CHAPTER 4

INVESTIGATION OF ALLELOPATHIC PROPERTIES

It is established that wild North American sunflowers have aggressive characteristics which include allelochemicals (Rice, 1968; Wilson and Rice 1968; Irons and Burnside 1982). Australian crop sunflowers, generally hybrid cultivars, are not good competitors against weeds (Dubbelde, personal communication, from extensive work with commercial growers), insects (Matheson, 1976) or pathogenic diseases (Brown *et al.* 1974; Allen *et al.* 1980). Putnam and Duke (1974) and Waller and Nowacki (1978) have suggested that allelopathic qualities have been bred out of crop plants unconsciously or, possibly, to make them more suitable for growing in monoculture and/or to remove any bitter or unpleasant taste in the harvested product.

Crop plants have been developed for maximum yields under stress-free cultural conditions. These conditions, however, predispose the crop to invasion by weeds which may seriously erode the yield potential if interference occurs. Herbicides and other weed management methods are frequently employed to reduce the impact of weeds. Putnam and Duke (1974,1978) are among workers who have suggested that crop plants containing the allelochemicals present in their wild progenitors, might maintain established yield levels whilst reducing crop protection costs through self-defence mechanisms.

The current project was designed to explore the hypothesis that the allelopathic aggressiveness found in the North American wild sunflowers was absent from an Australian cultivated type during growth, leaving it lacking in self-defence. An Australian biotype and three American biotypes were included for comparison.

The possibility of genetic manipulation of sunflower allelochemicals to promote self-defence was investigated. Preliminary analyses to identify the chemicals responsible and possible storage/release organs were carried out.

Statistical Analysis

The data were analysed using an Analysis of Variance (AOV) comparison program written by E.J. Burr, Professor of Computing Science, on the DEC system, at the University of New England's Computer Centre. The residual mean squares were plotted and examined to determine whether transformations of the data were necessary to stabilize the variance. No transformations were needed in the case of radicle and root length and coleoptile height data, but it was found that the arcsine root percentage transformation was appropriate for germination percentage data. When a trial was measured at different times, as in most of the germination figures, analyses were made within a time, as the variance was too large over all times to allow significant differences to show. Trials were, in most cases, replicated in time. Data presented are representative of trends consistent over multiple runs of the trials.

The figures were drawn by the Zeta plotter of the DEC system which creates a tensioned spline curve through a set of data points. It was necessary in some cases to tension the curve to create straight lines between the data points to reduce the error inherent in the Plot 79 package.

The AOV tables are included in Appendix I. Levels of significance are indicated on the graphical figures by use of letters and on the histogram figures by bars. All significant differences are at the 5% level, Studentized Range, unless otherwise specified. The bars on the histogram figures indicate the 5% LSD.

4.1 Determination of the Relative Toxicity of Chemicals Washed from Green Leaves and Leached Out of Green, Dried or Senesced Leaves of a Hybrid Sunflower (Suncross 53).

For allelotoxic chemicals to be important in the growing sunflower crop, their release must be easily effected, and by the most likely solvent, water, the presence of which is critical to the release and transfer of these compounds (Lovett, 1982b). Allelochemicals released from debris have been cited as the most potent (Sections 2.2.2, 2.3, 2.4.9.4) but these are of importance to the subsequent crop only (Irons and Burnside 1982). Leachates of debris would contain water soluble exogenous and cuticular chemicals as well as water soluble chemicals released during breakdown of the plant tissue, and possibly chemicals released by the micro-organisms involved in that breakdown. In the field, allelochemicals are likely to be washed from green plant material by rainfall, fog, dew or mist condensation and drip (Went, 1955); these agents may only be on the leaf surface for a short time, and hence produce low concentrations of allelochemicals, primarily from exogenous sources.

Leachings of green, senesced and green dried leaves were compared to green leaf washings. The hybrid Suncross 53 (Arthur Yates and Company Pty.Ltd., Narromine) was chosen for the tests. Suncross 53 is not a commercial line, although closely related to many cultivars, as field trials have found it to be non-uniform in appearance. Field trials by the Department of Agriculture, Victoria, however, have shown it to be a high yielding and promising production line (Jessop, personal communication). Linseed (*Linum usitatissimum* L.) was chosen initially, as the bioassay species because of its demonstrated sensitivity to plant toxins (Grümmer and Beyer 1960; Lovett and Sagar 1978).

4.1.1 Materials and Methods

Seeds of the hybrid sunflower, Suncross 53, were germinated in petri dishes on filter paper wetted with 5ml of distilled water at 24^{o}C in the dark. After 5-7 days the seedlings were transferred to 30cm diameter pots containing a standard potting mix (60% loam, 25% sand, 15% peat moss). The pots were kept in a glasshouse at a diurnal temperature range of 18^{o} to 30^{o}, with natural sunlight and daylength (10 to 14h), and watered daily. The plants were fertilised at intervals with a 'complete', soluble fertiliser. Sunflowers are susceptible to boron deficiency, it causing aberrent tissue expansion, thus, boron was added (as H_3BO_3 in solution, applied at the equivalent of the recommended 8kg/ha) at four weeks of age to ensure adequate status of this element.

Green and senesced leaves were harvested from the sunflower plants when the plants were nine weeks old. Some of the green leaves were dried in a microwave oven for five minutes so that leaves for testing could all be collected at once, and to minimise any loss of volatile leaf chemicals. Leaves were chosen to fit, approximately, a 9.0cm diameter petri dish; fresh leaf weight was 3.5 to 4.5g (dry weight 2.0 to 3.0g). The leaching treatments were: the green leaf sandwich (GS), senesced leaf sandwich (SS) and dried leaf sandwich (DS). These were set up by placing a 9.0cm sterile filter paper in a 9.0cm sterile petri dish, covering it with the appropriate type of leaf and another filter paper, wetted with 5ml of sterile water and pressed down gently to maximise surface contact between the leaf and the filter papers.

For the washing treatment (GW) green leaves were weighed, placed in a flask and sterile water added in the ratio 1:10 (leaf:water) w/w. The leaves were then washed by gentle inversion for ten minutes. Sterile

water was used to avoid introducing micro-organisms extra to those *in vivo*. The solution was then filtered through a Whatman No.1 filter paper using a Buchner funnel to remove any large debris, and 5ml of this solution pipetted onto sterile filter papers in petri dishes. Sterile water controls (C) were included, using 5ml of sterile water. Each of the five treatments was replicated three times.

Seeds of the bioassay species, linseed, were surface sterilised in commercial bleach ($NaOCl_2$ - 3 to 4% available chlorine) for three minutes, washed in sterile water for three minutes, drained on clean filter papers to remove excess water, and 25 were counted into each petri dish. The dishes were placed in an incubator in the dark at 24°C for 120h. Germination counts were carried out every 6h between 24h and 78h, and thereafter at 12h intervals until 114h. Germination was deemed to have occurred when the radicle length exceeded the larger width of the seed, circa 2mm. Destructive harvests (of three replicates per treatment) were carried out at 48h, 72h, 96h and 120h, when radicle lengths were measured.

4.1.2 Results

4.1.2.1 Germination

The germination percentage curves were, generally, similar for all the treatments. Germination began slowly from 24h with no statistically significant differences between treatments occurring until 54h, Figure 4.1. However, from 42h a trend was established for treatment C to exceed all treatments other than GS. DS treatment was generally significantly less than C treatment from 54h, and always lower than the other four treatments.

4.1.2.2 Radicle lengths

C treatment radicles were longer ($P < 0.05$) than all other

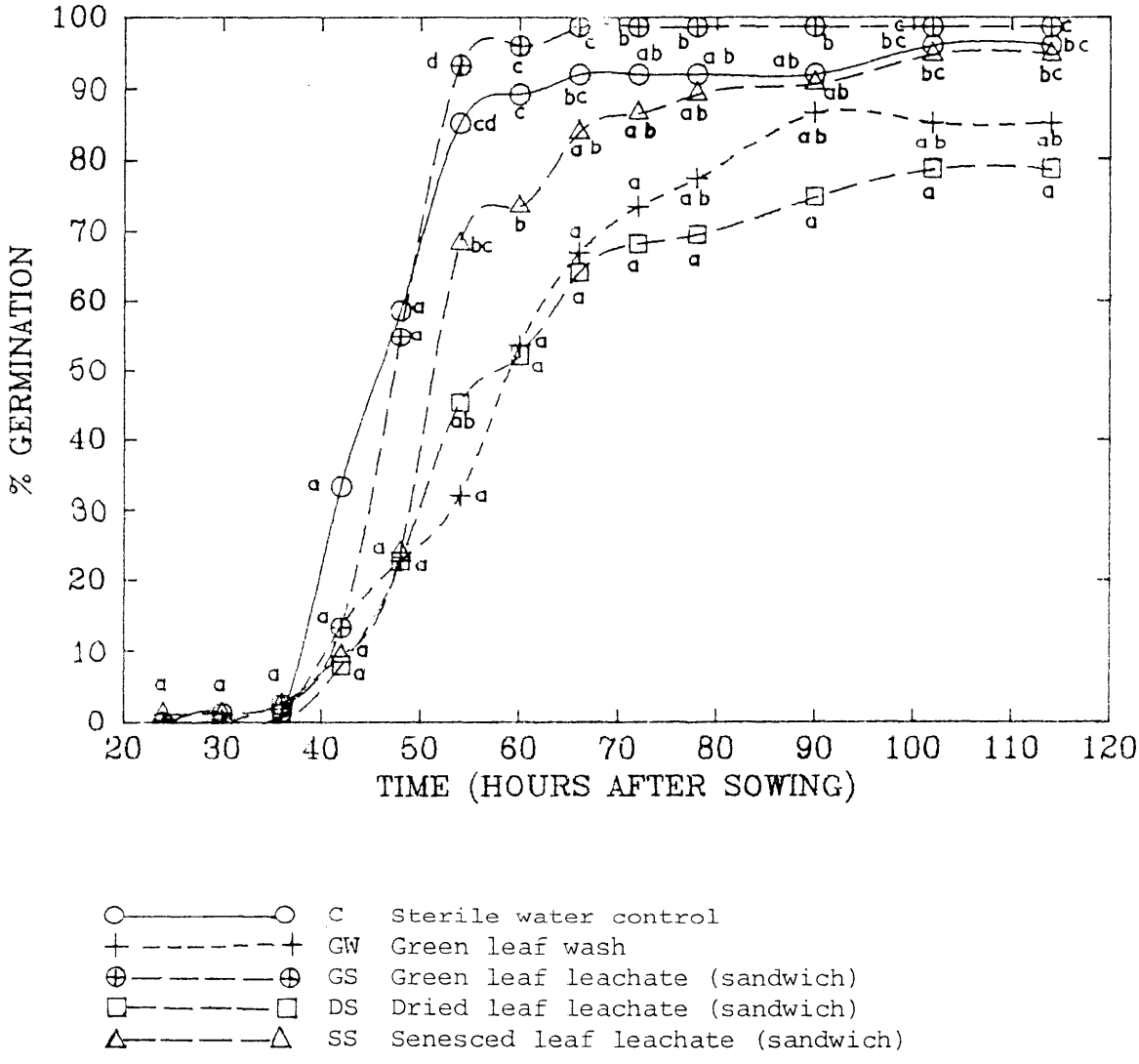


Figure 4.1: Germination percentage of linseed over 120h in solutions of Suncross 53 sunflower green leaf wash, leachates of green leaf, dried green leaf and senesced leaf, and sterile water.

treatments from 72h to 120h, Figure 4.2. GS treatment radicle growth was also rapid being longer ($P < 0.05$) than DS and GW treatment at 96h.

4.1.3 Discussion and Conclusions

Wilson and Rice (1968) reported that dead and decaying leaves of the wild type sunflower in the U.S.A. release allelochemicals which are inhibitory to the germination and seedling growth of sunflower itself and of many of its associated species. *Aristida oligantha* and *Croton glandulosus* were notable exceptions.

The present results indicate that senesced hybrid sunflower leaves (SS) inhibited early germination of linseed to a small extent but that radicle lengths were more sensitive to the presence of allelochemicals. These findings accord with those of Lovett and Jackson (1980) who used the same bioassay species in experiments with allelopathy in weeds.

Dried leaves (DS) caused a greater reduction in germination of linseed, while DS radicle lengths were greatly reduced when compared to C, but were similar to those of SS treatment, except at the 96h sampling. These results suggest that the dried leaf releases allelotoxic substances which are either more inhibitory to the process of germination or are present at a higher concentration than those released by the senesced leaf. In senescing leaves allelotoxic substances may be withdrawn by the plant for use elsewhere as nutritional substrates (Milthorpe and Moorby 1974). Allelochemicals remaining in the senesced leaf may be released through the "leaky" leaf surfaces which wet and leach more readily than younger leaves (Tukey 1969,1971a,b) or be metabolised by micro-organisms on, or in, the leaves. Investigations into the effects of the soluble compounds from leaf surfaces, in the presence and absence of phyllosphere micro-organisms, are discussed in

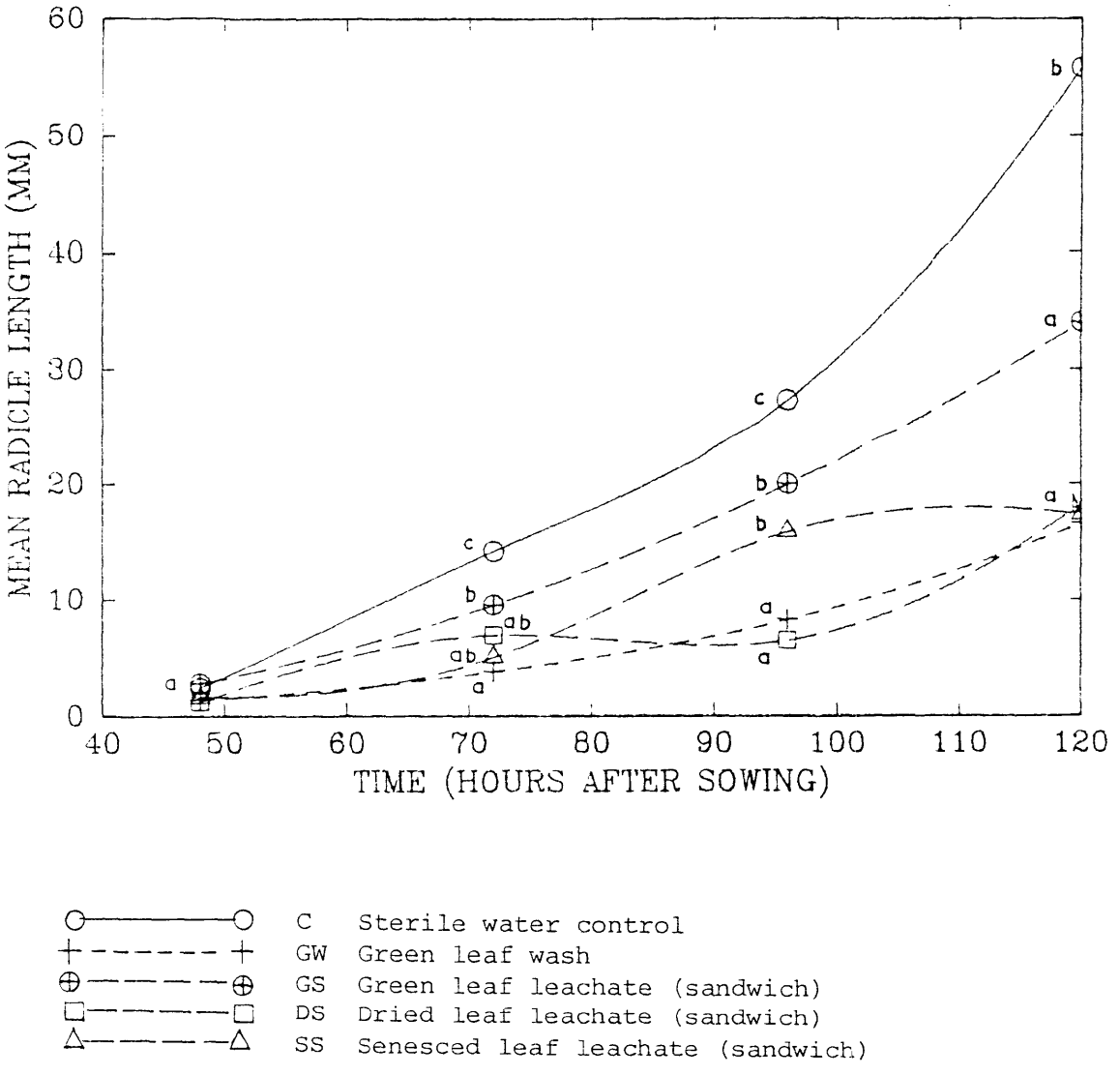


Figure 4.2: Mean radicle lengths of linseed over 120h when watered with solutions of Suncross 53 sunflower green leaf wash, leachates of green leaf, dried green leaf and senesced leaf, and sterile water.

section 4.4.

Leaves used in the GS treatment were similar to those dried for the DS treatment. GS caused no reduction in germination, but did reduce radicle length extension after 48h. The different effects of GS and DS may have been due to many factors, including differences in concentration of allelotoxic substances; chemical alterations during dehydration, resulting in increased allelotoxicity; differing micro-organism populations; respiration of the green leaves over the 120h of the trial, during which allelotoxins were metabolised to benign forms; or more ready release of allelochemicals from the dried leaves as a result of ruptured cell walls on drying.

The GW treatment should have contained, primarily, exogenous leaf chemicals and micro-organisms. Both germination and radicle extension were reduced to an extent similar to that induced by the highly allelotoxic DS treatment. These data support the findings by Rice (1964); Grant and Sallans (1964); Wilson and Rice (1968) and Bokhari (1978), that green aerial portions of some plants are more inhibitory to test species than other portions. This may mean either that the allelotoxic substances on the leaf surfaces are inherently more toxic or, that micro-organisms on the leaf can alter the phytochemicals to make them more toxic to the bioassay species. These green leaf allelochemicals could be counteracted by the internal substances or micro-organisms present in the GS treatment.

The leaves of the hybrid sunflower used for this trial showed allelopathic potential. The allelotoxicity of litter (senesced or dried leaves) was demonstrated on both germination and early growth of linseed, but washings of healthy, green leaves produced a very similar effect,

suggesting that allelopathy could play a role in a growing crop.

4.2 Determination of Relative Toxicity of Leaf Chemicals Washed from Five and Ten Week Old Sunflower (Suncross 53) Plants

Koeppe *et al.* (1970b) stated that older green leaves of sunflower had higher levels of the phenolics chlorogenic acid, 4-O-caffeoylquinic acid and neo-chlorogenic acid than young growing tips. Young leaves are hydrophobic (Cholodny, 1932), suggesting that there would be a greater likelihood of older leaves being leached. This has been confirmed by Tukey (1969,1971a,b) who showed that the peak of leachability occurred at senescence. When coupled with a higher concentration of allelochemicals, older leaves would be expected to have a more aggressive effect on competing species.

Five and ten week old Suncross 53 plants were compared for levels of allelotoxic chemicals in leaf washings. At five weeks the plants were still growing rapidly while at ten weeks the plants had virtually finished vegetative growth and inflorescences had initiated.

4.2.1 Materials and Methods

Healthy, entire leaves were collected from five and ten week old Suncross 53 plants selected from weekly sowings. The leaves were weighed and washed, and the solutions filtered as described in 4.1.1. 9.0cm diameter sterile petri dishes were set up containing a 9.0cm sterile filter paper and 2ml of the five or ten week plant washing (5W, 10W), or sterile water as the control (C). Linseed seeds were surface sterilised as described in 4.1.1, and transferred into the petri dishes. Four replicates of 25 seeds were set up per treatment. The dishes were incubated at 24^{o}C, in the dark, for 120h.

Three runs of the experiment were conducted. Germination counts

were made at 6 or 12h intervals from 24h to 96h, and at 120h. Radicle lengths were measured at 120h. Results from one run are presented.

4.2.2 Results

4.2.2.1 Germination

Germination was rapid for all treatments after 30h, Figure 4.3. C treatment had the highest ($P < 0.05$) germination percentage at 54h after which there were no significant differences between treatments.

4.2.2.2 Radicle lengths

Data for linseed radicle lengths at 120h are presented in Figure 4.4. The 5W treatment stimulated radicle extension, but was not statistically significantly longer than the other two treatments.

4.2.3 Discussion and Conclusions

Many toxic phytochemicals are the byproducts of plant metabolism (Whittaker and Feeny 1971; Levin, 1976) and may be the precursors of other phytochemicals (Taylor and Zucker 1966). The level of these chemicals is likely to fluctuate as the health, vigour and age of the plant changes. The chlorogenic acid and iso-chlorogenic acid commonly found in sunflower leaves (Wilson and Rice 1968) may be converted to a precursor of lignin (Taylor and Zucker 1966), and, if so, will be used rapidly by maturing plants. As plants age further and growth slows, free chlorogenic acids may be found once the rate of synthesis of these exceeds the rate of turnover to lignin.

Phyllosphere micro-organisms may also alter the potency of phytochemicals. The phyllosphere population may change with plant ageing, causing more, or less, allelotoxic substances to be released during the life of the plant. Elimination of these micro-organisms is investigated in 4.4.

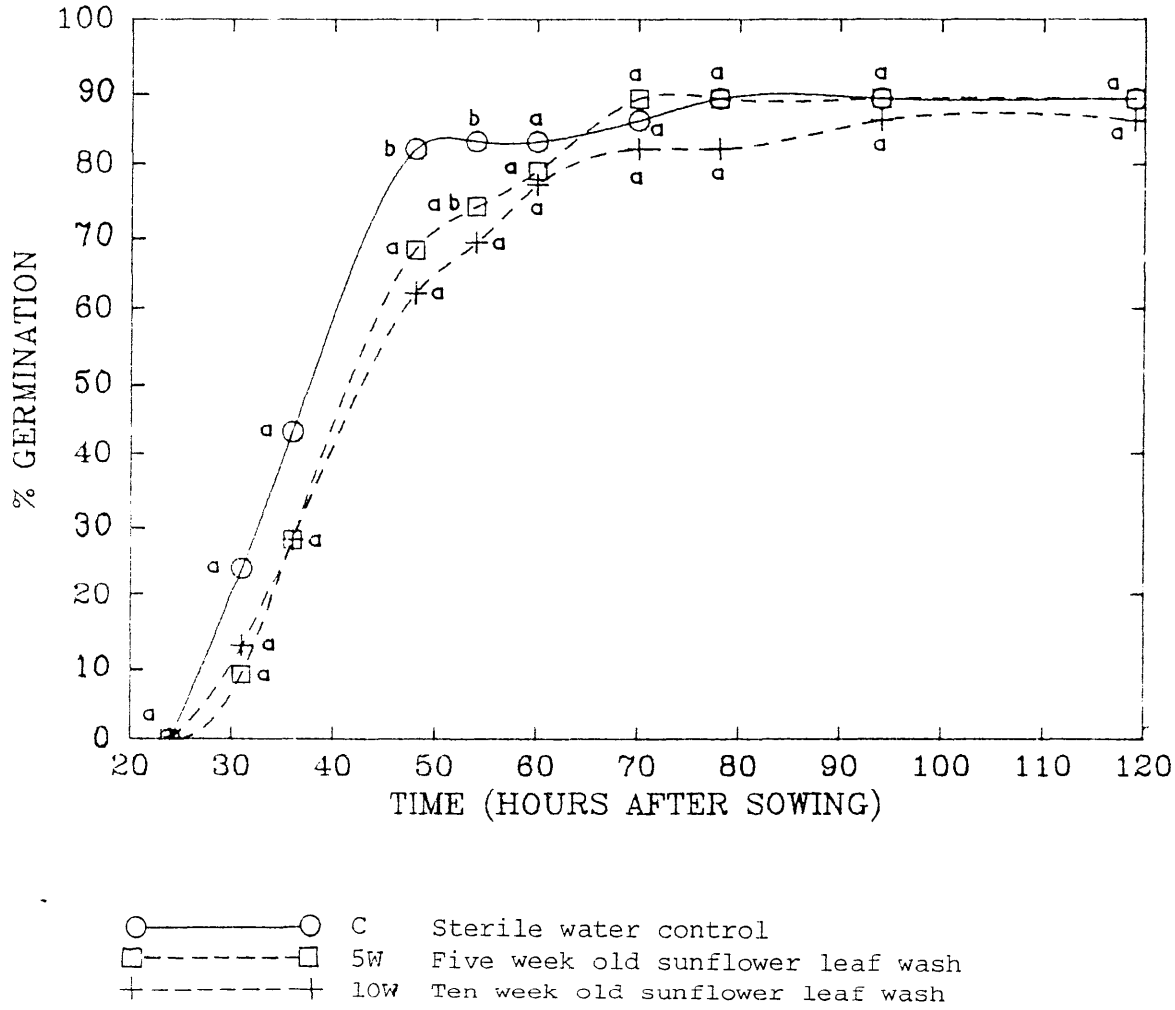
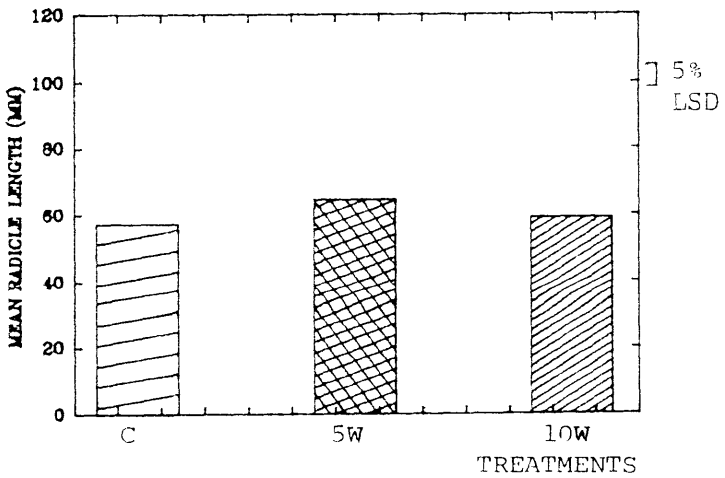


Figure 4.3: Germination percentage of linseed over 120h in solutions of five and ten week old Suncross 53 sunflower leaf wash, and sterile water.



C Sterile water control
5W Five week old sunflower leaf wash
10W Ten week old sunflower leaf wash

Figure 4.4: Mean radicle length of linseed at 120h when watered with leaf wash solutions of five and ten week old Suncross 53 sunflower, and sterile water.

The leaves harvested for examination were taken from all parts of five and ten week old sunflower plants. The data from all the trial runs indicated that 5W treatment caused a mild stimulation of early radicle growth of linseed, while germination rates were not affected. If chemicals were absent from 5W plants, the results should have been more like those of treatment C, hence it could perhaps be assumed that some chemicals were present, but at a low stimulatory, concentration (Evenari, 1949).

The data from all trial runs also indicated that 10W treatment produced a consistent but non-significant retardation of germination of linseed compared with C. 10W radicles were always shorter than 5W, while C results were not consistent. 10W plants may have been releasing some chemicals that slightly reduced germination, but were at too low a concentration in the petri dishes to affect radicle growth.

A juvenile sunflower crop is more liable to weed infestation than a mature one, as more light, water and nutrients are available to the invading species. It may be that the young sunflower further contributes to the competitive ability of weeds by releasing small amounts of allelochemicals that will actually stimulate their germination and early growth. As the crop plants mature their self-defence systems may come into play, the allelochemicals released being more toxic to the weeds and more readily leached under natural rainfall conditions.

4.3 Determination of Conversion Effects in Leaf Wash Solutions of Sunflower (Suncross 53) by Phyllosphere Micro-organisms Over a 24h Period

Allelochemicals may be released by the plant or converted to allelochemicals by micro-organisms, living in the phyllosphere, from substrates released by the leaf. Lovett and Sagar (1978) and Lovett and

Duffield (1981) found conversion to an allelochemical by bacteria on leaves of *Camelina sativa*, a species known to significantly reduce the yield of linseed (Grümmer and Beyer 1960), under field conditions which would favour the development of micro-organisms in the phyllosphere. To determine if micro-organisms altered the effect of sunflower leaf wash solution on bioassay species the solution was incubated for 24h and its effects compared to those of freshly collected leaf washings.

4.3.1 Materials and Methods

A healthy ten week old Suncross 53 sunflower plant was selected and leaves harvested from it on two consecutive days. The leaves were weighed and washed immediately after harvesting and the solution filtered as described in 4.1.1. The solution collected on the first day was bottled and incubated for 24h at 24°C. On the second day, 2ml of the appropriate solution, Fresh (F), 24h (24) or sterile water (C), were placed in a sterile 9.0cm petri dish containing a sterile filter paper. Four replicates of each treatment were set up. Twentyfive surface sterilised linseed seeds were placed in each dish and the dishes incubated at 24°C, in the dark, for 120h. There were three runs of the experiment. Germination counts were made at 6 to 18h intervals from 24h to 96h, and at 120h, and radicle lengths measured at 120h.

4.3.2 Results

Consistent results were obtained from the three runs of the experiment. These results are illustrated using data from one run.

4.3.2.1 Germination

Linseed germination data are illustrated in Figure 4.5. Germination in C treatment began first and had the highest percentage up to, and including, 46h. The difference was significant ($P < 0.05$) at 24h, 30h and 36h only.

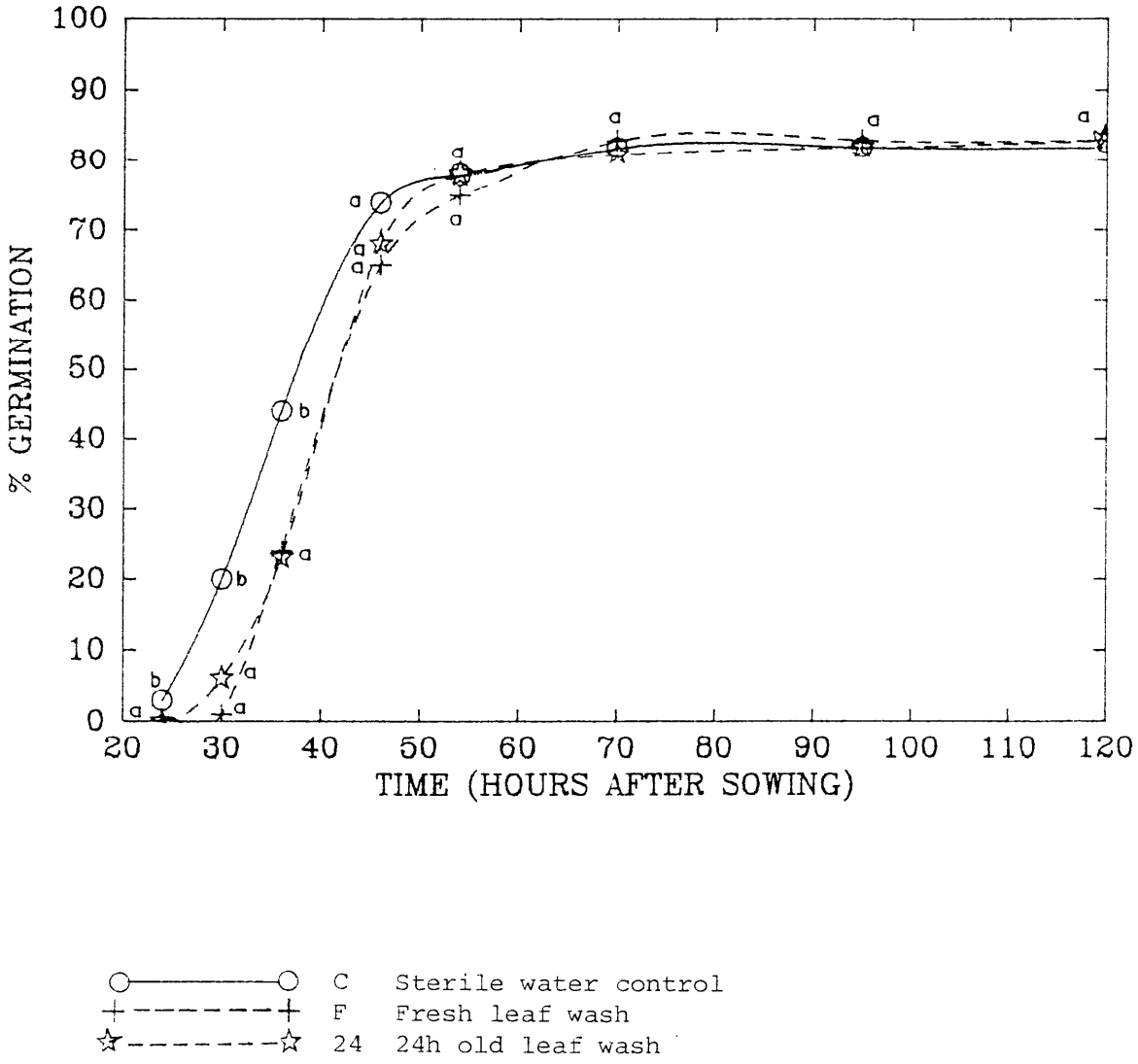


Figure 4.5: Germination percentage of linseed over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 24h old leaf wash, and sterile water.

4.3.2.2 Radicle lengths

24 treatment stimulated radicle elongation compared to C treatment, Figure 4.6, although this stimulation did not attain statistical significance. F treatment stimulated radicle elongation compared to C treatment, but the increase was not as great as that caused by 24 treatment. Differences were, again, not statistically significant.

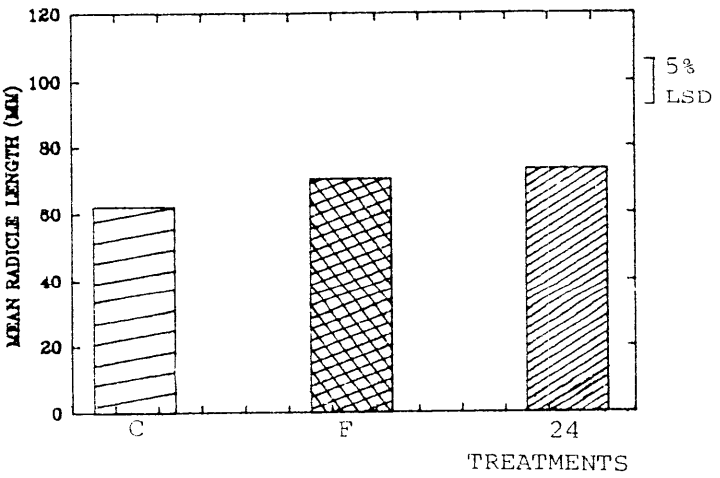
4.3.3 Discussion and Conclusions

The data from all the trial runs indicated that both 24 and F solutions caused germination of linseed to 48h to be slowed slightly, but may have stimulated early radicle growth. 24 solution tended to have a greater effect than F on radicle growth to 120h, although the effect was still not statistically significant.

At the concentration of washing used (1:10) the results obtained were not statistically significant between treatments. There appears to be little difference overall between F and 24 germination results. However, over all runs, 24 solution consistently produced longer radicles at 120h than F or C. There may be some microbial conversion of phyto-chemicals taking place, either rendering toxic substances less toxic, or enhancing the stimulatory effect of the substances.

4.4 Elimination of Phyllosphere Micro-organisms from Sunflower (Suncross 53) Leaf Wash Solutions

The results presented in section 4.3 showed that 24h incubated leaf wash slightly stimulated radicle growth of linseed as compared to freshly prepared leaf washings. In this experiment, bacteria and/or fungi were removed from the freshly collected leaf wash to ascertain which population, if any, was causing changes in the washings during incubation. A range of bioassay species was used.



C Sterile water control
F Fresh leaf wash
24 24h old leaf wash

Figure 4.6: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 24h old leaf wash, and sterile water.

4.4.1 Materials and Methods

Leaves were harvested from healthy ten week old Suncross 53 plants, weighed and washed, as in 4.1.1. Two-thirds of the collected solution was then passed through a sterile 1.2µm Gelman-Clemco Millipore filter, and half of the filtrate passed through a 0.2µm filter. All work was carried out in a laminar flow unit to minimise re-contamination of the solutions.

Petri dishes were set up as in 4.1.1, with 2ml of either sterile water as a control (C), 0.2 or 1.2 filtrates (0.2, 1.2), or fresh solution (F). Treatments were replicated four times.

A) 25 surface sterilised linseed seeds were placed in each dish and the dishes incubated at 24^{o}C, in the dark for 120h. The filter papers were kept moist as required with 2ml of sterile water.

Germination counts were conducted at 24h intervals in two runs, and at intervals of 6 to 12h to 96h in one run, and at 120h. Radicle lengths were measured at 120h for all three runs. Data from one run are presented.

B) The experiment was repeated using similar methods, and a range of bioassay species - wheat (*Triticum aestivum* L. cv. Songlen), perennial ryegrass (*Lolium perenne* L. cv. New Zealand), phalaris (*Phalaris aquatica* L. cv. Australian), sunflower (*Helianthus annuus* L. cv. Suncross 53), white clover (*Trifolium repens* L. cv. Ladino) and lucerne (*Medicago sativa* L. cv. Hunter River).

4.4.2 Results

4.4.2.1 Germination

A) Linseed

Germination began simultaneously for all treatments at approximately

24h, Figure 4.7. C treatment had the highest germination percentage to 48h (higher, $P < 0.05$, than 0.2 at 31h, higher, $P < 0.05$, than F and 1.2 at 48h, higher, $P < 0.05$, than 1.2 at 54h). Over the remainder of the 120h, C treatment germination percentage was equal to 0.2 treatment and not significantly higher than F and 1.2 treatments.

B) Bioassay species range

i) Wheat

0.2 treatment reached a germination of 100% by 48h, Figure 4.8a. Germination percentage in treatments C and F was similar, and not significantly less than 0.2 treatment through to 120h. 1.2 treatment germination percentage was less than 0.2 ($P < 0.05$) from 48h.

ii) Phalaris

Germination rates were much lower than for wheat and germination was still being observed at 120h, Figure 4.8b. Germination commenced at 20h with 1.2 treatment having the highest germination percentage until 84h. No significant differences were observed.

iii) White clover

1.2 treatment germination percentage was consistently higher than the other three treatments after 30h, Figure 4.8c. It was greater ($P < 0.05$) than C and 0.2 treatments at 48h only. F treatment germination also began rapidly whilst 0.2 and C treatments showed a similar germination percentage from 20h through to 120h.

iv) Sunflower

0.2 treatment germination percentage was the highest from 20h until 78h, Figure 4.8d. However, differences were at no time statistically significant.

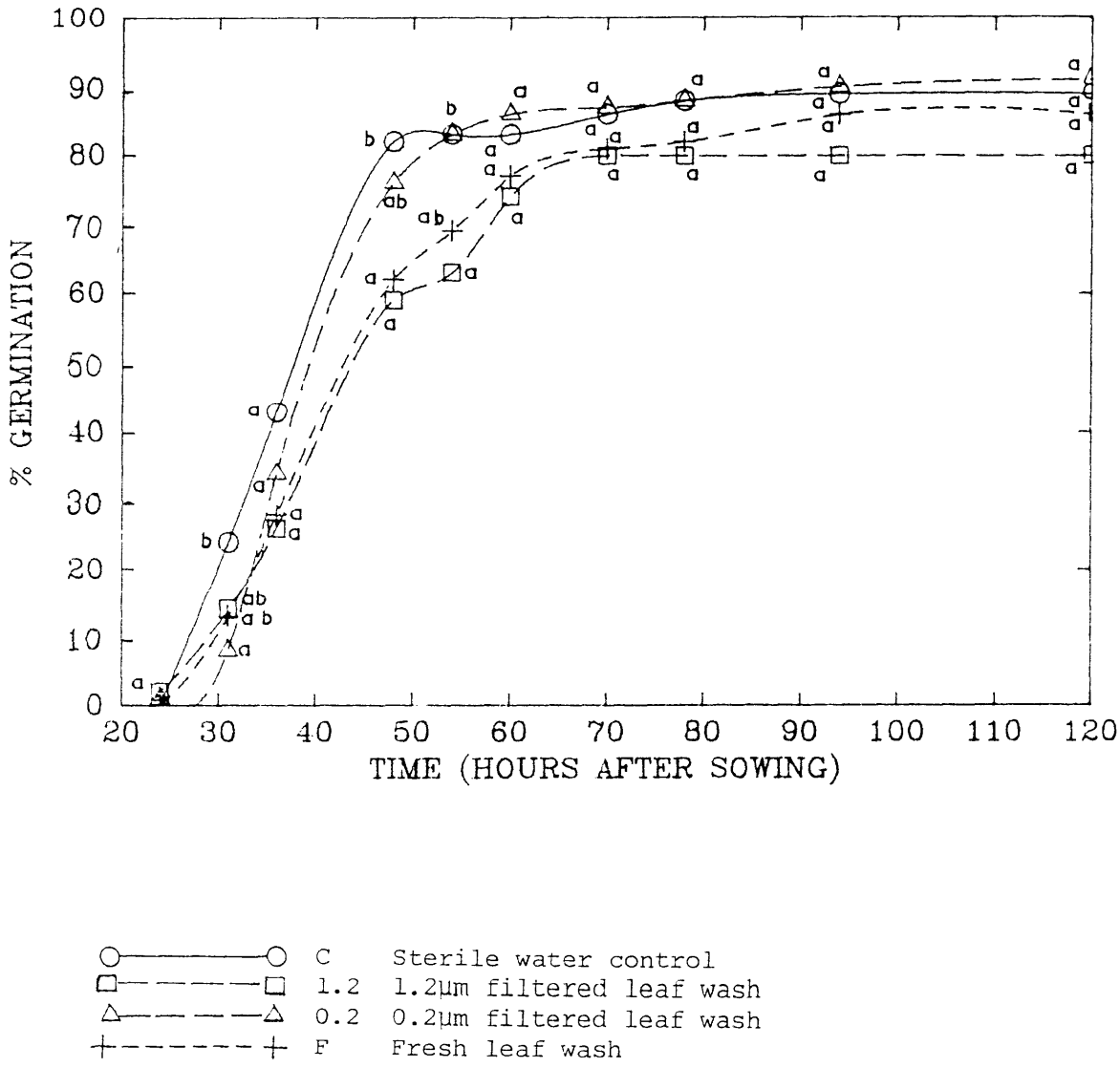


Figure 4.7: Germination percentage of linseed over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

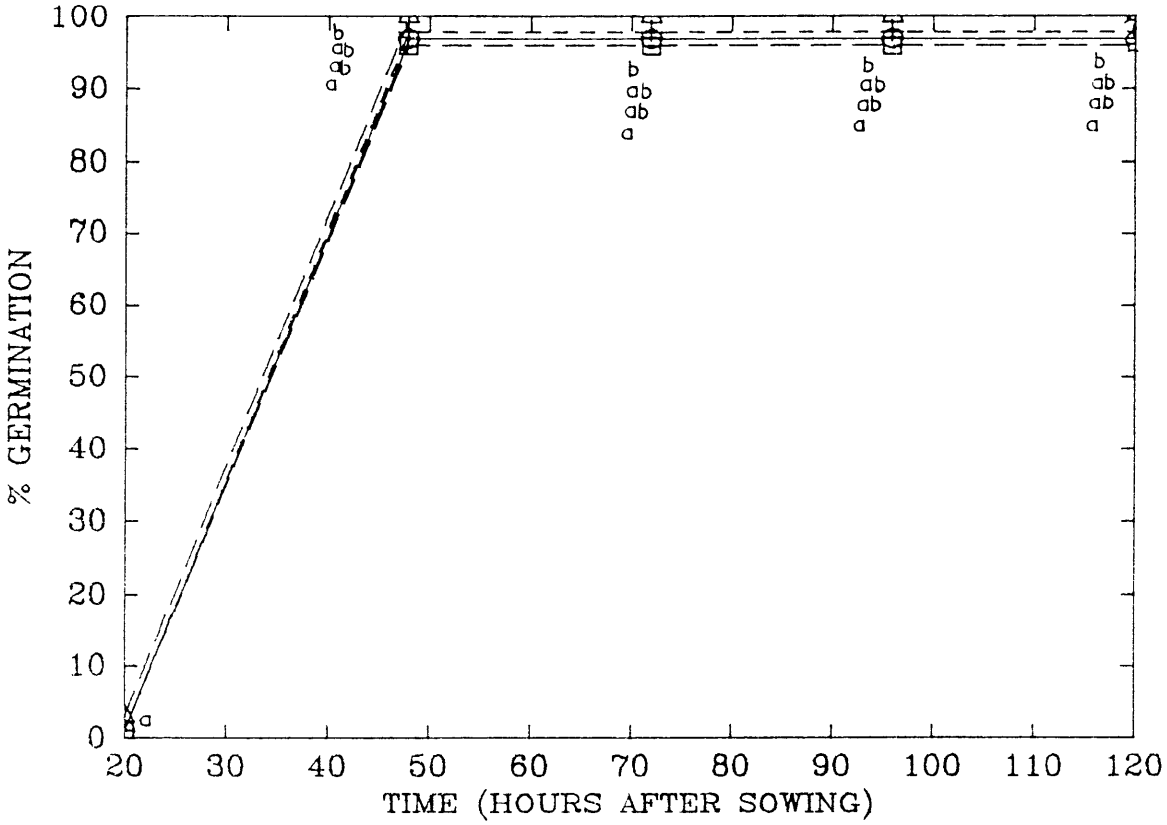


Figure 4.8a: Germination percentage of wheat over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

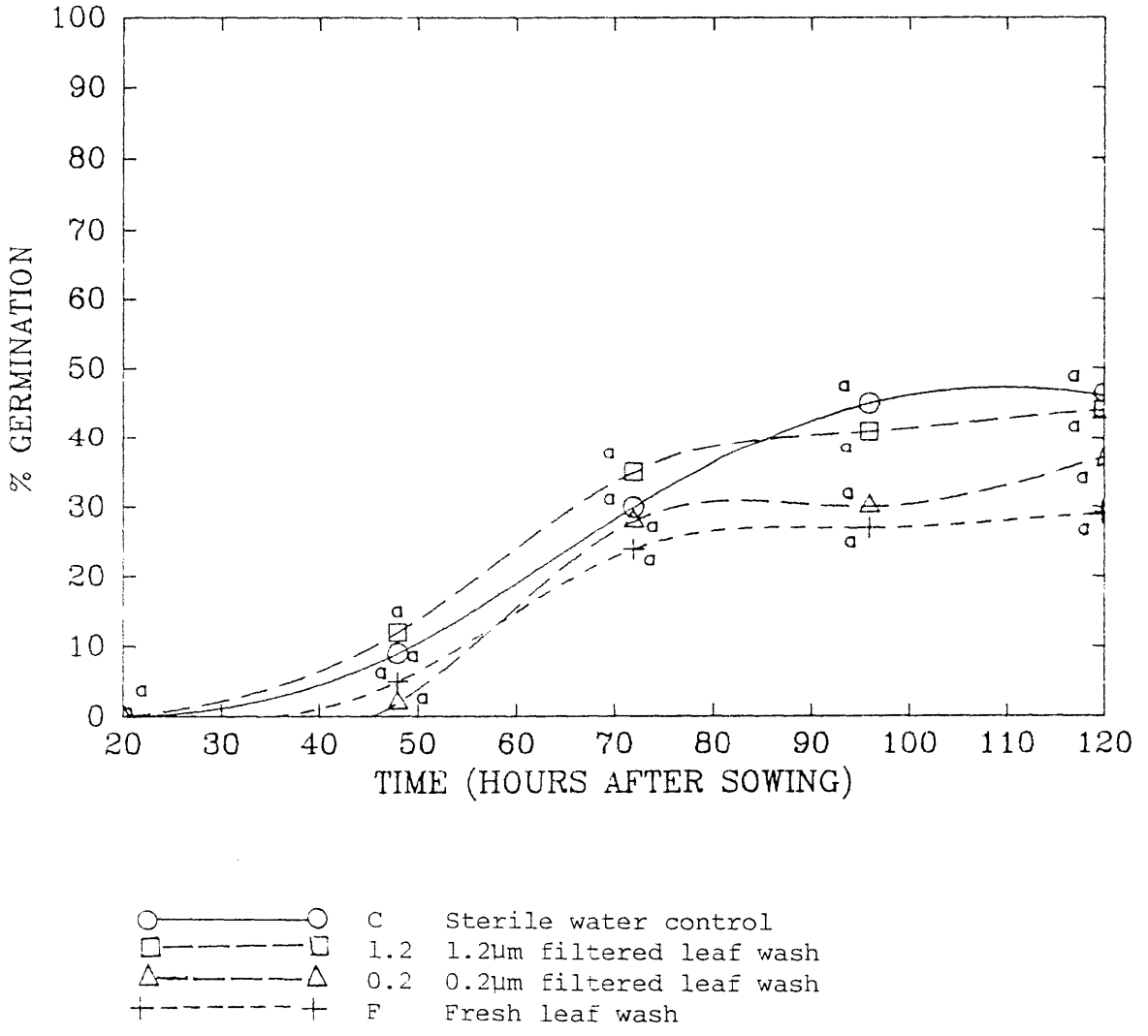


Figure 4.8b : Germination percentage of phalaris over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

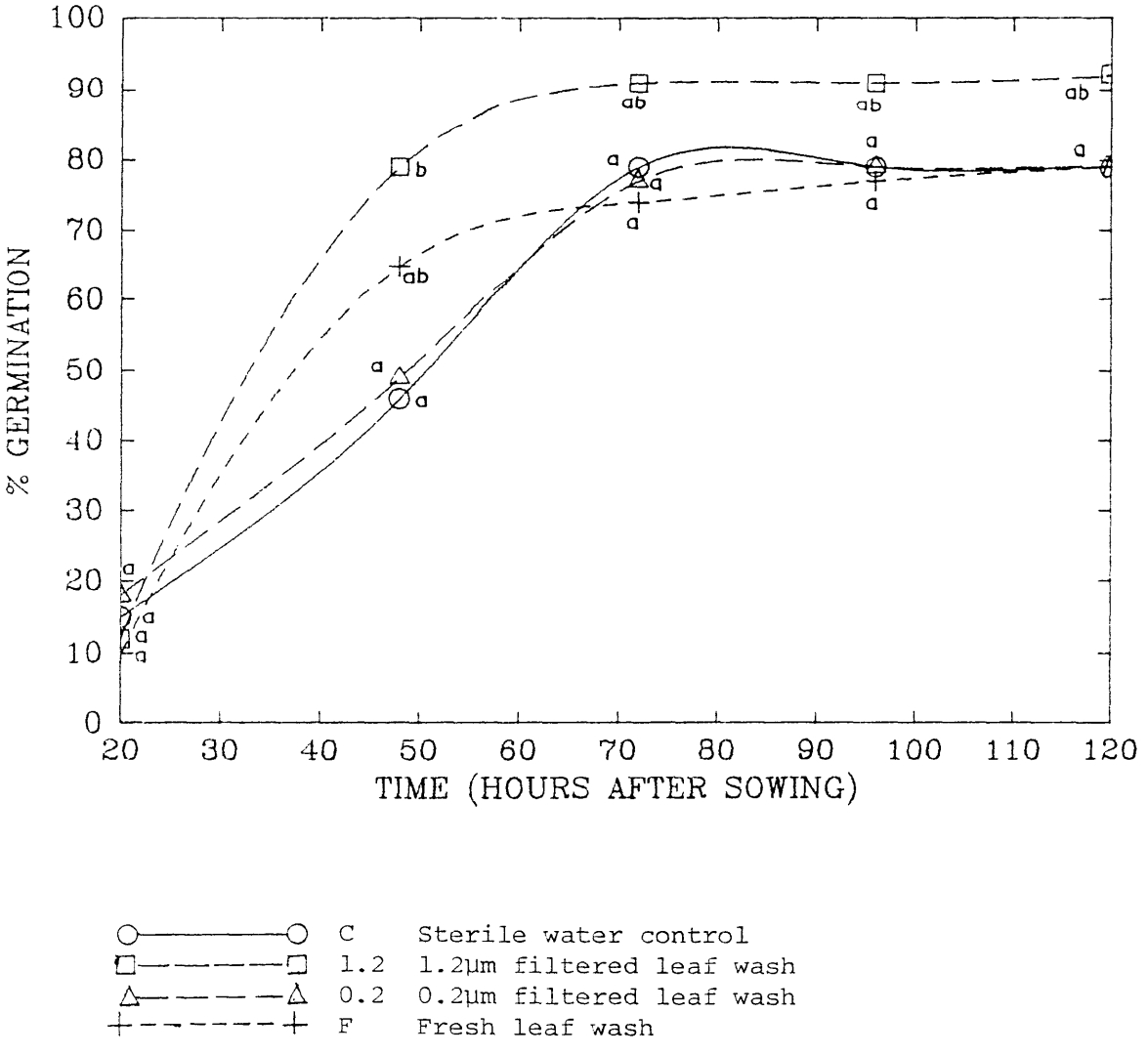


Figure 4.8c : Germination percentage of white clover over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

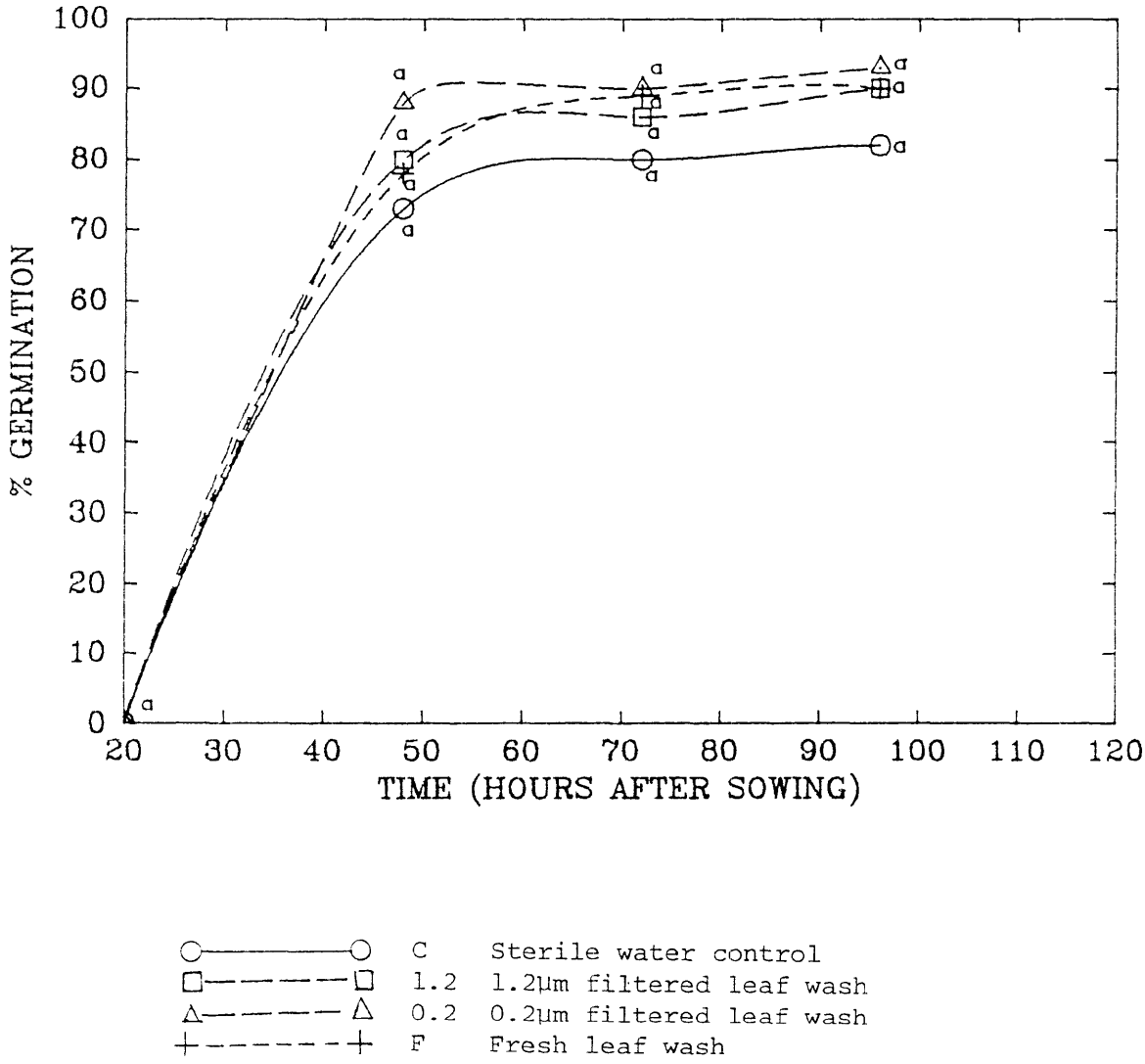


Figure 4.8d: Germination percentage of sunflower over 120h in solutions of Suncross 53 sunflower fresh leaf wash 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

No reading was taken at 120h as a fungal infection had destroyed the radicles.

v) Perennial Ryegrass

Germination commenced around 48h and was, effectively, complete by 120h, Figure 4.8e. C treatment had the highest germination percentage to 96h after beginning slightly later than F and 0.2 treatments. No differences were statistically significant.

vi) Lucerne

A trend for F treatment to have the highest germination and 0.2 treatment the lowest was maintained throughout the experiment, Figure 4.8f. F treatment germination percentage was significantly higher ($P < 0.05$) than C and 0.2 treatments at 120h.

vii) Sorghum

At 24h F treatment had a smaller ($P < 0.05$) germination percentage than 1.2 treatment, Figure 4.8g. At 30h, however, 0.2 treatment had the lowest germination percentage, being smaller ($P < 0.05$) than C treatment only. No significant differences occurred after 30h.

4.4.2.2 Radicle or first seminal root lengths

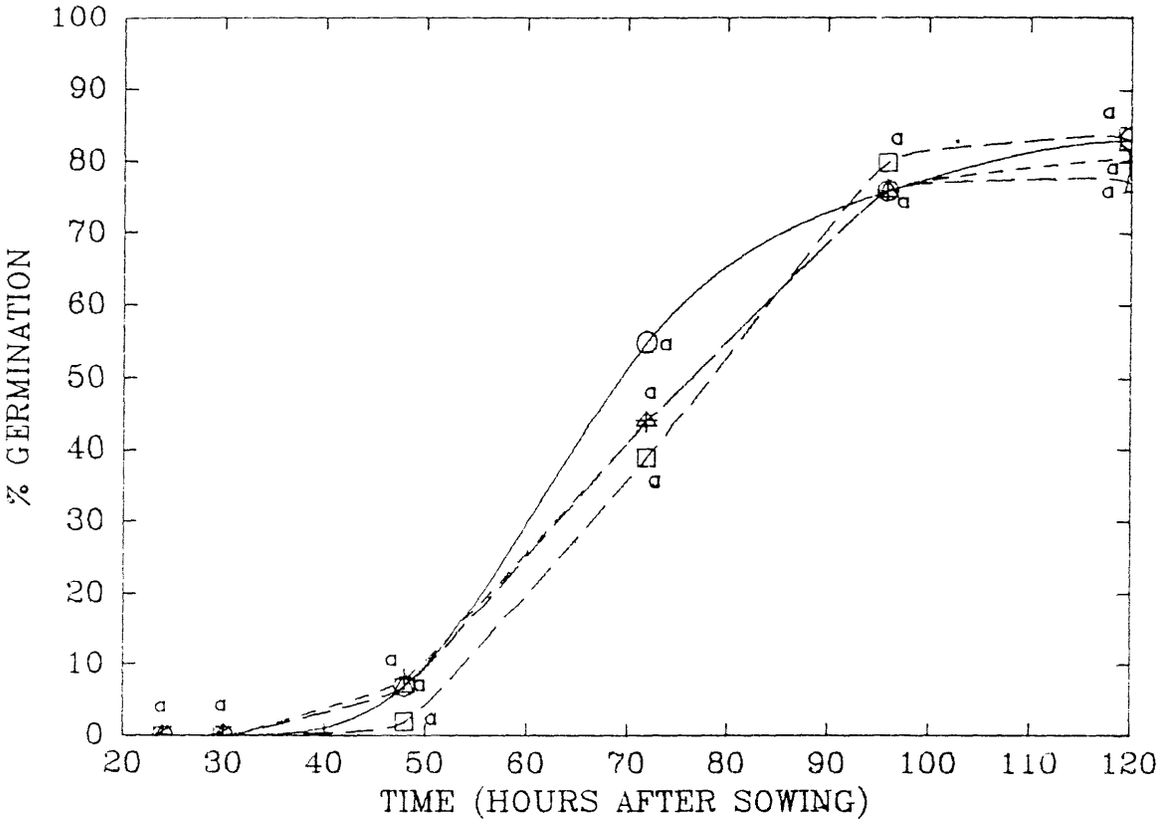
A) Linseed

Radicle length data for linseed at 120h are presented in Figure 4.9. C treatment radicles were similar to those of F treatment, and both were not significantly different to the radicles of the other two treatments. However, 1.2 treatment radicles were shorter ($P < 0.05$) than those of 0.2 treatment.

B) Bioassay range

i) Wheat

No significant differences existed at harvest at 120h, Figure 4.10a.



○——○	C	Sterile water control
□——□	1.2	1.2µm filtered leaf wash
△——△	0.2	0.2µm filtered leaf wash
+------+	F	Fresh leaf wash

Figure 4.8e: Germination percentage of perennial ryegrass over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

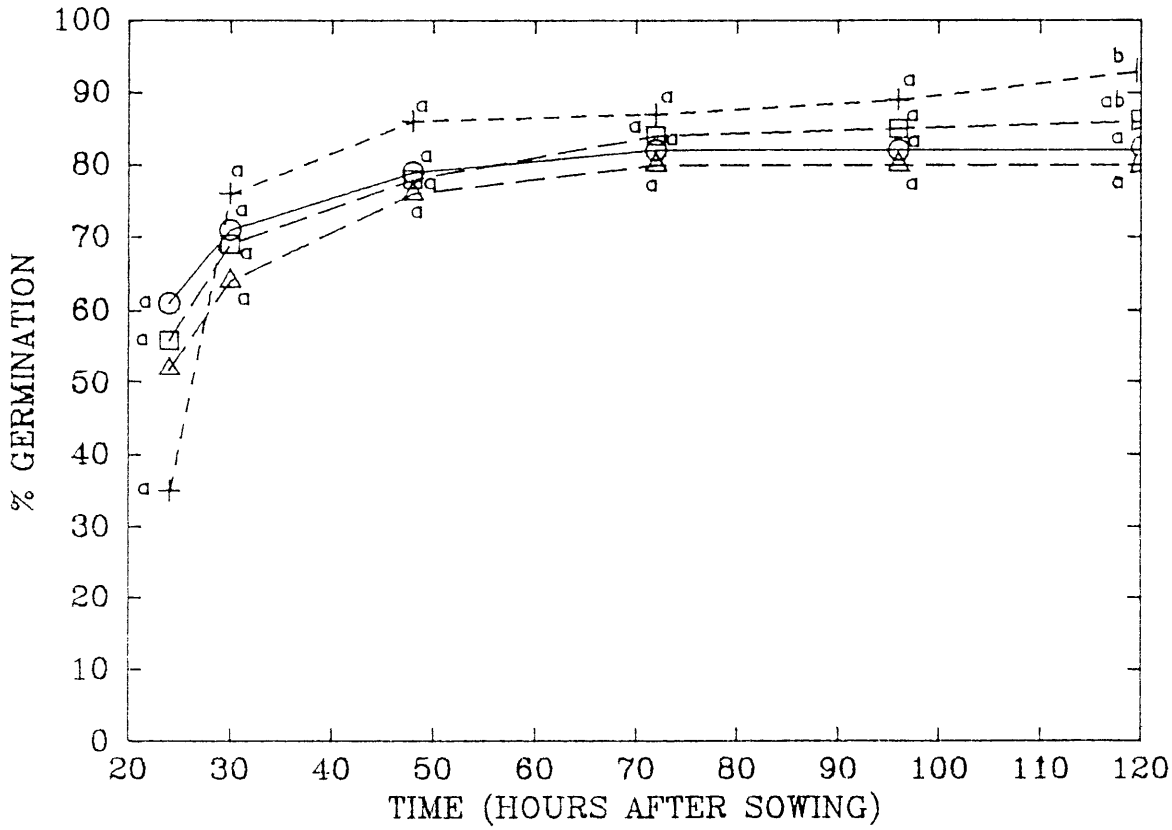


Figure 4.8f : Germination percentage of lucerne over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

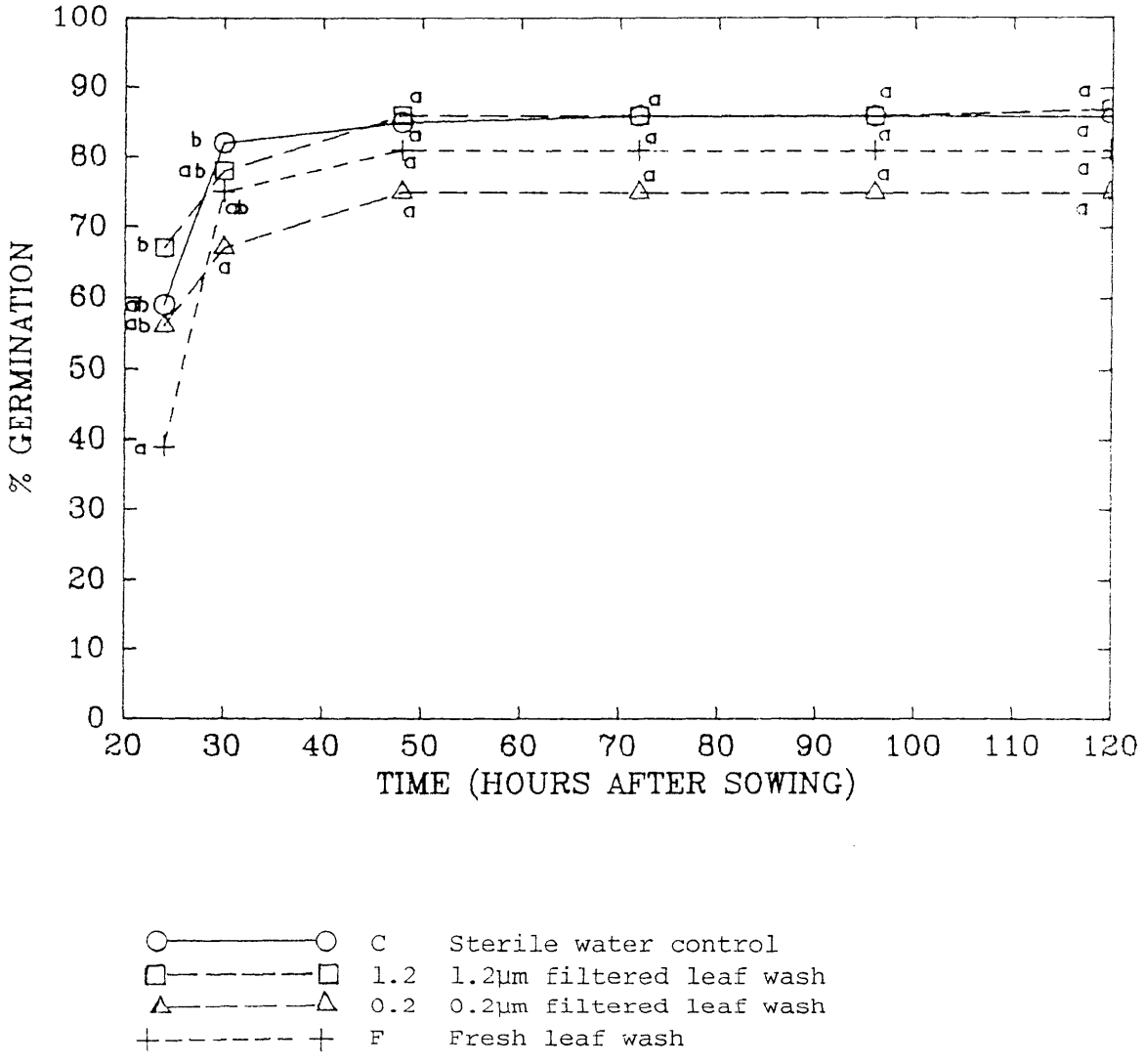


Figure 4.8g: Germination percentage of sorghum over 120h in solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

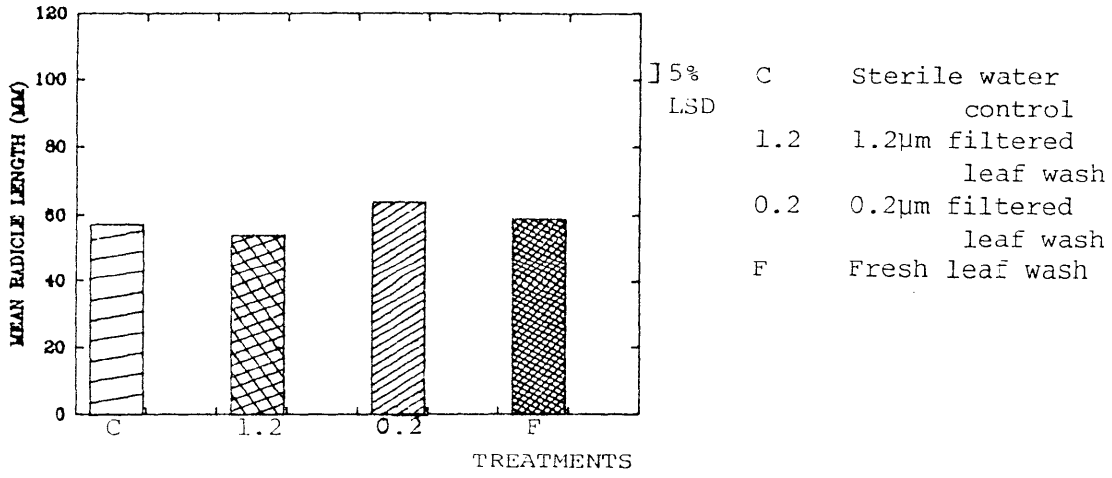


Figure 4.9: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

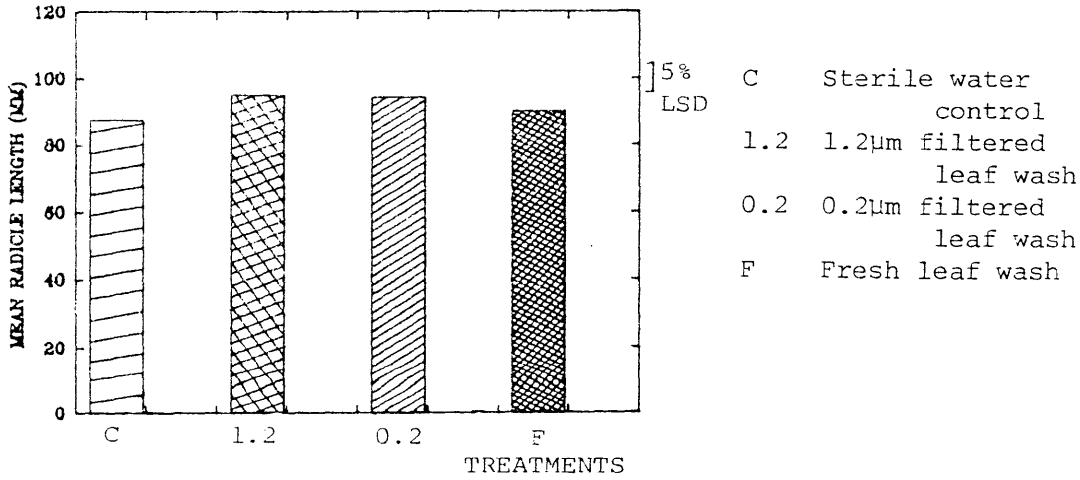


Figure 4.10a: Mean first seminal root length of wheat at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

ii) Phalaris

Again, no significant differences were noted, Figure 4.10b, although 1.2 solution treatment markedly retarded seminal root growth.

iii) White clover

C and 0.2 treatments produced similar radicle lengths, both shorter ($P < 0.05$) than F treatment radicle lengths, Figure 4.10c. 1.2 treatment radicle lengths were longer than those of C and 0.2 treatment, but shorter than those of F, although the differences were not statistically significant.

iv) Sunflower

No radicles were available for measurement.

v) Perennial Ryegrass

The seminal roots of treatment C were similar to those of treatment 0.2, and longer ($P < 0.05$) than those of treatments 1.2 and F, Figure 4.10d.

vi) Lucerne

C treatment had shorter radicle lengths ($P < 0.05$) than the other three treatments, Figure 4.10e. The radicles of treatment 0.2 were shorter ($P < 0.05$) than those of F and 1.2 treatments which were not significantly different from one another.

vii) Sorghum

C and 0.2 treatments had shorter seminal roots ($P < 0.05$) than treatments F and 1.2, Figure 4.10f. Treatment C seminal roots were not significantly longer than those of treatment 0.2.

4.4.3 Discussion and Conclusions

The involvement of phyllosphere micro-organisms in the production

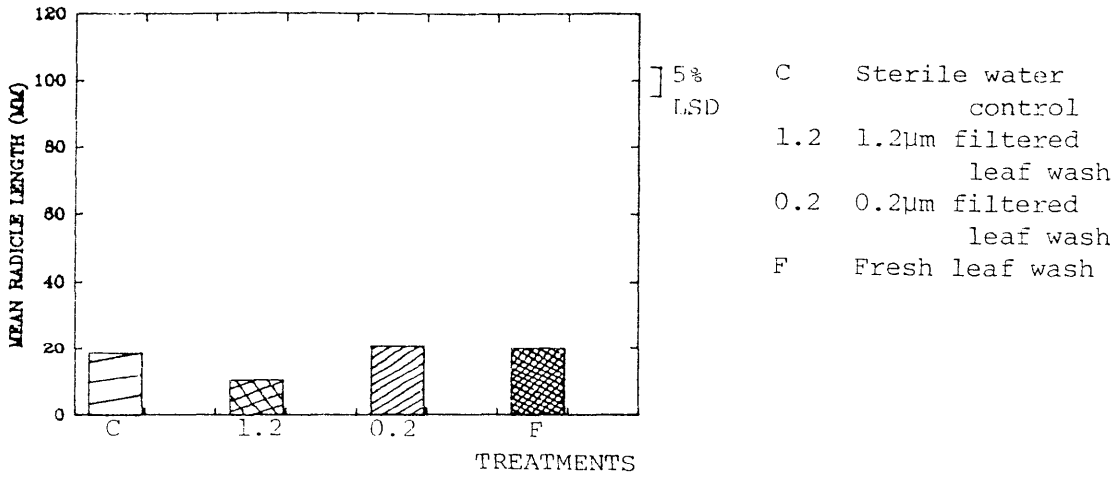


Figure 4.10b: Mean first seminal root length of phalaris at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

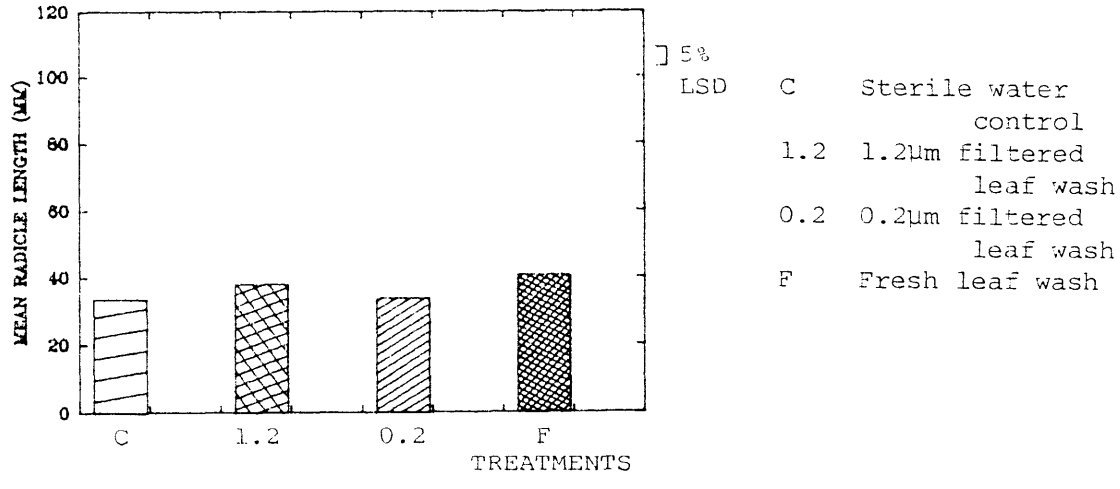


Figure 4.10c: Mean radicle length of white clover at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

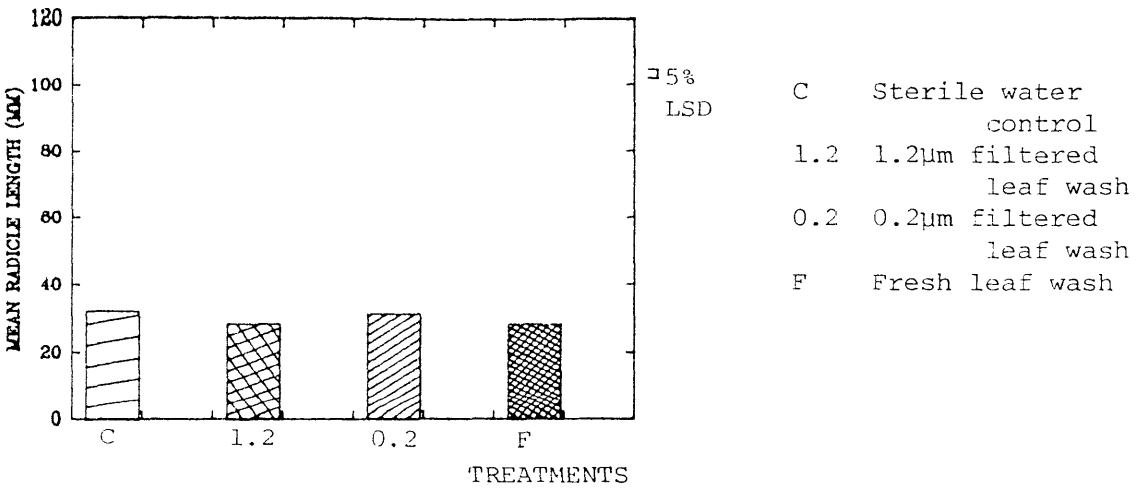


Figure 4.10d : Mean first seminal root length of perennial ryegrass at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

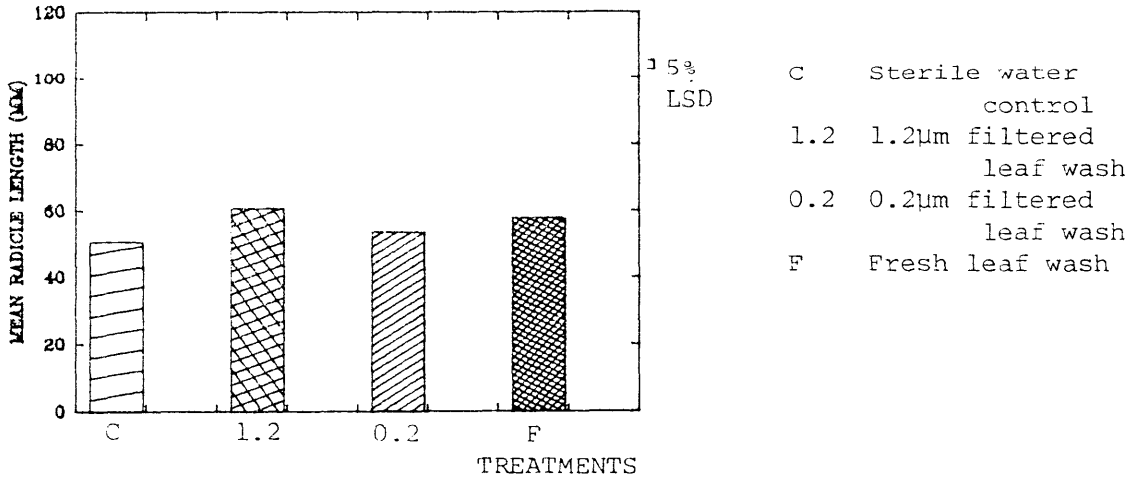
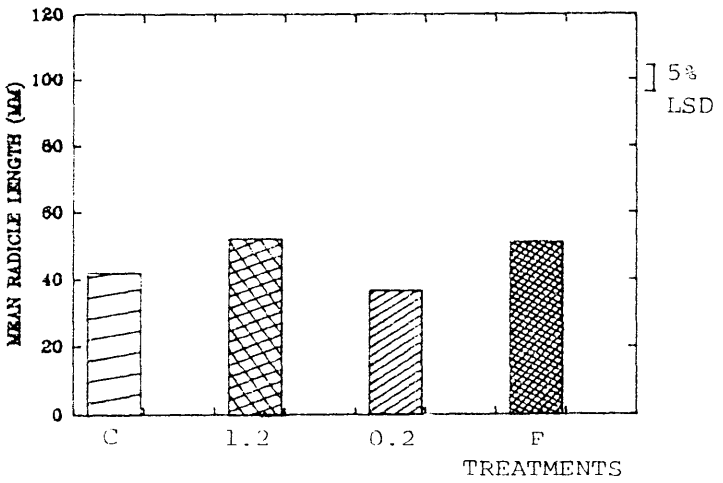


Figure 4.10e: Mean radicle length of lucerne at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.



C Sterile water control
1.2 1.2µm filtered leaf wash
0.2 0.2µm filtered leaf wash
F Fresh leaf wash

Figure 4.10f: Mean first seminal root length of sorghum at 120h when watered with solutions of Suncross 53 sunflower fresh leaf wash, 1.2 µm and 0.2 µm filtered leaf wash, and sterile water.

of an allelochemical has seldom been mentioned in scientific literature. Lovett and Sagar (1978) and Lovett and Duffield (1981) reported that a potent allelochemical (benzylamine) was produced by phyllosphere bacteria of *Camelina sativa* from a group of chemicals exuded by the leaves. When present at concentrations less than 10ppm, benzylamine stimulated radicle growth of germinating linseed seeds in both petri dishes and soil under controlled conditions, while larger concentrations were inhibitory (Lovett, 1979; Lovett and Levitt 1981).

Over the eight species examined, the three leaf wash solutions produced different results. Chemicals alone (0.2) stimulated lucerne root growth, while not affecting linseed, wheat and white clover germination or wheat, linseed, phalaris, white clover or perennial ryegrass root growth. Presence of bacteria (1.2) in the leaf wash solution maintained the stimulation of lucerne root growth, and also caused a stimulation of sorghum root growth and white clover germination. Again, linseed and wheat germination and wheat, linseed, and phalaris root growth were not greatly affected. Perennial ryegrass root growth was restricted. The presence of fungi, in addition to bacteria and chemicals in the leaf wash solution (F) again maintained the stimulation of lucerne root growth, and the stimulation of sorghum root growth. White clover root growth was stimulated. Linseed and wheat germination and root growth were still unaffected, as was phalaris root growth. Perennial ryegrass root growth was restricted.

The effects of the solutions on the other bioassay species were not statistically significant, however, sunflower germination was stimulated by all three solutions, while perennial ryegrass and sorghum germination was restricted by all three.

It was apparent that phyllosphere micro-organisms present in the leaf wash solutions were responsible for altering the potency of the chemicals in some of these solutions, bacteria generally making them less "toxic" to the bioassay species than bacteria and fungi together.

At the concentration used (1:10) chemicals in the leaf wash solution may have been present at stimulatory concentrations for some species, while either retarding or not affecting others.

4.5 Plating of Sunflower (Suncross 53) Leaf Wash Solutions, to Examine Phyllosphere Micro-organisms

In order to examine the components of the Suncross 53 phyllosphere microflora, and to ensure that the Millipore filters used in 4.4 were removing unwanted organisms from the solutions, leaf wash solutions prepared for 4.4 were plated onto agar plates.

4.5.1 Materials and Methods

Solutions prepared for section 4.4 (F, 1.2, 0.2) were plated onto sterile Tryptone Soya Agar (4% solution) plates, using standard microbiological techniques. Four replicates of each solution were included and the plates were incubated at 24^{o}C, in the dark, for 120h.

4.5.2 Results

F solution plates grew bacteria of the genera *Micrococcus* and *Enterobacter*, and another species identified only as a flavonoid bacterium. Fungi of the genus *Penicillium* were also present.

1.2 solution grew all of the above except *Penicillium*.

0.2 solution plates were clean.

4.5.3 Discussion and Conclusions

While three micro-organisms were identified at the generic level,

others may have also been present which were not detected on the medium or under the standard growth conditions used here. F plates, for example, produced a few colonies which were not identified. However, the frequency of *Micrococcus*, *Enterobacter*, the flavonoid bacterium and *Penicillium* indicate that they were the dominant components of the microflora. Billing (1976) cites *Micrococcus* as one of the common gram negative bacteria present on the phylloplane whilst *Enterobacter cloacae* (Jordan) Hormaeche and Edwards, a gram negative bacterium, was associated with allelochemical production in the phyllosphere by Lovett and Sagar (1978). *Penicillium* is one of 57 genera of Fungi Imperfecti commonly recorded on the phylloplane of higher plants (Dickinson, 1976).

From the results obtained in 4.4, and those reported here, it is suggested that phyllosphere micro-organisms were responsible for altering the chemical constituents of the leaf wash solutions. 0.2 filtered solutions contained no micro-organisms, yet germination and radicle growth results often differed from those of C, suggesting that chemicals in the leaf wash solution alone could have been affecting the germination and radicle growth of some of the bioassay species. More, or less, toxicity was added by the presence of bacteria and fungi, possibly those identified above.

4.6 Simulation of Direct Transmission of Sunflower (Suncross 53 and Biotypes) Leaf Washings to Receiver Plants

If allelochemicals are leached or washed from debris or erect plants they may be transferred to other plants via the imbibing seed, through soil/root interfaces, or through water drip onto foliage. Direct transmission is regarded as the more effective means as the allelochemicals are not taken up by soil micro-organisms or colloids and immobilised

(Rice, 1964).

To examine the effects of allelochemicals on imbibing seeds, linseed seeds were soaked in low concentration Suncross 53 leaf wash solutions for periods up to 24h. Subsequently, seedlings were grown in sand, watered with leaf wash solution, to determine if allelochemicals could affect the receiver plant if taken up by roots.

At this stage an Australian sunflower biotype (an escapee crop or bird seed type growing in self-sown patches in northern New South Wales) was included in the investigation. This biotype shows the ability to colonise wasteland and develop tall (up to 3m), dense stands, however, it is not considered to be a serious weed (Matheson personal communication) as it is easily eradicated. For comparison with work carried out in the U.S.A. (Wilson and Rice 1968), seeds of three accessions (self-sown biotypes) of *Helianthus annuus* were obtained from the C.S.I.R.C., Canberra. These were collected in the Dakotas, in central North America, as were the collections studied by Rice (1964,1968,1971a,b,1974,1979) and Wilson and Rice (1968). Leaf washings from all four biotypes were compared to Suncross 53 washings. Wheat was included as a bioassay species at this stage as it is often grown in rotation with sunflower and hence could be affected by substances leached from sunflower debris in field situations.

4.6.1 Materials and Methods

4.6.1.1 Imbibition of hybrid sunflower (Suncross 53) leaf chemicals by bioassay species seeds

Leaves were harvested from ten week old Suncross 53 plants, weighed, washed and filtered as in 4.1.1. Linseed seeds were soaked in this solution for 0, 1, 3, 6, 12, 18 or 24h. Four replicates were set up for each treatment consisting of 25 surface sterilised linseed seeds

on a sterile filter paper in a petri dish, as described in 4.1.1. The initial watering consisted of 2ml of sterile water, with a further 1-2ml as required. The Oh soak was the control (C). the dishes were incubated in the dark at 24°C for 120h. Germination counts were made at 12h intervals from 48h to 96h, and again at 120h. Radicle lengths were measured at 120h.

4.6.1.2 Seedlings grown in sand watered with hybrid sunflower (Suncross 53) leaf wash

Linseed seeds were germinated in distilled water in petri dishes at 24°C in the dark. Two day old seedlings were transplanted, five per pot, into 30cm diameter plastic pots filled with washed river sand (Treatment 1: DW1, SS1). Two weeks later, more linseed seeds were soaked for one hour prior to germination in distilled water (Treatment 3: DWS, SSS), while others were not soaked (Treatment 2: DW2, SS2). All seeds were germinated in distilled water, as described above, and transplanted into pots of washed river sand at two days. The seedling pots were watered daily with either 100ml of distilled water (DW1, DW2, DWS) or 100ml of freshly collected sunflower leaf wash (SS1, SS2, SSS).

Plant height and leaf number were measured every two days, and plant top and root dry weights measured after a destructive harvest at eight days. Root length measurements at harvest were made on DW2, SS2, DWS and SSS treatments.

4.6.1.3 Comparison of effects of leaf chemicals of hybrid (Suncross 53) and Australian biotype sunflowers on early seedling growth

Seeds were collected of the biotype sunflower growing on wasteland in the Inverell (northern New South Wales) district, and grown under conditions described in 4.1.1. The seeds were incubated in the light

as they had an apparent light requirement for germination. When the plants were ten weeks old, leaves were harvested, weighed, washed and filtered as in 4.1.1, for each plant type used. Petri dishes were set up as in 4.1.1, with 25 surface sterile linseed seeds.

A) Control (sterile water) (C) compared with leaf wash solution from Australian biotype (W). Treatments were replicated five times.

B) Control (sterile water) (C) compared with leaf wash solutions from both hybrid (SC) and Australian biotype (W). The treatments were replicated five times.

The seeds were watered with 3ml of the appropriate solution and incubated in the dark at 24^{o}C for 120h. Subsequent waterings were with 1ml of sterile water, as required.

Germination counts were conducted at approximately 24h intervals, and radicles measured at 120h for all runs conducted. Data from one run are presented.

4.6.1.4 Comparison of effects of leaf chemicals of hybrid (Suncross 53), Australian biotype and some North American biotype sunflowers on early seedling growth

Seeds of three biotype sunflowers from the United States of America, were obtained from the Plant Introduction Service, C.S.I.R.O., Canberra. These were Ll467 (CPI 66636, North Dakota No. 724, collected at De Smet, S.D.), Ll468 (CPI 66671, North Dakota No. 72219, from Box Elder, S.D.), and Ll469 (CPI 66672, North Dakota No. 77236, from Sykeston, N.D.). These were germinated and grown under similar conditions as before (4.6.1.3), and leaves from them, the Australian biotype (W), and the hybrid Suncross 53 (SC) were collected, weighed, washed and filtered as before, when the plants were ten weeks old. 25 surface sterile linseed seeds were placed in petri dishes as in 4.1.1, watered

with 3ml of the appropriate solution, and placed in an incubator in the dark at 24°C for 120h. The treatments were replicated five times. Subsequent waterings were with sterile water. Germination counts were conducted at 24h intervals, and radicles were measured at 120h. Data from one run are presented.

4.6.2 Results

4.6.2.1 Imbibition of hybrid sunflower (Suncross 53) leaf chemicals by bioassay species seeds

4.6.2.1.1 Germination

Linseed germination data, after soaking in hybrid sunflower leaf wash solution for times varying from 0h to 24h, are presented in Figure 4.11. No statistically significant differences between the treatments were recorded.

4.6.2.1.2 Radicle lengths

Radicle lengths of linseed presoaked in hybrid sunflower leaf wash, after 120h of growth in sterile water are presented in Figure 4.12. C treatment radicles were shorter than all other treatments except the 6h soak, although only significantly shorter than the 1h and 24h soak. All other treatments were not significantly different.

4.6.2.2 Seedlings grown in sand watered with hybrid (Suncross 53) sunflower leaf wash

4.6.2.2.1 Plant height

Two week old seedlings (Treatment 1) gained height marginally, but not significantly, more quickly when watered with sunflower solution (SS1) than with distilled water (DW1), Figure 4.13. Zero week old

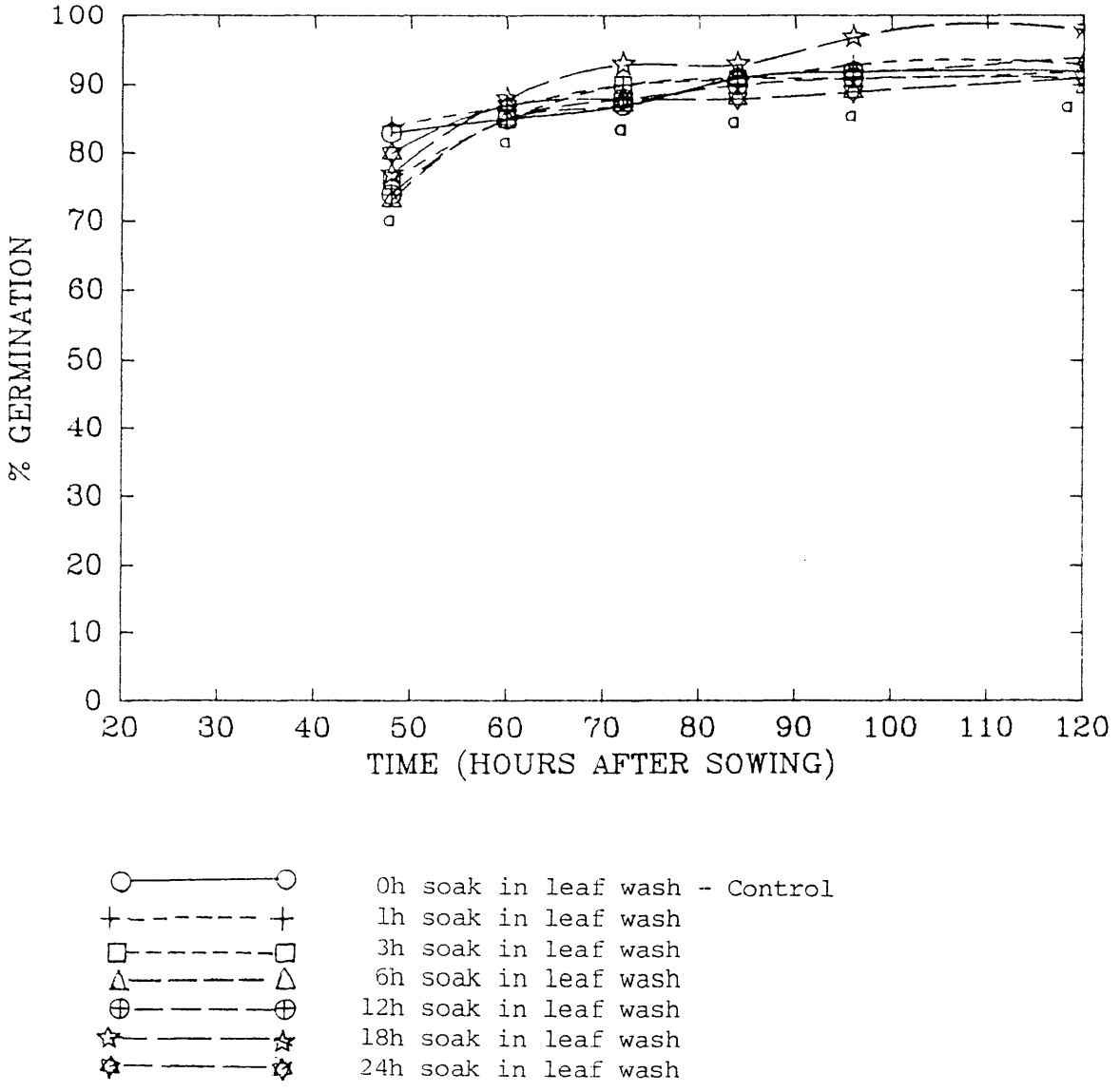
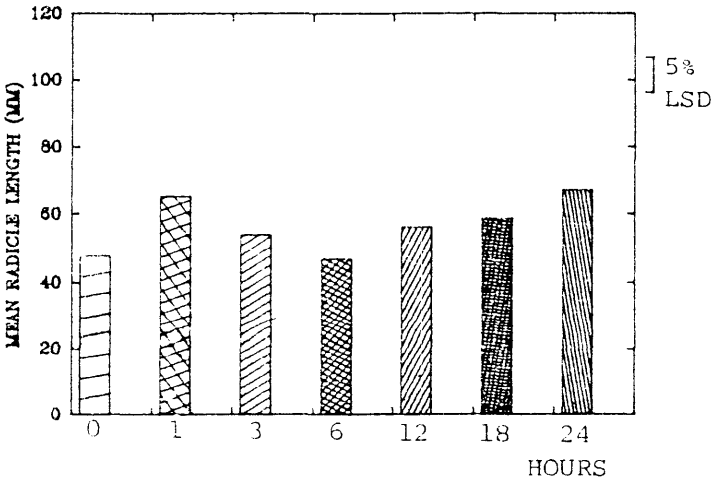


Figure 4.11: Germination percentage of linseed over 120h in sterile water, after soaking in Suncross 53 sunflower leaf wash solution for periods of 0h to 24h.



0h soak in leaf wash - Control
1h soak in leaf wash
3h soak in leaf wash
6h soak in leaf wash
12h soak in leaf wash
18h soak in leaf wash
24h soak in leaf wash

Figure 4.12: Mean radicle length of linseed at 120h, when watered with sterile water, after soaking in Suncross 53 sunflower leaf wash solution for periods of 0h to 24h.

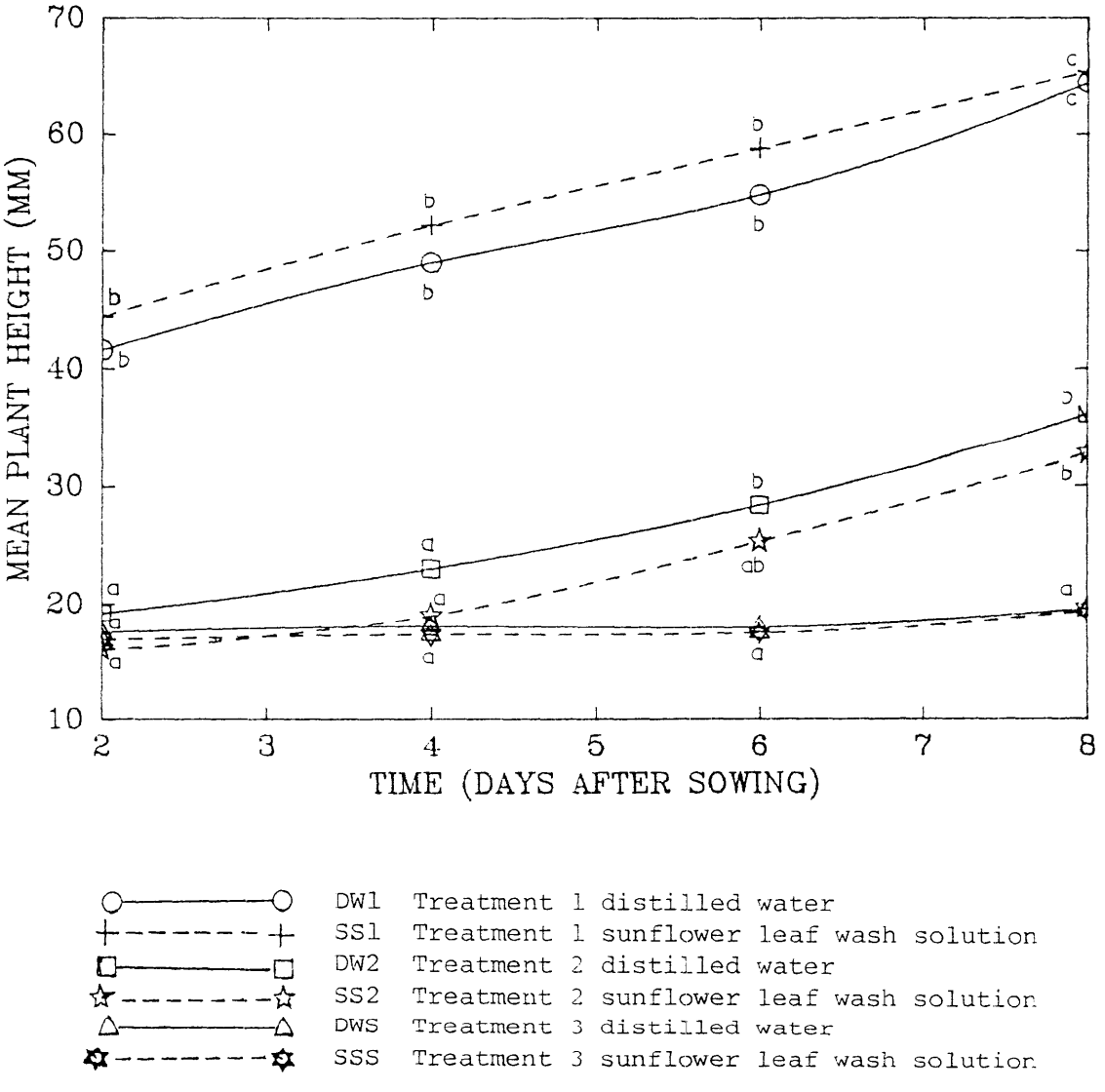


Figure 4.13: Mean height of linseed seedlings over eight days when watered with Suncross 53 sunflower leaf wash solution, and distilled water.

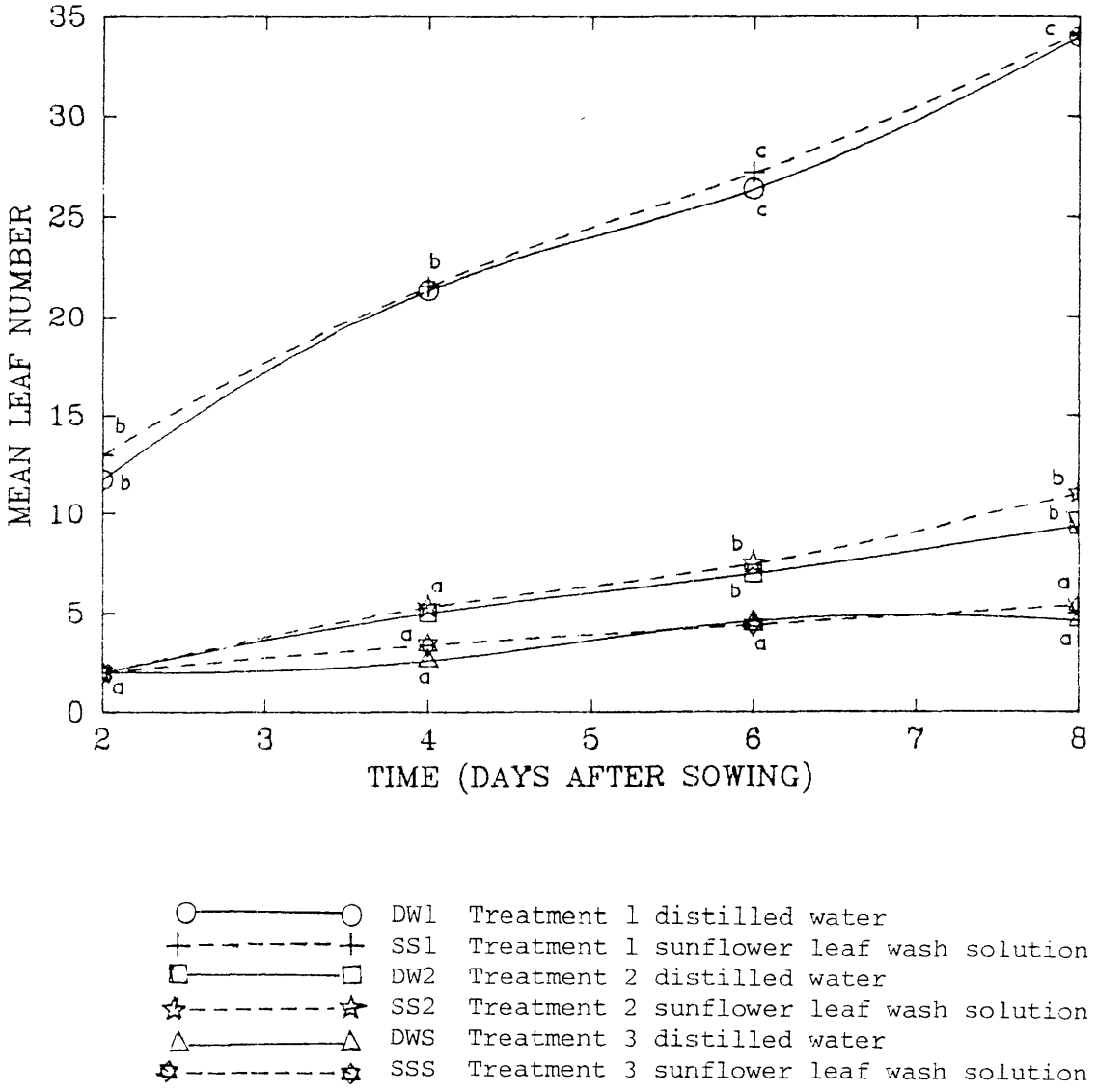


Figure 4.14 : Mean leaf number of linseed seedlings over eight days when watered with Suncross 53 sunflower leaf wash solution, and distilled water.

seedlings (Treatments 2 and 3) gained height more quickly with distilled water (DW2, DWS), but there were no significant differences. Presoaked seeds (DWS, SSS) resulted in less vigorous seedlings ($P < 0.05$ at Day 8) than non-soaked seeds, with the zero week old (DW2, SS2) seedlings gaining height at a similar rate to the two week old seedlings (DW1, SS1). Differences between sunflower solution and distilled water were greater for the non-soaked seed seedlings than for presoaked, but were still non-significant. Gain of height in Treatment 3 (soaked) was minimal over the trial period.

4.6.2.2.2 Leaf number

In all three treatments leaf number was slightly, but not significantly, higher in SS pots than DW pots, Figure 4.14. Presoaked seed seedlings (DWS, SSS) developed leaves more slowly than the non-soaked seeds DW1, DW2, SS1 and SS2. DW2 and SS2 developed leaves at a rate only slightly lower than the advanced plants in DW1 and SS1.

4.6.2.2.3 Plant tops dry weight

Treatment 3 (DWS, SSS) was less well developed after eight days growth than Treatment 2 (DW2, SS2), Figure 4.15, with the SSS treatment showing the largest reduction. The differences were not, however, statistically significant. In both Treatments 1 and 2, the leaf wash solution (SS1, SS2) produced slightly, but not significantly, heavier tops weights than the distilled water (DW1, DW2).

4.6.2.2.4 Root dry weight

A similar trend is seen to that of the plant tops weight for all treatments, Figure 4.16.

4.6.2.2.5 Root length

The distilled water treatment (DW2, DWS) root lengths were shorter

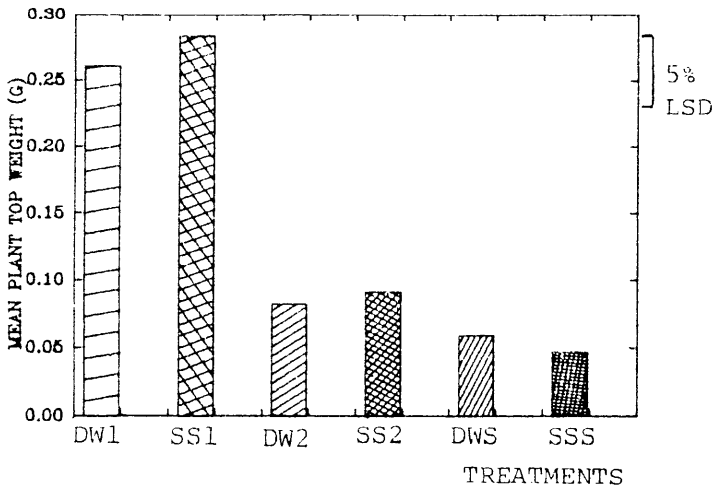


Figure 4.15 : Mean plant top weight of linseed seedlings at eight days when watered with Suncross 53 sunflower leaf wash solution, and distilled water.

Legends for: Figs. 4.15,4.16: DW1 Treatment 1 distilled water
SS1 Treatment 1 sunflower leaf wash
DW2 Treatment 2 distilled water
SS2 Treatment 2 sunflower leaf wash
DWS Treatment 3 distilled water
SSS Treatment 3 sunflower leaf wash

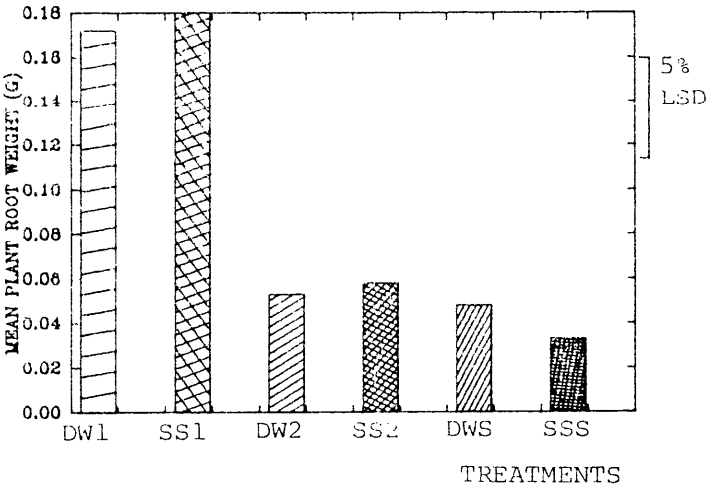


Figure 4.16: Mean plant root weight of linseed seedlings at eight days when watered with Suncross 53 sunflower leaf wash solution, and distilled water.

than the sunflower leaf wash solution treatments SS2 and SSS, although not significantly so, Figure 4.17. The presoaked seeds seedling roots (DWS, SSS) were shorter ($P < 0.05$) than the non-soaked DW2 and SS2.

4.6.2.3 Comparison of effects of leaf chemicals of hybrid (Suncross 53) and Australian biotype sunflowers on early seedling growth

4.6.2.3.1 Germination

A) Sterile water compared with leaf wash solution from Australian biotype

C treatment had higher germination percentages than W treatment over 120h, Figure 4.18, although the differences were generally not statistically significant.

B) Sterile water compared with leaf wash solutions from both hybrid and Australian biotype

Little difference was seen between the three treatments, Figure 4.19.

4.6.2.3.2 Radicle lengths

A) Sterile water compared with leaf wash solution from Australian biotype

The radicle lengths of C and W treatments were not significantly different, Figure 4.20.

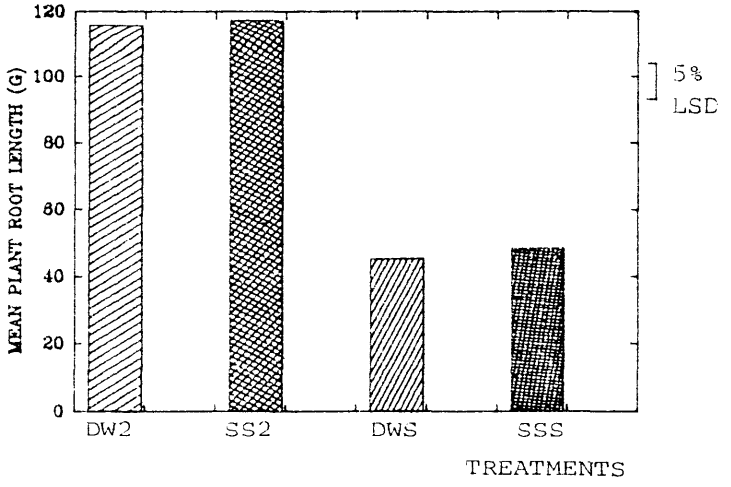
B) Sterile water compared with leaf wash solutions from both hybrid and Australian biotype

W treatment tended to stimulate linseed radicle growth compared to C treatment, Figure 4.21. SC treatment also stimulated growth compared to C treatment. The differences were not, however, statistically significant.

4.6.2.4 Comparison of effects of leaf chemicals of hybrid (Suncross 53), Australian biotype and some North American biotype sunflowers on early seedling growth

4.6.2.4.1 Germination

The germination percentages were similar for all the treatments



DW2 Treatment 2 distilled water
SS2 Treatment 2 sunflower leaf wash solution
DWS Treatment 3 distilled water
SSS Treatment 3 sunflower leaf wash solution

Figure 4.17: Mean plant root length of linseed seedlings at eight days when watered with Suncross 53 sunflower leaf wash solution, and distilled water.

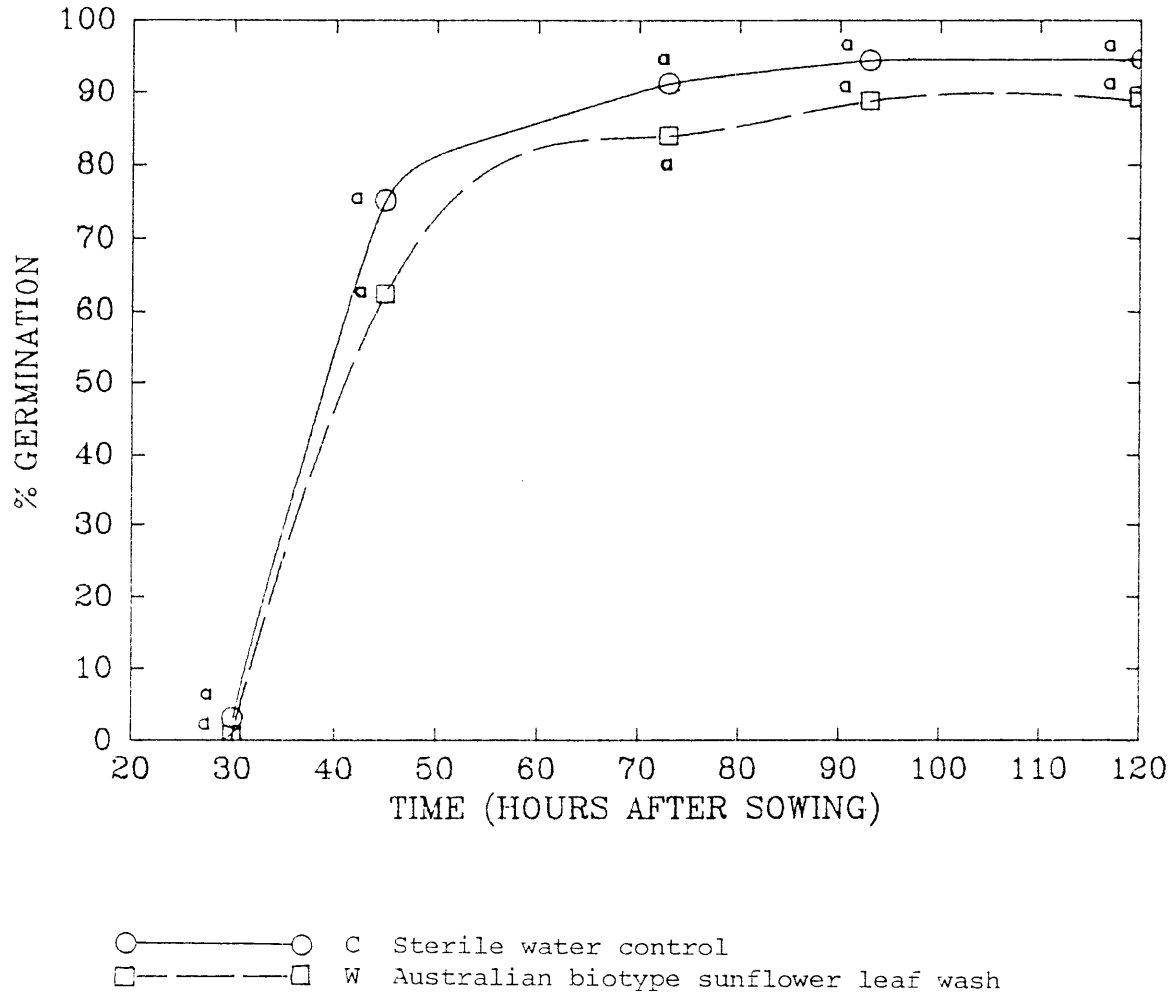


Figure 4.18: Germination percentage of linseed over 120h in solutions of the Australian biotype sunflower leaf wash, and sterile water.

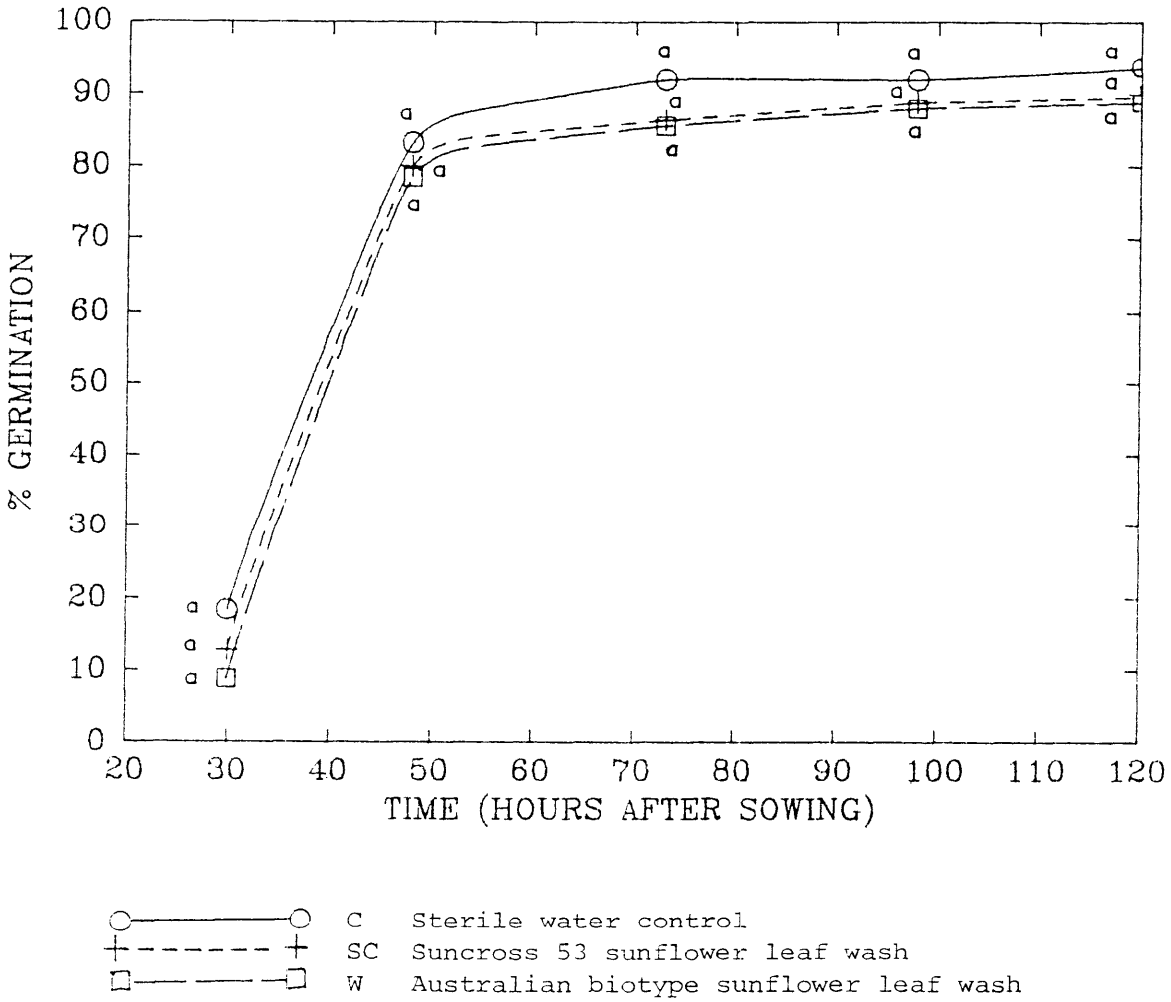


Figure 4.19: Germination percentage of linseed over 120h in solutions of Suncross 53, and the Australian biotype sunflower leaf wash, and sterile water.

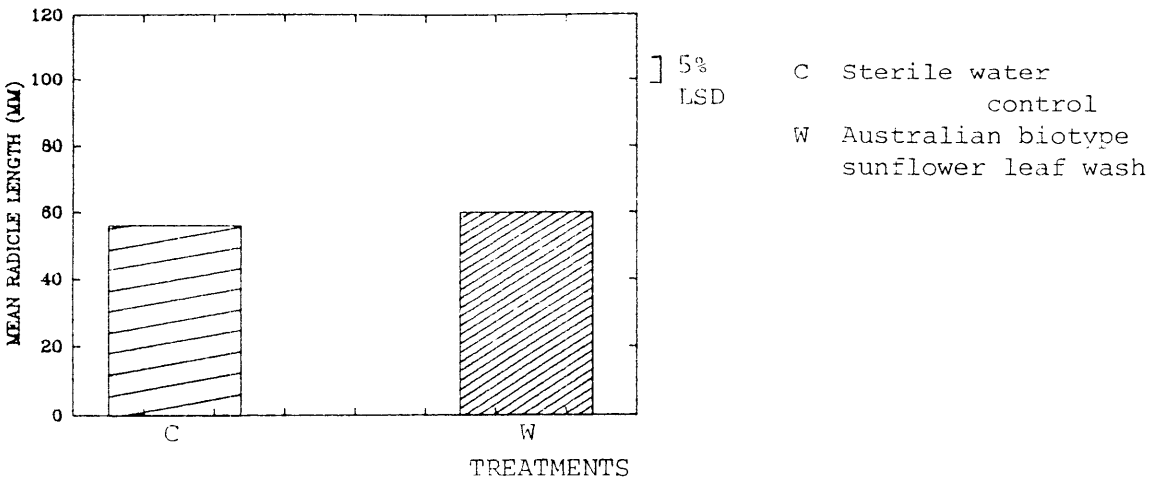


Figure 4.20: Mean radicle length of linseed at 120h when watered with solutions of the Australian biotype sunflower leaf wash, and sterile water.

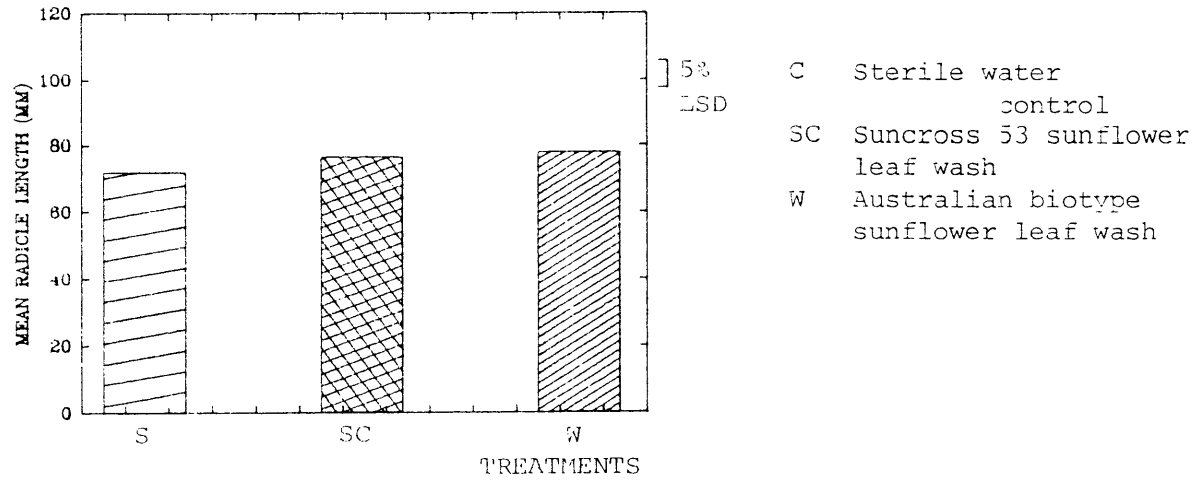


Figure 4.21: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53, and the Australian biotype sunflower leaf wash, and sterile water.

over 120h, Figure 4.22.

4.6.2.4.2 Radicle lengths

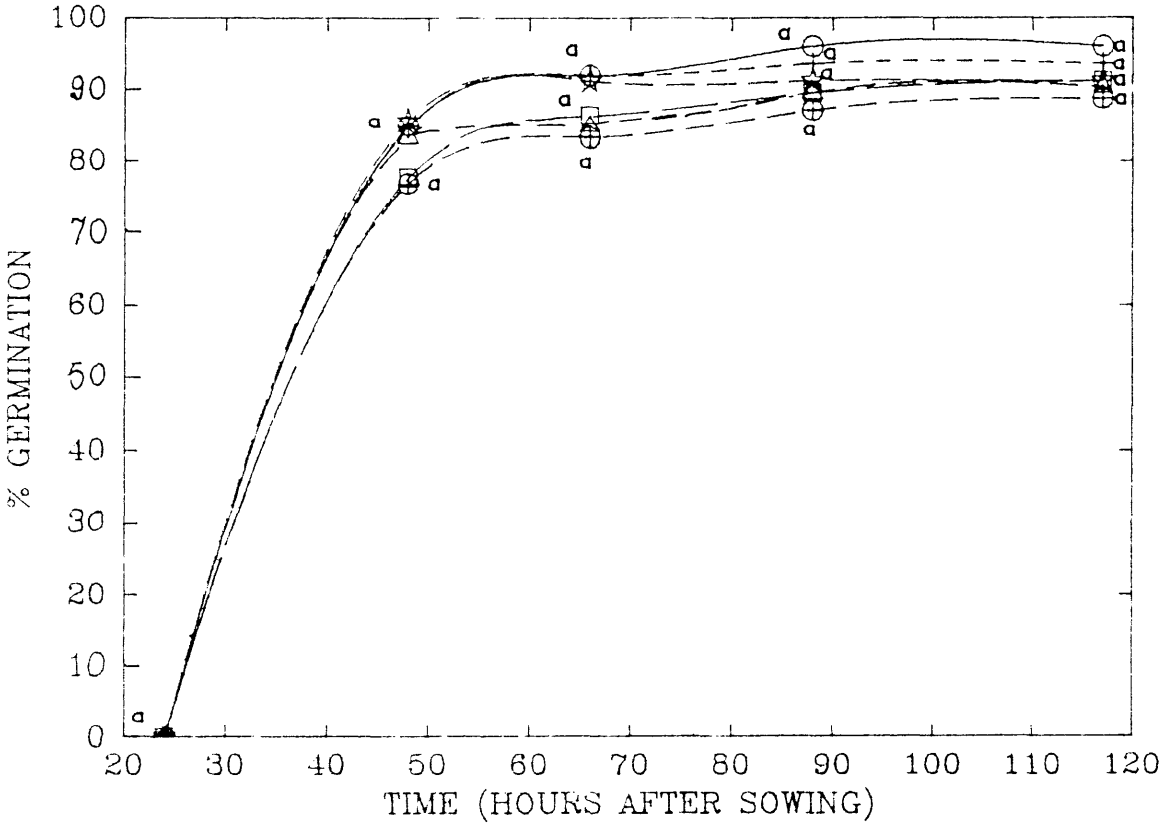
C treatment radicle lengths at 120h were similar to those of the other treatments, Figure 4.23.

4.6.3 Discussion and Conclusions

Many phytochemicals have been found to stimulate plant growth at low concentrations, but to damage plant tissue and function at higher concentrations (Evenari, 1949). The low concentration leaf allelochemical trials conducted here reflect this phenomenon as the data from all the trial runs indicated that radicle extension of linseed at 120h was generally slightly stimulated by the application of leaf wash solutions of SC, W and the North American biotype 1469. 1467 and 1468 did not greatly affect radicle growth, but along with SC and W, slightly restricted early germination. At 120h, SC, W and 1469 germination was generally similar to C.

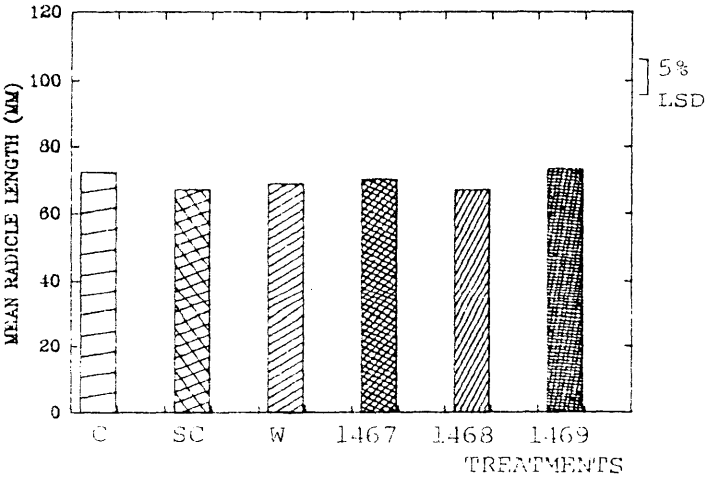
Extrapolating these data to the field, it may be that, at low concentrations, both Suncross 53 and the Australian biotype sunflower leaf allelochemicals will marginally slow germination of nearby seeds, but may stimulate faster radicle growth. It appears, however, that these effects exist only in the early stages of plant growth as seedlings zero to one week and two to three weeks old are less affected than seedlings zero to five days old.

Even though the concentration of leaf allelochemicals used in these experiments was relatively low (1g leaf material:10g water), the concentration released from the plant in the field during a rain shower could be much lower, and hence have no affect on the receiver plants.



○——○	C	Sterile water control
+------+	SC	Suncross 53 sunflower leaf wash
□——□	W	Australian biotype sunflower leaf wash
△———△	1467	U.S.A. biotype sunflower leaf wash
⊕———⊕	1468	U.S.A. biotype sunflower leaf wash
☆———☆	1469	U.S.A. biotype sunflower leaf wash

Figure 4.22: Germination percentage of linseed over 120h in solutions of Suncross 53, Australian biotype, and U.S.A. biotype (1467, 1468, 1469) sunflower leaf wash, and sterile water.



C	Sterile water control
SC	Suncross 53 sunflower leaf wash
W	Australian biotype sunflower leaf wash
1467	U.S.A. biotype sunflower leaf wash
1468	U.S.A. biotype sunflower leaf wash
1469	U.S.A. biotype sunflower leaf wash

Figure 4.23: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53, Australian biotype, and U.S.A. biotype (1467, 1468, 1469) sunflower leaf wash, and sterile water.

However, fog, mist or dew condensation drips may contain an allelochemical concentration similar to that used in sections 4.1 to 4.6 as the water remains on the leaf for a short time and the volume dripping from the leaf can be small at times (Went, 1955).

Soaking seeds for one hour prior to germination in Suncross 53 leaf wash solution resulted in mild stimulation of germination and radicle extension over 120h. Soaking for one hour in distilled water prior to germination resulted in unthrifty plants overall with plant height, leaf number, top weight, root weight and root length all being depressed over the eight days of the trial. Watering with sunflower leaf wash solution during the eight days caused further small restrictions to top weight and root weight.

Soaking seeds for longer lengths of time in sunflower leaf wash solution produced variable results. It appears that soaking in leaf wash solution for periods up to 24h will not greatly affect germination but will stimulate radicle growth, most stimulation being achieved with either a one h or a 24h soak.

In the types of trials carried out here, the low concentration of leaf wash solutions used produced variable results. Allelochemicals in the leaf washes had greater effects on younger seedlings; these effects were often mildly stimulatory, and root systems were generally more affected than germination or plant top growth. These findings are in accord with those of workers such as Lovett and Lynch (1979) who have studied allelochemicals liberated from leaves of weed species. Such substances, however, generally appear to be more active than those present in the sunflower material.

4.7 The Effects on Wheat and Linseed Germination and Early Growth of High Concentration (1:2) Leaf Washings of Hybrid Sunflower (Suncross 53), Australian Biotype Sunflower and North American Biotype Sunflower.

To test the hypothesis that sunflower phytochemicals may produce retardation of growth and development of bioassay species at high concentrations while causing stimulation at low concentrations, some experiments were repeated using higher concentration leaf washes than those used in sections 4.1 to 4.6.

Reviews by Rice (1974,1979) indicate that many workers in the field of allelopathy have used ground or macerated plant preparations, sometimes extracted with solvents, in their experiments. Accordingly, a comparison of aqueous sunflower leaf washings, prepared with minimal damage to the leaf, was made with ground leaves leached with water. By this means any changes in toxicity to the bioassay species attributable to release of phytochemicals endogenous to the leaf should be detected.

4.7.1 Materials and Methods

The sunflower plants were grown to ten weeks of age under standard conditions. Leaves were harvested, weighed and washed, and the solutions filtered as before (4.1.1). 25 surface sterilised seeds (linseed or wheat) were placed on sterile filter papers in sterile petri dishes as before, and watered with the appropriate solution. The dishes were incubated in the dark at 24^{o}C for 120h.

4.7.1.1 Comparison of high concentration leaf washings (1:2) of the hybrid (Suncross 53) and the Australian biotype sunflowers

Leaves were harvested from Suncross 53 (SC) and Australian biotype (W) sunflowers and washed in the ratio of 1g leaf material per

2g water. Treatments were replicated four times using linseed as the bioassay species. The initial watering was with 3ml of the appropriate solution, with subsequent waterings of 1ml sterile water as required. Germination counts were conducted at 48h, 72h and 120h. Radicle lengths were measured at 120h.

4.7.1.2 Comparison of high concentration leaf washings (1:2) of the hybrid (Suncross 53), the Australian biotype, and some North American biotype sunflowers

Leaves of the hybrid (SC), the Australian biotype (W) and two U.S.A. biotype (1468,1469) sunflowers were harvested, weighed and washed in a 1:2 ratio. Each treatment was replicated four times. A sterile water control (C) was included, and wheat and linseed used as bioassay species. The initial watering consisted of 3ml of the appropriate solution, with sterile water being used later as required. Germination counts were carried out at 24h, 36h, 45h and 120h in the first run, and at 22h, 28h, 36h, 46h, 52h, 75h and 120h in the second run. Linseed radicles in Run 1, linseed radicles and first seminal roots of wheat in Run 2 were measured at 120h. Wheat in Run 1 was destroyed by a fungus. Run 2 data are presented.

4.7.1.3 Comparison of internal and external leaf chemicals of hybrid (Suncross 53) and Australian biotype sunflowers on early seedling growth

Leaves of Suncross 53 (SC) and the Australian biotype (W) were harvested, weighed and washed in a 1:2 ratio. More leaves were ground with a mortar and pestle and diluted with a similar volume of water as the washed leaves (w/w). All solutions were filtered with a Buchner funnel to remove large debris. Linseed and wheat were used as the bioassay species in Run 1, wheat only in Run 2. 5ml of the appropriate solution were used in the first watering, with 2ml of sterile water at

subsequent waterings. The treatments were replicated four times. Germination counts, lengths of linseed radicles, first seminal root length and coleoptile height of wheat were measured at 120h, while germination was counted at 24h intervals, and first seminal root and coleoptiles measured at 120h.

4.7.1.4 Comparison of leached and washed, internal and external leaf chemicals of the hybrid (Suncross 53) and the Australian biotype sunflowers on early seedling growth

Leaves of the hybrid (SC) and the Australian biotype (W) sunflowers were harvested, weighed and washed in a 1:2 ratio as before. Sandwich treatments of dried, senesced and green leaves for each sunflower type were set up as in 4.1.1, to estimate the level of toxicity of chemicals in them compared to washed and ground leaves, as in 4.7.1.3. Treatments were replicated four times using wheat as the bioassay species. A sterile water control (C) was included. Dishes were initially watered with 5ml of the appropriate solution, thereafter with sterile water as required. Germination counts were conducted at 24h intervals from 24h to 120h, and first seminal root length and coleoptile height measured at 120h.

4.7.2 Results

4.7.2.1 Comparison of high concentration leaf washings (1:2) of the hybrid (Suncross 53) and the Australian biotype sunflower

4.7.2.1.1 Germination

Initially, germination of linseed in C treatment was greater than in treatments SC and W, but by 64h was surpassed by them both, Figure 4.24. Differences were not statistically significant.

4.7.2.1.2 Radicle lengths

SC treatment caused a greater stimulation of radicle extension

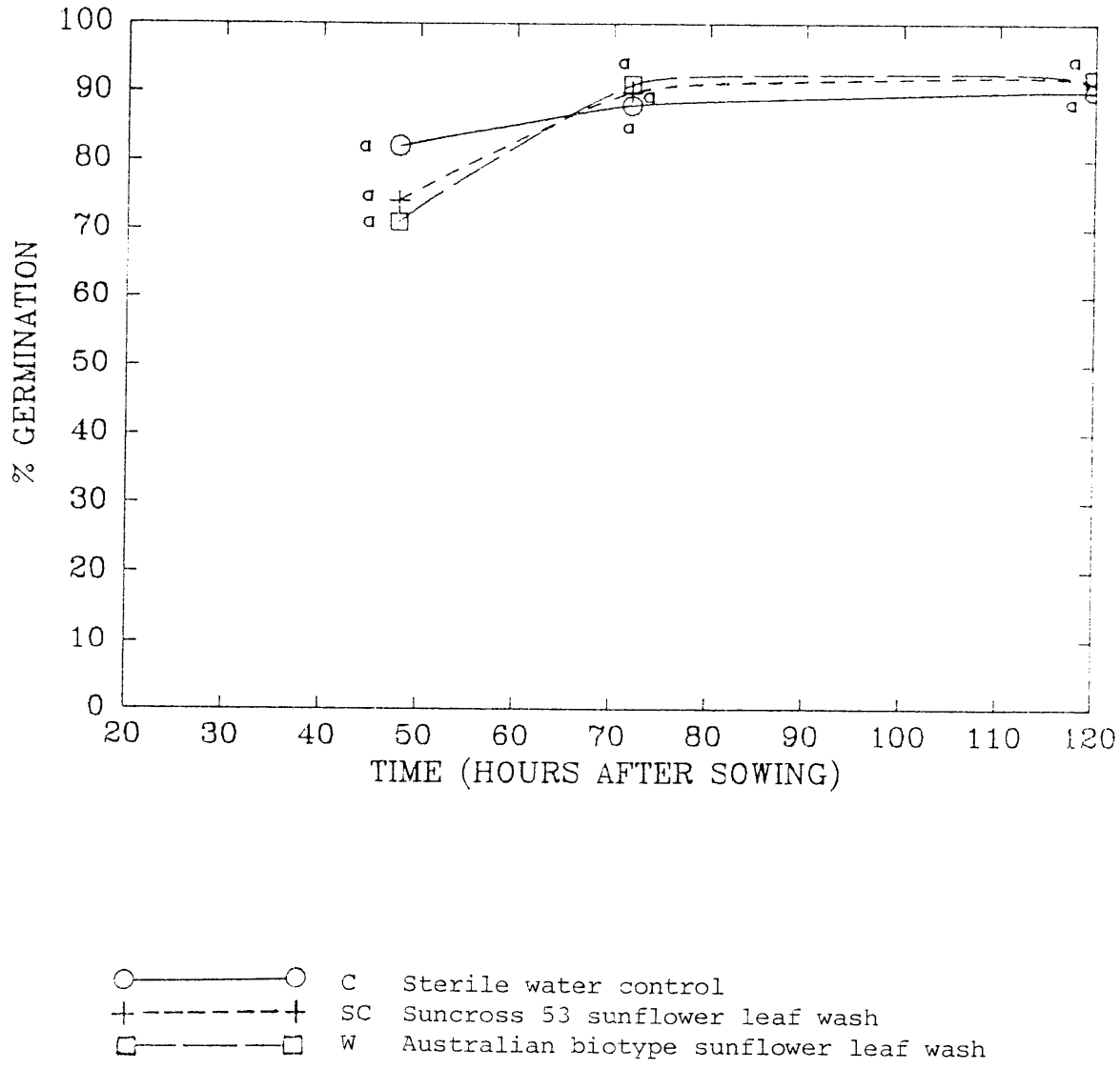


Figure 4.24: Germination percentage of linseed over 120h in solutions of Suncross 53, and Australian biotype sunflower leaf wash, and sterile water.

than W treatment when compared to C treatment, but the difference was not significant, Figure 4.25.

4.7.2.2 Comparison of high concentration leaf washings (1:2) of the hybrid (Suncross 53), the Australian biotype, and some North American biotype sunflowers

4.7.2.2.1 Germination

Linseed

Germination data of linseed over 120h are presented in Figure 4.26. C treatment had a higher germination percentage than all other treatments through to 120h, however, the percentages were significantly higher ($P < 0.05$) only at 36h.

Wheat

C treatment had a significantly higher early germination percentage ($P < 0.05$) at 24h, Figure 4.27. By 36h all treatments were similar and not significantly different from one another.

4.7.2.2.2 Radicle and seminal root lengths

Linseed

At 120h W treatment was significantly longer ($P < 0.05$) than the other four treatments, Figure 4.28.

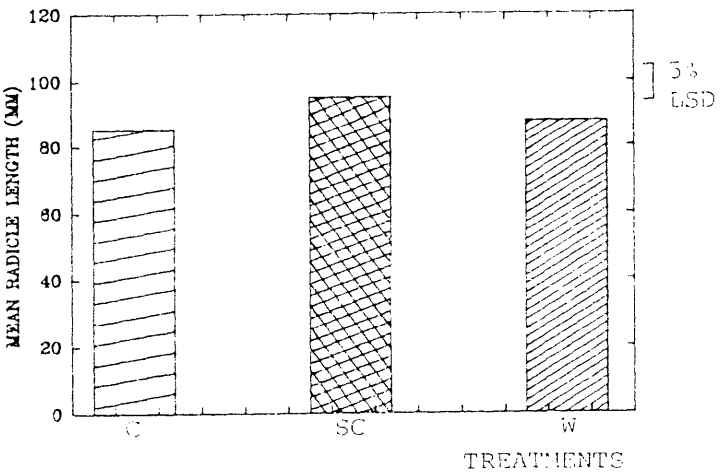
Wheat

At 120h C, 1468 and W treatment seminal roots were similar in length and shorter ($P < 0.05$) than those of SC treatment, Figure 4.29. 1469 was not significantly longer than these, while being not significantly shorter than SC treatment.

4.7.2.3 Comparison of internal and external leaf chemicals of hybrid (Suncross 53) and Australian biotype sunflowers on early seedling growth

4.7.2.3.1 Germination

Linseed and wheat germination percentage data at 120h are



C Sterile water control
SC Suncross 53 sunflower leaf wash
W Australian biotype sunflower leaf wash

Figure 4.25: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53, and Australian biotype sunflower leaf wash, and sterile water.

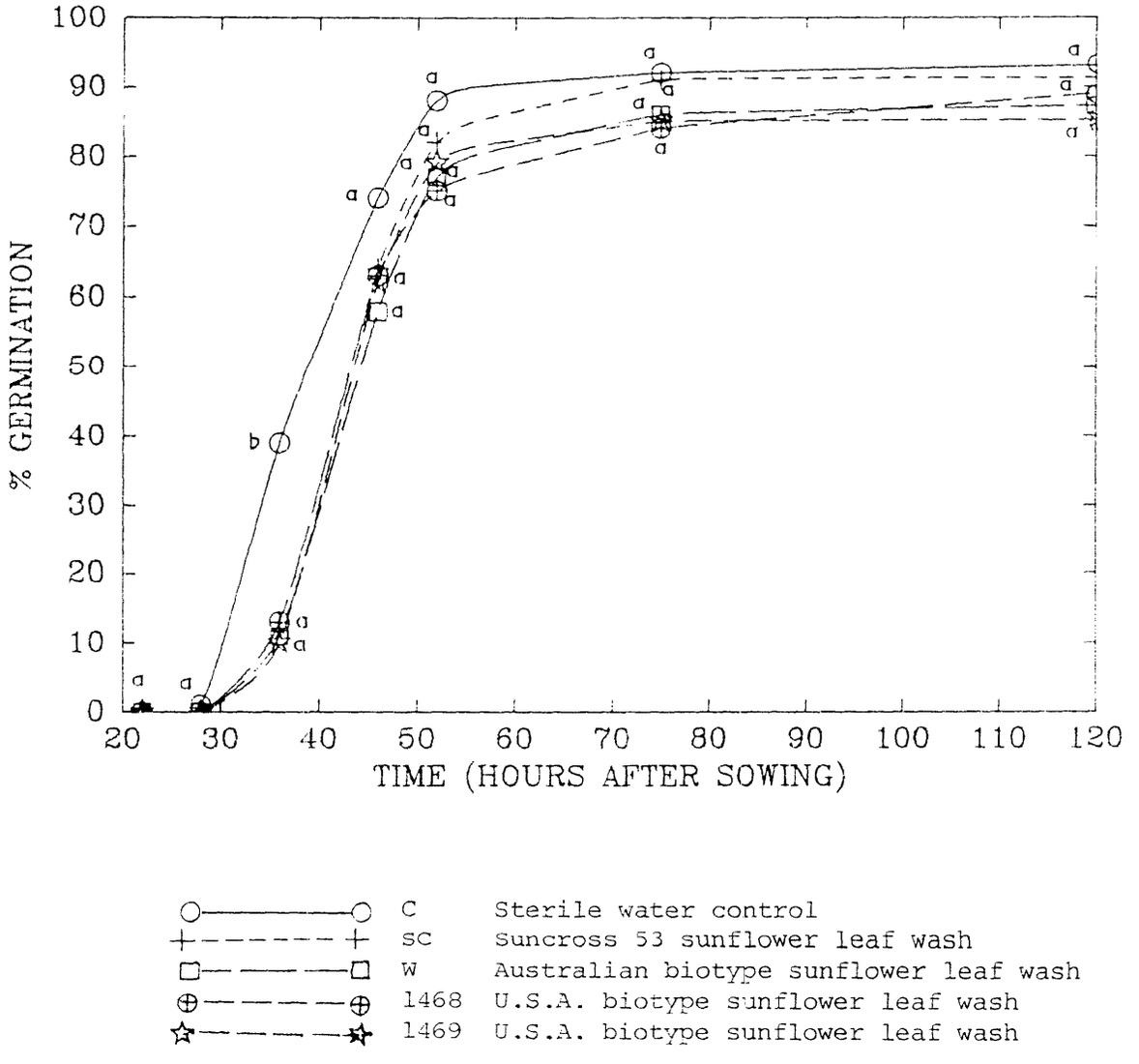


Figure 4.26: Germination percentage of linseed over 120h in solutions of Suncross 53, the Australian biotype, and U.S.A. biotype (1468, 1469) sunflower leaf wash, and sterile water.

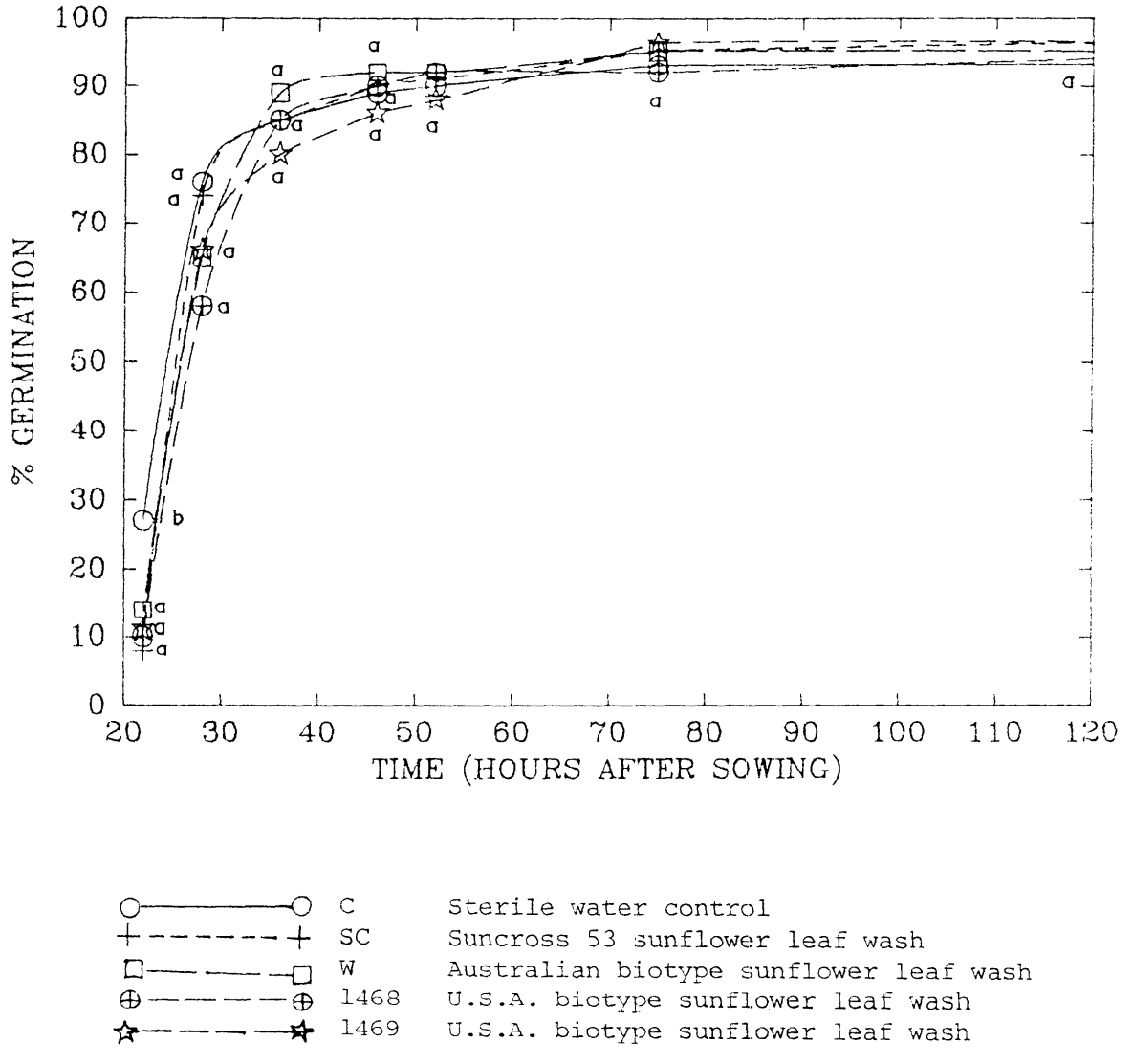


Figure 4.27: Germination percentage of wheat over 120h in solutions of Suncross 53, the Australian biotype, and U.S.A. biotype (1468, 1469) sunflower leaf wash, and sterile water.

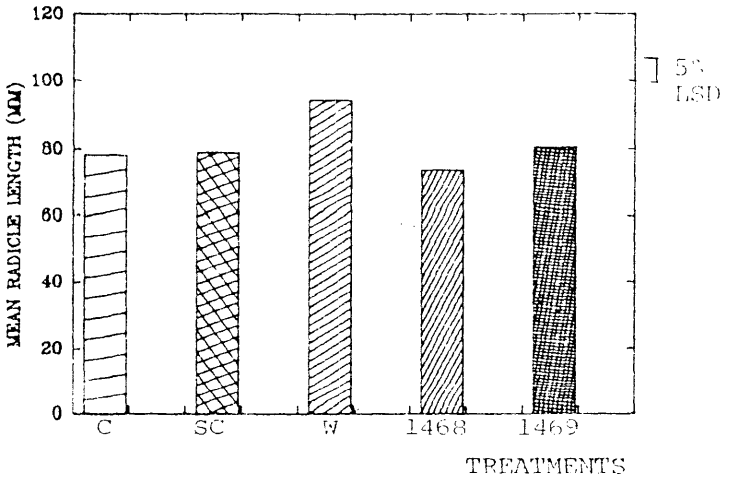


Figure 4.28: Mean radicle length of linseed at 120h when watered with solutions of Suncross 53, the Australian biotype, and U.S.A. biotype (1468, 1469) sunflower leaf wash, and sterile water.

Legends for: Figs. 4.28,4.29.

C	Sterile water control
SC	Suncross 53 sunflower leaf wash
W	Australian biotype sunflower leaf wash
1468	U.S.A. biotype sunflower leaf wash
1469	U.S.A. biotype sunflower leaf wash

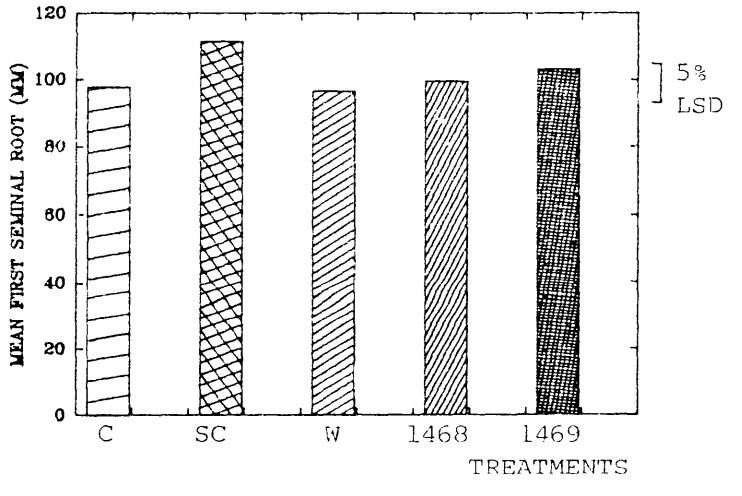


Figure 4.29: Mean first seminal root length of wheat at 120h when watered with solutions of Suncross 53, the Australian biotype, and U.S.A. biotype (1468, 1469) sunflower leaf wash, and sterile water.

presented in Figure 4.30a,b.

Linseed

Both SC treatments (SCW - leaf wash, SCM - ground leaf) and WM treatment (wild type - ground leaf) caused reductions ($P < 0.01$) in germination at 120h compared to C and WW (wild type leaf wash treatments), Figure 4.30a. The two ground leaf treatments resulted in very large restrictions in germination with WM being less than SCW ($P < 0.05$) and SCM less than WM ($P < 0.05$).

Wheat

At 120h, Figure 4.30b, both WW and WM treatments showed no difference from C treatment. Both hybrid sclution treatments (SCW, SCM) reduced germination compared to C treatment ($P < 0.01$).

4.7.2.3.2 Radicle and seminal root lengths

Linseed

At 120h radicle lengths in C and WW treatments were similar and longer than treatments SCW, WM and SCM ($P < 0.01$), Figure 4.31a.

Wheat

At 120h, Figure 4.31b, C and WW treatments were similar and longer ($P < 0.05$) than the other three treatments. SCM treatment was shorter than SCW and WM treatments ($P < 0.05$).

4.7.2.3.3 Coleoptile heights

At 120h C and WW treatments were similar and taller ($P < 0.05$) than the other three treatments, Figure 4.32. The order of the treatments was similar to that seen for both linseed and wheat germination at 120h, Figure 4.30a and b, and root length at 120h, Figure 4.31a and b.

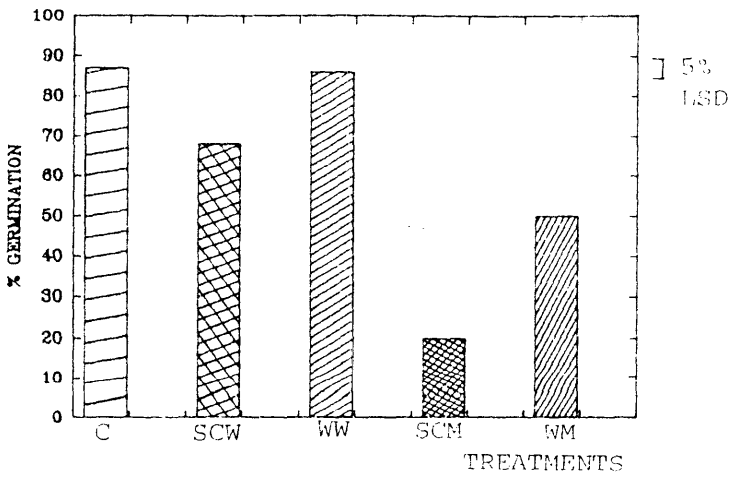


Figure 4.30a: Germination percentage of linseed at 120h when watered with leaf wash and ground leaf solutions of Suncross 53,and the Australian biotype sunflower, and sterile water.

Legends for: Figs. 4.30a,b; 4.31a,b; 4.32.

C	Sterile water control
SCW	Suncross 53 sunflower leaf wash
WW	Australian biotype sunflower leaf wash
SCM	Suncross 53 sunflower macerated leaf
WM	Australian biotype sunflower macerated leaf

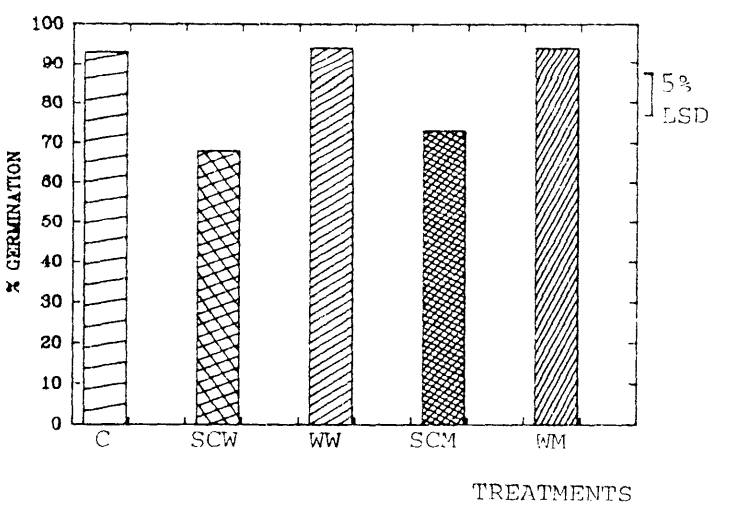


Figure 4.30b: Germination percentage of wheat at 120h when watered with leaf wash and ground leaf solutions of Suncross 53,and the Australian biotype sunflower, and sterile water.

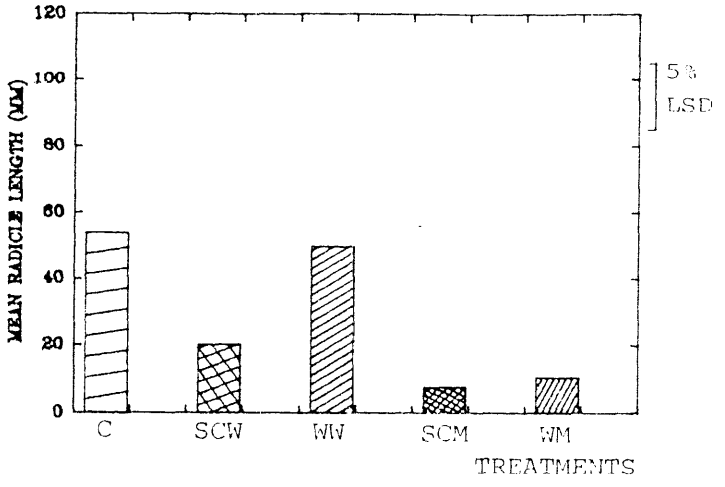


Figure 4.31a: Mean radicle length of linseed at 120h when watered with leaf wash and ground leaf solutions of Suncross 53, and the Australian biotype sunflower, and sterile water.

Legends for: Figs. 4.30a,b; 4.31a,b; 4.32.

C Sterile water control
SCW Suncross 53 sunflower leaf wash
WW Australian biotype sunflower leaf wash
SCM Suncross 53 sunflower macerated leaf
WM Australian biotype sunflower macerated leaf

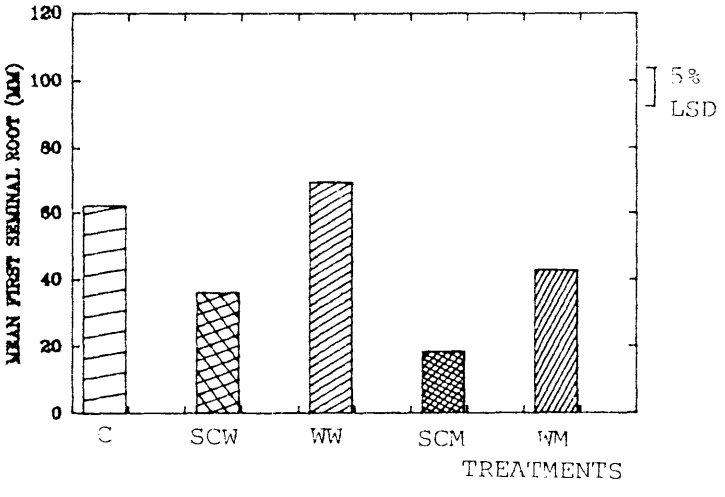
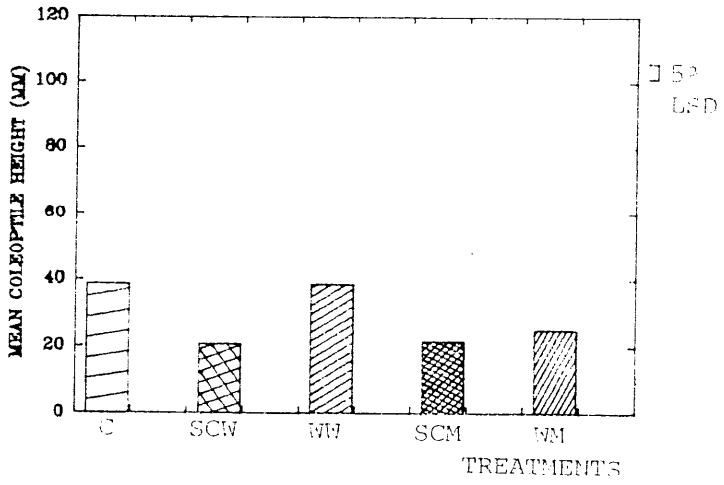


Figure 4.31b: Mean first seminal root length of wheat at 120h when watered with leaf wash and ground leaf solutions of Suncross 53, and the Australian biotype sunflower, and sterile water.



Legends for: Figs. 4.30a,b; 4.31a,b; 4.32

C Sterile water control
SCW Suncross 53 sunflower leaf wash
WW Australian biotype sunflower leaf wash
SCM Suncross 53 sunflower macerated leaf
WM Australian biotype sunflower macerated leaf

Figure 4.32: Mean coleoptile height of wheat at 120h when watered with leaf wash and ground leaf solutions of Suncross 53, and the Australian biotype sunflower, and sterile water.

4.7.2.4 Comparison of leached and washed, internal and external leaf chemicals of the hybrid (Suncross 53) and the Australian biotype sunflowers on early seedling growth

4.7.2.4.1 Germination

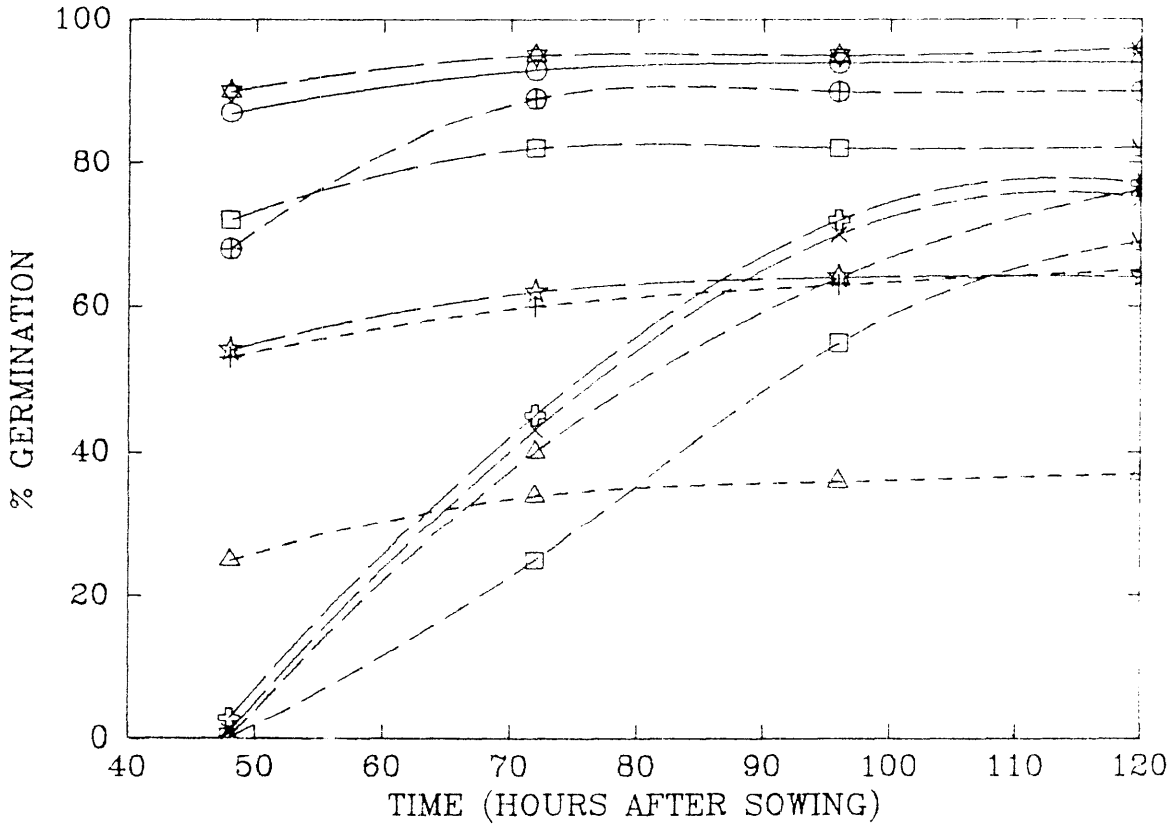
The data presented in Figure 4.33 contain internal and external treatments (SCW, WW, SCM, WM) similar to that of Figure 4.30b in addition to leached treatments (SCG, WG, SCD, WD, SCS, WS). There appear to be two sets of relationships:

a) the green leaves, whether they be external chemicals only (wash), leached internal chemicals (sandwich) or intracellular chemicals as well (ground),

b) the leached treatments of dried (SCD, WD) and senesced (SCS, WS) leaves of both sunflower types.

Initiation of germination was slow for senesced and dried sandwich treatments (SCS, WS, SCD, WD). At 48h germination percentages were very low (0-4%) while the other treatments had reached a level of more than fifty percent of their final germination. Subsequently, germination of SCD, WD, SCS and WS was rapid up to 120h, tending to slow in the last 24h of the trial period. WD, WS, SCS and SCD germination percentages were not significantly different from each other for the period 48h to 120h, although SCD was generally the lowest after 48 h.

WG treatment germination percentage was higher than C treatment from 48h while SCG treatment was similar, but slightly less than C treatment from 72h. WW, SCW, WM and SCM treatments had similar shaped curves to those of C and WG, but WW treatment was approximately 12% lower than C treatment, SCW and WM treatments about 30% lower ($P < 0.05$), and SCM treatment 60% lower ($P < 0.05$) throughout the 120h. SCM treatment was lower ($P < 0.05$) than all other treatments at all times except 72h.



C	Sterile water control
SCW	Suncross 53 sunflower leaf wash
WW	Australian biotype sunflower leaf wash
SCM	Suncross 53 sunflower macerated leaf
WM	Australian biotype sunflower macerated leaf
SCG	Suncross 53 sunflower green leaf leach (sandwich)
WG	Australian biotype sunflower green leaf leach (sandwich)
SCD	Suncross 53 sunflower dried leaf leach (sandwich)
WD	Australian biotype sunflower dried leaf leach (sandwich)
SCS	Suncross 53 sunflower senesced leaf leach (sandwich)
WS	Australian biotype sunflower senesced leaf leach (sandwich)

Figure 4.33: Germination percentage of wheat over 120h in leaf wash and ground leaf solutions, and sandwich treatments of green, green dried and senesced leaves, of Suncross 53 and the Australian biotype sunflower, and sterile water.

4.7.2.4.2 Seminal root lengths

C and WW treatments had the longest first seminal root lengths at 120h, ($P < 0.05$), Figure 4.34. However, WG and SCG treatments were restricted in radicle elongation, SCG being less ($P < 0.05$) than WG and both being shorter ($P < 0.05$) than C and WW treatments. Both the dried (SCD, WD) and senesced (SCS, WS) leaf sandwich treatments caused very low extension rates, similar to SCG treatment. SCW and WM treatments were similar and less ($P < 0.05$) than C treatment. SCM treatment roots were very short and not significantly different in length from SCG and the dried and senesced leaf sandwich treatments.

4.7.2.4.3 Coleoptile heights

Results for coleoptile measurements at 120h were similar, but the differences were not so large as for the root length data, Figure 4.35. Again, WW treatment was not significantly different from C treatment, but SCW and WM treatments were also not significantly different from C. SCG and WG treatments were, again, less ($P < 0.05$) than the wash treatments SCW and WW, with SCG being shorter ($P < 0.05$) than WG. The dried and senesced leaf sandwich treatments were shorter ($P < 0.05$) than all other treatments, but SCM treatment coleoptiles were relatively taller than in the root length data, while still being shorter ($P < 0.05$) than the wash treatments and WM.

4.7.3 Discussion and Conclusions

Results with 1:2 leaf wash solution were still somewhat variable although the data from all the trial runs indicated that the sterile water controls tended to have more rapid and higher germination rates, longer root lengths and taller coleoptiles at 120h than any of the leaf wash solutions. The Suncross 53 leaf wash solution tended to produce lower germination and radicle or root growth than the Australian biotype,

Legends for:

Figure 4.34: 4.35

C	Sterile water control
SCW	Suncross 53 sunflower leaf wash
WW	Australian biotype sunflower leaf wash
SCM	Suncross 53 sunflower macerated leaf
WM	Australian biotype sunflower macerated leaf
SCG	Suncross 53 sunflower green leaf leaf (sandwich)
WG	Australian biotype sunflower green leaf leach (sandwich)
SCD	Suncross 53 sunflower dried leaf leach (sandwich)
WD	Australian biotype sunflower dried leaf leach (sandwich)
SCS	Suncross 53 sunflower senesced leaf leach (sandwich)
WS	Australian biotype sunflower senesced leaf leach (sandwich)

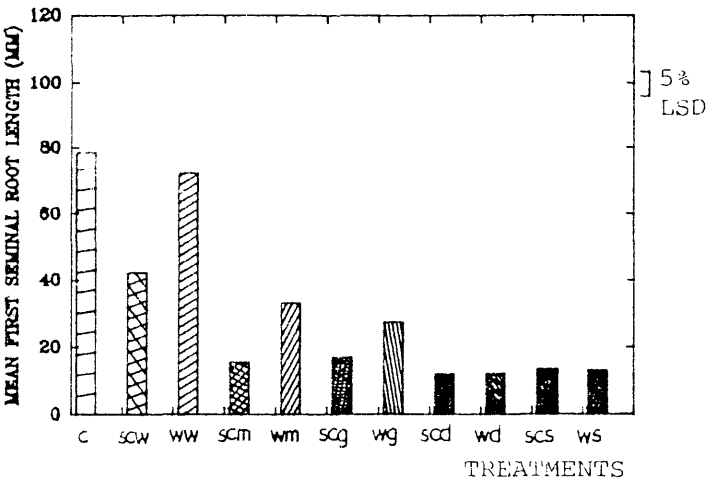


Figure 4.34: Mean first seminal root length of wheat at 120h when watered with leaf wash and ground leaf solutions, and leachates of green, green dried and senesced leaves, of Suncross 53 and the Australian biotype sunflower, and sterile water.

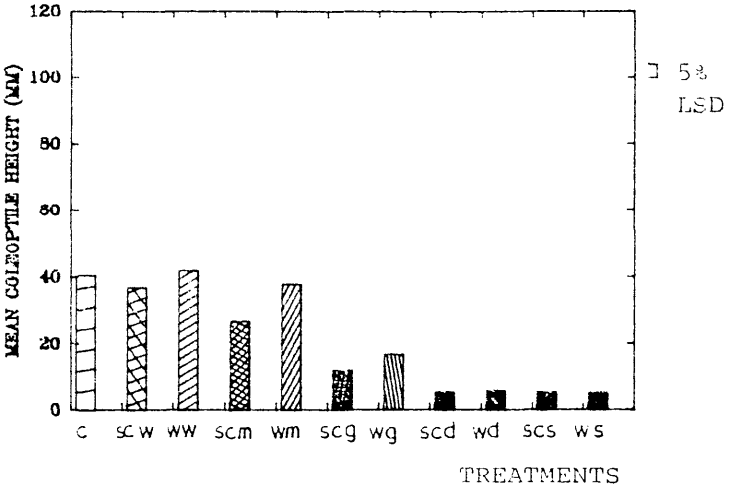


Figure 4.35: Mean coleoptile height of wheat at 120h when watered with leaf wash and ground leaf solutions, and leachates of green, green dried and senesced leaves, of Suncross 53 and the Australian biotype sunflower, and sterile water.

although the difference was often small, particularly with wheat. 1468 and 1469 were similar to W.

Despite the use of standard materials and methods, there was considerable variation in results between experiments. The experiments were carried out at different times of the year, and while plants were always the same age at leaf collection times, the hours of daylight and the maximum temperature during the growth period varied. It is possible that the phyllosphere micro-organism populations also differed through the year, altering the toxicity of the leaf chemicals, as noted by Lovett and Sagar (1978). Results tended to be more consistent when the experiments were repeated over a short time span.

Comparing the results of this trial with the low concentration leaf wash results described in section 4.6, it appeared that W and 1468 treatments produced a similar retardation of germination for both concentrations, whilst SC and 1469 treatments stimulated germination in the low concentration (SC mildly) while retarding in the high concentration (1469 early). Root lengths did not seem to be greatly affected although 1468 treatment may have caused mild retardation in the high concentration while not having an effect in the low concentration.

The macerated leaf treatments were carried out in an attempt to determine the relative toxicity of exogenous plus endogenous allelo-chemicals compared with chemicals liable to be released from leaves under field conditions (that is, primarily the exogenous fraction). The germination and radicle data showed that the macerated leaf treatments were always more allelotoxic than the washed leaf treatments for the same plant.

The macerated leaf treatments did not, however, cause such severe restrictions to germination and radicle growth as did the dried and senesced leaf leachates (a comparison with 4.1). Both SC and W,

D and S treatments slowed early germination of wheat. Germination did not commence until after 48h when normally it would have been near completion. However, after 48h germination was rapid so that by 120h restrictions were not so large. The seminal root lengths and coleoptile heights of wheat at 120h were likewise greatly retarded, with the young wheat plants showing very reduced vigour. Leached green leaves produced results similar to 4.1.

From these results it can be concluded that the Australian biotype sunflower would not become such a weed problem as *Helianthus annuus annuus* has in the U.S.A. as it lacks the strong allelopathic potential of its U.S.A. counterpart. This may reflect its original selection as a crop type. The hybrid, Suncross 53, showed allelopathic potential in the petri dish trials carried out, which may be useful for exploitation in a breeding program to increase self-defence of the crop sunflower.

The allelopathy shown by Suncross 53, particularly from the senesced leaf material, could seriously restrict the growth and development of subsequent crops if debris were allowed to remain in the field through sowing, and/or if rainfall had been insufficient to leach the allelotoxic chemicals from the debris. Trials conducted by Purvis (unpublished data) have shown similar results with litter of the hybrid cultivar Hysun 31, which greatly reduced the leaf area, dry weight, tiller number and grain weight of wheat (cv. Songlen) when applied at the rate of five tonnes per hectare, prior to the sowing of the wheat. This problem may have to be overcome to reduce any deleterious effects to the subsequent crop. Simply allowing time between the sunflower crop and the subsequent crop may be necessary to allow soil microflora to breakdown or soil colloids to inactivate the debris and its allelotoxic

chemicals. Promoting active soil microflora may speed the breakdown and inactivation process.

CHAPTER 5

GENETIC INHERITANCE OF ALLELOPATHIC PROPERTIES

5.1 Germination and Early Seedling Growth Experiments

Following the discovery of allelopathic activity of green leaf washings of the hybrid Suncross 53, members of Arthur Yates and Co. produced data which indicated that germinating seeds of the male parent of the hybrid exhibited self-inhibition in petri dish germination trials. However, when sown in the field for growth trials, the self-inhibition was greatly reduced (MacPherson and White, personal communication).

5.1.1 Materials and Methods

Seeds of the male parent of the hybrid (OR1) were obtained, pre-germinated and grown under standard conditions as described in 4.1.1. At ten weeks of age leaves of OR1, the hybrid (SC) and the Australian biotype (W) were harvested, weighed, washed and filtered as in 4.1.1. Petri dishes and filter papers were set up as in 4.1.1, with 25 surface sterilised wheat seeds. 5ml of the appropriate solution were applied to the dishes, which were then incubated in the dark at 24^{o}C for 120h. Treatments were replicated four times. Subsequent waterings were with sterile water as required.

Germination was counted at 24h, 48h and 120h, and the first seminal root length and coleoptile height were measured at 120h.

5.1.2 Results

5.1.2.1 Germination

C treatment germination was rapid and largely complete by 60h, Figure 5.1. All of the leaf wash solutions caused germination restriction

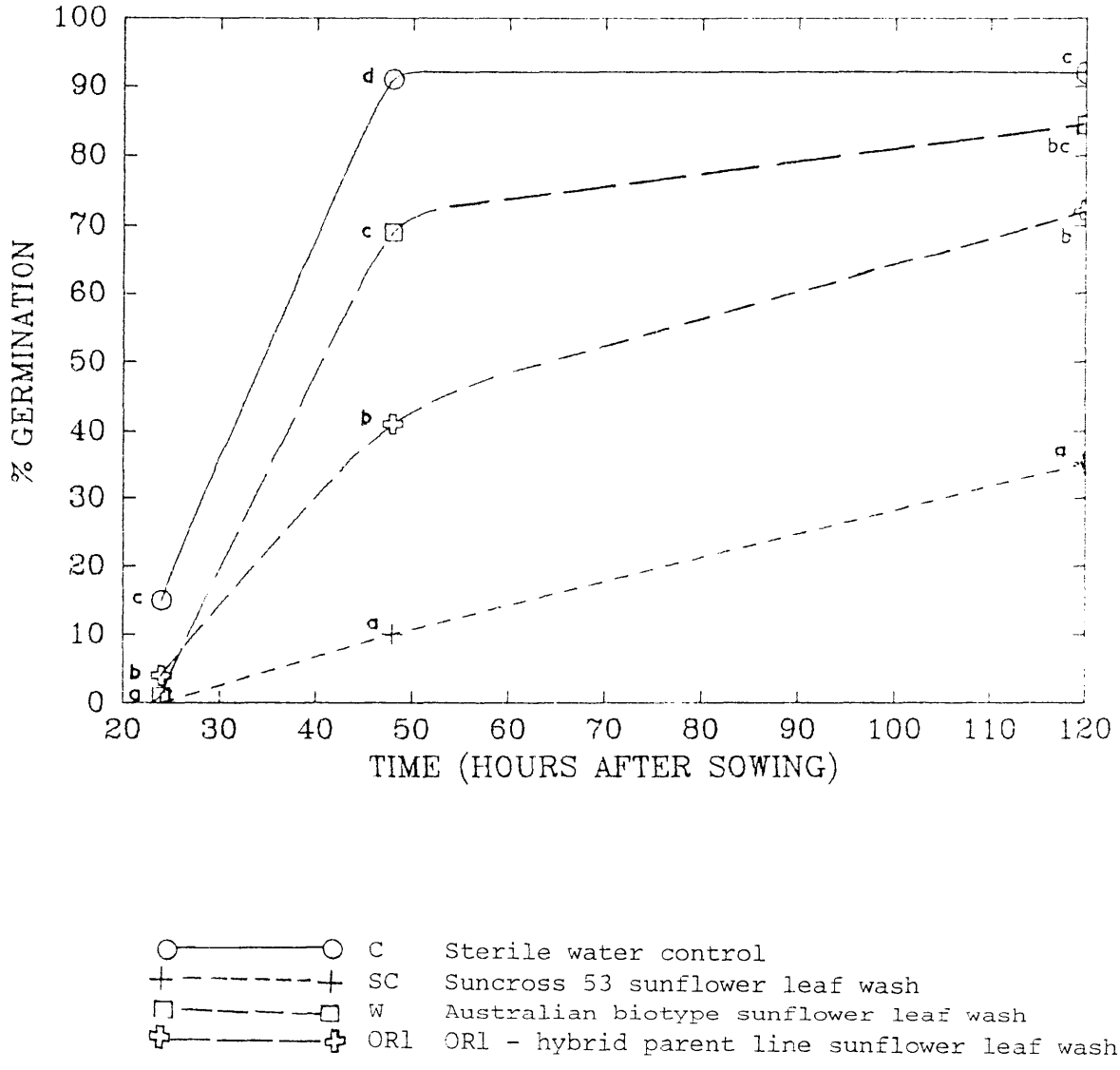


Figure 5.1: Germination percentage of wheat over 120h in solutions of Suncross 53, the Australian biotype and the hybrid parent line OR1 sunflower leaf wash, and sterile water.

($P < 0.05$ from 24h), W treatment producing the smallest restriction, becoming not significantly different from C treatment at 120h.

5.1.2.2 First seminal root length

SC and ORl treatments produced restrictions to first seminal root growth ($P < 0.05$), Figure 5.2, SC causing a larger ($P < 0.05$) reduction than ORl. W treatment root lengths were similar to those of C.

5.1.2.3 Coleoptile heights

C and W solutions produced the tallest coleoptiles at 120h, Figure 5.3. SC and ORl solutions restricted coleoptile growth ($P < 0.05$), SC more markedly ($P < 0.05$) than ORl.

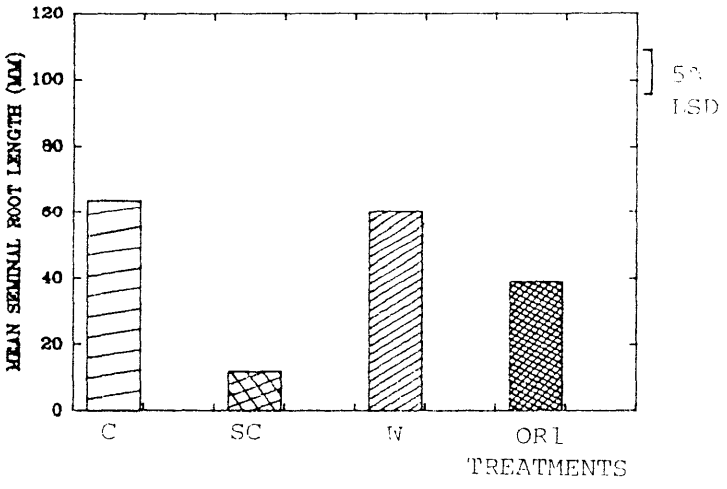
5.1.3 Discussion and Conclusions

As seen in the previous trials (4.7) there were no large differences between the control treatments and W solutions for wheat germination, first seminal root length or coleoptile height. ORl and SC reduced all three growth parameters, SC more so than its parent.

Fay and Duke (1977) and Putnam and Duke (1978) suggest that either the ability to produce allelotoxins, or their presence, may be heritable, and hence could be utilised, via a breeding program, to enhance a crop plant's competitive ability if the ability or presence could be found in appropriate germplasm. Both SC and ORl demonstrated allelopathic properties to wheat. Section 5.2 covers the analyses of chemical components of their leaf wash solutions in an attempt to determine if the toxins in both plants are the same.

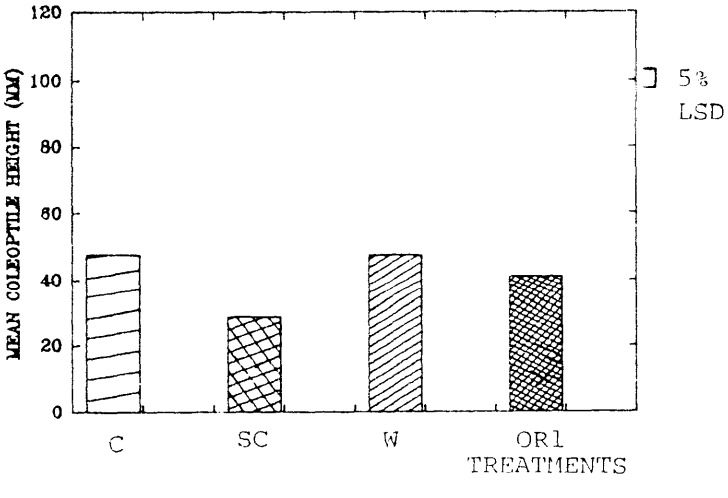
5.2 Chemical Analyses of Leaf Wash Solutions

Extracts of green leaves of the North American wild sunflower



C Sterile water control
SC Suncross 53 sunflower leaf wash
W Australian biotype sunflower leaf wash
ORl ORl - hybrid parent line sunflower leaf wash

Figure 5.2: Mean first seminal root length of wheat at 120h in solutions of Suncross 53, the Australian biotype and the hybrid parent line ORl sunflower leaf wash, and sterile water.



C Sterile water control
SC Suncross 53 sunflower leaf wash
W Australian biotype sunflower leaf wash
ORl ORl - hybrid parent line sunflower leaf wash

Figure 5.3: Mean coleoptile height of wheat at 120h in solutions of Suncross 53, the Australian biotype and the hybrid parent line ORl sunflower leaf wash, and sterile water.

Helianthus annuus, have been found to contain high levels of the chlorogenic acids and scopolin (Koeppe *et al.* 1969; Koeppe *et al.* 1970a,b). However, Wilson and Rice (1968) found only a naphthalene or α-naphthol derivative and scopolin in leaf drips collected after light, artificial rain on green leaves of similar material. Chemical analyses were carried out on leaf washings and leaf extracts of the hybrid Suncross 53 (SC) and the Australian wild type (W) sunflowers, on leaf washings of the hybrid's parent (ORl), and three, six and eight week old hybrid and ORl plants, the latter to examine the theory that chemical content varies with age of the plant (Koeppe *et al.* 1970a,b).

5.2.1 Materials and Methods

5.2.1.1 Leaf wash solutions

Leaves were harvested from nine week old SC, W and ORl plants grown under standard conditions, weighed and washed as described in 4.1.1, but in the ratio of lg of leaf material to 2g of water. The solutions were filtered through 0.2μm Millipore filters to remove any fungi or bacteria which may have been present.

5.2.1.2 Macerated leaf solutions

Leaves were harvested from SC and W plants, as described above, and weighed. They were then ground with a mortar and pestle and water added in the ratio of lg of leaf material to 2g of water. The solution was filtered through a Buchner funnel to remove large debris, then through a 0.2μm Millipore filter to remove any micro-organisms.

5.2.1.3 Leaf chemical changes with plant ageing

Leaves were harvested, weighed and washed, in the ratio 1:2, as

described in 4.1.1, from three, six and eight week old SC and OR1 plants. The solutions were passed through 0.2µm Millipore filters to remove any micro-organisms.

5.2.1.4 Chemical analysis

20ml of each of the leaf solutionswere acidified to pH 1 with 2M HCl and lyophilised to dryness. The residues were derivatised by heating with BSTFA (N,O - bis trimethylsilyl-trifluoroacetamide; Pierce Chemical Company, Rockford, Illinois) to convert N-H and O-H to $N\text{-}Si(CH_3)_3$ and $O\text{-}Si(CH_3)_3$, in anhydrous pyridine (50µl) at 100°C for 30 minutes. After cooling, aliquots (5µl) were injected into the gas chromatographic/ mass spectrometric (GCMS) system.

Chemical analyses were carried out on a Finnigan Model 3200 chemical ionisation gas chromatograph quadrupole mass spectrometer interfaced to the same manufacturer's Model 2300 Incos Data System. Methane (flow = 20ml per minute) served as the GC carrier gas and chemical ionisation reagent gas (ion source pressure = 0.8 Torr). The gas chromatographic columns were packed with 2% OV-17 on Gas Chrom Q (100 - 120 mesh). The column temperature was programmed one minute after sample injection from 100° to 300°C at 10°C per minute.

The RIC (Finnigan designation for TIC, total ion current) shows the total ion intensity or total number of ions, summed over some 600 two second scans of the mass spectrometer (scanning between, usually, mass 60 and mass 700), plotted against scan number. These are to be found in Appendix II.

Mass chromatogram printouts illustrate the intensity of a single mass ion. They are useful to locate specific compounds amongst a mass

of overlapping GC peaks (Appendix III).

Figures on both RIC and mass chromatogram peaks refer to the scan number only.

5.2.2 Results

5.2.2.1 Leaf wash solutions

5.2.2.1.1 SC and W plants (July sampling)

The RIC printouts for this analysis show that SC and W leaf wash solutions have an essentially similar compound composition, although SC solution had higher concentrations of most compounds. SC contained a compound at scan 389 which did not appear in the W solution. No chlorogenic acid, vanillic acid, ferulic acid or scopoletin was present in either solution.

5.2.2.1.2 SC, OR1 and W plants (April sampling)

The RIC printouts showed that there were very many compounds present in the OR1 and W leaf wash solutions, but very few in the SC solution. The concentration of these compounds was generally higher in the OR1 solution. The five compounds present in SC at moderately high concentrations were present also in OR1, as were many of the lower concentration compounds.

All of the leaf wash solutions had some compounds at scan 129, or very near, that is, an ion mass of 127. The three samples fall into two categories: W and OR1 have more intensity of ion mass 127, while SC has less (mass chromatogram figures). W and OR1 are very similar when viewed from the perspective of ion 127 (mass chromatogram figures).

5.2.2.2 Macerated leaf solutions (July sampling)

The SC macerated leaf solution contained a number of compounds

at high concentrations. Three of these were found in the W solution. W solution also contained a compound similar to inositol, and some related compounds, possibly sterioisomers. The other compounds were present at low concentrations.

5.2.2.3 Leaf chemical changes with plant ageing (June sampling)

Looking at SC solution analyses over the three samples (RIC, Appendix II), almost all the peaks coincide. All the peaks, except glycerol, were short in the three week old plant leaf wash solution, becoming taller in the six week old plant leaf wash, and taller again in the eight week old plant leaf wash.

The three ORl leaf wash solutions contained similar compounds, but the concentration tended to be higher in the eight week old plant.

The three week old plant leaf wash solution analyses showed that SC contained a few more compounds than ORl, although the concentrations of these were similar. There were two compounds in ORl at moderately high concentrations that were at much lower concentrations in SC. At six weeks SC again contained more compounds than ORl, and two of these were at much higher concentrations than in ORl. At eight weeks, SC again had more compounds, and at generally higher concentrations than ORl.

5.2.3 Discussion and Conclusions

The leaf washings analysed in 5.2.2.1 and 5.2.2.3 (Appendix II), were collected in April, June and July. The hybrid had similar compounds present at like concentrations at the three collection times. The Australian biotype was analysed in April and July; there were fewer compounds in smaller amounts in July. This may have been due to lowered

production of allelotoxic chemicals when the plants were under less environmental stress in July. The hybrid appeared, from germination and growth trials, to be very allelotoxic, and may produce a higher concentration of chemicals than the more innocuous biotype all the year round.

The Australian biotype and the hybrid parent had many compounds present in the RIC printouts (Appendix II), and a higher intensity of ion mass 127 in the mass chromatogram printouts (Appendix III). The hybrid had fewer compounds at high concentration and less intensity of ion mass 127 than its parent or the biotype, but was more allelotoxic to germination and root growth than either of them.

The ion mass 127 could be a member of the naphthyl group. This is similar to the compound found by Wilson and Rice (1968) in green leaf washings of the North American biotype sunflower.

The analyses of the macerated leaf solutions of the hybrid and the Australian biotype indicated that the hybrid leaf contained many more compounds at high concentrations than the biotype, while both contained more compounds than either of the leaf wash solutions. This was reflected in the germination and early growth of linseed and wheat (4.7.2.4) when the hybrid leaf solution produced a greater toxicity to both linseed and wheat than the biotype whether macerated or washed, but markedly more so when macerated. This is confirmed by the findings of many workers (for example, Rice 1964,1974; Muller and Chou 1972; McCahon *et al.* 1973; del Moral *et al.* 1978) of the allelotoxicity of aqueous extracted macerated or ground material. This technique of extraction disrupts cell membranes and may release substances from the material which would not be released under natural field conditions

(Putnam and Duke 1978).

The analyses of the three, six and eight week old hybrid and hybrid parent leaf washings suggest that there may be a genetic link between the two for presence of certain chemical compounds. Similar compounds were present in both the sunflower types by three weeks of age. The concentration of these increased over the five weeks, more quickly for the hybrid. The number of compounds also increased more in the hybrid. However, many of these substances were also present in the Australian biotype. Koeppe *et al.* (1969) and Koeppe *et al.* (1970a,b) found that certain compounds (phenolics) increased in concentration as sunflower leaves and plants aged.

The eight week old plant analyses suggest that the hybrid contained more compounds than its parent (June sampling), while the nine week old OR1 (April sampling, previous year) contained more than the hybrid. The number and concentration of chemicals present in the leaf wash solutions are dependent on many factors including the stage of growth of the plant, the health of the plant, the composition of the phyllosphere micro-organism population, the time of the year, the prevailing temperature and light regimes, and possibly many more, all of which increase the variability of the concentration of chemicals released, and hence the allelotoxic potential of the plant for interference.

The results suggest that the presence of the chemicals may be responsible for the allelotoxicity of the leaf wash solutions to wheat, however, the number and concentration of the compounds do not appear to be proportional to the level of allelotoxicity seen in the petri dish trials in 5.1. Direct comparisons cannot be made confidently between

the concentration of the chemical compounds by comparing the heights of the peaks in the RIC and mass chromatogram figures as these give only an estimation of the amounts present. Generally, however, a taller peak implies that more of that compound is present.

If there are one, or more, chemicals common to the hybrid and its parent causing the allelotoxicity, the analyses have not shown it. More analyses are needed, with repeats at frequent intervals to confirm the consistency of occurrence of individual compounds, whether they change in concentration over the life of the plant, with health or over time, and are peculiar to the hybrid and its parent line only.

5.3 Leaf Surface structures

Thurston *et al.* (1966) found that many *Nicotiana* species, including *N. tabacum*, stored alkaloids, principally nicotine, in trichomes, to aid in preventing insect attack; Schildknecht (1981) found similar structures in the *Urticaceae;* Cutter (1976) reported that *Cannabis* species store tetrahydro-cannabinol, an hallucinogen, in trichomes.

Trichomes are unicellular or multicellular projections on the surface of plants, which may or may not have a secretory function (Cutter, 1976). They may be used by the plant to store toxic chemicals to prevent damage to its own tissues, while holding the chemicals in readiness against attack by predation or competition from other plants (Whittaker, 1971). Trichomes of *Salvia reflexa* have been found to collapse and release allelochemicals upon wetting (Lovett and Speak 1979).

5.3.1 Materials and Methods

5.3.1.1 Light microscopy

Sections of healthy, green sunflower leaves (SC, ORl and W),

from plants grown under standard conditions, were taken, and examined under the light microscope using fibre optics to reduce dehydration and damage of the leaf tissues. Photographs were taken at magnifications x 2.5, x 4 and x 10 of both adaxial and abaxial surfaces.

5.3.1.2 Scanning electron microscopy (SEM)

1cm discs were cut from healthy, green sunflower leaves (SC, OR1 and W), mounted on stubs, dried and gold coated. SEM photographs were taken at magnifications of x 39 to x 900, of both adaxial and abaxial surfaces. Leaf structures were counted over 3 1μm^2 fields on the x 39 prints.

5.3.2 Results

5.3.2.1 Light microscopy

Trichomes were seen on both leaf surfaces of SC, OR1 and W plants, Plates 1, 2 and 3 (Type 1 - elongated glandular trichomes, Type 2 - spherical glandular trichomes, and leaf hairs - non-glandular trichomes). Type 1 trichomes appeared to be more prevalent on SC than on OR1 or W, and more prevalent on the abaxial surface. OR1 and W had few Type 1 trichomes on either surface. SC also appeared to have more leaf hairs than OR1 and W on the abaxial surface. OR1 and W appeared to have a similar number of leaf hairs on both surfaces. Type 2 trichomes were again more prevalent on SC than on OR1 and W, and on the abaxial surfaces of SC, OR1 and W than on the adaxial surfaces.

5.3.2.2 SEM

The three 1μm^2 field counts are summarised in Table 5.1 (Average of two disc prints).

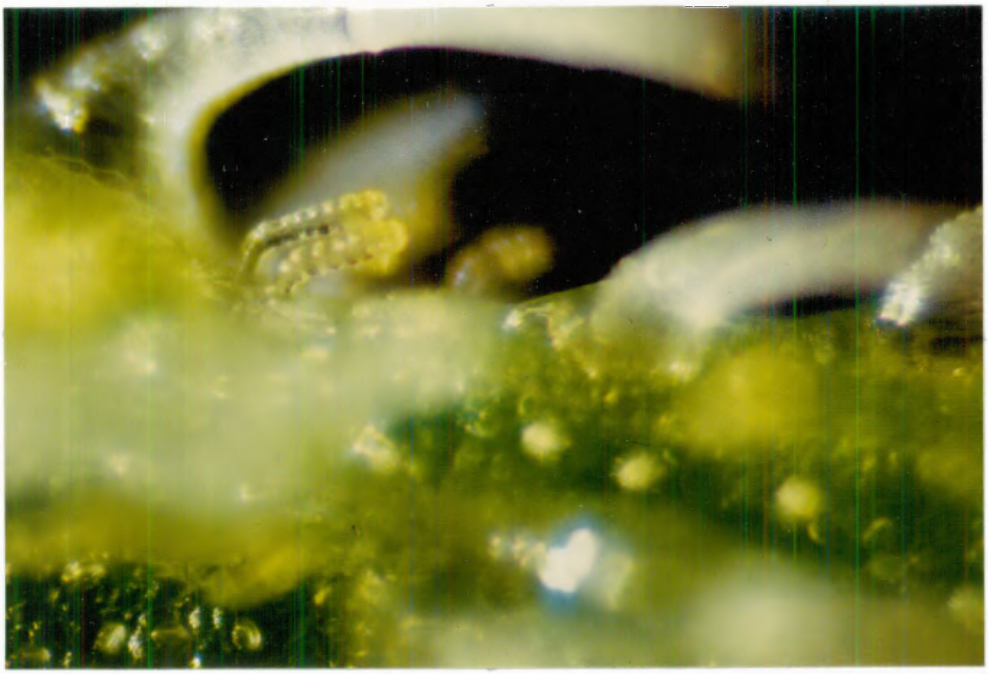


Plate 1: Elongated (Type 1) glandular trichomes and non-glandular leaf hairs on Suncross 53 sunflower adaxial leaf surface (x 10).

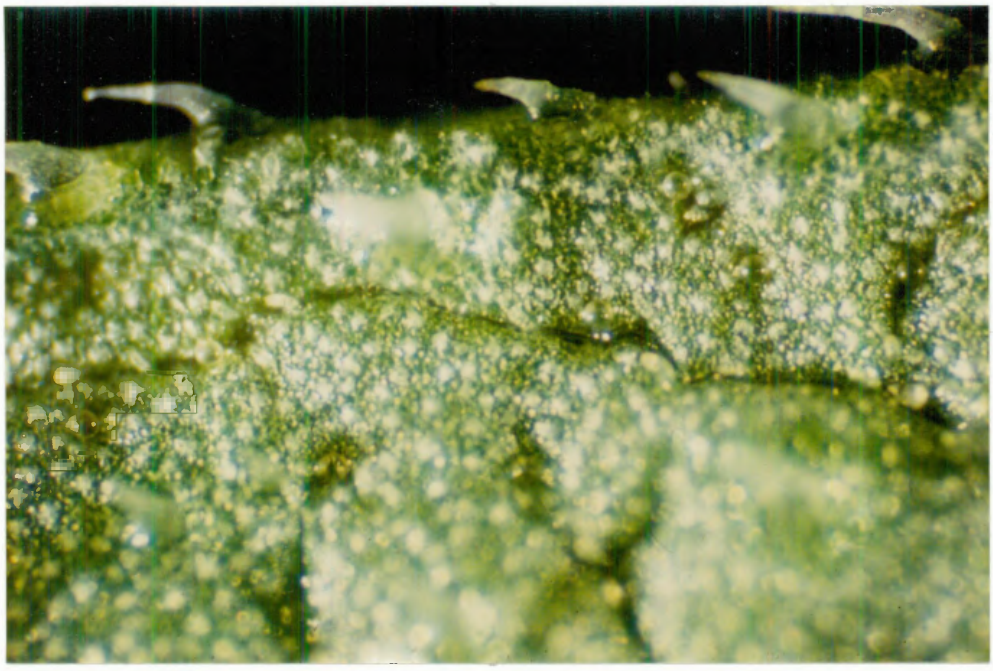


Plate 2: Spherical (Type 2) glandular trichomes and non-glandular leaf hairs on Australian biotype sunflower abaxial leaf surface (x 4).

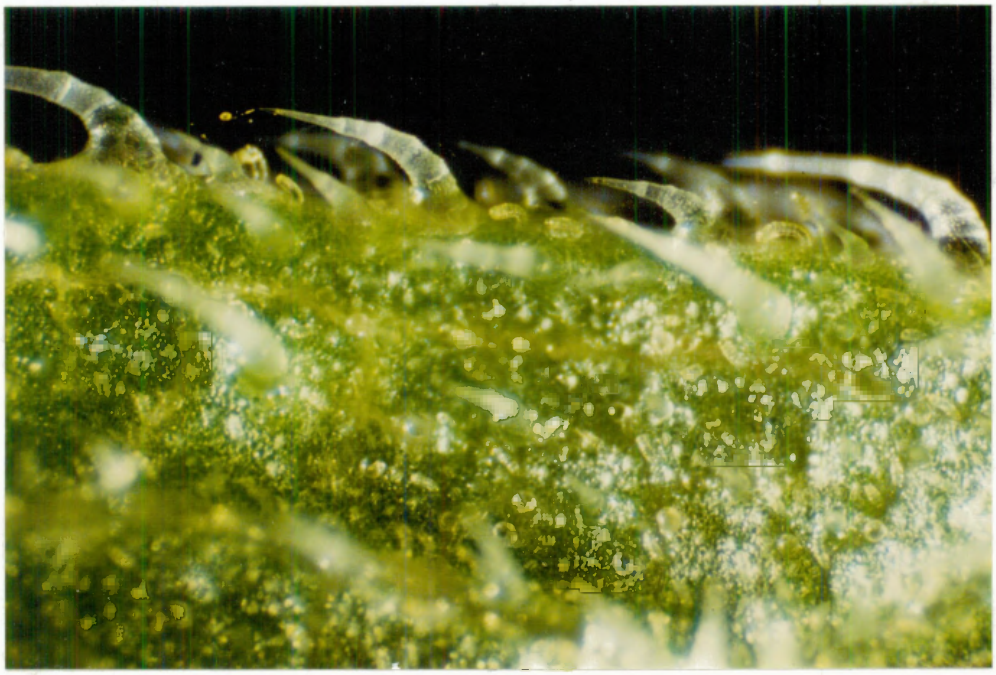


Plate 3: Elongated (Type 1) and spherical (Type 2) glandular trichomes and non-glandular leaf hairs on Suncross 53 sunflower abaxial leaf surface (x 4).

Table 5.1: Leaf structure counts of sunflower SEM leaf prints of the hybrid Suncross 53, the hybrid's male parent line ORl, and an Australian biotype sunflower (three 1μm^2 fields per print, averaged for two prints)

Sunflower type	Adaxial surface Hairs	Adaxial surface Type 1 trichomes	Adaxial surface Type 2 trichomes	Abaxial surface Hairs	Abaxial surface Type 1 trichomes	Abaxial surface Type 2 trichomes
SC	20.5	37.5	6	47	63.5	25
ORl	15	18	0	14	17	4
W	14.5	15.5	0	17.5	17	4

The data show that SC had more hairs and trichomes (both types) on both surfaces than ORl and W, and more of each structure on the abaxial surface than on the adaxial surface. W and ORl had similar numbers of hairs and Type 1 trichomes on both their surfaces, while having mo Type 2 trichomes on the abaxial surfaces.

The two types of trichomes collapsed slightly on dehydration during preparation of the leaf disc stubs (Plates 4,5,6 and 7).

5.3.3 Discussion and Conclusions

If trichomes are a storage organ for allelotoxic chemicals as suggested by Thurston *et al.* (1966), Lovett and Speak (1979), Lovett and Levitt (1981), Schildknecht (1981) and others, both the presence of the trichomes and the ability to remove the substances for storage in them would be an advantageous feature for plant interference. The chemicals could be collected over a period of time, and released when necessary, for example, if the plant should become stressed (Koeppe *et al.* 1969,1971; Lehman and Rice 1972; Koeppe *et al.* 1976). The manufacture of trichomes and the storage of the toxic substances requires energy (Levin, 1976), suggesting that the ability to store chemicals in this manner must be purposeful, and to the plant's evolutionary advantage.

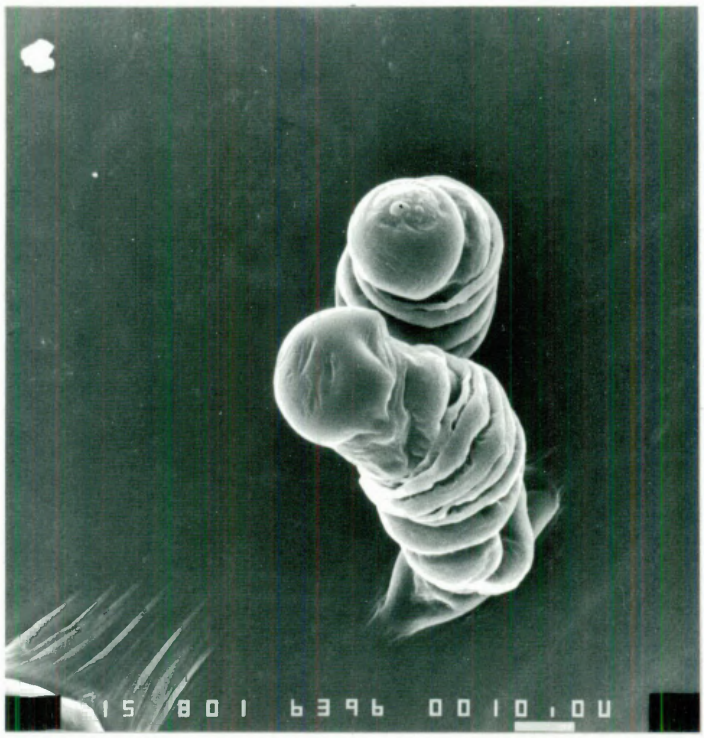


Plate 4: Elongated (Type 1) glandular trichomes on the hybrid parent line OR1 sunflower adaxial leaf surface (x 800).

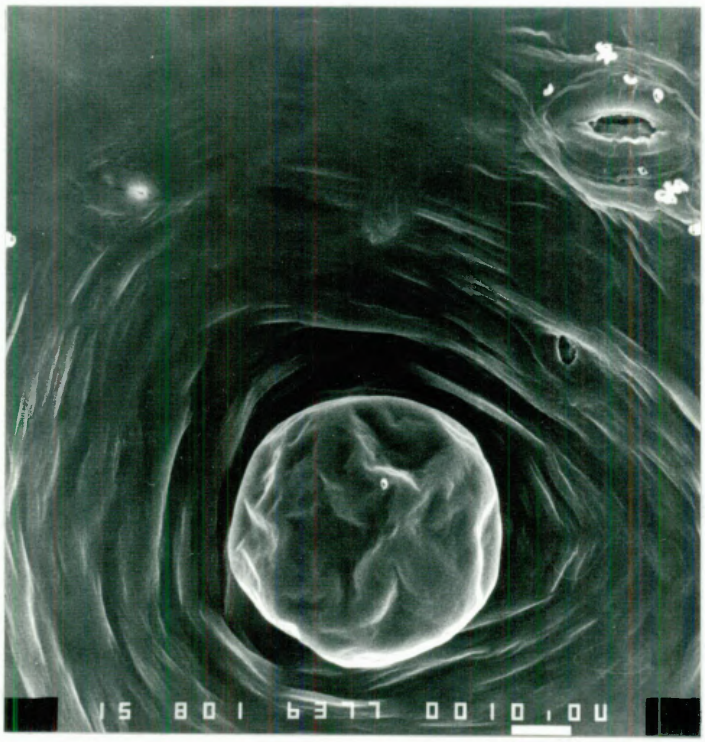


Plate 5: Spherical (Type 2) glandular trichome on Suncross 53 sunflower adaxial surface (x 800).

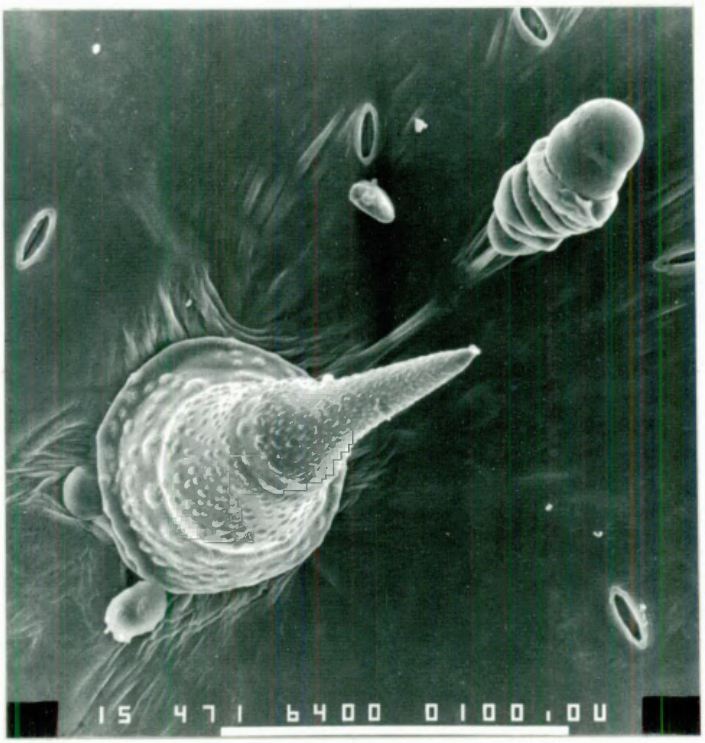


Plate 6: Elongated (Type 1) glandular trichome and non-glandular leaf hair on the hybrid parent line ORl sunflower adaxial leaf surface (x 470).

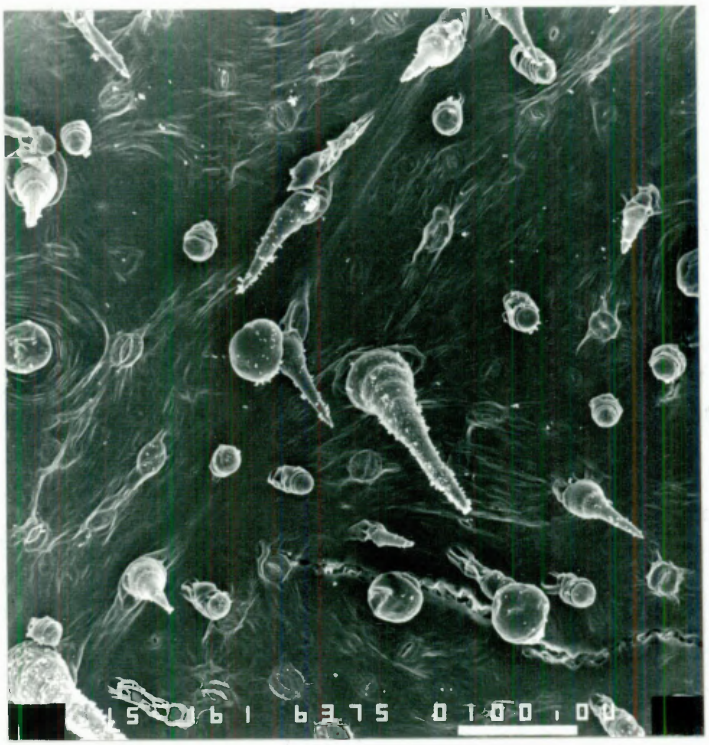


Plate 7: Elongated (Type 1) and spherical (Type 2) glandular trichomes and non-glandular leaf hairs on Suncross 53 sunflower adaxial leaf surface (x 160).

Often the substances are released when the leaves become wet, the trichomes burst or collapse and release their contents into the environment.

In the data examined in 5.1, the hybrid leaf washings were found to be quite allelotoxic to wheat germination and early growth. Washings contained several unidentified substances, plus a small concentration of ion mass 127 which could be a member of the naphthyl group, similar to that reported in the North American wild sunflower (Wilson, 1968; Wilson and Rice 1968). The hybrid had many leaf surface glandular trichomes, which may have been the sites for storage of these chemicals. The glandular trichomes did not, however, collapse completely on wetting. The hybrid also had many leaf hairs, which can act as physical deterrents to attack from animal and insect predators (Levin, 1973).

ORl, the male parent line of the hybrid, also showed allelotoxic properties to wheat germination and early growth. Chemical analysis showed that many substances were present in the leaf washings, including ion mass 127 at a higher concentration than in the hybrid, but similar to that of the Australian biotype sunflower. The number of leaf hairs and glandular trichomes was similar to the biotype, which showed only marginal restrictions to germination and early growth of wheat compared to the sterile water controls.

The trichomes seen in the light microscopy and SEM prints may be the sites for storage of these allelotoxic chemicals. On washing (gentle inversion in water for ten minutes) and examination, the trichomes appeared unaltered. SEM work conducted by Allen (personal communication) on sunflower leaf surfaces, has shown the spherical trichomes were not present after the leaf discs were prepared by the "Critical point drying"

technique (involving washing of the leaves for some hours in buffer solutions). Perhaps a surface wash is not sufficient to cause collapse of the trichomes, but will release sufficient chemicals to produce allelotoxic effects in sterile petri dish experiments. The elongated trichomes were still seen in Allen's leaf prints. Both types of glandular trichome collapsed slightly on dehydration for the SEM work, suggesting that they may release their contents in the manner postulated by Lovett and Speak (1979) with *Salvia reflexa*.

CHAPTER 6

INTEGRATING DISCUSSION AND CONCLUSIONS

Allelopathy, as an adjunct to competitive ability, has commonly been identified with weed species (Lovett and Levitt 1981). Putnam and Duke (1974) and Fay and Duke (1977), however, identified allelopathic potential in accessions of the crop plants cucumber and oats, respectively. Both of these species have the ability to suppress the growth of several weeds. Waller and Nowacki (1978) and other workers have suggested that the allelopathic potential of crop plants may have been reduced relative to their weed ancestors.

Accordingly, in the current work it was postulated that the allelotoxicity found in the North American wild sunflower (Wilson and Rice 1968; Rice, 1974) would be absent in Australian sunflower cultivars. This hypothesis was not confirmed as the hybrid, Suncross 53, displayed toxicity to bioassay species. With the confirmation of allelopathic properties in the hybrid, the investigation was broadened to include its male parent, OR1. Allelopathic properties, although not as pronounced as in Suncross 53, were identified in OR1 as were chemicals and leaf surface storage organs common to it and to the hybrid offspring. Both chemicals and storage organs were identified also in other lines.

The investigation also included an Australian sunflower biotype which, by analogy with its North American counterparts, was expected to show allelotoxicity. There was no consistent evidence for the existence of such properties. Matheson (1976) suggests that these multi-headed biotypes are descendants of bird-seed, confectionery or ornamental varieties and it may be that allelopathic properties were reduced during

their original selection, particularly if they were related to palatability. It may also be signficant that the North American biotypes also showed little evidence of allelopathic activity in these experiments. Although collected in a similar area it may be that this material differed from that used by Wilson and Rice (1968) and Rice (1974) and which displayed allelotoxic activity. Conversely, the relatively non-limiting conditions for growth employed in the current experiments may not have been conducive to the production of allelochemicals by biotypes accustomed to normal constraints on environmental resources such as water and nutrients.

Clearly, a further appraisal of the genetics of allelopathy in sunflower and of the genotype x environment X allelopathy interactions, is required if these preliminary data are to be placed in proper perspective.

Whilst the original hypothesis was not supported by the data collected, the investigations have highlighted a number of areas of considerable significance in studies of allelopathy. These include:

6.1 Type of Experiment

Much of the investigation of inter-plant relationships involving allelochemicals has been carried out on a small scale, in petri dishes (as here), or in small pots of soil or sand (Rice, 1974). The most serious limitation in this type of work is the lack of reality, particularly in sterile petri dish experiments where no micro-organisms exist to mediate the chemical effects which may occur on receiver plant surfaces. These trials are, however, necessary in order to demonstrate the existence of the chemicals and to gain some idea of their relative toxicities. Ideally, the next step is field trials, so designed as to eliminate the effects of competition for environmental resources such as nutrients,

water and light, which may mask relatively inconspicuous phytochemical interactions (Whittaker, 1970).

6.2 Change in Chemical Identity and Concentration

Stresses on the plants such as high levels of ultra-violet light, long daylight hours and high maximum daily temperatures, frequently cause variation in numbers and quantities of allelotoxic substances produced (Koeppe, 1968; Koeppe *et al.* 1969; Dorrell, 1976b). Changes in concentration of allelochemicals may also affect the response of bioassay species, low concentrations acting as stimulants and high concentrations as growth retardants (Lovett, 1982a). In the current work, this phenomenon was demonstrated in the trials in 4.2, 4.3, 4.4 and 4.6 where low concentrations were stimulatory. Conversely, the sandwich treatments of 4.1, which caused relatively high levels of allelochemicals to be released over a 120h period, plus the trials of 4.7 and 5.1 produced restriction of linseed and wheat germination and retardation of early seedling growth.

6.3 Type and Treatment of Plant Material

The green leaves of the hybrid, Suncross 53, released some chemical(s) on washing that was (were) toxic to the bioassay species. This was seen clearly in 4.1, although the chemical solution was of a relatively low concentration (1:10), and in 4.7 and 5.1 where the concentration was higher. The male parent line of the hybrid, OR1, also showed allelotoxic effects at high concentration but were not as restricting as those the hybrid. The Australian and North American biotype sunflower leaf washes were never very toxic to the bioassay species.

When damaged, the green leaf releases many chemical substances, some of which cause allelotoxic effects in bioassay species. In 4.7.3,

when leaves of the hybrid and the Australian biotype were macerated and washed with water, both sunflowers caused large restrictions to germination and early seedling growth, with the hybrid producing the larger restriction. Analysis of the two solutions (5.2.2.2) showed that the hybrid contained a large number of chemicals, many of them in high concentration, while the Australian biotype had a similar number of peaks but the concentrations were much lower than those of the hybrid.

The three week old hybrid leaf wash contained a few chemicals at only low concentrations. Both number and concentration were increased in the six and eight week old leaf. The nine to ten week old leaf wash analysis (5.2.2.1) showed further increases in concentration. Chemical analyses of leaf wash solutions of the male parent line (OR1) showed little increase in number and concentration of chemicals with age eight weeks, but did show that there were many more chemicals present than in the hybrid, particularly at nine to ten weeks when many of the compounds were higher in concentration than those in the hybrid leaf wash. This finding did not correlate with the allelotoxicity noted in the petri dish trials, suggesting that further analyses, repeated at frequent intervals in association with petri dish trials, need to be conducted before any assumptions can be made regarding the concentration and number of chemicals and the allelotoxicity of the leaf washes.

6.4 Seasonal Effects

Chemical analyses of the Australian biotype leaf wash solutions (5.2.2.1) showed many compounds present in the Total Ion Current when the plants were sampled in April, but most were at low concentration. Very few were present when the plants were sampled in July, and these

were again at low concentration. These findings do correlate with the noted allelotoxicity in the petri dish trials, that is, the biotype was not very restricting to the bioassay species.

6.5 Effects of Micro-organisms

The allelotoxic effects of the leaf chemicals of the hybrid were inherent to the chemicals themselves as the data presented in 4.3 and 4.4 showed that the presence of leaf micro-organisms, predominantly *Penicillium*, *Micrococcus* and *Enterobacter*, often reduced the allelotoxicity of the chemicals. The green leaf leaching (sandwich) treatment (4.1) also showed little toxicity to the bioassay species, compared to the green leaf wash, supporting this theory.

The dried and senesced leaf leachates (sandwiches) (4.1) showed a toxicity similar to the leaf wash solution, suggesting that either their chemicals were more allelotoxic than that of the green leaf, or that ameliorating micro-organisms were no longer present, a definite possibility if the micro-organisms are indigenous to the phylloplane of green leaves. Irons and Burnside (1982) and Lovett and co-workers (Lovett 1982a,b; Lovett *et al.*, 1982; Purvis, unpublished data) have demonstrated the allelotoxic effects found with dead and decaying leaf material incorporated into the media in the vicinity of germinating seeds and growing plants. The phytochemicals may be more allelotoxic due to changes in their nature on dehydration, or to concentration effects. They may also be more readily released through the ruptured cell walls of the dried or senesced leaves than through intact cell walls of the green leaf.

6.6 Presence of Trichomes

If trichomes are a storage site for allelochemicals (Thurston

et al. 1966; Schildknecht 1981), the large number found on the hybrid and the significantly smaller number found on the hybrid parent OR1, correlate well with allelotoxicity in the petri dish trials. However, the biotype, which showed less allelotoxicity than either the hybrid or OR1, had similar numbers of both types of glandular trichome and the non-glandular leaf hairs as OR1 on the abaxial surface, but a few less glandular trichomes on the adaxial surface. Glandular trichomes (both spherical and elongated) may store the allelochemicals, which could be released when the leaves are washed. The data suggest that the hybrid may have more of these storage sites although fewer types of chemical stored in them than OR1.

6.7 Time of Application of Allelochemicals

The time at which the phytochemicals were applied to the bioassay species appeared to have some bearing on the effects. Application of phytochemicals through the imbibition/germination period caused some small effects, while up to 120h, radicles were often markedly effected. Coleoptile measurements did not vary as much as the radicle measurements between the treatments. As the seedlings aged (4.6.1.2), the large differences were significantly reduced. The faster germinating species (for example, wheat) often escaped restriction from the leaf wash solutions in the low concentration experiments (4.4), while being retarded when the chemicals were present in a higher concentration (4.7, 5.1).

Overall, it appeared that the most consistent parameter for assessing the allelotoxicity of the leaf chemicals was radicle length at 120h. Germination over 120h was useful in the higher concentration trials. The relative sensitivity of radicle elongation and germination

in these experiments agrees with previously published data (Lovett and Lynch 1979).

Winter (1961) has suggested that the visible effects of phytotoxins are secondary manifestations of primary events. Such events are discussed by Rice (1974,1979). In the present experiment, it seems most likely that the secondary manifestations, as discussed above, may represent effects on primary events such as cell elongation and membrane permeability during the intense metabolic activity of the germination phase.

CONCLUSION

The hypothesis (Page 60) which has been tested by the work reported here was that the allelopathic aggressiveness found in the North American wild sunflowers was absent from an Australian cultivated type during growth, leaving it lacking in self defence. This hypothesis has essentially been confined in that statistically significant allelopathic activity has not been consistently demonstrated. However, trends in the data strongly suggested that a level of allelopathic activity, possibly associated with different phytochemicals, is present. The level of activity is, however, much lower than that identified in wild sunflower by Rice and his co-workers, which leads to the conclusion that there has been conscious or unconscious selection against allelopathic activity in the development of current commerical sunflower.

The finding of some common chemical and morphological features between the hybrid and one of its parent lines and the indications that the hybrid had greater allelotoxicity than its parent are encouraging, even though some of these features were not exclusive to these particular lines. Putnam and Duke (1978) and other workers have suggested that allelotoxicity is a potentially manipulable component of self-defence in plants and the data, here reported, suggest that further investigations of the allelopathic potential of cultivated sunflower would indicate the scope for genetic manipulation of allelopathic potential to the benefit of this crop