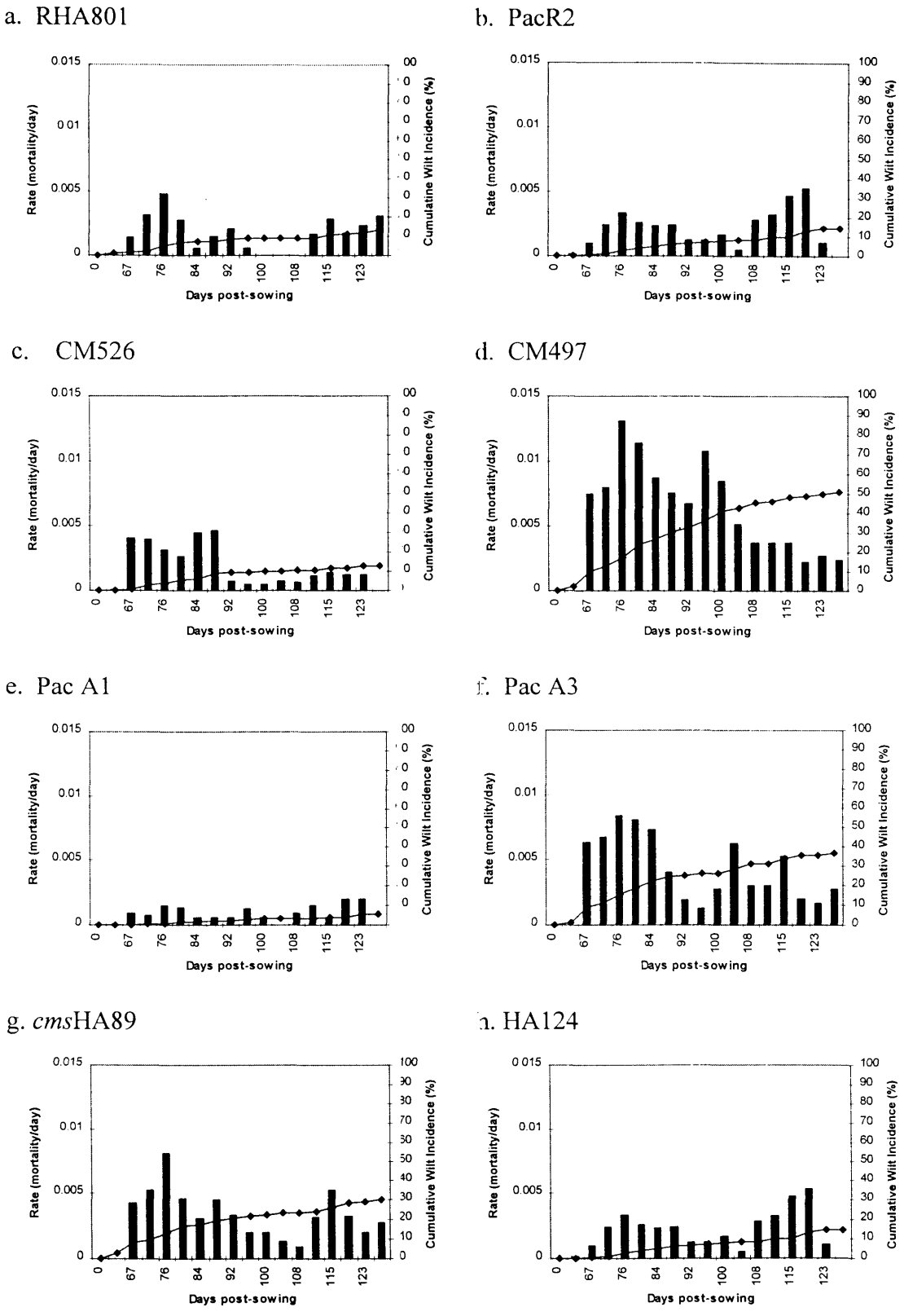

Figure 4.2 Disease progress curves and estimates of relative rates for development of *Sclerotinia minor* wilt in eight inbred sunflower lines at the LD91 field screening site.

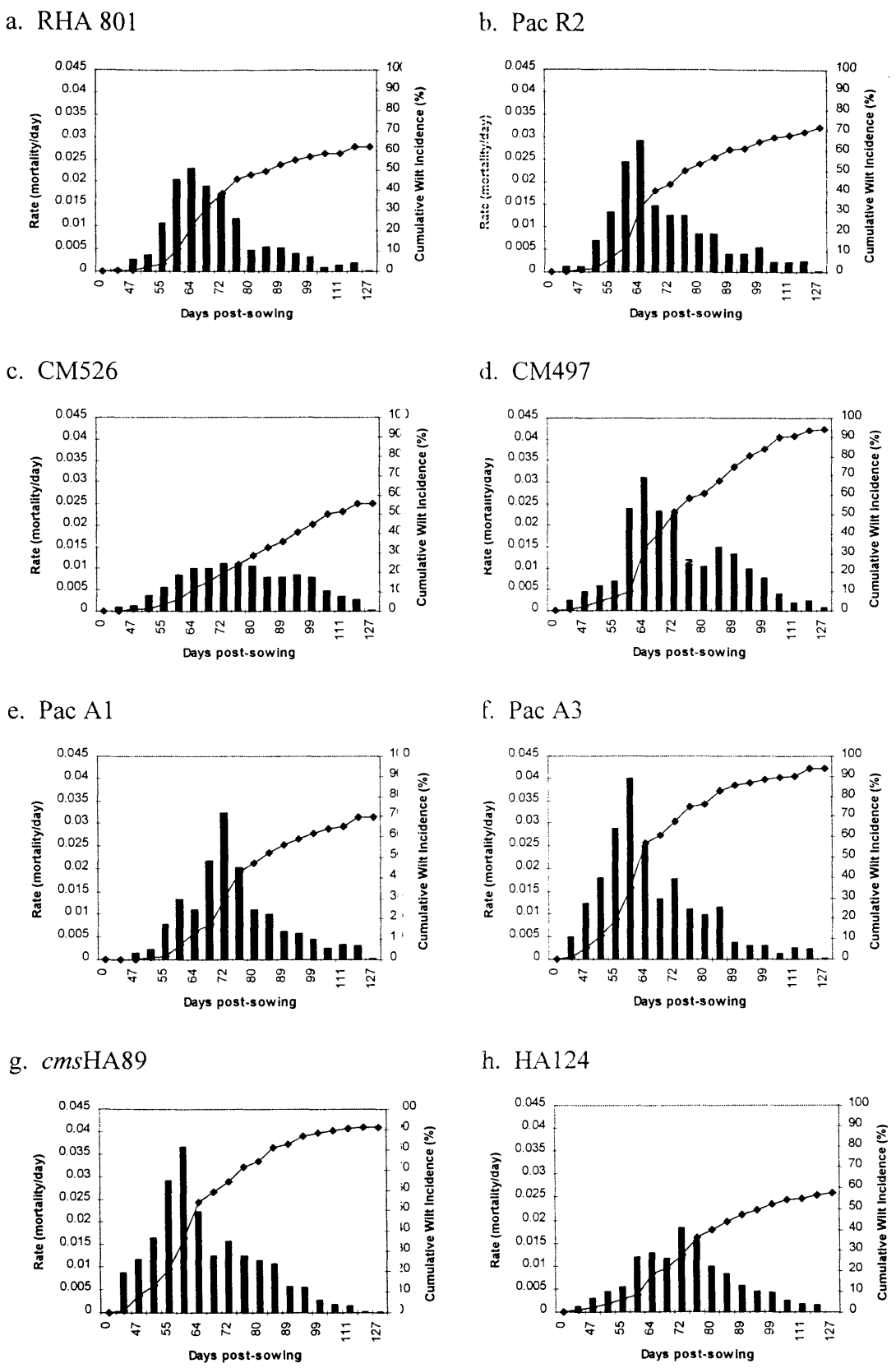

Figure 4.3 Disease progress curves and estimates of relative rates for development of *Sclerotinia minor* wilt in eight inbred sunflower lines at the LD92 field screening site.

Table 4.1 Comparison of models used to estimate progress of sclerotinia wilt caused by *Sclerotinia minor* in eight inbred sunflower inbreds at two screening sites.

Site	Line	Model												
		Logistic			Gompertz			Monomolecular			Weibull			
		Rate	R^2	P	Rate	R^2	P	Rate	R^2	P	Rate	Shape	R^2	P
LD91														
	RHA801	0.085	0.790	***	0.018	0.878	***	0.0013	0.832	***	0.0013	1.205	0.764	***
	Pac R2	0.093	0.802	***	0.019	0.901	***	0.0033	0.830	***	0.0027	1.205	0.628	***
	CM526	0.098	0.747	***	0.017	0.814	***	0.0014	0.805	***	0.0028	1.904	0.837	***
	CM497	0.101	0.815	***	0.026	0.931	***	0.0071	0.835	***	0.0072	2.682	0.924	***
	Pac A1	0.105	0.648	***	0.015	0.705	***	0.0005	0.634	***	0.0024	2.499	0.902	***
	Pac A3	0.096	0.776	***	0.022	0.880	***	0.0041	0.866	***	0.0041	1.346	0.858	***
	*cms*HA89	0.092	0.764	***	0.019	0.863	***	0.0034	0.874	***	0.0046	1.877	0.938	***
	HA124	0.094	0.848	***	0.017	0.912	***	0.0015	0.761	***	0.0014	2.022	0.402	***
LD92														
	RHA801	0.088	0.790	***	0.030	0.881	***	0.011	0.851	***	0.0089	2.285	0.847	***
	Pac R2	0.104	0.706	***	0.035	0.857	***	0.014	0.867	***	0.0099	2.56	0.867	***
	CM526	0.098	0.738	***	0.029	0.914	***	0.009	0.865	***	0.0078	2.75	0.947	***
	CM497	0.106	0.901	***	0.049	0.964	***	0.029	0.852	***	0.0118	4.18	0.966	***
	Pac A1	0.119	0.714	***	0.037	0.859	***	0.014	0.845	***	0.0093	2.79	0.888	***
	Pac A3	0.100	0.823	***	0.048	0.946	***	0.030	0.891	***	0.0133	3.61	0.940	***
	*cms*HA89	0.097	0.805	***	0.045	0.939	***	0.027	0.884	***	0.0133	3.87	0.949	***
	HA124	0.081	0.787	***	0.027	0.931	***	0.010	0.865	***	0.0082	2.34	0.891	***

1. Probability of significance of coefficient of determination as determined by Student's t-test, *** $P<0.001$.

Table 4.2 Summary of the results of screening eight inbred sunflower lines for resistance to sclerotinia wilt caused by *Sclerotinia minor* at two field screening sites.

	LD91				LD92			
	D.F.$_{50}$[1]	Mortality[2]		AUDPC	D.F.$_{50}$	Mortality		AUDPC
Line	(days)	Anthesis	Final		(days)	Anthesis	Final	
RHA801	84	7.33 (55.5[3])	13.2	130.0	80	47.8 (77.1)	62.0	602.0
Pac R2	84	15.13 (56.2)	26.9	300.0	75	50.1 (70.1)	71.5	686.3
CM526	88	9.02 (71.0)	12.7	137.2	90	36.3 (64.9)	55.9	448.0
CM497	96	36.23 (70.8)	51.2	570.0	100	89.9 (95.5)	94.1	867.4
Pac A1	84	1.66 (29.1)	5.7	43.9	82	52.5 (75.4)	69.6	613.5
Pac A3	84	21.98 (59.9)	36.7	408.0	92	85.3 (90.9)	93.8	991.7
*cms*HA89	92	22.45 (75.3)	29.8	331.2	92	86.6 (95.2)	91.0	978.4
HA124	84	4.98 (34.3)	14.5	122.2	88	47.2 (82.2)	57.4	528.1
L.S.D.	(P=0.05)	15.42	16.43	208.6		26.62	28.12	298.3

1. D.F.50- days from sowing to 50% anthesis. 2. Means in column analysed after angular sine transformation but presented as untransformed data. 3. Percentage of total or final mortality present at 50% anthesis.

4.3.2 Correlations between glasshouse and field tests used to screen eight inbred sunflower lines for resistance to sclerotinia wilt caused by Sclerotinia minor.

In this experiment the pot base inoculation method described in the previous chapter and the method described by Sedun and Brown (1989) were used to screen eight inbred sunflower lines and the results compared to those obtained from the field.

Materials and method

The eight inbred sunflower lines used in this study were RHA801, PacR2, CM526, CM497, PacA1, PacA3, *cms*HA89 and HA124. Twenty plants of each line were grown in a controlled environment supplying a photon flux density of 420μM.m^{-2}.sec^{-1} in 14 hour photoperiods and a temperature regime of 22/18°C light/dark. At thirty-five days after sowing, ten plants of each line were inoculated by the method described by Sedun and Brown (1989) and ten by the pot base inoculation method. Plants inoculated with either method were completely randomised in opposite ends of the controlled environment cabinet.

The time taken from inoculation to the first appearance of a lesion at the stem base (ie. delay) was recorded as well as the length of the subsequent stem lesion so that the rate of lesion extension could be calculated. Mortality was assessed as the number of plants that had developed stem lesions, wilted and died by 21 days after inoculation.

A '*Sclerotinia* resistance' index was calculated for each inbred sunflower line from this data as follows:

$$SRI = (t - d). r. p$$

where t was the cut-off for assessment (ie when new disease was no longer expected to appear which, in the present experiments, was 21 days), d was mean delay in days, r was linear rate of lesion extension and p was the proportion of plants killed. The underlying assumption behind this formula was that longer delays between inoculation and lesion appearance, slower rates of lesion extension and lower mortality were all measures of higher resistance.

Results

The experiment was repeated once and the combined results presented in Table 4.3. Inoculation using the method of Sedun and Brown (1989) killed fewer plants of RHA801 and PacA1 while lowest mortality of plants inoculated with the pot base method was seen in HA124 and *cms*HA89. The time taken from inoculation to lesion appearance was longer for HA124, RHA801 and *cms*HA89 with the pot base method while there were no significant differences ($P<0.05$) among the lines inoculated with the method of Sedun and Brown (1989). The rate of lesion extension was slowest on RHA801, CM526, HA124 and PacA1 with the pot base inoculation method and RHA801 and CM526 with the method of Sedun and Brown (1989).

Combining these individual parameters of disease assessment into an index of resistance produced similar rankings. The most resistant lines were RHA801 and HA124, then PacA1 and *cms*HA89, and CM526, PacA3, CM497 and PacR2.

Correlation analyses were conducted between the parameters used to assess resistance to sclerotinia wilt in this experiment as well as with results from the two field trials described in the previous experiment. Mortality as determined with the pot base method was negatively correlated with time to lesion extension ($P>0.05$) and positively correlated to mortality resulting from inoculation with the method Sedun and Brown ($P<0.10$) (Table 4.4) but was not significantly correlated to any of field results ($P>0.10$). Similarly, the number of plants killed following inoculation with method of Sedun and Brown (1989) was not correlated to any of the field results. Time taken from inoculation to development of basal stem lesions using either inoculation method were not significantly correlated with any field results ($P<0.10$). The rate of lesion extension following inoculation with the pot base method was positively correlated with the rate following inoculation with the method of Sedun and Brown (1989) ($P<0.05$) and with field mortality and AUDPC's ($P<0.01$ - $P<0.10$). In contrast, the rate of lesion extension produced with the method of Sedun and Brown (1989) showed only significant with the results of the LD92 field screening trial ($P<0.1$). The use of the resistance indices did not improve the correlations with field results although they were correlated with each other ($P<0.10$). The field

results from the two screening trials provided strong correlations particularly between mortality at 50% anthesis ($P<0.01$).

Table 4.3. Comparison of two inoculation methods used to screen eight inbred sunflower lines for reaction to *Sclerotinia minor* wilt.

Line	Pot Base Method				Sedun and Brown (1989)			
	Mortality (/10)	Delay (d)	Rate of lesion extension ($mm.d^{-1}$)	SRI[1] (Rank)	Mortality (/10)	Delay (d)	Rate of lesion extension ($mm.d^{-1}$)	SRI (Rank)
RHA801	7	12.75	7.70	44.2 (2)	4	6.50	8.65	50.2 (1)
PacR2	9	7.57	13.96	168.7 (8)	9	8.13	13.89	160.9 (7)
CM526	9	10.88	8.15	74.2 (5)	9	9.11	9.36	100.1 (6)
CM497	9	11.89	14.13	115.9 (7)	6	9.17	13.79	97.9 (5)
PacA1	7	10.86	9.53	68.4 (4)	5	8.60	14.00	86.8 (3)
PacA3	9	11.86	12.34	101.5 (6)	10	6.88	14.97	211.4 (8)
cms HA89	6	13.00	11.75	56.4 (3)	6	9.20	13.00	92.0 (4)
HA124	5	14.60	8.44	27.0 (1)	6	8.50	11.30	84.8 (2)
L.S.D. (P=0.05)	4.3	2.893	2.35		5.1	3.106	2.266	

1. SRI- *Sclerotinia* resistance index refer to text for calculation

4.3.3. Field screening of twelve sunflower hybrids for resistance to Sclerotinia minor.

Commercial sunflower production in Australia is based on the use of hybrid cultivars. The opportunity was taken therefore to field screen a selection of commercial and pre-commercial (or experimental) hybrids. Included among these hybrids are Pac 3435, Pac 9454 and Pac 7622 which are F_1 hybrids with the pedigrees of PacA1/RHA801, PacA1/PacR1 and PacA3/PacR2, respectively.

Materials and method

The Site used was a part of the field infested with *S. minor* as described for Site 2 (LD92) in Experiment 4.3.1. The screening trial consisted of 4 row plots each 10m long with an inter-row spacing of 50cm arranged in a randomised complete block design with 4 replicates. Plots were oversown and then manually thinned to 4 plants.m^{-1} to give an equivalent plant density of 80000.ha^{-1}. Plants were examined periodically for the appearance of wilt symptoms.

Table 4.4. Correlation matrix of parameters used to assess resistance to sclerotinia wilt in eight inbred sunflower lines in glasshouse and field screenings.

	Pot	Base		Sedun and Brown (1989)				LD91		LD92	
	Mortality	Delay	Rate	SRI[1]	Mortality	Delay	Rate	SRI	Mortality (anthesis)	AUDPC	Mortality (anthesis)	AUDPC
	1	2	3	4	5	6	7	8	9	10	11	12
1	1.0	-0.702**	0.503ns	0.788**	0.645*	-0.079ns	0.251ns	0.573ns	0.451ns	0.491ns	0.135ns	0.164ns
2		1.0	-0.477ns	-0.863***	-0.493ns	-0.034ns	-0.322ns	-0.450ns	-0.043ns	-0.098ns	0.195ns	0.085ns
3			1.0	0.814**	0.386ns	0.190ns	0.801**	0.598ns	0.796**	0.846***	0.692*	0.716**
4				1.0	0.587ns	0.032ns	0.560ns	0.648*	0.477ns	0.556ns	0.207ns	0.294ns
5					1.0	-0.012ns	0.331ns	0.864***	0.230ns	0.315ns	0.057ns	0.176ns
6						1.0	0.140ns	-0.246ns	0.216ns	0.121ns	0.091ns	-0.066ns
7							1.0	0.665*	0.471ns	0.542ns	0.649*	0.685*
8								1.0	0.342ns	0.464ns	0.348ns	0.495ns
9									1.0	0.985***	0.850***	0.769**
10										1.0	0.837***	0.779**
11											1.0	0.954***
12												1.0

1.SRI- disease index calculated as described in the text. 2. Level of significance of correlation: ns P>0.10, * 0.10>P>0.05, ** 0.05>P>0.01, *** P<0.01

Results

Disease assessments commenced at 40 days after sowing when floral buds were first visible. Less than 1% of plants of any of the hybrids had developed sclerotinia wilt at this stage. Disease subsequently developed rapidly and by flowering most of plants that were going to die had died (Table 4.5). Disease incidence at physiological maturity is also presented in Table 4.5 since in commercial production situations any disease occurring after this time would not directly affect the yield of infected plants although yield loss through indirect consequences of the disease such as lodging cannot be wholly discounted. Disease incidence at physiological maturity was determined arbitrarily as being the incidence of disease at the first assessment after 3 weeks post-anthesis.

The hybrids differed significantly ($P<0.05$) in the incidence of plants with basal stem lesions and wilt at anthesis and physiological maturity (Table 4.5). Mortality at anthesis ranged from 45.2% (Pac 3435) to 92.4% (Agseeds AgX 8740) and at physiological maturity from 49.7% to 97.4% for the same two hybrids. There were a number of experimental hybrids with greater resistance than the three commercial hybrids tested as well as some which were more susceptible.

Table 4.5 Summary of results of screening 12 sunflower hybrids for resistance to sclerotinia wilt caused by *Sclerotinia minor*.

Hybrid	Status (Commercial / Experimental)	D.F.$_{50}$ (days)	Mortality[1]		AUDPC
			Anthesis (%)	Physical Maturity (%)	
Pac 9433	E	91	55.8	67.1	598.4
Pac 3435	E	84	45.2	49.7	541.0
AgX 9340	E	94	67.1	73.9	727.5
AgX 3740	E	89	77.7	88.3	879.5
Pac 9454	E	76	66.1	83.5	873.0
AgX 9040	E	95	75.7	87.1	742.0
Suncross 40+	C	94	77.0	84.2	784.9
Suncross 40R	C	89	76.7	86.2	813.4
Hysun 33	C	90	86.2	94.1	979.2
Pac 7622	E	83	89.4	93.6	1072.6
Dk 3903	C	94	83.8	95.5	1002.3
AgX 8740	E	94	92.4	97.4	1025.4
	L.S.D. (P=0.05)		33.1	25.7	71.09

1. Means in column analysed after angular sine transformation but presented as untransformed data.

4.3.4 Correlations between glasshouse and field tests used to screen twelve sunflower hybrids for resistance to sclerotinia vilt caused by Sclerotinia minor.

The objective of this experiment was to further examine the ability of a glasshouse screening method to discriminate between sunflower germplasm for resistance to *Sclerotinia minor* and compare these results with field reactions of the same germplasm.

Materials and method

The twelve sunflower hybrids used were Pac 3435, Pac 9433, Pac 9454, Pac 7622 and Hysun 33 (Pacific Seeds Pty Ltd), AgX 3740, AgX 8740, AgX 9040, AgX 9340, Suncross 40+, Suncross 40R (Agseeds Research and Dk 3903 (Dekalb). Ten plants of each line were grown in a controlled environment cabinet supplying a photon flux density of 400μ $M.m^{-2}.sec^{-1}$ in 14 hour photoperiods and a temperature regime of 22/18°C light/dark. At thirty-five days after sowing plants were inoculated using the pot base inoculation method. The time taken from inoculation to the first appearance of a lesion at the stem base (ie. delay) was recorded as well as the length of the subsequent stem lesion so that the rate of lesion extension could be calculated. Mortality was assessed as the number of plants that had developed stem lesions, wilted and died by 21 days after inoculation. A 'Sclerotinia resistance' index was calculated for each inbred sunflower line from this data as described in Experiment 4.3.2. Regression analysis was conducted between the parameters of disease assessment to test whether the ranking of susceptibility in the glasshouse was similar to that which was observed in the field.

Results

The experiment was repeated three times and the combined results are presented in Table 4.6. The mean number of plants killed ranged from 5.7 per 10 inoculated for Pac 9433 to 8.3 per 10 for Dk 3903. Suncross 40R had the lowest mortality (6.0 per 10) of any of the commercial hybrids. The mean delay from inoculation to appearance of basal stem lesions ranged from 10.1 days for Hysun 33 to over 14 days for Pac 9433 and Suncross 40+. The mean rates of lesion extension also differed significantly ($P<0.05$) among the hybrids form the slowest of 7.24mm.d^{-1} (Suncross 40R) to the quickest of 13.11mm.d^{-1} (Pac 9454). Among the commercial hybrids the rate of lesion extension was quickest for Hysun 33 (11.87mm.d^{-1}). Combining the individual parameters of disease assessment produced a range of resistance indices from 31.33 for Pac 9433 to 103.2 for Hysun 33. The most 'resistant' hybrid was Suncross 40R (SRI- 40.1) and the most 'susceptible' experimental hybrid was Pac 7622 (SRI- 92.6).

Table 4.6. Reaction of twelve sunflower hybrids to inoculation with *Sclerotinia minor* using the pot base inoculation method (ranked by increasing *Sclerotinia* resistance indices).

Line	Mortality (/10)	Delay (d)	Rate of lesion extension ($mm.d^{-1}$)	Sclerotinia Resistance Index
Pac 9433	5.7	14.2	8.34	32.33
Suncross 40R	6.0	11.8	7.24	40.14
AgX 8740	6.3	14.2	10.02	43.12
Pac 3435	6.0	12.6	9.07	45.69
Suncross 40+	7.3	14.4	9.67	46.59
AgX 9340	7.7	12.7	9.35	59.83
AgX 9040	8.0	13.1	9.76	61.45
Pac 9454	5.3	11.3	13.11	67.39
AgX 3740	7.7	11.0	10.35	79.46
Pac 7622	8.0	11.7	12.40	92.06
Dekalb Dk3903	8.3	10.6	10.85	93.84
Hysun 33	8.0	10.1	11.87	103.22
L.S.D. (P=0.05)	4	2.98	2.03	

Among the parameters used to measure the reaction of the sunflower hybrids in the glasshouse there was a negative correlation between delay and rate ($P<0.10$) (Table 4.7). Between the glasshouse parameters and field parameters there were significant correlations between mortality in the glasshouse and mortality at anthesis and AUDPC in the field ($P<0.10$) while delay was negatively correlated with AUDPC ($P<0.05$) and rate was positively correlated with AUDPC ($P<0.05$). Resistance index was correlated with mortality in the field at anthesis and physiological maturity ($P<0.10$) and highly correlated with AUDPC ($P<0.01$).

4.3.5. Effect of plant growth stage on susceptibility to sclerotinia wilt caused by **Sclerotinia minor.**

In the screening optimisation experiments described in the previous chapter seedlings were inoculated at 35-40 days after sowing when plants were still vegetative or in the early reproductive stages of development. This time is convenient for small pot experiments but how the susceptibility of plants at this growth stage compares to alternative growth stages is uncertain especially since most disease in the field occurs after budding. Attempts to grow plants to maturity in small pots was successful but screening these plants with the pot base inoculation method was unsuccessful. A field trial was conducted therefore to ascertain the relative susceptibility of sunflower plants at different growth stages.

Table 4.7. Correlation coefficient matrix between parameters of sclerotinia wilt disease assessment in glasshouse and field experiments.

	Pot Base Inoculation				Field		
	Mortality	Delay	Rate	SRI[1]	Mortality (anthesis)	Mortality (phys.mat.[2])	AUDPC
	1	2	3	4	5	6	7
1	1.0	-0.346ns[3]	0.266ns	0.708**	0.551*	0.484ns	0.539*
2		1.0	-0.501*	-0.813***	-0.244ns	-0.324ns	-0.626**
3			.0	0.746***	0.377ns	0.441ns	0.624**
4				1.0	0.516*	0.539*	0.787***
5					1.0	0.962***	0.835***
6						1.0	0.858***
7							1.0

1. SRI Sclerotinia resistance index calculated as described in text 2. Physiological maturity 3. Level of significance of correlation: ns P>0.10, * 0.10>P>0.05. ** 0.05>P>0.01, *** P<0.01

Materials and method

Serial plantings of two hybrid sunflower cultivars, Dekalb Dk3903 and Agseeds Suncross 40R, were conducted at the University of New England's Laureldale Research Farm during the 1990/1991 and 1991/1992 summer seasons. Dk3903 was considered to be highly susceptible and Suncross 40R less so under natural disease conditions in Victoria (Dr. I. Porter, *pers. comm*) and as indicated in Experiment 4.3.4. A split-plot design was used with five replicate main plots planted on six occasions at 10 day intervals. Sub-plots consisted of Dk3903 and Suncross 40R plots planted side-by-side. Individual plots were 5m long, separated by 1m gaps, interrow spacing within the plot was 75cm, intrarow spacing was 25 cm, and plots were orientated east-west to minimise shading effects between plots.

Plants were inoculated 30 days after the last serial planting. A soil core sampler was used to remove a 2cm diameter by 7.5 cm deep core at a distance 2.5cm from the base of the stem. The hole was filled with moist millet grain inoculum and a 2cm long soil core pressed into the hole to provide a seal. The site was provided with an equivalent of 25mm irrigation with overhead sprinklers after inoculation was completed. The number of plants that developed basal stem lesions, the day on which the lesions were first visible and the subsequent length of lesions were recorded at daily intervals for 28 days after inoculation.

Results

The growth stages of the plants of the two hybrids were similar for each planting date at the time of inoculation for the two years of the experiment (Table 4.8). Growth stages ranged from vegetative, through stages of bud development, flowering and seed fill.

The effect of year, plant age and host line were compared by factorial analyses. Year did not have a significant effect on mortality ($P>0.05$) over all plant ages. The only exception was the high mortality for 30 day old plants of Dk3903 inoculated in 1990/1991 (Figure 4.4 A). The effect of plant age on mortality was highly significant ($P<0.001$) with greater numbers of older plants of both lines being killed (Figure 4.4 A,B). Mortality was not significantly different between the two sunflower hybrids ($P>0.05$).

Table 4.8. Growth stage of two sunflower hybrids at inoculation with *Sclerotinia minor*[1].

Plant Age (days from sowing)	Growth Stages 1990 / 1991 Dk 3903	1990 / 1991 Suncross 40R	1991 / 1992 Dk 3903	1991 / 1992 Suncross 40R
30	V9-V10	V9-V10	V9-V10	V9-V10
40	V14-R1	V14-R1	R1	V14-R1
50	R2	R2	R3	R2-R3
60	R4	R4	R4	R4
70	R5.4	R5.5	R5.4	R5.5
80	R6	R6	R6	R6

1. Growth Stages after Schneiter and Miller (1981).

The time taken for appearance of basal stem lesions was not significantly different ($P>0.05$) between years. Plant age had a significant effect ($P<0.05$) on the time taken for lesions to appear at the stem bases for both hybrids. The trend for Dk3903 was for more rapid appearance of lesions on older plants (Figure 4.5 A) while for Suncross 40R the longest delays occurred in plants at anthesis (Figure 4.5 B). This difference between lines was significant ($P<0.05$). For Dk3903 it would seem that older plants were more susceptible while the reverse was true for Suncross 40R.

The rate of lesion extension on stems decreased with plant age for both hybrids (Figure 4.6 A,B). This effect was more pronounced for Suncross 40R than Dk3903 and was highly significant ($P<0.001$). The rate differed significantly ($P<0.05$) between years with lesion extension being more rapid in the 1991/1992 season. When rate of lesion extension was used as a measure of susceptibility/resistance the older sunflower plants were considered more resistant to *Sclerotinia minor*. It is questionable whether this parameter can be used to differentiate between lines since the rate of lesion extension was not significantly different for the two lines over two years.

A. Dekalb 3903 B. Suncross 40R

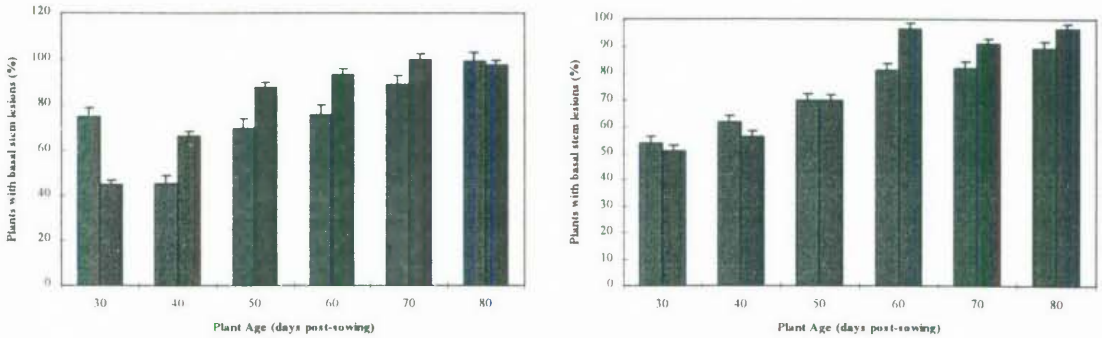

Figure 4.4. Effect of plant age on percentage of plants developing stem base lesions following inoculation of two sunflower hybrids with *Sclerotinia minor* during the 1991 (bars on left) and 1992 (bars on right) seasons. (Standard error of means are represented).

A. Dekalb 3903 B. Suncross 40R

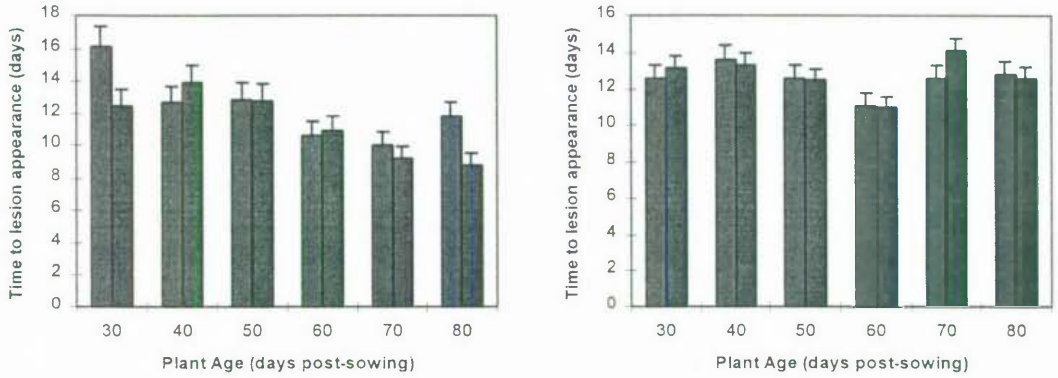

Figure 4.5. Effect of plant age on the time taken for stem base lesions to appear following inoculation of two sunflower hybrids with *Sclerotinia minor* during the 1991 (bars on left) and 1992 (bars on right) seasons. (Standard error of means are represented).

A. Dekalb 3903 B. Suncross 40R

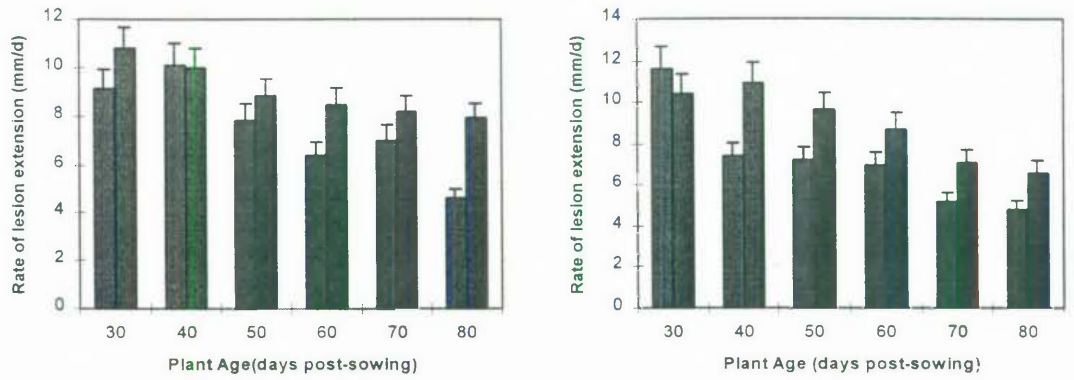

Figure 4.6 Effect of plant age on the linear rate of lesion extension following inoculation of two sunflower hybrids with *Sclerotinia minor* during the 1991 (bars on left) and 1992 (bars on right) seasons. (Standard error of means are represented).

4.3.6. The use of potting mix incorporating sclerotia of Sclerotinia minor *to screen inbred sunflower lines for resistance to sclerotinia wilt.*

The acquisition of field data on the relative susceptibility of sunflower lines provided a basis for critical assessment of screening methods different to those already described. Three of these alternative methods are described in the following experiments.

Materials and method

Sclerotia of *S. minor* were prepared by incubating flasks of millet grain inoculated with *S. minor* isolate UNE#3 for 30d at 20°C. The mixture of sclerotia and colonised grains was dried in flat trays in the air stream of a laminar flow hood for 96h. Once dry, the mixture was sieved through 1mm and 250µm pore size sieves. Free sclerotia trapped between the screens were collected and stored in glass bottles in a refrigerator at 4-5°C for 60d.

Sclerotia were incorporated into the standard potting mix at a rate of $1.cm^{-3}$ as estimated by calculating the average weight of a sclerote and adding the appropriate weight to 60L of potting mix. Mixing was performed by a combination of manual mixing and mixing in a concrete mixer. The inoculated potting medium was dispensed in $500cm^3$ lots to 10cm diameter plastic pots. Each pot contained approximately 500 sclerotes.

Seed of each of the sunflower inbred lines RHA801, Pac R2, CM526, CM497, Pac A1, Pac A3, *cms*HA89 and HA124 were planted in 15 pots containing inoculated potting medium on 13 October (ie spring). Six control pots of each sunflower line were prepared by sowing seeds into uninoculated potting medium. The experiment was conducted in the glasshouse with the pots arranged in a completely randomised design. The experiment was conducted for 120d when the last line matured. A daily watering was increased to twice daily as plants grew and transpiration increased.

Plants were observed for occurrence of wilt and the concurrent stage of plant development recorded.

Results

No plants growing in the uninoculated potting medium had developed symptoms of wilt by the time they reached maturity. Mortality of the plants growing in inoculated media ranged from 2/15 to 8/15 (Table 4.9). The relationships between incidence of mortality and the other parameters assessed shows the tendency for those inbred lines with greatest mortality to have the average time to lesion expression between budding and 50% anthesis.

Regression analysis of 'Time to Lesion Appearance' and 'Mortality' gave a coefficient of correlation of r = -0.952 (P = <0.001). That is, greater mortality was related to reduced time to expression of disease. Most of the mortality of highly susceptible host lines occurred between budding and flowering while the delayed expression of disease in the more resistant lines occurred at or after anthesis. In this experiment the most resistant lines as determined by lowest mortality and longest time to appearance of disease were Pac A1, HA124, CM526 and RHA801 while the most susceptible were PacA3, PacR2, *cms*HA89 and CM497. This distinction was well correlated with the findings of the field screening trials.

Table 4.9. Comparison of differences in growth stage and mortality of 8 inbred sunflower lines growing in potting media containing sclerotia of *Sclerotinia minor*.

Line	Mortality (/15)	Rank	Mean Time to Lesion Appearance (d)	Mean Time to G.S. R1[1] (d)	Mean Time to G.S. R5.5 (d)
Pac A1	2	1	84.0	47.0	79.6
HA124	3	2	85.3	42.3	73.8
CM526	4	3	72.5	44.8	74.3
RHA801	5	4	70.6	42.6	69.3
Pac A3	6	5	67.3	46.5	75.7
Pac R2	6	5	60.3	43.3	77.5
*cms*HA89	6	5	63.7	46.7	77.3
CM497	8	8	57.3	48.9	83.0
L.S.D.(P=0.05)	5		16.3		

1. G. S. R1 is reproductive bud first visible and G.S. 5.5 is 50% anthesis.

4.3.7. The use of Martinson's (1992) method to assess the resistance of 8 inbred sunflower inbred lines for resistance to Sclerotinia minor.

Martinson (1992) described an indirect method involving the use of petioles to screen sunflower plants for resistance to *Sclerotinia sclerotiorum*. This method was tested here using *S. minor*.

Materials and method

The inbred sunflower lines screened were RHA801, Pac R2, CM526, CM497, Pac A3, *cms*HA89 and HA124. Plants growing in the guard rows of test plots at the LD92 field trial (Experiment 4.3.1.) were used as the source of petioles. Petioles were harvested from one symptomless plant in each of the 6 replicate plots of each inbred sunflower line.

Petioles were collected from the tenth youngest leaf (here-on designated top) and from a leaf at mid-plant height. The criteria in choosing petioles were similar to those used by Martinson(1992); 1) petioles were at least 8 cm long, 2) they were straight and 3) no obvious injuries or disease lesions were present. The petioles were excised with a scalpel at the stem and leaf laminar before being placed in marked paper packets and stored in a cooled container until returned to the laboratory were they were processed in a laminar flow hood.

The petioles were surface sterilised in 70% ethanol for 45sec and then rinsed in two changes of sterile distilled water. A sterile scalpel was used to remove short sections of the ends of the petioles damaged by cutting in the field and immersion in alcohol. The petioles were placed into 16 x 150mm glass test tubes containing 2mL solidified V-8 juice agar on which *S. minor* UNE#3 had been growing for 2d. The fungus had covered the surface of the agar but sclerotial initials were not visible. The test tubes were sealed with aluminium caps and placed in test tube racks. These were incubated on a laboratory bench at room temperature (22^{o}C) for 24hr and then transferred to darkness in an incubator at 30±1^{o}C for 24hr. After removal from the 30^{o}C environment the lengths of the lesions were measured by inspection through the tubes. These measurements gave the initial lesion lengths. The petioles were then incubated at room temperature for 24hr to allow further lesion development. The tubes were placed in an incubator at 35^{o}C for 4hr which aided subsequent measurement of lesions by causing the water-soaked regions of pathogenic activity to become more conspicuous relative to the healthy tissue. The lesion lengths measured provided the final lesion lengths. The net lesion length (NLL) was calculated by subtracting the initial lesion length from the final lesion length. The average nett lesion length (ANLL) was calculated by combining and averaging the nett lesion lengths obtained from the upper leaves and the middle-plant leaves.

Results

The experiment was repeated with the same sunflower inbred lines on two occasions (Table 4.10). Significant differences ($P<0.05$) existed between the average net lesions lengths produced on the eight sunflower inbred lines. The descending ranked order for average net lesion length (ANLL) changed only slightly between the two sampling times.

At the first sampling date the descending order of average nett lesion length was Pac R2, CM497, CM526, *cms*HA89, Pac A3, RHA801, HA124 and Pac A1 while for the second sample the order was Pac R2, RHA801, CM497, *cms*HA89, Pac A3, CM526, HA124 and Pac A1. This change in ranking may be a result of the change in plant maturity between the two sampling dates. At the second date RHA801 had completed anthesis and had entered grain fill stage of growth and had become relatively more susceptible in terms of lesion

development on petioles. This increased susceptibility was manifested in both the mid-plant and upper petioles so it seemed to be a whole plant phenomenon.

Regression analysis revealed no relationship between the calculated average nett lesion lengths for the eight inbred lines and field susceptibility as measured by mortality at 50% anthesis (r = 0.238 P=1.0, 0.023 P=1.0) or AUDPC(r = 0.236 P=1.0, r = 0.163 P= 1.0).

Table 4.10 Net lesion lengths on petioles of 8 inbred sunflower lines after inoculation with *S. minor*.

	Petiole Position			Petiole Position			
Inbred	Growth Stage	Mid-Plant	Upper	ANLL[1]	Growth Stage	Mid-plant	Upper	ANLL
Pac A1	R5.1	2.83	4.83	3.83	R5.9	4.0	3.80	3.90
HA124	R4	7.0	5.00	6.00	R5.5	6.83	6.00	6.42
RHA801	R5.5	6.17	6.0	6.09	R6	11.17	11.67	11.42
Pac A3	R4	7.33	5.17	6.25	R5.2	9.17	7.83	8.49
cms HA89	R4	6.5	6.83	6.67	R5.3	8.0	9.50	8.75
CM526	R4	8.0	5.67	6.84	R5.5	6.67	7.17	6.96
CM497	R3	11.0	5.67	8.34	R4	10.67	9.83	10.25
Pac R2	R5.8	6.33	11.0	8.67	R6	19.0	17.00	18.00
L.S.D. (P=0.05)				2.44				2.78

1. ANLL- average nett lesion length calculated as mean of lesion lengths from mid- and upper plant samples.

4.3.8. Screening eight sunflower inbred lines for resistance to Sclerotinia minor *using the method of Bazzalo et al. (1985).*

Bazzalo *et al.* (1985) and Bazzalo *et al.* (1991) inoculated a number of sunflower lines by simply placing rice grain colonised by *Sclerotinia sclerotiorum* at the base of the stem and recording time to plant death and the length of lesions. This method was used here to screen eight inbred lines of sunflower for resistance to *S. minor*.

Materials and method

The eight inbred sunflower lines used were RHA 801, Pac R2, CM526, CM 497, Pac A1, Pac A3, *cms*HA89 and HA124. Seed of each line was sown into standard potting mix contained in twelve 10cm diameter pots. Seedlings were raised in the glasshouse. Inoculum was produced by inoculating pearl millet grain (150g grain + 100 mL water) autoclaved in 250 mL flasks with *S. minor* #3. Ten seedlings of each line were inoculated at a late vegetative stage of growth when 8-10 leaves (>4cm long) were present on most

plants (Experiment 1) or slightly later when some lines had entered the reproductive phase (Experiment 2). Inoculation consisted of placing 3 colonised pearl millet grains in a small depression 5mm deep dug at the base of the stem and covering with potting mix. The remaining two seedlings of each line were inoculated with three autoclaved uncolonised pearl millet grains. Plants were watered twice each day; once following evaluation of symptoms in the morning and the second after 1800h so that plants were moist over-night.

Inoculated plants were examined daily for the presence of a water-soaked lesion at the stem base. The length of lesions was measured daily until the plants were considered dead. Death was considered to have occurred when all leaves (>2cm long) on the plant showed affects of damage such as flaccidity or permanent wilting when examined at 0800h. At this time any plant showing total wilt were considered incapable of recovering turgidity and, therefore, physiologically dead.

Results

Seedlings inoculated with sterile millet grain did not develop symptoms. Lesions were first visible on the stem bases above soil level at 36-48h after inoculation. The lesions then progressed quickly up the stems at rates approaching 20mm.d^{-1} on some lines (Table 4. 11). The rate of lesion extension was slowest on RHA801 and most rapid on CM497 in both experiments. Seedlings were incapable of maintaining turgor when lesions were approximately 3cm in length. The time taken for RHA801 to be killed was the longest in both experiments. While on average the first to be killed were seedlings of *cms*HA89 and Pac A3.

Table 4.11. Reaction of eight inbred lines of sunflower after inoculation with *Sclerotinia minor* using the method of Bazzalo *et al.* (1991).

	Experiment 1			Experiment 2		
	Growth Stage	Occurrence of Death (d)	Rate of Lesion Extension (mm.d^{-1})	Growth S Stage	Occurrence of Death (d)	Rate of Lesion Extension (mm.d^{-1})
RHA 801	V9	5.44	12.39	R1	9.8	11.93
HA124	V9	4.71	13.17	R2	6.7	13.68
Pac A1	V9	4.63	14.88	V11	7.0	13.75
CM497	V9	4.57	15.74	V10	7.7	18.69
CM526	V10	4.33	14.14	R2	8.6	14.90
Pac R2	V8	4.20	14.35	R2	5.8	17.74
Pac A3	V10	4.17	15.36	V12	5.5	17.95
cms HA89	V9	3.83	14.50	V12	5.9	15.67
L.S.D. (P = 0.05)		0.374	2.947		0.999	2.297

Regression analysis of these parameters showed significant negative correlations between the time to death and the rate of lesion extension (Experiment 1 r=-0.625 P<0.10, Experiment 2 r=-0.575 P=0.136). That is, higher rates of lesion extension were associated with reduced times to seedling death. There were no significant correlations (P>0.10) between the time to death and rate of lesion extension in either experiment and the field measures of susceptibility for the same eight inbred lines.

4.5 Discussion

The experiments described in this chapter dealt with the generation of field data on the relative susceptibility of a number of inbred sunflower lines and hybrids to wilt caused by *Sclerotinia minor*. These findings were compared with data generated in glasshouse and laboratory experiments. There was no doubt that various glasshouse screening methods can demonstrate differences in the reaction of sunflower lines to sclerotinia diseases. However if these methods were to be adopted in breeding programs they must have a demonstrable capacity to discriminate among germplasm in a way that produces similar rankings to that produced under field conditions.

Two notable features of the field epidemics generated in these experiments were the rapid disease development following budding and the sigmoidal disease progress curves. Sigmoidal disease progress curves suggest that the underlying epidemic is polycyclic. However, it would seem unlikely that in the *Sclerotinia minor* pathosystem would have been effectively disseminated from initial infections during the growing season. Movement of microsclerotia of *Macrophomina phaseolina* within fields has been attributed to cultivation during the inter-crop fallow (Mihail, 1989). Plant-to-plant spread of *Sclerotinia sclerotiorum* may occur via root contact but at the interrow spacing of 75cm used in the field trials reported in this chapter these would have been limited to the single dimension, along the row (Hoes and Huang, 1985; Nelson *et al.*, 1989). Such single dimensional movement of disease should in theory be unable to sustain logistic disease increase. Descriptions of the disease progress curves using the Weibull probability distribution model showed that most did not conform to either the monomolecular or logistic models but lay somewhere in between. This may be a result of the restricted spread of the disease. The development of sclerotinia wilt of sunflower caused by *S. sclerotiorum* at varying plant populations was also found not to conform to either the monomolecular or logistic models (Nelson *et al.*, 1989).

It has long been recognised that the fitting of models can give misleading ideas on the underlying biology of epidemics (Huisman, 1982, Pfender, 1982). Possible explanations for the sigmoidal curves produced in these experiments, where low disease development

was followed by rapid increase before reaching an asymptote, probably lie with plant growth especially of the roots, changes in plant susceptibility with age, environmental changes over the duration of the field trials and predetermined levels of inoculum or, most likely, combinations of these factors.

There were a limited number of sclerotia in field soil at the time of sowing and this number would not have increased unless successful host colonisation had occurred. Young plants are susceptible to the disease but may escape infection as was demonstrated in Experiment 4.3.5.. When inoculum was inserted into the soil at only 2.5cm from the stem base and to a depth from 0-7.5cm only 50% of plants developed stem base lesions whereas up to 90% of older plants (later than the R4 growth stage) inoculated at the same time developed lesions. Young plants may have escaped infection through limited exposure to the inoculum. In other words, low density of lateral roots may have resulted in low frequency of contact with inoculum of *S. minor*. An alternative explanation is that young sunflower plants may possess the ability to slough off infected roots and so prevent infections moving from lateral roots to the tap root or stem base (Nelson and Christianson, 1993). The rapid disease progress between budding and anthesis in naturally infected fields coincided with the greatest proliferation of root mass in sunflower (Sobrado and Turner, 1986; Leach and Foale, 1987). During this stage plants were susceptible to *S. minor* in terms of the time from inoculation to appearance of lesions (Experiment 4.3.5.). Rapid increase in root length would increase the probability of interception of inoculum and present more infection courts. The slowing of the epidemics after anthesis may be a product of cessation of new root growth which reduced the opportunity for new infections, reduction in the number of sclerotia capable of germinating to initiate infections, reduction in the susceptibility of host tissue, and/or environmental changes. It is difficult to study the dynamics of sclerotia behaviour in the soil but the experiments in this chapter demonstrated that the rate at which the fungus was able to colonise stem bases and stems decreased after anthesis.

The role of the environment in disease expression should not be discounted. The slowing of disease progress in the inbred sunflower lines at the LD91 site coincided with the period when the mean daily air and soil temperatures approached and exceeded 25°C. In pot trials this temperature was shown to reduce the number of sunflower plants infected by *S. minor* (Experiment 3.3.3) compared to lower temperatures. A late increase in disease incidence in this trial occurred after a period of heavy rain and cooler temperatures. At the second field site the reverse was true. Air and soil temperatures were decreasing in such a way that the period from budding to anthesis (40-90d) occurred when mean daily air temperatures were less than 20°C and soil temperatures were around 20°C. Following anthesis temperatures possibly became too cool for further disease development.

The difficulties in interpreting the actual biology of epidemics under field conditions explain to a great degree why plant breeders seek alternative means of screening germplasm for resistance to plant pathogens. However, the transfer of results from screening procedures that use the fungus under laboratory conditions or even those more indirect methods that avoid use of the pathogen altogether to field situations can be difficult. This is especially true when disease resistance in the field is measured by disease incidence, which in itself is a population parameter, whereas in glasshouse experiments disease is assessed on an individual plant basis. Field screening includes the whole growth cycle of the plants whereas in the glasshouse tests plants of a single age are preferred and, for convenience, the preference is for young seedlings. The area-under-the-disease-progress-curve can be minimised by delay in the onset of the disease and/or lower disease incidences. It is not known whether the mechanisms that cause delays in development of symptoms and subsequent severity of symptoms on seedlings are the same as those that operate on mature plants in the field.

In the experiments conducted in this chapter the most consistent relationship between the ranking of field and glasshouse resistance to *Sclerotinia minor* wilt was between disease incidence at anthesis and AUDPC and the linear rate of lesion extension on seedlings in pots. This was true for the eight inbred sunflower lines and twelve sunflower hybrids tested. This might be explained by the observation that plants are not killed until the stem is girdled. Colonisation of a major lateral root may result in wilting of those leaves supplied by that root but the remainder of the plant survives. It is only after the fungus has progressed along the root into the tap root and starts to progress up the stem that the plant shows severe symptoms. The rate at which this colonisation of the stem occurs dictates when a plant will be killed.

Placement of inoculum at a set distance from the stem base, as achieved in the pot base inoculation method reduces variability in the delay from inoculation to lesion appearance. Sedun and Brown (1989) dismissed this parameter because it was too variable when assessed with their inoculation method. The time between inoculation and appearance of a lesion at the stem base is a result of the rate at which the fungus is able to colonise roots. The resistance of roots should be a major determinant of the how quickly plants are killed under field conditions. Correlation analysis between glasshouse and field experiments did not support the use of this parameter in ranking the performance of inbred sunflower lines. It did however, give a slightly better correlation with the AUDPC values in the twelve hybrids than did linear rate of lesion extension. The correlation was also negative, that is, sunflower lines with longer delays between inoculation and lesion extension in pots had lower AUDPC values in the field.

Three other glasshouse screening procedures were examined- sowing seed in potting mix incorporating sclerotia, placing inoculum directly at the stem base and the use of detached petioles. Growing seedlings in the presence of sclerotia of *S. minor* produced results of relative susceptibility similar to the field results. However, this procedure has the disadvantage that the plants must be grown through the whole growth cycle or at least to physiological maturity which may take over 100 days. The pot base inoculation method in contrast is finalised by 60 days after sowing. Placement of inoculum at the stem base of seedlings as described by Bazzalo *et al.* (1991) produced rapid results with most plants colonised and even the most resistant lines were killed by 10 days after inoculation. The method is quick and easy to perform but did not produce results that could be related to the relative susceptibility of the lines in the field. Also this inoculation procedure does not involve exposing the roots, the natural infection courts, to infection. Martinson (1992) suggested that detached petioles could be used to screen sunflower for resistance to *S. sclerotiorum* and that differences between lines was heritable. The use of this procedure to screen sunflower for resistance to *S. minor* also showed differences in nett lesion length between lines. However, those differences were not related to differences in field performance of the donor lines. The method depends on the production in the petioles of compounds inhibitory to *Sclerotinia* during an incubation at 30°C which prevents fungal growth. For sclerotinia wilt it would be better to test the ability to induce the production of inhibitory compounds in roots.