RESISTANCE IN SUNFLOWER TO *SCLEROTINIA MINOR*.

by

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ABSTRACT

Sclerotinia sclerotiorum and *Sclerotinia minor* infect sunflower crops in Australia. The first fungus is mostly associated with head rots of sunflower crops growing in Queensland and northern New South Wales during cooler times of the year. *S. minor,* in contrast, is limited to root and basal stem rots leading to wilt in the irrigation areas of northern Victoria and southern New South Wales as well as small areas of the Liverpool Plains area of New South Wales. It can occur throughout the growing season. Damage caused by head rot can be reduced by ensuring that crops are not flowering during periods when the likelihood of conditions favourable for carpogenic germination of sclerotia is high. Control of sclerotinia wilt caused by *S. minor* has not been adequately achieved in Australia. Plant breeding is one approach that might help to control of this disease.

A review of the literature showed that many different methods have been used to screen sunflower for resistance to sclerotinia wilt caused by *Sclerotinia sclerotiorum.* These methods could readily divide sunflower lines into groups on the basis of susceptibility. However, no single screening method has gained general acceptance as being the most efficient and effective means of selection for resistance to sclerotinia wilt. To increase the amount of useful data that could be collected from each inoculation, inoculum was placed in the bases of pots after careful removal of the root ball which was then replaced. The time taken from inoculation to the appearance of the a basal stem lesion, the rate of lesion extension on the stem and the proportion of the population killed were recorded. Incubation temperature, inoculum age and plant maturity were shown to affect the susceptibility of sunflower to *S. minor* but quantity of inoculum used did not.

Data from controlled laboratory and glasshouse experiments were compared to those obtained from field trials. Disease progress of *S. minor* wilt was characterised by very little disease before budding. Rapid disease increase occurred after budding so that most plants that were killed had become diseased by anthesis. Disease progress was best linearised by the Gompertz model. However 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 area under the disease progress curve (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 for the eight inbred sunflower lines from the two trials were very similar. The eight inbred sunflower lines were screened using the pot base inoculation method or the method described by Sedun and Brown (1989). 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 only one field trial.

The pot base inoculation method was tested as a technique to select plants for increased resistance to *Sclerotinia minor* in recurrent phenotypic selection of a sunflower population. Plants not displaying stem lesions at 28 days after inoculation were inter-mated to constitute the next generation (Cycle). Over 3 cycles of screening the percentage mortality of the population compared to the mean of the four check lines decreased from 100.4% to 27.4% while the rate of lesion extension and the mean time from inoculation to expression of basal stem lesions showed little change. The non-destructive screening method of Castaño *et al.* (1992) was applied to the Cycle 3 population in an attempt to select for increased resistance of shoot tissue to *S. minor*. The detached petiole test of Martinson (1992) was applied to field grown plants. The results obtained suggested that the leaves of the Cycle 3 population had enhanced levels of resistance to *Sclerotinia minor*.

The Cycle 4 population was as resistant to *S. minor* as the resistant inbred RHA 801 if the parameter 'rate of lesion extension' was best correlated with field resistance. However, the other parameters mortality and delay from inoculation to appearance of basal stem lesions indicated that the population was more susceptible than RHA 801. The screening cycles did not result in the selection of plants with decreased root density. Over ninety partially inbred (S_2 and S_3) families were screened at two field sites as single row replicates in a modified augmented design. The second site consisted of head-to-row plots selected from the first site. There was a distinct increase in the number of test plots with adjusted area under the disease progress curve less than RHA801 in the second trial indicating that selection for increased resistance was still necessary and possible during inbreeding. Some of these lines demonstrated good resistance to head rot caused by *Sclerotinia sclerotiorum* when screened in Argentina by Dr Maria Bazzalo.

Attempts were made to identify plant traits correlated with resistance to sclerotinia wilt that could be used in plant breeding. In the field there were various correlations between physical characters of plants (eg height, leaf area, days to flower, root length density and estimated average lateral root diameter) but the only measure with a significant correlation with disease incidence at anthesis was days to flower at both sites ($r=0.78$, $P<0.05$; $r=0.67$, $P<0.10$). The physical quantity of roots in the soil did not seem therefore to be a primary determinant of the final incidence of sclerotinia wilt, at least for the eight sunflower inbred lines used in this study. The role of the shoot in modifying the susceptibility of plants to sclerotinia wilt was demonstrated by grafting different combinations of four sunflower inbred lines.

Calcium has been implicated as a factor in the resistance of plant tissue to pathogenesis by soft rot organisms. Growth of *S. minor* in culture was not affected by calcium concentrations over the range 125-5000μM although at higher concentrations harvested mycelia was heavier. Susceptibility of sunflower varied when grown in both sand and solution culture and supplied with nutrient solutions differing in calcium concentration. Increasing concentrations of calcium chloride increased levels of tissue calcium but reduced resistance to *S. minor* while supplying seedlings with calcium nitrate also increased tissue calcium levels and did increase resistance to *S. minor*. Plants supplied with strontium were more resistant than plants supplied with calcium which, in turn, were more resistant than plants supplied magnesium.

No correlation was found between calcium content and disease incidence in field grown plots of eight sunflower inbred lines nor were correlations found for sodium, magnesium, or sulphur. There were, however, significant positive correlations between phosphorus content of roots and the susceptibility of the eight inbred lines at both field sites. Addition of superphosphate in another two trials increased disease incidence but the addition of diammonium phosphate, which was used at one site only, did not.

Species of *Sclerotinia* release oxalic acid during pathogenesis. The sensitivity of sunflower to oxalic acid may provide an indirect means of selecting host germplasm for resistance to *Sclerotinia*. Excised sunflower seedlings that were fed oxalate solutions developed a number of symptoms including necrosis of the stem base at low pH and wilting and leaf vein darkening at higher pH. pH4 was chosen for screening eight sunflower inbred lines for tolerance to oxalate. This pH was also close to that measured in stem lesions of field grown plants infected with *Sclerotinia minor*. No significant correlations ($P>0.05$) were obtained between tolerance to oxalate and resistance to *S. minor* in either glasshouse or field screening experiments. The method of Tu (1980) was used to assess membrane stability in leaf discs and a modification of this method were tested for the eight inbred sunflower lines. When the leaf discs were soaked in 2.5 mM oxalic acid (pH2.9) the resultant decreases in conductivity recorded for the eight inbreds produced significant correlations with measures of resistance in glasshouse screening and in one case a low level of correlation with field results ($r=0.666$, $P<0.10$). This suggests that membrane stability *per se* may not be a good indicator of resistance to *S. minor*.

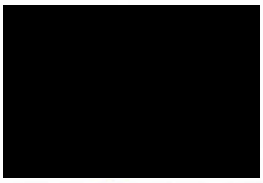
Oxalate oxidase activity was not found in sunflower seedling roots either constitutively or on induction with oxalate but was present in high quantities in the roots of barley. A number of other plant species considered to be either oxalate tolerant or sensitive were inoculated with *S. minor*. On the whole, species not capable of producing or tolerating oxalate were more susceptible to *S. minor*.

Low molecular weight phenolic compounds have been implicated in contributing to the partial resistance of sunflower to sclerotinia diseases. Two highly fluorescent compounds were detected in plants infected with *Sclerotinia minor*. The High Performance Liquid Chromatography (H.P.L.C.) retention times (R_t) and Thin Layer Chromatography (T.L.C.) R*f* values of the extracted compounds were comparable to those of commercial scopoletin (6-methoxy-7-hydroxycoumarin) and synthesised ayapin (6,7-methylene-dioxycoumarin). These compounds are known to be produced in sunflower in response to a number of biotic and abiotic stresses but this was the first time they have been shown to be produced in sunflower stems in response to infection by either *Sclerotinia sclerotiorum* or *S. minor*. These compounds were not detected in extracts from healthy plants. The antifungal activity of these compounds and other phenylpropanoid compounds was demonstrated *in vitro*.

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



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