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ALLELOPATHIC ACTIVITY OF CULTIVATED SUNFLOWERS.

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ABSTRACT

Workers in North America have documented allelopathic activity in several species of weed-type sunflower. Such activity has been identified with allelochemicals produced by living plants and from decaying debris in the field. Autotoxicity, resulting from allelochemicals of sunflowers, has also been reported.

In Australian experiments, conducted over a three-year period, little evidence for allelopathic activity by naturalised (multi-head) roadside populations of sunflower, or by open-pollinated cultivars, was identified. However, in studies of an unreleased hybrid cultivar, evidence was consistently obtained for the production of the allelochemicals by living, senescing and dried foliage. Similar activity was identified in the male parent line.

Gas chromatographic/mass spectrometric analyses indicated the presence of similar organic compounds in washings of living foliage of the hybrid and its parent. The possible nature of these compounds and their relationship with allelochemicals previously identified in sunflower is discussed. The evidence is evaluated in the context of allelochemicals as agents which may contribute to the defence and/or competitive ability of those plants which possess them in active concentrations.

INTRODUCTION

In his review of defence substances in higher plants, Schildknecht (1981) refers to the "many hundreds of substances emitted day and night above and below ground by higher plants" as a means to protect themselves against other organisms, including other plants. This defensive capability is often better developed in weed than in crop species, Lovett (1982).

Cooper and Stoesz (1931) reported that *Helianthus scaberrimus* (Rough Sunflower), in common with other weed-type sunflowers, produces rhizomes which contribute to the development of a "fairy ring" effect. These authors cite an example in which a fairy ring was estimated to develop at the rate of 1 metre per annum over a period of 6 — 7 years. The centre of the ring was barren. In the absence of seed of *H. scaberrimus,* tests for allelopathic activity were conducted using seed of *Helianthus annuus* and *Triticum aestivum.* Allelopathic effects were confirmed although no suggestion as to the nature of the allelochemicals present was made.

Studies of allelopathy in various other sunflower species were made by Curtin and Cottam (1950). Such activity was, again, linked with the production of rhizomes in *H. rigidus, H. occidentalis, H. grosseserratus* and *H. tuberosus.* Of these species *H. rigidus* and *H. occidentalis* were found to be the most active. Anderson, Katz and Anderson (1978) further identified allelopathic activity in *H. mollis.* With many of these species of *Helianthus* autotoxic, as well as allotoxic, activity has been reported.

Wilson and Rice (1968) identify *H. annuus* as an important dominant in plant succession in abandoned fields in parts of Oklahoma and Kansas. Associated species often show reduced growth which cannot be attributed to competitive effects, as defined by Harper (1977). Leaf leachates, extracts of decaying leaves, root exudates and extracts of soils from the vicinity of *H. annuus* plants all inhibited germination and early growth of many species, including *H. annuus.* Chlorogenic acid and isochlorogenic acid were present in all extracts of organs of *H. annuus.* A suspected α-naphthol derivative and scopolin from leaf leachates were other possible phytotoxins. Wilson and Rice (1968) note that the type of phytotoxin varies with the plant part which produces it.

The fact that the allelochemicals of weed-type sunflowers are frequently autotoxic may contribute to the fact that such species often represent only a fairly brief phase in plant succession. Succeeding species, for example, *Aristida oligantha* in the case of *H. annuus,* are often not affected by sunflower allelochemicals (Wilson and Rice, 1968).

In reviewing allelopathic effects of sunflower, Rice (1974) notes that allelochemicals have differential effects on competing species, enter the soil, and show greater activity after the accumulation of plant debris than during active growth. This latter observation is probably a consequence of the concentration of allelochemicals, as discussed by Tukey (1969).

Whittaker (1970) takes the view that almost all plants have the ability to produce chemicals for their defence. Allelopathic activity is, however, often better developed among natural plant populations than is true of crop plants. Lovett and Levitt (1981) suggest that, given the close relationship between many weeds and crops, genetic manipulation to enhance the content of allelochemicals, which may perform defensive functions not only against plants but also against other organisms, may be possible.

To date, there are few reports of allelopathic activity being manifested by sunflower cultivars. Experiments of Tang and Young (1979) confirmed that most phytotoxins isolated from sunflowers were phenolics. In glasshouse experiments, Koeppe, Southwick and Bittell (1976) studied the effects of phosphorus nutrition on levels of chlorogenic acid in cv. Russian Mammoth and on leaching of phenolic compounds from living and dried plant parts. Caffeoylquinic acid and scopolin were identified as two of the possible allelochemicals present. In studies of the same cultivar, Hall and Blum (1977) varied nutrient and density levels in studies of allelopathy between sunflower and a weed species, whilst Leather and Forrence (1979) evaluated thirteen cultivars of sunflower for allelopathic effects upon populations of weeds. Weeds showed differing responses to aqueous extracts of dried sunflower leaf and stem material in controlled experiments. In the field, a sunflower-oat-sunflower rotation was shown to reduce weed cover.

In a comprehensive study of populations of multi-headed *H. annuus* naturalised in Australia over several decades, open-pollinated cultivars and hybrid cultivars, Fraser (unpublished data) was unable to find consistent evidence for allelopathic activity from living material. Recently, such activity has been identified in an unreleased hybrid cultivar of *H. annuus* and in its male parent. Residues of cv. Peredovic have also consistently shown allelopathic activity. The implications of these findings are discussed in this paper.

MATERIALS AND METHODS

(a) Experiments with living plants.

Seeds of an unreleased hybrid cultivar and of its male parent line, which had shown some evidence of autotoxicity in experiments conducted under controlled conditions, were supplied by Arthur Yates and Co., seed producers and merchants. Seeds were pre-germinated at 20 — 24°C in the light and sown into a loam, sand, peat mix in 20 cm diameter pots. The pots were placed in a glasshouse, diurnal temperature range 18°C to 35°C, under natural light conditions. Plants were watered daily and fertilised regularly with a soluble complete fertiliser. Leaves were harvested for testing when the plants were 9 — 10 weeks old.

Intact foliage was gently washed in distilled water (2 ml per g foliage) for 10 minutes. 5 ml of foliage washings was applied to Whatman No. 1 filter paper in 9 cm diameter petri dishes containing 25 seeds of the test species, *Triticum aestivum* cv. Songlen. Sterile water was employed as a control. Experiments were replicated four times. Petri dishes were incubated for periods up to five days in the dark at 24°C constant temperature. The experiment was repeated on several occasions with similar results. Two experiments are reported here. Numbers germinating were recorded at the conclusion of incubation. In Experiment 1, germination counts were also made at 24 h intervals as the experiment progressed. At the conclusion of the experiments, height of the coleoptile and length of the longest (usually the first) and shortest seminal roots was recorded.

(b) Experiments with plant residues.

Helianthus annuus cv. Peredovic was grown in the field at Armidale under normal commercial conditions. Plant material was harvested during senescene when some green foliage was still available and an initial experiment set up in the laboratory in which green, decaying and air-dried foliage samples of equivalent dry weight were placed between Whatman No. 1 filter papers in 9 cm diameter petri dishes to form foliage "sandwiches". The sandwiches were wetted up with 10 ml sterile water, in the case of the decaying foliage this operation taking place 48 h before the commencement of the experiment. A further 2 ml sterile water was applied 48 h later. A sterile water control was included in sandwich experiments. *T. aestivum* cv. Songlen was used as a test species, as described above, but using 20 seeds per petri dish and 5 replicates. Incubation was for three days after which percent germination and length of coleoptile and first seminal root were recorded.

(c) Chemical analyses.

Chemical analyses were performed on aqueous plant washings (20 ml) which were acidified to pH 1 with 2M HCl and lyophilised to dryness. The residue was derivatised by heating with BSTFA (N,O— bis trimethylsilyl-trifluoroacetamide; Pierce Chemical Company, Rockford, Illinois) in anhydrous pyridine (50 μl) at 100°C for 30 minutes. After cooling, aliquots (5 μl) were injected into the GCMS system.

Chemical analyses were recorded by a Finnigan Model 3200 chemical ionisation gas chromatograph quadrupole mass spectrometer interfaced to the same manufacturer's Model 2300 Incos Data System. Methane (flow = 20 ml per min) 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 1 minute after sample injection from 100° to 300°C at 10°C per min.

RESULTS

(a) Living plants (Experiments 1 and 2).

In Experiment 1, conducted over five days, germination was significantly retarded in the early phases by washings of foliage of both the hybrid and its parent, Table 1. After 24 h the hybrid had retarded germination more than its parent but this effect ceased at 48 h and, after 72 h, there were no significant differences in percent germination.

Table 1. The effect of washings of green sunflower foliage on the germination of wheat.

Time of incubation (h)	Germination (X/25 seeds) Control	Hybrid	Parent	P
24	21.6a	11.0b	14.0c	<0.001
48	24.0a	21.0b	20.4b	<0.01
72	24.4	22.8	23.0	N.S.
96	24.4	23.2	23.6	N.S.
120	24.4	23.2	23.8	N.S.

Means identified by a common letter are not significantly different at the 5% level, Studentized Range Test.

Both the hybrid and its parent significantly reduced the length of the longest seminal root, usually the first seminal root, and the height of the coleoptile after 5 days (Table 2). There were no differences in the average number of seminal roots present but the length of the shortest seminal root was significantly greater where foliage washings had been applied (Table 2).

Table 2. The effects of washings of green sunflower foliage on coleoptile height and root length of wheat after 120 h incubation.

Plant Part	Height or length (mm) Control	Hybrid	Parent	P
Coleoptile	47.0a	41.0b	40.0b	<0.001
Longest seminal root	68.3a	61.2b	59.8b	<0.001
Shortest seminal root	24.7a	29.4b	27.7b	<0.05

Means identified by a common letter are not significantly different at the 5% level, Studentized Range Test.

Experiment 2 was conducted over a 3-day period. In common with Experiment 1, there were not significant differences in germination after 72 h. Neither the height of the coleoptile nor the length of the shortest seminal root showed statistically significant differences after 3 days' growth, Table 3. The length of the longest seminal root was, however, significantly depressed by washings of foliage of the hybrid.

Table 3. The effects of washing of green sunflower foliage on coleoptile height and root length of wheat after 72 h incubation.

Plant Part	Height or length (mm)			P
	Control	Hybrid	Parent	
Coleoptile	20.6	18.8	20.2	N.S.
Longest seminal root	46.9a	28.9b	49.5c	<0.001
Shortest seminal root	12.2	10.2	10.1	N.S.

Means identified by a common letter are not significantly different at the 5% level, Studentized Range Test.

(b) Plant residues (Experiment 3).

In common with Experiments 1 and 2, no significant effects of sunflower residues on germination were found after 3 days' incubation, Table 4. All residues significantly ($P<0.001$) depressed height of the coleoptile and length of the longest seminal root. Decaying residues were most severe in respect of the former and dried residues in respect of the latter.

Table 4. The effect of washings of green, decaying and dry sunflower foliage on the germination and early growth of wheat.

	Control	Green	Decaying	Dry	P
Germination (x/20 seeds)	18.4	18.6	18.0	19.2	N.S.
Height of coleoptile (mm)	18.7a	8.70b	6.40c	8.14b	<0.001
Length of longest seminal root (mm)	38.9a	18.4b	21.6b	13.2c	<0.001
Root/shoot ratio	2.09a	2.12a	3.38b	1.64c	<0.001

Means identified by a common letter are not significantly different at the 5% level, Studentized Range Test.

Green residues had no effect upon root/shoot ratio but decaying residues significantly ($P<0.001$) increased this parameter whilst dry residues invoked a significant decrease.

(c) Chemical analyses.

Chemical analyses were conducted on aqueous washings of live foliage of the cultivar and its male parent line. Examination of the results indicated a close similarity between each analysis within lines and between the hybrid and its male parent. A number of compounds were present of which the most abundant and readily identifiable was glycerol (identified as its tri-TMS derivative by GC retention time and the coincidence of its methane CIMS with an authentic standard). The analyses showed no significant differences between the hybrid and its male parent line which could be assigned to a possible allelochemical.

DISCUSSION

The allelopathic activity of weed-type sunflowers is well documented (Rice, 1974) but there is less evidence for such activity in cultivars of the species. Data presented in this paper suggest that, in living sunflower material, and allelopathic potential may exist but be expressed only in certain cultivars.

Experiments with washings of living foliage of a sunflower hybrid and its male parent indicated that germination of wheat was retarded in the initial stages and that this retardation was further expressed, in a 5-day experiment, in decreased height of the coleoptile and length of the longest seminal root, relative to controls. In this experiment the length of the shortest seminal root was increased by washings of both hybrid and parent, indicating a compensatory effect.

In a 3-day experiment, whilst the washings of the hybrid significantly reduced the length of the longest seminal root, the height of the coleoptile was unaffected. In this experiment, slight stimulation in the length of the longest seminal root by washings of the parent may indicate a concentration effect of the type discussed by Lovett (1982).

These data, involving a major crop species as a phytometer, are unique in reports of allelopathy in sunflower to date. The indication is that an allelopathic potential exists in some cultivated sunflowers and that it may be manipulated genetically, for example, through a hybrid breeding programme.

The chemical data strongly suggest that similar compounds are present in both the hybrid and its parents. Although glycerol was the most abundant compound identified it is not suggested that it is, itself, an allelochemical. None of the other compounds present resembled substances such as chlorogenic acid, isochlorogenic acid or scopolin, all of which have been identified as allelochemicals in wild-type sunflowers (Wilson and Rice, 1968).

Some data, for example, germination at 24 h in the 5-day experiment and the length of the longest seminal root in the 3 day experiment, suggest that the allelopathic effect of the hybrid is greater than that of its parent and this may be related to a greater concentration of allelochemicals. This supposition, however, remains to be substantiated.

Data from the residue experiment indicate that allelochemicals are produced by residues of the common sunflower cultivar Peredovic. No consistent evidence for such activity in living plants of this cultivar has been found (Fraser, unpublished data). As was the case with washings of living foliage, leachings of residues had no significant effect upon germination after 3 days' incubation, however, significant effects were recorded for parameters of early growth. Whilst height of the coleoptile was most susceptible to chemicals produced by decaying residues of sunflower, root length was most susceptible to chemicals liberated by dried residues. Whether these phenomena are the result of differing concentrations of the same chemical or to different chemicals remains to be determined. Comparisons with the compounds present in Experiments 1 and 2 have also to be made.

Since sunflower and wheat may occur as summer and winter crop combinations in Australian agriculture, and as the latter may germinate in proximity to residues of the former, allelopathy may be a significant factor in successful establishment of a wheat crop.

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POST-EMERGENCE WEED CONTROL IN SUNFLOWER.

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ABSTRACT

There are many occasions when pre-emergent herbicides can be used for weed control and if properly used they are quite satisfactory. After crop emergence, we have not been able to use any herbicides to control dicotyledons in our trials, but certain anti-graminae herbicides could be used. These were alloxydime-Na, fluazifop-butyl or dic-lofop methyl. It seems that these herbicides are only economical if used quite early in crop growth.

INTRODUCTION

Although sunflower is widely grown in our country — we expect some 200,000 ha to be grown in 1982 — little research has been conducted into herbicide requirement for this crop. As a matter of fact, none of the products generally used on sunflower were developed for that crop first. Now we have shown, and there is general agreement among crop specialists, that weed control in sunflowers is quite profitable.

Some 10 products are used for weed control in sunflower crops in France (Table 1). These are normally applied after sowing and are therefore dependent on rainfall to be effective. Moreover, farmers may find some weed species which they did not know occurred in their field. Lastly, except in the case of trifluralin which has a wide action, it is not possible to increase the dosage of most products to improve efficacy.

Taking these facts into account, we decided to examine postemergence weed control.