CHAPTER 1

INTRODUCTION

Plant communities, undisturbed or managed by Man, are dynamic congregations of individuals, often encompassing many different species. In both monocultures and mixtures, individual plants constantly "interfere" with one another, both positively and negatively, by altering the shared environment (Harper, 1977). The term "interference" includes the depletion of resources (competition) to the detriment of other plants in the environment either simultaneously or sequentially (Muller, 1968,1970), the addition of chemical compounds (allelopathy), and changes in the physical conditions of the environment (Harper, 1977).

Molisch (1937) coined the term "allelopathy' literally meaning "mutual harm", to cover both beneficial and detrimental interactions between all types of plants including micro-organisms (Rice, 1974). In the past many workers have tended to use allelopathy to describe only detrimental interactions between higher plants, but Molisch's interpretation is now generally employed (Rice, 1979).

In this thesis the terms "phytochemical" and "allelochemical" are used to describe the plant-derived chemicals which may have negative or positive (allelopathic) effects on plants and, sometimes, other living organisms.

Phytochemicals which produce a negative effect are termed allelotoxins or allelotoxic chemicals, and these may be effective against plants of other species (allotoxic), against plants of the same species (autotoxic), or both (allelotoxic).

The significance of phytochemicals has been disputed since the earliest reports of their existence by workers such as de Candolle (1813). In the light of many well-documented examples in the literature it is, however, difficult to escape the conclusion that phytochemicals play a role in the organisation of plant communities. It is, for example, the relative tolerance of plants to released phytochemicals that has given rise to the principles of companion planting, recognised in horticulture and now extending into agriculture (Putnam and Duke 1978). These workers suggest the idea of "exploiting allelopathy in agriculture" by determining which plants display allelopathic properties, and introducing these into breeding programs of crop plants, to promote the "self-defence" of the crop.

Most annual crops are developed from weeds or ruderals. Waller and Nowacki (1978) state that many of these crop ancestors contain allelochemicals which are useful for the plant's self-defence against plant competition, attack by predators and invasion by pathogens. It should, therefore, be possible to incorporate self-defence mechanisms into crop types through breeding programs which include their "wild" ancestors.

This thesis examines the types of plants which produce allelochemicals, particularly the sunflower (*Helianthus armuus* L.), their identity and how useful they can be in plant protection. The sunflower is known in both weed and crop forms. It originated in what is now the western United States of America, and was an important food source for early man. He spread the plant through the eastern United States, where it is thought to have been domesticated from its "wild" multibranched, small-headed form to a single branched, large head type. From North America it was spread eastward to Europe as a minor crop plant.

It was readily accepted as a crop in Russia, and selection for higher oil yield began (Lovett, Harris and McWilliam 1979).

Crop sunflowers are now grown in many countries, but problems exist in the areas of weed, pest and pathogen control. The "wild" sunflower biotype still exists in central and western U.S.A. (Wilson and Rice 1968; Rice, 1974) as a prolific weed, which shows pronounced allotoxic and autotoxic properties (Wilson and Rice 1968; Rice, 1974; Irons and Burnside 1982).

As crop types of sunflower, particularly oilseed cultivars, have been developed, they have changed from the non-uniform weed types to plants of similar characteristics, often single stemmed and tall for ease of mechanical harvesting, and which produce high yields when sown in relatively high density monocultures. This process may have involved breeding out any allelochemicals, consciously or not, to improve the yield, and perhaps, the palatability of the harvested product. In turn this may have rendered the crop types open to competition by pest organisms.

Russell (1978) has estimated that as much as 50 per cent of the yield potential of crop species is lost annually to the depredations of weeds, insects and diseases despite wide scale use of costly "control" strategies. Thus, the potential for enhancing possible inherent crop plant defences against pest organisms, is worthy of further exploration.

CHAPTER 2

PHYTOCHEMICALS

Production and release of phytochemical compounds is an ubiquitous, albeit inconspicuous, phenomenon of natural plant communities (Whittaker, 1970). These compounds are released into the environment through "leaky" plant surfaces: the leaching of substances from leaf surfaces and glands, volatilization from leaves, exudation or excretion of water soluble chemicals from roots, and through leaching, with or without decomposition by micro-organisms, of above and below ground plant parts (Whittaker and Feeny 1971; Putnam and Duke 1978). Tukey (1971a,b) states that "no plant yet studied cannot be leached".

Most allelochemical interactions involve secondary compounds (Whittaker, 1970), which are by-products of metabolic pathways in the plant (Whittaker and Feeny 1971; Levin 1976) (Figure 2.1).



Figure 2.1: Biosynthetic relationships of secondary plant products. (after Levin, 1976).

Primary compounds may also be released, but are nutritional and generally stimulatory (Godfrey, 1976). Some of the primary compounds known to be released from plants are listed in Table 2.1.

Table 2.1: Nutritional (stimulatory) substances released from plants by rain washing or dew/fog condensation and drip leaching

Substa	ance	Found in	Reference
Amino	acids: lysine alanine threonine aspartic acid glutamic acid histidine phenylalanine glycine serine isoleucine	Foliage of Douglas Fir (<i>Pseudotsuga douglasii</i> Lindl.) Carr.	Jones, 1976
Amino	acids: 22 most abundant - homoserine threonine glutamine asparagine alanine	21 day old pea root (<i>Pisum sativum</i> L.)	Rovira, 1956
Amino	acids: 14 most abundant - glycine serine lysine asparagine leucine	21 day old oat root (Avena sativa L.)	Rovira, 1956
Fructo	ose and glucose	10 day old oat and pea root	Rovira, 1956
Amino	acids	Mulberry leaves (<i>Morus indica</i> L.)	Vasantharajan and Bhat 1968
Tyrosi	ine	Tomato (<i>Lycopersicon</i> <i>esculentum</i> Mill.) and Red pepper (<i>Capsicum annuum</i> L.) root at fruiting	Harmsen and Jager 1962

Table 2.1 continued.

Substance	Found in	Reference
Gibberellic acid like compounds	Stem and leaf of 'Princess Anne' chrysanthemum (<i>Chrysanthemum</i> <i>morifolium</i> Ramat.)	Kozel and Tukey 1968
Organic acids: butyric valeric acetic proprionic	Wheat (<i>Triticum aestivum</i> L.) roots up to tillering	Riviére, 1960
Minerals: K Ca Mg Mn	Many species	Tukey, 1971a
N	Douglas Fir leaves	Jones, 1970,1976
Ν	Tropical plant species	Ruinen, 1965,1970, 1975
Ν	Coffee (<i>Coffea arabica</i> L.) Banana (<i>Musa cavendishii</i> Lamb.) Cacao (<i>Theobroma cacao</i> L.)	Tukey, 1971b
Carbohydrates	Mulberry leaves	Vasantharajan and Bhat 1968
Carbohydrates	Tropical plant species	Ruinen, 1970,1975
Carbohydrates	Plant foliage	Tukey, Wittwer and Tukey 1957

The effect of the released phytochemical on phytometer species may vary with the concentration. Lovett (1982b) found that allelochemicals released from litter of certain weeds produced inhibition of crop plant growth and/or development, while green leaf washings of the same weed species often produced mild stimulation of the phytometer species. This type of effect has long been known for other phytochemicals such as auxins and gibberellic acids.

2.1 Types of Phytochemicals

Among chemicals released from living plants the two most common groups are the phenolics and the terpenes (Muller, 1966), with the alkaloids also being prevalent (Whittaker, 1970). Other compounds are released by fewer plant species (Whittaker, 1970; Swain, 1977), including tannins (Swain, 1977), isoflavonoids (Newman and Rovira 1975), and flavonoids (Lodhi, 1979). Scopoletin is thought to be the most prevalent coumarin (Robinson, 1963). Among all living things, plants contain the widest variety of phenolics. These usually occur in combined form, for example, as glycosides, (Swain, 1969). The number and range of chemicals identified to date is extensive, Table 2.2.

2.2 Changes in Phytochemical Release

Release of chemicals from plants over time is not constant. Changes occur both in amount and composition with environmental variations, age, and physiological state of the plant. Chemical concentration may also differ between parts of the same plant. The following sections describe these changes in detail, but largely exclude sunflower references as these are cited in Chapter 3.

2.2.1 The climatic factors

While plants are constantly producing, sequestering and/or releasing compounds into the environment, the important role of water in aiding release becomes apparent. In the plant environment water is the most likely solvent and many plants have evolved to exploit the presence of water as rain, mist, fog or dew to rid themselves of unwanted byproducts (Went, 1955; Tukey, 1966; del Moral and Cates 1971; Jones, 1976) be they toxic wastes or nutritional substances. Ruinen (1961) emphasises the importance of water as an aid to the release of phytochemicals.

Chemical	Plant	Plant part released from:	Cited in:
Aldehydes & Ketones:			
Acetaldehyde	Pisum sativum	seeds	Evenari 1949
Artemisia keton <mark>e</mark>	Ar <i>temisia californica</i> Less. (California sagebrush)	green, litter	Halligan 1975
Terpenoids & steroids:			
Arbutin	<i>Arctostaphylos glauca</i> Lindl. (Bigberry manzanita)	green, litter	Muller 1970,1971
Arbutin	Arctostaphylos sp. (Adans.) (manzanita)	green, litter	Hanawalt 1971
Borneol	Artemisia californica	green, litter	Halligan 1975
Camphene	Salvia leucophylla Greene (a sage), S. mellifera Greene (black sage), S. apiana Japs (white sage)	leaf, atmosphere above macerated leaf	Muller and Muller 1964
Camphene	S. leucophylla	atmosphere around plant	Muller 1970, 1971
Camphor	5. leucophylla, 5. mellifera 5. apíana	Leaf, atmosphere	Muller and Muller 1964
Camphor	S. leucophylla, 3. mellifera	atmosphere	Muller 1965
Camphor	5. leucophylla	a tmos phere	Muller 1970, 1971 Revnolds 1975
Camphor	Internigia californica	areen. litter	Halligan 1975
Carvophyllene	1 zalifornica	green, litter	Halligan 1975
Civeole	A. californica	green, litter	Hallican 1975
	Salvia lewonbulla	leaf.	Muller and
	3. mellijera 5. apiana	atmosphere	Muller 1964
Cineole	s. leucophylla S. mellijera	atmosphere around plant	Muller 1965
Cineole	5. leucophylla	atmosphere around plant	Muller 1970, 1971 Reynolds 1975
Dipentene	5. leucophylla 5. mellifera 5. apiana	leaf, atmosph ere	Muller and Muller 1964
α-pinene	5. leucophylla 5. mellifera 5. apiana	leaf, atmosphere	Muller 1970, 1971 Muller and Muller 1964
2 pinana	C laurente lla	loaf	Muller 1970
P-husue	5. teleophytta 5. mellijera 5. apiana	atmosphere	Muller and Muller 1964
		1	Hallier 1904
β-pinene	Artemisia californica	green, litter	Halligan 1075
Sesquiterpene hydrocarbon	A. californica	green, litter	Hallingan 1975
Sesquiterpene lactone	Artemisia tridentata Nutt. var. vaseyana (sagebrush)	macerated]eaf	McCahon, Kelsey, Sheridan and Shafizadeh 1973
- absinthin	Artemisia absinthium L.	leaf	Börner 1960
- absinthin - absinthin	(wornwood) A. absinthium A. absinthium	not specified (n.s.) root hairs	Reynolds 1975 Holm 1971

Table 2.2: Chemicals identified from plant exudates and phyllosphere environments

Benzoic acid and derivatives:

Gallic acid	Euphorbia виріпа Raf. (a spurge)	green parts	Blum and Rice 1969
Gallic acid	grassland climax communities	n.s.	Rice and Pancholy 1973
Gallic acid	Eucalyptus baxteri (Benth.) Maiden & Blakely (Brown stringybark) Eucalyptus obliqua L'Herit. (Messmate stringybark)	<pre>itter</pre>	del Moral, Willis and Ashton 1978
Gallic acid	<i>Quercus borealis</i> Michx. (red oak) <i>Q. alba</i> L. (White oak)	litter	Lodhi 1978a
Gentisic acid	Euphorbia supina	green parts	Ðlum and Rice 1969
Gentisic acid	Eucalyptus baxteri	foliage, litter	del Moral et al. 1978
p-hydroxybenzoic acid	Hordeum vulgare L. (barley), Secale cereale L. (rye), wheat	straw, roots	Collison 1925
p-hydroxybenzoic acid	<i>Camelina alyeвum</i> Mill. Thellung. (Camelina)	leaf	Grümmer and Beyer 1960, Grümmer 1961
p-hydroxybenzoic acid	Zea mays L. (corn), wheat, oat, <i>Sorghum</i> vulgare Pers. (sorghum)	residues	Guenzi and McCalla 1966
p-hydroxybenzoic acid	n.s.	straw, stubble	Wang, Yang and Chuang 1967
p- nydroxybenzoic acid	Avena fatua (wild oat)	straw	Tinnin and Muller 1972
p-hydroxybenzoic acid	White oak	litter	Lodhi 1978a
Salicyclic acid	<i>Medicago sativa</i> L. (Alfalfa/lucern e)	hay	Collison 1925
Syringic acid	corn, wheat, oat, sorghum	litter	Guenzi and McCalla 1966
Syringic acid	n.s.	straw, stubble	Wang et al. 1967
Syringic acid	Avena jatua	straw	Tinnin and Muller 1972
Vanillic acid	barley, rye, wheat	straw, roots	Collison 1925
Vanillic acid	barley	husks	van Sumere Hilder so n and Massart 1958
Vanillic acid	Camelina alyssum	leaf	Grümmer and Beyer 1960 Grümmer 1961
Vanillic acid	corn, wheat, oat, sorghum	residues	Guenzi and McCalla 1966
Vanillic acid	n.s.	straw, stubble	Wang et al. 1967
Vanillic acid	Avena fatua	straw	Tinnin and Muller 1972
Homovanillic acid	White oak	litter	Lodhi 1978a
Vanillin	Alfalfa	hay	Collison 1925
Cinnamic acid and derivatives:			
Caffeic acid	Helianthus annuus L. (sunflower)	seeds	Cater, Gheyasuddin and Mattil 1972
Caffeic acid	grassland climax communities	litter	Rice and Pancholy 1973
Caffeic acid	Eucalyptus baxteri	litter	del Moral et al. 1978

Caffeic acid	<i>Celtis occidentalis</i> L. (Hackberry)	litter	Lodhi 1978a
Caffeic acid	Platanus occidentalis L. (Sycamore)	litter	Lodhi 1978a
Caffeic acid	Red and White oak	. litter	Lodhi 1978a
Caffeic acid	Kochia ecoparia Schrad. (Chenopodium ecoparia L. - summer cypress)	litter	Lodhi 1979
Glucose ester of caffeic acid	Euphorbia supina	green parts	Blum and Rice 1969
Chlorogenic acid (3-0-caffeoylquinic acid)	Sunflower	all parts	Rice 1965 Wilson 1968 Wilson and Rice 1968
Chlorogenic acid (3-0-caffeoylquinic acid)	Sunflower	leaf	Watanabe, Chorney, Skok and Wender 1964 Koeppe, Rohrbaugh, and Wender 1969 Koeppe, Rohrbaugh, Rice and Wender 1970b
Chlorogenic acid (3-0-caffeoylquinic acid)	Sunflower	leaf, stem	Lehman and Rice 1972
Chlorogenic acid (3-0-caffeoylquinic acid)	Sunflower	seeds	Joubert 1955, Lane 1965; Cater <i>et al.</i> 1972; Dorrell 1976a,b
Chlorogenic acid (3-0-caffeoylquinic acid)	Sunflower	n.s.	Koeppe, Southwick and Bittell 1976
Chlorogenic acid	Nicotiana tabacum L.	leaf	Koeppe et al.
(3-0-caffeoylquinic acid)	(tobacco)	stem,leaf, root	1969 Zucker and Ahrens 1958
Chlorogenic acid (3-0-caffeoylquinic acid)	Xanthium penneylvanicum (Wall.) (Xanthium)	leaf	Taylor and Zucker 1966
Chlorogenic acid (3-0-caffeoylquinic acid)	Solanum suberoвит L. (potato)	tuber	Taylor and Zucker 1966
Chlorogenic acid (3-0-caffeoylquinic acid)	Red oak	litter	Lodhi 1978a
Chlorogenic acid (3-0-caffeoylquinic acid)	Sycamore	litter	Lodhi 1978a
Chlorogenic acid (3-0-caffeoylquinic acid)	Kocnia scoparia	litter	Lodhi 1979
Chlorogenic acid (3-0-caffeoylquinic acid)	Eucalyptuв obliqua	foliage, litter	del Moral <i>et al.</i> 1978
Chlorogenic acid (3-0-caffeoylquinic acid)	Euphorbia supina	green parts	Blum and Rice 1969
4-0-caffeoylquinic acid (Band 510)	Sunflower	all parts	Lehman and Ríce 1972
4-0-caffeoylquinic acid (Band 510)	Sycamore	litter	Lodhi 1978a

	neo-chlorogenic acid (5-0-caffeoylquínic acid)	Sunflower	leaf, root, stem	Lehman and Rice 1972
	neo-chlorogenic acid (5-0-caffeoylquinic acid)	Sycamore	litter	Lodhi 1978a
	tso-chlorogente actd (3-5-dicaffeoylquinte actd)	Sunflower	all parts	Rice 1965, Wilson 1968, Wilson and Rice 1968
	tso-chlorogentc actd (3-5-dicaffeoylquinic acid)	Sunflower	leaf	Koeppe et al. 1970b
	iso-chlorogeníc acíd (3-5-dicaffeoylquinic acid)	Sunflower	seeds (during imbibition)	Lane 1965
	iso-chlorogenic acid (3-5-dicaffeoylquinic acid)	Sunflower	seeds	Mourgue, Lanet, Blanc and Steinmetz 1975
	iso-chlorogenic acid (3-5-dicaffeoylquinic acid)	Sycamore	litter	Lodhi 1978a
	iso-chlorogenic acid (3-5-dicaffeoylquinic acid)	Euphorbia supina	g reen parts	Blum and Rice 1969
	unspecified (total) chlorogenic acid(s)	Sunflower	leaf	Koeppe <i>et al</i> . 1969
	unspecified (total) chlorogenic acid(s)	tobacco	leaf	Koeppe et al. 1969
	Cinnamic acid	Parthenium argentatum Gray. (guayule)	roots	8onner 1946, 1950
	Coumaric acid	barley, wheat, rye	straw, roots	Collison 1925
	p-coumaric acid	corn, wheat, oat, sorghum	residues	Guenzi and McCalla 1966
	p-coumaric acid	n.s.	straw, stubble	Wang et al. 1967
	p-coumaric acid	Avena fatua	strāw	Tinnin and Muller 1972
	p-coumaric acid	Hackberry	litter	Lodhi 1978a
	Ferulic acid	barley, rye, wheat	straw, roots	Collison 1925
	Ferulic actd	corn, wheat, oat, sorghum	litter	Guenzi and McCalla 1966
	Ferulic actd	n.s.	straw, stubble	Wang et al. 1967
	Ferulic actd	Euphorbia supina	green parts	Blum and Rice 1969
	Ferulic acid	Avena fatua	straw	Tinnin and Muller 1972
	Ferulic acid	grassland climax communities	straw	Rice and Pancholy 1974
	Ferulic actd	Red and White oak	litter	Lodhi 1978a
	Ferulic acid	Kochia seoparia	litter	Lodhi 1979
	Hydroxycinnamic acid	barley	husks	van Sumere et al.1958
	Hydroxycinnamic acid	Arctostaphylos sp.	green, litter	Hanawalt 1971
	Quinic acid	Tomato, sunflower, lettuce (Lactuca sativa L.), radish (Kaphunnus sativas L.)	leaf	Perkins and Aronoff 1956
	Quinic acid	Sunflower	seeds	Cater <i>et al</i> . 1972 Mourgue et al. 1975
	Sinapic acid	Eucalyptus baxteri	litter	del Moral et al. 1978
Counar	rins:			
	Loumarin	barley	nusks	van Sumere et al 1930
	Coumartn	Melilotum spp. (Mill.) (sweet clover)	litter	McCalla and Army 1961

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	Coumarin	Coumarouna odorata Aublet.	seeds	Valto 1973
	Coumarin	Anthoxanthum odoratum L. (sweet vernal grass)	n.s.	Reynolds 1975
	Coumarin derivative	Arctostaphylos sp.	litter	Hanawalt 1971
	Esculin	Sunflower	leaf	Watanabe et al. 1964
	Esculin	Artemisia tridentata	macerated leaf	McCahon et al. 1973
	Scopoletin	tobacco	leaf	Best 1944
	Scopoletin	oat	roots	Martin and Rademacher 1959
	Scopoletin	sunflower	leaf	Watanabe <i>et al.</i> 1964
	Scopoletin	wheat	green plant	Fay and Duke 1977
	Scopoletin	Red oak	litter	Lodhi 1978a
	Scopoletin	Hackberry	litter	Lodhi 1978a
	Scopolin	tobacco	whole plant treated with 2,4-D	Rohrbaugh, Thiesfeld and Wender 1964
	Scopolin	tobacco	leaf	Armstrong 1968
	Scopolin	sunflower	leaf	Wilson 1968 Wilson and Rice 1968
				Lehman and Rice 1972
	Scopolin	Red oak	litter	Lodhi 1978a
	Scopolin	Sycamore	litter	Lodh1 1978a
	Scopolin	Hackberry	litter	Lodh1 1978a
Flay	ono1ds:			
	Myricetin	grassland climax communities	litter	Rice and Pancholy 1974
	Myricetin	Koshia ecoparia	litter	Lodhi 1979
	Isoquercitrin	sunflower	leaf(boron deficient)	Watanabe Bt al. 1964
	Quercitin	Kochia scoparia	litter	Lodhi 1979
Tanr	ins:			
	Digallic acid	grassland climax communities	n.s.	Rice and Pancholy 1973
	Digallic acid	Red oak	litter	Lodhi 1978a
	Ellagic acid	grassland climax communities	n.s.	Rice and Pancholy 1973
	Ellagic acid	Eucalyptus baxteri E. obliqua	foliage, litter	del Moral st al. 1978
	Ellagic acid	Red and White oak	litter	Lodhi 1978a
	Tannic acid	Euphorbia supina	green parts	Blum and Rice 1969
	Tannins	Eucalyptus baxteri 5. obliqua	litter foliage, litter	del Moral st al. 1978

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Alkaloids: Overland 1966 Gramine barley green parts Juglans nigra L. (walnut) canopy Böde 1958, Börner 1960, Hydrojuglone Went 1970, Whittaker 1970 Lovett, Levitt, Duffield and Smith 1981 Datura stramonium L. seeds, leaves (thorn-apple) Hyoscyamine Lovett, Levitt, Duffield and Smith seeds, leaves D. strumonium Scopolamine 1981 Phenolic glycoside:_ del Moral et al. 1978 Eucalyptus baxteri E. obliqua foliage, litter unresolved litter Amine: Linum usitatissimum L. (linseed, flax) Lovett and Duffield 1981 leaves, stems Benzylamine Wilson 1968 sunflower leaf α -napthol derivative Rice, personal communication

Koeppe *et al.* (1970b) have suggested that some compounds may remain in the leaf as innocuous substances with the biochemical change to allelochemicals being stimulated by leaching.

Water is necessary to the survival of phyllosphere microorganisms, which may be responsible for the production of compounds. *Azotobacter, Aerobacter, Beijerinckia, Pseudomonas* and *Spirillium* use plant exudates as carbon substrates to convert nitrogen, often supplied as amino acids, to usuable forms on the leaves of many tropical plant species (Ruinen, 1956,1961,1965). Mulberry (*Morus indica*) (Vasantharajan and Bhat 1968) and Douglas Fir (*Pseudotsuga douglasii*) (Jones, 1970,1976) also support phyllosphere populations which convert nitrogen to usable forms. The nitrogen is released into the environment through leaching by water. Lovett and Sagar (1978) illustrated the need for the presence of water for activity of bacteria which produce allelochemicals in association with the weed *Camelina sativa* (L.) Crantz. Water is also essential to maintain populations of micro-organisms involved in plant litter decay – an important source of allelochemicals (Lovett, 1982b).

The intensity and duration of rainfall may determine the amount and toxicity of chemicals washed off or out of plants (Grümmer and Beyer 1960). Light drizzle will wash plants more effectively than a short, heavy downpour (Lausberg, 1935; Schloch, 1955; Stenlid, 1958). Leaves that are hydrophobic do not wet well, and will therefore not be leached greatly (Cholodny, 1932). Hydrophoby tends to decrease with age, hence older parts of the plant are likely to leach more than younger parts (Cholodny, 1932; Tukey, 1969).

Long periods of rain will tend to reduce the concentration of the leached chemicals in solution, the concentration remaining in or on the plant, or in the litter (Gleissman and Muller 1972), but may increase the concentration of chemicals in the soil.

Drought stress, in the short or long term, may accentuate the allelotoxicity of chemicals released from plants. Drought stress is necessary for the volatile phytochemicals of the Californian chaparral to express their full toxicity (Muller, 1970). These chemicals are adsorbed onto dry soil colloids (Muller, 1968) and must reach a critical level before release to create an allelotoxic effect to maintain dominance. Rainfall and mulching reduce the toxicity, and it is thought that improved soil water regimes promote the growth of micro-organisms that degrade the chemicals (Muller, 1970).

Similarly, periods of low rainfall allow an increase in the concentration of allelotoxin in *Arctostaphylus* plants. Hence the first rains of autumn contain a large concentration of toxin washed from the plants (Hanawalt, 1971). Large amounts of allelotoxin are also released from *Brassica nigra* (L.) Koch. (black mustard) debris and litter with the first autumn rains, in the Californian annual grasslands (Bell and Muller 1973).

Tukey, Wittwer and Tukey (1957) have stated that carbohydrate available for leaching from plants varies with the light impingeing on the leaf, with very little leaching potential occurring in the dark. Increasing light results in an increase in photosynthetic rate, which in turn increases the carbohydrates present in the leaf, and leakage of these may occur. Chlorogenic acid is synthesised in leaves only in the light (Zucker and Ahrens 1958).

Koeppe, Rohrbaugh and Wender (1969,1971) demonstrated the increased levels of scopolin in tobacco and sunflower, and chlorogenic

acid in sunflower with high levels of ultra-violet (U.V.) radiation. They suggest that high U.V. levels result in stress in the plants, which produce more "defence" chemicals to withstand any possible competition, or attack, while they are weakened in the stressed state.

Bhan, Wallace and Lunt (1959) have stated that wind buffeting will increase the leaching potential of rain. This may be due to damage caused to the leaves which would increase leakage from plant surfaces.

2.2.2 Variability with plant part

Tables 2.1, 2.2 and 2.3 (section 2.4.9) include a list of parts of plants that contribute allelochemicals to the environment. They include leaves, stems, roots, tubers and rhizomes, husks, seeds, pollen, and debris or litter. The most toxic plant part seems to vary with the plant under discussion. Del Moral and Cates (1971) state that the litter fraction of the Californian chaparral (Salvia Leucophylla, S. mellifera, S. apiana and Artemisia spp.) are generally more toxic than other fractions. Lovett (1982a,b) has found that decomposing foliage of weeds such as Sorghum almum (L.) Pers. (Columbus grass), Stevia eupatoria Willd. (Kempton's weed), Salvia reflexa Hornem (mintweed), Datura stramonium L. (common thornapple), and Camelina sativa inhibit crop plant growth and development, while washings of live foliage of these often produce mild stimulation of the phytometer species. This is possibly due to the concentration effect of the allelochemicals by drying of the plant and the "leakiness" of the debris as breakdown begins. Contrary to this, Rice (1964), Wilson and Rice (1968) stated that green leaves of Helianthus annuus were more toxic than any other part of the plant; Fletcher and Renney (1963) found a similar reaction in Centaurea spp.; Grant and Sallans (1964) found that of the eight grass and legume species they tested, aerial portions of

all, except lucerne, were more inhibitory to the eight test species than root allelotoxins, and Bokhari (1978) found a similar effect with leaves of blue grama grass (*Bouteloua gracilis* (H.B.K.) Lag.) and western wheat grass (*Agropyron smithii* Rydb.), compared with litters.

Plant allelochemicals are often autotoxic (that is, toxic to the producing species) and must be stored safely in the plant if they are not to be released immediately. Some workers, for example, suggest that such storage represents a waste disposal system. However, many plants have evolved specialised storage structures such as trichomes. The ability to concentrate chemicals over time by storage in trichomes could enhance the toxic effect on release from the plant and may imply an active evolutionary purpose in their development. For example, Thurston, Smith and Cooper (1966) demonstrated the storage of alkaloids, principally nicotine, in trichomes of all the wild *Nicotiana* species and strains of *N. tabacum* that they tested. These substances help to ward off insect attack, and Schildknecht (1981) showed a similar modus operandi in the *Urticaceae* (stinging nettles).

Trichome storage may aid plants in semi-arid areas by allowing chemicals to remain in the plant until water becomes available to flush them out. Atriplex vesicaria Benth. (bladder saltbush) stores salt for removal using this method, and Salvia reflexa bladders collapse on wetting to release allelochemicals (Lovett and Speak 1979).

2.2.3 Concentration changes with plant ageing and health

A well reported example of a phytochemical changing with plant age is that of chlorogenic acid. Zucker and Ahrens (1958) noted that chlorogenic acid content of tobacco leaves decreased in concentration from the apex to the base of the plant, that is, as the leaves grew older. Koeppe, Rohrbaugh, Rice and Wender (1970a) noted a similar change with chlorogenic acid in tobacco, but that scopolin levels showed the reverse. They indicated that, while overall the total amount of phenolics in tobacco increased with age, the concentration per unit area decreased as plant tissues expanded. Koeppe *et al.* (1970b) found that chlorogenic acid, 4-O-caffeoylquinic acid and neo-chlorogenic acid concentration in sunflower stems showed a similar pattern with the concentration decreasing from the apex to about the fifth node, with an additional slight increase in the bottom leaves of the plant. However, these compounds increased in leaves to the fifth or sixth node, and decreased after the sixth to eighth node. Morgan (1964) reported a decrease in total phenolic concentration in older leaves of cotton (*Gossypium hirsutum* L_o) compared with younger leaves, and Hamidi and Wanner (1964) reported a decrease in concentration, as well as in total amount, of phenolics in coffee (*Coffea arabica*) with age.

Zucker and Ahrens (1958) suggest that as chlorogenic acid is synthesised in leaf tissue, the gradient of chlorogenic acid content from tip to base may indicate the physiological difference in the ability of older tissue to synthesise chlorogenic acid, but Koeppe *et al.* (1970a,b) suggest that chlorogenic acid and its isomers (iso- and neo-chlorogenic acid) may be lignin precursors and that in mature leaves the rate of synthesis approximates the rate of utilization (Taylor and Zucker 1966). Chlorogenic acid is converted to 3-5-dicaffeoylquinic acid (iso-chlorogenic acid) which is made up of two caffeoyl moities. Taylor and Zucker (1966) suggest that this compound may be converted to free caffeic acid which is then utilised in the manufacture of lignin at a faster rate in older leaves than young ones (Koeppe *et al.* 1970a,b).

Injuries to plants caused by viruses or mechanical means can

increase the production, accumulation, and release of chemicals. Helder (1956) and Tukey and Morgan (1963) have found that injured leaves release more substances than uninjured ones. Best (1944) found that tobacco plants infected with Tomato Spotted Wilt increased their scopoletin concentration. Koeppe *et al.* (1970a), also working with tobacco, showed that physical injury to the plant resulted in accumulation of both scopolin and scopoletin in plant tissues. Levin (1976) comments that production of secondary compounds places an energy demand on the plant, suggesting that the production is purposeful, not merely a leakage through damaged tissue.

2.2.4 Soil fertility status

Nutrient deficiencies may cause actual injury to plants. A soil deficiency may also stress a plant and promote its competitive ability, manifested perhaps by the increased production and release of allelotoxic compounds. The work by Lehman and Rice (1972) on nitrogen, potassium and sulphur, and by Koeppe *et al.* (1976) on phosphorus deficiencies in sunflower are reported in Chapter 3, but other plant species have also been investigated.

Armstrong (1968) noted that nitrogen and boron deficiencies in tobacco plants resulted in increases in scopolin concentration in the plants; Perkins and Aronoff (1956) reported increases in caffeic and quinic acids in boron deficient plants; Watanabe, McIllrath, Skok, Chorney and Wender (1961) reported increases in scopoletin in boron deficient tobacco - up to twenty times the normal levels; and Watanabe *et al.* (1964) reported increases in scopolin in boron deficient sunflower. These workers suggest that boron is involved in lignin synthesis (Watanabe *et al.*, 1961), and that low boron levels result in low lignin production,

and hence a build up in lignin precursors. These precursors, toxic to other plants, may be useful as self-defensive chemicals, to reduce competitive stress and to improve the boron supply to surviving plants.

2.3 The Importance of the Phyllosphere in Phytochemical Production

Although phytochemicals may be released from all parts of the plant, some workers have reported that leaf chemicals are more potent in certain plant species than chemicals released from other parts of the same plant (Grant and Sallans 1964; Wilson and Rice 1968; del Moral and Cates 1971). Decaying plant material has often caused problems in agricultural soils by releasing, during decomposition, allelotoxic chemicals which may either directly inhibit subsequent crops (Schreiner and Sullivan 1909; Ahlgren and Aamodt 1939; McCalla and Duley 1949; Patrick and Koch 1958; Börner, 1960; McCalla and Army 1961; Guenzi and McCalla 1962; Patrick, Toussoun and Snyder 1963; Patrick, Toussoun and Koch 1964; Guenzi and McCalla 1966; Guenzi, McCalla and Norstadt 1967; Megie, Pearson and Hiltbold 1967; Schlatterer and Tisdale 1969; McCalla, 1971; Bokhari, 1978), inhibit nitrogen fixation for the subsequent crop (Rice, 1964; Munro, 1966a,b; Rice, 1968,1971a,b; Rice and Pancholy 1972,1973,1974; Murthy and Ravindra 1974,1975; Murthy and Nagodra 1977; Murthy and Shihora 1977) or limit phosphorus uptake in the subsequent crop (Newman and Miller 1977). However, the effects of leached allelochemicals in soil may be short-lived as they can be readily adsorbed onto clays (Rice, 1964), and/or modified by soil texture (del.Moral and Cates 1971; Drost and Doll 1980). Both allelotoxic chemicals and nutrients released into soil are quickly attacked by soil micro-organisms and broken down (Woods, 1960) into innocuous, often useful, compounds which can be utilised by subsequent crop plants or by soil micro-organisms.

The more potentially active compounds tend, therefore, to be those washed out, or from, the surface of leaves, as these are often more potent initially and may be transmitted directly to the foliage of other plants through droplet splash, avoiding soil microbial breakdown. Work by Grümmer and Beyer (1960) and Grümmer (1961) illustrated this phenomenon in the association of *Camelina* spp. with flax, with rain splash being the important transmission method.

Allelochemicals may be produced by the plant itself; by the plant but modified by micro-organisms living on or in the leaf, or by the leaf or phyllosphere micro-organisms themselves from substrates produced in the plant. Lovett and Sagar (1978) and Lovett and Duffield (1981) found that a potent allelochemical is produced by bacteria living on the leaves of *Camelina sativa* from a complex chemical exuded by the leaves. In small concentrations this allelochemical (benzylamine) is able to disrupt the functioning of cell membranes.

2.4 Roles of Naturally Produced Compounds

Chemical inhibitors are produced by many members of the plant kingdom. These chemicals may play one, or more, roles on production and/or release.

2.4.1 Micro-organism/micro-organism interaction

Micro-organisms may produce antibiotics which are effective against other micro-organisms (Evenari 1961; Swain 1977). These include a wide range of organisms used in both human and animal health care. Antibiosis in soil systems is well known and is being used in several areas of Australia to combat microbial attacks on trees and plants. For example, in the native Jarrah forests (*Eucalyptus marginata* Sm.) in

Western Australia, and in the Eucalypt forests of Victoria, it is being employed against the dieback fungus *Phytophthora cinnamomi* Rands (Pratt, 1971; Broadbent and Baker 1973; Hopkins, 1973; Marks, Kassaby and Fagg 1975).

2.4.2 Micro-organism/plant interaction

Marasmins are chemicals produced by micro-organisms which are effective against higher plants (Grümmer and Beyer 1960; Rice, 1974). These substances can restrict germination and growth of plants in the region of chemical release. *Penicillium expansum* Link. and *P. urticae* Bainer produce a toxic chemical, patulin, from apple bark in apple orchards (Börner, 1971; McCalla, 1971). This chemical severely inhibits apple seedling growth and constitutes the apple nursery soil sickness problem.

2.4.3 Plant/micro-organism interaction

2.4.3.1 Anti pathogen

Plants may protect themselves against microbial attack by the production of chemicals - phytoncides (Grümmer and Beyer 1960; Rice, 1974). These chemicals can act as deterrents in host/parasite relationships, by ensuring that unadapted micro-organisms cannot exist on host plants, or cannot penetrate the cuticle to establish themselves (Evenari, 1961). Production of cyanides, alkaloids, phenolics and tannins has been shown to inhibit growth and development of microbial disease on plant surfaces, cell walls and within cells (Levin, 1971; Whittaker and Feeny 1971; Deverall, 1972; Swain, 1977). Timonin (1941) reported that flax roots exude hydrogen cyanide (HCN) which retards development of several species of pathogenic fungi. Ducker and Knox (1978) suggest that some species of seagrass release antibiotics which limit the frequency and composition of algal epiphytes.

Harborne (1977) reported that phenolic compounds are universally distributed among higher plants and are often toxic to micro-organisms (*in vitro*) at concentrations of 10^{-4} to 10^{-6} M. He also described the infection process, and stated that phytoalexins produced by the plant on attack by a fungal organism can limit the development of the fungi in hypersensitive tissues, that is, those in contact with the parasite.

Florence and Crocker (1962) have examined blackbutt (Eucalyptus pilularis Sm.) domination of blackbutt forest soils and have suggested that the domination strength is related to the ability of plant exudates, primarily, leached from debris to suppress the growth of soil micro-organisms antagonistic to blackbutt seedling establishment.

Naqvi and Chauhan (1980) have reported a case involving chillis (Capsicum annuum L.), where the ability of root exudates to suppress the pathogenic fungus, Fusarium oxysporum f. sp. capsici (authority not cited), is the deciding factor as to whether the plant is a susceptible or resistant variety to the pathogen. The susceptibility or resistance is, however, further enhanced as the exudates of the resistant lines promote the spore germination of soil fungi antagonistic to F. oxysporum, while exudates from the susceptible lines generally increase the germination of rhizosphere fungi, but reduce the spore germination of the antagonistic fungi.

Toussoun and Patrick (1963) discovered that aqueous extracts of residues of barley, wheat, rye, timothy grass (*Phleum pratense* L.), broccoli (*Brassica oleracea* L.) and broad bean (*Phaseolus vulgaris* L.) promoted the growth of conidia of *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Synd. & Hans. in the bean plant, causing disease

symptoms to develop far more quickly than in control plants.

These examples demonstrate how a plant may resist a pathogen by using chemical methods. However, specific pathogens may actually evolve sufficiently so as to use the chemical exudate as a substrate, as may be the case with the chillis and beans, above.

2.4.3.2 Nitrogen fixation

Microbial nitrogen fixation can contribute nitrogen to a community for continued plant growth. Higher plants may directly compete with neighbouring plants by limiting the supply of nitrogen through the release of chemicals toxic to nitrogen fixing micro-organisms. A well studied case is that of old-field succession in Oklahoma where the dominant Stage 1 plant is Helianthus annuus, a prolific weed. Rice (1968,1971a,b) demonstrated that aqueous leachates of leaves and roots of H. annuus reduced the number and size of nitrogen fixing nodules formed by red kidney bean (Phaseolus vulgaris L.), Korean lespedeza (Lespedeza stipulacea Maxim.), and white clover (Trifolium repens L.). Two phenolics identified in the extracts, gallic and tannic acids, can, when applied individually, also cause reductions of nodule size and number of these three species (Blum and Rice 1969), suggesting that these are toxic components of the leachate. Leghaemoglobin contents tend to be lower in the nodules of the plants treated with H. annuus leachate than in control plants (Rice, 1971a,b). The extracts were shown to reduce the growth and function of the nitrogen fixing Nitrosomonas, and to a lesser extent Nitrobacter (Rice, 1964), and to be toxic to Rhizobium and Azotobacter (Rice, 1965), and blue-green algae (Parks and Rice 1969). The overall effect from these compounds will be that nitrogen levels in the soil are kept at a low level, primarily as NH_A^+ ; later plant stages comprising higher nitrogen

requiring species (Rice, Penfound and Rohrbaugh 1960), will be retarded in their speed of invasion.

Murthy and co-workers have noted a similar phenomenon with Aristida adscensionis L. Leachates from A. adscensionis are toxic to Rhizobium (Murthy and Nagodra 1977; Murthy and Shihora 1977) and Azotobacter (Murthy and Ravindra 1975), resulting in low nodule numbers and low leghaemoglobin levels in the test plants (Murthy and Ravindra 1974), and subsequent low levels of nitrogen in the soil (Murthy and Ravindra 1975).

Lodhi (1978b) commented on toxic litter extracts from forests which inhibit *Nitrosomonas* and *Nitrobacter*, and hence maintain NH_4^+ at high levels in the soil, and Munro (1966a,b) noted the same in a perennial grass system where exudates from grass roots were the controlling agents, managing the system so as to slow further species progression.

2.4.4 Plant/insect interaction

Plants may utilise their exuded chemicals to ward off attack by insects. Green peach aphid (*Myzus persicae* Sulz.) is repelled by the nicotine in tobacco plants (Self, Guthrie and Hodgson 1964; Thurston *et al.* 1966), hence the destruction potential of the aphid is minimised.

Some plant chemicals known to be toxic to insects have been employed as insecticides, for example, pyrethrins from *Chrysanthemum cinerariifolium* (Rev.) Vis. (*Tanacetum cinerariifolium* (Trev.) Sch. Bip.) (pyrethrum), rotenoids from legume roots (Harborne, 1977), and nicotine from tobacco (Thurston *et al.* 1966).

While acting as repellents to some insects, certain phytochemicals may be attractants, or feeding stimuli, to other insects. Some species of milkweed (Asclepiadaceae), for example, Ascelpias curassavica (L.),

contain several cardiac glycosides which are both bitter and toxic, but are fed on by the Monarch butterfly (*Danaus plexippus* L.) and four other danaid butterflies as a preferred plant. The Monarch butterfly caterpillar adapts to cope with the glycosides which are sequestered during feeding and stored within the body (Whittaker and Feeny 1971, Rothschild, 1972; Roeske, Seiber, Brower and Moffitt 1976). Similarly, the Tiger (*Arctia caja* L.) and Cinnabar (*Tyria jacobaeae* L.) moths preferentially feed on *Senecio* species which contain a series of pyrrolizidine alkaloids, by sequestering them during feeding. These insects then use the presence of these phytochemicals in their bodies as protection against predation themselves (see section 2.4.8). The Tiger and Cinnabar moths further the use of the phytochemicals by passing them on in their eggs, to protect the offspring (Harborne, 1977).

The mulberry (Morus alba L.)/silkworm (Bombyz mori L.) relation is also based on phytochemicals. An olfactory attractant, a monoterpene mixture, attracts the larvae to the mulberry. Once feeding, other chemicals promote continuation of feeding (Harborne, 1977). The weevil Sitonia cylindricollis Fahraeus is likewise attracted to Melilotus albus Medik. by coumarin (Akeson, Haskins and Gorz 1969).

Cucurbitacins are produced by the *Cucurbitaceae* which contain twenty tetracyclic triterpenes. These will attract the cucumber beetles *Acalymma trivittata* (Mannerheim) and *Diabrotica undecimpunctata howardii* Barber, but repel most other insects (Chambliss and Jones 1966). There is a trend in plants that increasing complexity of the phytochemicals will reduce the numbers of insects feeding on it as few insects would be able to detoxify all of the compounds in the chemical (Harborne, 1977). Polyphagous insects have high levels of oxidase activity to cope with

chemicals that they may encounter in feeding from several types of plants (Wasserman, 1979).

Methods of protection vary greatly. Reese (1978) suggests that protective phytochemicals may work in one of three ways:

- (a) they block the nutrient availability to the insect systems, for example, oak leaf tannins form a complex with insect proteins
- (b) digestibility is reduced, for example, creosote resins
- (c) they may reverse growth by affecting the hormone system, for example, certain diterpene acids react with cholesterol if cholesterol is present in large amounts.

2.4.5 Plant/animal (predator) interaction

As in defence against insects, phytochemicals can be employed by plants to evade destruction by vertebrate predators. Cyanogens, alkaloids and tannins may all be utilised to reduce palatability to the potential predator by adding a bitter taste (Harborne, 1977; Swain, 1977). Cyanogenic glycosides are only toxic once broken down by the animal digestion, as HCN or prussic acid are produced. Birdsfoot trefoil (Lotus corniculatus L.) and white clover (Trifolium repens) are examples of plants which contain cyanogens (Jones, 1972). Some species of slugs and snails (Agriclimax reticulatus Müller, Arianta arbustorum L., Helix aspersa Müller) and the vole Microtus agrestis L., selectively eat the cyanogenic form of L. corniculatus and T. repens, and must therefore be adapted to cyanide in their diet (Jones, 1962; Harborne, 1977). There is also much evidence of detoxification systems in sheep and cattle: the enzyme rhodanase converts cyanide to thiocyanate which is harmless and passes out of the animal's system. Should a mild HCN poisoning occur, sheep will cease feeding until the toxin clears from their systems

(Harborne, 1977). Death may ensue if a single large intake occurs and the detoxification system cannot cope.

Ehrlich and Raven (1965) suggest that the presence of toxic phytochemicals has been largely responsible for the generation of terrestrial species diversity, as different animal species adapted to cope with different phytochemicals. The preceding examples lend some weight to this theory, as adapted species can ingest these plants without danger unless an excess suddenly occurs, while many other herbivore species cannot without danger of death.

2.4.6 Plant/plant/animal interaction

Three way interactions involving allelotoxic chemicals occur which reduce the competitive ability of a plant. An example quoted by the National Academy of Science (1971) is that of Amaranthus hybridis L.. Exudates from neighbouring Ambrosia artemisiifolia L. (low ragweed) resulted in stunted plants of Amaranthus which in turn were selectively grazed by field voles (Microtus), and did not regrow.

2.4.7 Plant/plant/weather interaction

National Academy of Science (1971) cites another example of reduced competitive ability after allelotoxins had played a role. Goldenrod (*Solidago* spp.) reduced the growth of tulip poplar (*Liriodendron tulipifera* L.) seedlings to about 2.5 to 5cm, compared to 40 to 45cm for control plants. In winter, frost-heaving eliminated both tall and short plants, but the stunted ones were more vulnerable.

2.4.8 Plant/insect/predator interaction

Two examples of this involvement of phytochemicals have already been mentioned in section 2.4.4. They involve alkaloids eaten, sequestered

and stored by the Tiger and Cinnabar moths, and cardiac glycosides likewise treated by the Monarch butterfly and the Tiger moth. The adults use coloration to warn potential predators of their stored body allelotoxins. The compounds are bitter, and cause vomiting so that once the predator tastes one, it will not try another, so affording protection to the species (Harborne, 1977). The coloration is often mimiced by other insects that do not contain the toxins. In Australia this type of mimicry (Batesian) occurs with the Coleopteran *Metriorrhynchus rhipidius* (Macl.), a distasteful insect, being mimiced by four other Coleopteran and one Lepidopteran insects (Norris, 1970).

2.4.9 Plant/plant interaction

Higher plant species may produce chemicals which are directly effective against other higher plants - allelochemicals (Rice, 1974). Table 2.3 lists some examples of these.

2.4.9.1 Enhancement of competitive ability

Instances of enhanced competitiveness have been reported since early in the 19th Century, with Ahlgren and Aamodt (1939) presenting good evidence to suggest negative root interactions as a cause of reduced growth of some grasses when grown in mixed plots. They showed that, when grown with red top (Agrostis alba auct. non L.), the growth of timothy grass (Phleum pratense L.) and Kentucky blue grass (Poa pratensis L.) was reduced. Growth of Canada blue grass (Poa compressa L.) was reduced by the presence of Kentucky blue grass. They eliminated competition for water, light and nutrients, and temperature stress as possible causes of the results.

Other examples of allotoxic effects include both crop and weed plant species. Some of these are listed in Table 2.3.

Chemical released from:	Area	Toxic to:	Cited in:
Oat	roots	Sinapis arvensus L.	Martin and
Rye	roots	(charlock) Matricaria marisima L. (scentless mayweed)	Kademacher 1999
Hordeum sativum Pers. (barley)	n.s.	weeds	Went, 1970
Guayule	n.s.	itself	Bonner, 1950 Went, 1970
Tobacco	leaf, stem	n.s.	Dieterman et 11., 1964
Camelina гіуввит	leaf	flax (linseed)	Grümmer & Beyer 1960 Grümmer, 1961
Cumelina sativa	tops	linseed	Lovett, 19825
Sorghum halepense (L.) Pers. (Johnson grass)	n.s.	n.s.	Abdul-Wahab & Rice,1967
Parthenium lysterophorus L. (carrot weed)	pollen	tomato, chilli, french bean (<i>Phaseolus vulgaris</i> L.)	Char, 1977
Helianchus scaberrimus Ell. (Prairie sunflower)	rhizomes	itself <i>H. occidentalis</i> Riddel	Curtis & Cottom 1950
3. хтаз	all parts	itself Smigeron canadensis L. (horseweed), Digitaria sanguiralis (L.)Scop. [G] (crab grass), Haplopappus ciliatus (Nutt.) DC	Wilson, 1968 Wilson & Rice, 1968
Salvia reflexa	decomposing foliage	n.s.	Lovett, 1982b
Artemisia abeinthium	leaf	n.s.	Bonner, 1950 Börner, 1960 Reynolds, 1975
Artemisia tridentata	tops	wheat	Weaver & Klarich, 1977
Brassica nigra	standing dead stalks and leaf in soil	Avena fatua, 3rcmus rigidus auct. non Roth (great brome) Bromus mollis L. (soft brome)	Bell & Muller, 1973

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Table 2.3: Crop, weed, shrub, grass and tree species known to release allelochemicals

Chemical released from:	Area	Toxic to:	Cited in:
Larrea divaricata Cav. (creosote bush)	tops	itself	Went, 1978
Encelia farinosa Gray. (brittle bush)	leaves	understorey spp.	Gray & Bonner 1948
Pholistoma auritum (Lindl.) Lilja. (a waterleaf)	litter	A. fatua Bromus diandrus Roth (great brome)	Parker and Muller 1979
Salvia leucophylla	leaf	annual grassland spp.	Muller, 1968, Muller & Muller 1964, Muller and Hauge 1967
S. mellifera	leaf	annual grassland spp.	Muller & Muller 1964
S. apiana	leaf	annual grassland spp.	Muller & Muller 1964
Sweet vernal grass	tops	other grass spp.	Newman & Rovira 1975, Reynolds, 1975
Holchus lanatus L. (Yorkshire fog grass)	tops	other grass spp.	Newman & Rovira 1975
<i>Lolium perenne</i> L. (Perennial ryegrass)	tops	other grass spp.	Newman & Rovira 1975
Sestura amundinacea Schreb. (Fescue)	tops	Loblolly pine (<i>Pinus taeda</i> L.)	Wheeler & Young 1979
Phalaris arundinacea L. (Reed canary grass)	tops	other grass spp.	Grant & Sallans 1964
Bromus spp. (brome grass)	tops	other grass spp.	Grant & Sallans 1964
Lucerne	tops	other grass spp.	Grant & Sallans 1964
Red Clover	tops	other grass spp.	Grant & Sallans 1964
Ladino clover	tops	other grass spp., itself	Newman & Rovira 1975
Walnut	canopy	understorey spp.	Börner, 1960 Went, 1970

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Went, 1970 Reynolds, 1975

2.4.9.2 Autotoxicity

Some of the species cited in Table 2.3 are autotoxic, for example, sunflower (Wilson and Rice 1968), *Salvia* spp. (Muller, 1966), guayule (Went, 1970), creosote bush (Went, 1978), red clover (*Trifolium pratense* L.) (Grant and Sallans 1964) and white clover (Grant and Sallans 1964; Newman and Rovira 1975), and inhibit their germination and/or growth markedly. The "fairy ring" phenomenon demonstrates the autotoxicity of some species to their own seedlings. These bare rings are caused by leachates from the living plant, and from senesced plants and debris. They have been reported in *Helianthus* (Cooper and Stoesz 1931; Garb, 1961; Muller, 1969; Audus, 1972), *Bromus* and *Hieracium* (Muller, 1969), and *Antennaria*, *Aster* and *Erigeron* (Audus, 1972). Del Moral *et al.* (1978) reported a similar effect, a bare area around an existing individual, in *Eucalyptus baxteri* forests in Australia.

2.4.9.3 Smother crop effects

Some cereal crops are particularly noted for their smother crop effect in defeating weed competition. Barley is a well known example, the effect generally being regarded as the result of competition for water, nutrients or light. Overland (1966) has reported, however, that while barley is a good competitor for the mentioned resources, it also exudes an alkaloid - "gramine", which enhances the ability of barley to reduce weed growth.

Mann and Barnes (1952) reported that barley, even at a low sowing density, reduced the growth of clover sown with it by over 50%. Clover reduced the barley growth but the difference was non-significant. The data here add support to the allelotoxin theory as the growth reduction in clover was achieved while barley densities were low but did not alter when barley densities were markedly increased.

Fay and Duke (1977) reported allotoxicity in *Avena* germplasm, with reductions in growth of Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), red root pigweed (*Amaranthus retroflexus* L.), wild mustard (*Brassica kaber* (D.C.) L.C. Wheeler var. *pinnatifida* (Stokes) L.C. Wheeler) and oats.

2.4.9.4 Crop residue effects

Soil sickness problems have been noted in horticultural and agricultural areas for many years. In fact, crop rotations have been suggested, often unknowingly, because of this effect. The first recorded suggestion of phytochemical interactions was by Plenk (1795), and this was elaborated by de Candolle (1813,1832). Pickering (1914) suggested that grass toxins released on decay of litter caused "soil fatigue". He was supported by Russell (1914) and Whitney (1909) who reported soil sickness in wheat fields in the U.S.A.. Collison (1925) conducted experiments with wheat and oat straws, and timothy grass and alfalfa hays, finding that aqueous leachates from them all were toxic to barley seedlings in sand culture. He identified two components of the leachate as vanillin and salicylic acid, but did not confirm their toxicities. Schreiner and Sullivan (1909) working with cowpeas (*Vigna spp.*) and soil fatigue also demonstrated the toxicity to cowpeas of cowpea litter leachates. No chemicals were identified.

More recently, the allelotoxicity of cereal straws has been examined further. McCalla and Duley (1949) showed that aqueous extracts of wheat straw inhibited the growth of corn; Guenzi and McCalla (1962) and Guenzi, McCalla and Norstadt (1967) demonstrated the toxicity to sorghum, wheat and corn seedlings of wheat and oat straw, soybean and sweet clover hay and corn and sorghum straws and stalks, and Audus (1972) published data on the toxicity to seedlings of wheat, oat, rye and barley of aqueous leachates from all four of these cereals. The data of Tinnin and Muller (1972) suggested toxicity of *Avena fatua* leachates to itself, *Bromus rigidus*, *Silybum marianum* (L.) Gaertn. (variegated thistle) and *Centaurea melitensis* L. (cockspur thistle). Direct contact with barley, rye and wheat residues, grown with or without vetch (*Vicia* spp.), reduces the growth of lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea* L.) seedlings (Patrick *et al.* 1963).

Other crops have also been studied. McCalla and Army (1961) noted toxicity of sweet clover (*Melilotus* spp.) residue to corn germination and seedling growth, Patrick and Koch (1958) showed toxicity of leachates of timothy grass, corn, rye and tobacco plants, and Megie *et al.* (1967) suggested that cotton residue increased the NH_4^+ nitrogen in the soil, which in turn raised the pH, and decreased subsequent growth of alfalfa, sudan grass (*Sorghum sudanense* (Piper) Stapf) and peanuts (*Arachis hypogaea* L.).

In a recent review (Lovett, Hoult, Jessop and Purvis 1982) the point is made that crop residues in stubble retention systems promote both positive and negative effects. The former include improved organic matter content in soils and improved infiltration of moisture, the latter relate particularly to the effects of phytotoxins.

In natural communities litter from shrub species has been shown to be allelotoxic to grass, herb and forb species in the Californian grasslands and chaparral areas (Muller and Muller 1964; Muller, 1966,1970, 1971; Muller and Chou 1972; Parker and Muller 1979). Börner (1971) and McCalla (1971) have both reported that bark residues in apple (*Malus* spp.) orchards can result in the production of an inhibitory toxin, patulin, by *Penicillium* spp. (*P. urticae* and *P. expansum*), which may result in the soil sickness problem, and inhibition of apple seedlings and replants.

Lodhi (1978a) found that litter leachates of red and white oak, sycamore and hackberry trees inhibited the nitrification process in the soil, which may indirectly limit growth of other plant species or individual plants.

2.4.9.5 Plant succession

Went (1970) has suggested that the growth of plants in arranged communities is a result of the presence of organic chemicals, rather than a consequence of physical effects such as shading. Communities of species tend to be constant in some areas (for example, the Californian and soft chaparral in California, old-fields and wasteland in western and central U.S.A.) with predictable species compositions. These three examples appear to employ allelochemicals in interference, and tolerant species only survive. The soft chaparral dominants are *Salvia leucophylla*, *S. apiana*, *S. mellifera* and *Artemisia* spp. The *Salvia* spp. produce volatile substances (see Table 2.2) which accumulate and inhibit the growth of other shrubs and grasses during dry periods of the year. Rain reduces the toxicity by leaching compounds off soil colloids and through the profile (Muller and Muller 1964; Muller, 1966,1970,1971; Muller and Chou 1972). The toxins produced are autotoxic, and will eventually destroy the *Salvia* spp. when the concentration becomes sufficiently high.

Californian chaparral dominants are Salvia mellifera, Lepechinia calycina (Benth.) Epl. in Munz (pitcher sage), Adenostoma fasciculatum H. & A. (Chamise) and Arctostaphylos glauca and A. glandulosa Eastw. (Eastwood manzanita) (Muller, 1970,1971; Chou and Muller 1972; Muller and Chou 1972). Adenostoma fasciculatum and Arctostaphylos glauca produce water soluble allelotoxins which again limit the viability of understorey grasses and forbs. Removal of the overstorey, usually by fire, is necessary to eliminate the allelotoxins and allow grasses and herbs to regrow. The shrubs are the "climax" stage, which will return four to five years after a fire.

Not all dominant species use chemical means to outcompete neighbouring plants. Montenegro, Rivera and Bas (1978) reported that the dominants in the Chilean matorral, a community similar to the Californian chaparral, do not exude allelotoxins and note that the annual species diversity and abundance are much greater than in the chaparral.

Kochia scoparia (Lodhi, 1979) acts in a similar fashion to Salvia spp., in old mining areas in North Dakota. These areas are established with this species within one to three years after abandonment. By the time the species has been established for one year, the plant dominates the community (51 to 100%), and is up to 1m tall. In the second year large numbers of seeds germinate, but the plant reaches only 3 to 6cm tall. By the third to fourth years the species has completely autotoxified itself out of the community.

The succession of plant species in abandoned cropping fields is well discussed by Rice and co-workers (Rice, 1964,1968,1971b; Wilson, 1968; Wilson and Rice 1968). *Helianthus annuus* is the dominant species in Stage 1 which secretes allelotoxic chemicals that inhibit other Stage 1, 2 and later species except *Croton glandulosus* L. (Stage 1) and *Aristida oligantha* Michx., the dominant in Stage 2. The allelotoxic chemicals also indirectly slow succession by inhibiting nitrogen fixing soil microflora (Rice, 1964; Rice, 1971b). Murthy and Ravindra (1975) report a similar indirect effect with *Aristida adscensionis* which produces chemicals that inhibit nitrogen fixation and hence availability, to regulate the rate of succession.

From the literature it appears that very many, if not all, plants produce allelochemicals, primary (nutritional) and secondary. Many of the allelochemicals produced are involved directly with insect/ predator deterrence and/or insect/predator feeding stimuli (including humans as predators), but this thesis is concerned with the allelochemicals in plant interference, that is, their allelopathic effect.

Even though plants do constantly produce allelochemicals, the timing of release of them is extremely important in determining their effect. As mentioned in section 2.2.1, the time of rains may affect the concentration of allelochemicals released. The health and age of both the producer and the receiver plants may also change the situation; a damaged producer may release more allelochemical, a non-vigorous receiver may succumb more quickly, or to a lower concentration of allelochemical; an older plant may produce more allelochemicals; a younger plant may be less resistant to them. It often appears that in allelochemical interactions, a set of particular circumstances must exist to enhance the effect of the aggressor plant's survival methods.

CHAPTER 3

THE SUNFLOWER

The sunflower is a member of the Asteraceae, the largest plant family, and one generally regarded as being the oldest family of contemporary plants. Many members of the family have become established as weeds (Matheson, 1976), some very important ones being *Kanthium* spp. (L.), *Senecio* spp. (L.), *Onopordum* spp. (L.), *Cirsium* (Mill.) and *Carduus* (L.) spp., while many others are cultivated as vegetables, including lettuce, endive (*Cichorium endiva* L.), Jerusalem artichoke (*Helianthus tuberosus* L.) and globe artichoke (*Cynara cardunculus* L.), as oil seed crops such as sunflower and safflower (*Carthamus tinctorius* L.), or for rubber yielding latex as in *Taraxacum bicorme* Dahlst. (Clapham, Tutin and Warburg 1962; Lovett, Harris and McWilliam 1979). Relatively few Asteraceae members have developed into useful field crop plants (Heiser, 1976).

The subfamily Liguliflorae or Cichorioideae contains the latex producing species, and *Helianthus* belongs to one of the ten tribes of this subfamily, the *Heliantheae* (Clapham *et al.*, 1962; Matheson, 1976). *Helianthus* contains approximately 70 species, separated into four groups. *Helianthus annuus* and 13 other species comprise the Annui section, all of which are diploid, generally annuals, and tap-rooted (Heiser, 1976). Two forms have achieved domesticated status as ornamentals or as seed and forage crops; the remaining forms are weed types (Matheson, 1976) found in most regions of the United States of America (Irons and Burnside 1982).

3.1 Morphology

The cultivars and ecotypes of the sunflower vary greatly in

stature, outer ray floret colour and leaf form (Lovett *et al.*, 1979). Generally, the plant is an erect, hirsute, annual herb from 0.7m tall, for example, in the Romanian hybrid cultivars, to more than 3.5m for the giant Russian open pollinated types. A strong tap root to a depth of 3m is accompanied by a large spread of lateral surface roots. Stems are generally unbranched in cultivars but profuse branching occurs in some ecotypes and in the older varieties, for example, bird-seed types (Figure 3.1A). The branching habit produces heads that mature over a long period, making them unsuitable as crops (Clapham *et al.*, 1962; Purseglove, 1968; Beadle, Evans and Carolin 1972; Lovett *et al.*, 1979).

Stems are at first round, becoming thick, angular and woody with age. The xylem becomes abundant, the pith often hollowing. Lower leaves are cordate and opposite, becoming ovate and alternate in spiral with two-fifths phyllotaxy. The petiole is long, decussate; the laminae have three main veins (Figure 3.1A), are sinate-toothed, hispid with stiff appressed hairs on both sides. Leaf dimensions range from 10 to 30cm in length by 5 to 20cm wide, with the tip acute or acuminate (Clapham *et al.*, 1962; Purseglove, 1968; Beadle *et al.*, 1972; Lovett *et al.*, 1979).

In the crop types, the stem terminates in a capitulum 7 to 50cm in diameter, often drooping. The receptacle is flat or dilated and convex; involucral bracts are ovate or ovate-lanceolate, acuminate, ciliate and arranged in three rows. Outer ray florets (Figure 3.1B) are neuter with ligulate, elliptic corolla, which are showy and yellow in most cultivars. Colours may range through orange to red and purple hues in some ecotypes. These florets are strongly two-nerved, deciduous and about 6cm long by 2cm wide. Disc florets (Figure 3.1C,3.1D) are numerous



Figure 3.1: Helianthus annuus: sunflower.

- A: flowering shoot $(x \frac{1}{2})$; B: ray floret (x 1);
- C: portion of capitulum in longitudinal section $(x l_{2}^{l});$
- D: disc floret in longitudinal section $(x \ 4)$;
- E: achene (x 3)

(After Purseglove, 1968).

and spirally arranged, hermaphroditic, about 2cm long and subtended by a bract. There are two chaffy, deciduous pappus scales. The corollas (Figure 3.1D) are tubular, dilated at the base, somewhat hairy and fivelobed. There are five stamens with free, flattened filaments; the anthers are long, connate, ending in a triangular appendage and often dark brown in colour. The ovary is inferior, pubescent, with a single basal ovule; the style is slender and the stigma two-lobed (Purseglove, 1968; Lovett *et al.*, 1979).

The seed (Figure 3.1E) from the cultivars is an obovoid, compressed achene, variable in size but seldom less than lcm long. The ecotype seed is often less than lcm long (down to 0.4 to 0.5cm). Colour ranges in all types from the most common black, through brown, cream and white, to white or grey with black stripes or spots (Clapham *et al.*, 1962; Purseglove, 1968; Lovett *et al.*, 1979).

The sunflower is generally self-incompatible, and requires insects to effect cross fertilisation. Absence of honey and bumble bees can severely limit the seed set of the crop (Matheson, 1976).

3.2 History, Origin and Distribution

The evolutionary history of sunflower is unclear due to the lack of fossil evidence, but the early forms are thought to have originated in the northern foothills of the Central Highlands of Mexico (Matheson, 1976). It is thought that this pioneer type - *Helianthus annuus jaegeri* Heiser, was a short, winter growing, tap-rooted, semi-indeterminate annual that produced small, cross-pollinated, self-incompatible heads (Heiser, 1976; Matheson, 1976).

Dispersion started with the adoption of the plant as an oil crop by the nomadic American Indian tribes, who took the plant with them

across what is now southern U.S.A. Mutations resulted in a longer life cycle and subsequently an enlarged vegetative form and increased reproductive efficiency. These types were more suited to favorable environments and were taken, by man, north along the western foothills of the Rocky Mountains toward the present Canadian border. The tribes had begun to realise the food crop value of the plant and began selection for a higher oil type (Heiser, 1976; Matheson, 1976). This type was a mutation, "transitional" *Helianthus annuus macrocarpus* (DC.)Ckll.. Figure 3.2 illustrates the possible migration of *H. annuus* across northern America (Heiser, 1976).





In 1510 sunflowers were introduced into Spain from Mexico (Purseglove, 1968). They reached Spain also via the eastern U.S.A., and from here went eventually to France and England (Matheson, 1976). In Europe the plant was generally regarded as an ornamental (Heiser, 1976; Matheson, 1976). The value of the seed was not realised until the plant reached Russia in 1830 where it was readily accepted as a crop, and selection for high oil content began seriously (Heiser, 1976; Matheson, 1976). After the success of the new types (the true form of *H. annuus macrocarpus*) in Russia, it was re-introduced to North America, and introduced to India, China, Africa, Australia and other countries (Matheson, 1976). However, it was not until the early 1960's, when Russia released a number of new high seed and oil yielding cultivars, that sunflower became a major world oil seed crop (Matheson, 1976).

3.3 World Status

3.3.1 As a crop

Improvement of sunflower began in earnest in Russia in 1860 (Purseglove, 1968; Heiser, 1976) with selection for higher seed oil content. Later, types were bred for large single heads; dwarfness for ease of mechanical harvesting; low hull content and resistance to rusts and other diseases and pests, including birds (Purseglove, 1968). In the early 1960's Russia released several cultivars with good crop characteristics and sunflower production expanded in many parts of the world (Matheson, 1976).

Russia is the largest producer of sunflower seed (Table 3.1) with over four million hectares sown to the crop, and a total annual seed production of almost five million tonnes. Following rapid expansion of the industry in the early 1970's the U.S.A. has been, since 1979, the second largest producer. Argentina is now ranked third. Production there began during the Spanish Civil war when outside supplies of olive oil were cut off (Purseglove, 1968; Heiser, 1976; Matheson, 1976). China has been expanding her sunflower industry with both increased areas sown to the crop and improved yields, and ranks fourth. Other major producing countries are Romania, Turkey, Hungary, South Africa, France, Bulgaria, Spain, Yugoslavia, Canada and Australia, with many other countries

	Area	harveste	d 1000	На	Yield		Kg,	/На	Producti	on	10	00 MT
	1969-71	1979	1980	1981	1969-71	1979	1980	1981	1969-71	1979	1980	1981
World	8413	12280	12 309	11661	1173	1266	1109	1193	9872	15544	13656	13908
U.S.S.R.	4682	4334	4353	4358	1293	1249	1069	1124	6055	5414	4652	4900
U.S.A.	110	2305	1597	1584	1101	1511	1118	1.354	121	3484	1786	2145
Argentina	1283	1557	1900	1280	739	918	868	984	949	1430	1650	1260
China	81	356	600	620	873	1053	1550	1613	71	375	930	1000
Romania	562	519	508	506	1370	1711	1608	1582	769	888	817	800
Turkey	347	445	575	450	1104	1326	1304	1278	383	590	750	575
Hungary	98	228	273	260	1243	1837	1671	2000	122	419	456	520
Sth Africa	140	306	288	300	757	1047	1143	1651	106	320	329	495
France	31	83	110	171	1768	2082	2475	2480	55	172	273	424
Bulgaria	277	230	247	230	1697	1854	1535	1478	471	426	380	340
Spain	179	638	699	720	813	790	736	450	146	504	492	324
Yugoslavia	199	257	180	210	1679	2043	1678	1381	334	525	302	290
Canada	48	161	136	122	809	1350	1219	1234	39	218	166	150
Australia	38	261	221	201	680	714	641	731	26	186	142	147

Table 3.1: Sunflower production - world figures (F.A.O. Monthly Bulletin of Statistics, 1982)

producing smaller yields (F.A.O. 1982).

90% of the seed produced is crushed for oil, producing a stable, well flavored oil which is high in polyunsaturated linoleic acid. Because of its high linoleic content, the oil is particularly suited to the manufacture of polyunsaturated margarines, its principal end use in Australia, while lesser quantities are used in cooking margarines and oils, salad oils, paints and varnishes, and cosmetics and soaps (Purseglove, 1968; Matheson, 1976). The oil cake residue after crushing is high in protein (37%), is of high biological value and digestibility and hence $\langle \stackrel{\text{is}}{a}$ valuable stock feed (Purseglove, 1968). The seed kernels may be eaten raw, roasted or salted, or grown for bird seed, or used as a high energy addition to horse rations. The plant may also be grown for fodder and silage for stock, and can be used as a green manure crop (Purseglove, 1968).

3.3.1.1 Weed problems

The crop types of sunflower, while being high producers, need intensive management in order to achieve their potential yields. Weed infestation is a common yield eroder as the crop types are extremely weed susceptible due to their single stem habit and relatively low plant densities, particularly in the early stages of growth (up to initiation) (Matheson, 1976; Lovett *et al.*, 1979).

The most common weeds are summer grasses (Lovett *et al.*, 1979) which can be satisfactorily controlled by the pre-emergent herbicides Dalapon or Dowpon (2,2 DPA) (2,2-Dichloroproprionic acid), or the presowing weedicide Treflan (trifluralin) (Swarbrick, 1974). As sunflowers are susceptible to damage from the hormone type weedicides such as 2,4-D (2,4-Dichlorophenoxyacetic acid) and MCPA (methyl-chlorophenoxyacetic acid), broad leaf weeds such as Bathurst burr (*Xanthium spinosum* L.), Noogoora burr (*Xanthium pungens* Wallr.) and *Datura stramonium* are almost impossible to control in the growing crop (Matheson, 1976; Lovett *et al.*, 1979). The most commonly used weed control measures are fallowing and inter-row cultivation (Matheson, 1976; Lovett *et al.*, 1979).

3.3.1.2 Insect pests

The crop is attractive to many insect pests. The most common are the Rutherglen bug (*Nysius vinitor* Bergr.), the Green Vegetable bug (*Nezara viridula* (L.)), budworms (*Heliothis* spp.) and cutworms (*Agrotis* spp.) (Matheson, 1976; Lovett *et al.*, 1979). The cutworms feed at night and may chew young plants off at ground level, or strip the leaves between the veins (Matheson, 1976). *Heliothis* spp. feed on leaves, buds and flowers and can completely destroy young buds and severely damage flowering heads (Matheson, 1976; Lovett *et al.*, 1979). The sucking bugs can result in loss of vigour in the plant, and subsequent moisture stress conditions can cause severe losses (Matheson, 1976; Lovett *et al.*, 1979).

At times either of the two bugs or the *Heliothis* can assume plague proportions in individual crops. Control by chemical insecticides can result in death of pollinating insects and hence poor seed set (Matheson, 1976).

3.3.1.3 Pathogenic diseases

Sunflower crops can be attacked by a range of pathogens that may severely reduce seed yields. In Australia, rusts caused by *Puccinia helianthi* Schw. and *Albugo tragopogonis* Pers. ex S.F. Gray are widespread

and common (Brown, Kajornchaiyakul, Siddiqui and Allen 1974) in New South Wales (Middleton, 1971) and Queensland (Stovold and Moore 1972), but their potential to reduce yields has been minimised by the production of rust "resistant" hybrid lines (Brown, personal communication). The Noogoora burr rust (Puccinia xanthii Schw.) has become increasingly prevalent in recent years, being found on P. helianthi resistant cultivars (Allen, Brown and Kochman 1980), but at a low incident rate on adult plants. Charcoal rot (Macrophomina phaseolina (Tassi) Goid) is also widespread but more damaging in late summer crops (Allen et al., 1980), while Alternaria blight (Alternaria helianthi (Hansf.) Tubaki and Nishihara) has caused very large yield losses in restricted areas of Queensland (Allen et al., 1980). Head rots caused by Rhizopus sp., Alternaria sp. and Botrytis sp., Sclerotinia sclerotiorum (Lib.) de By. head and stem rot, root rots caused by Fusarium sp. and a leaf spot caused by Septoria helianthi Ellias and Kellerman have been observed in isolated fields but are not considered to be important to the industry as a whole. Allen (1972) also reported damage to individual plants by Verticillium wilt (Verticillium dahliae Kleb.), Fusarium wilt (Fusarium oxysporum Schlecht) and bacterial wilt (Pseudomonas solonacearum E.F. Smith), but states that these are uncommon in Australia. Other diseases include Powdery Mildew (Erysiphe cichoracearum D.C. ex Merat), Cercospora leaf spot (Cercospora sp.), stem rot associated with the Phomopsis sp., seed rot (Aspergillus sp.), bacterial leaf spot (Pseudomonas sp.) and dampingoff by Pythium sp. (Brown et al., 1974; Allen et al., 1980). Rigid quarantine regulations have been imposed to prevent the introduction of the serious overseas pathogen Downy mildew (Plasmopara halsteadii (Farl.) Berl. and Toni) (Brown et al., 1974).

3.3.1.4 Animal pests

Other pests commonly attacking sunflower crops include birds (which can reduce seed numbers per head by feeding, or decapitate plants and reduce the overall yield) and rodents (Purseglove, 1968; Matheson, 1976).

3.3.2 As a weed

While the single headed sunflower has become one of the world's major crop plants, the multi-headed type has remained a weed, particularly near its centre of origin, in the western and central United States of America (Rice 1968,1971a,b; Wilson and Rice 1968; Heiser 1975,1976).

The multi-headed sunflower is a minor weed in parts of Australia also, but generally only on roadsides and wastelands (Matheson, personal communication). It is thought that these weeds have reverted from birdseed and crop types.

3.3.2.1 Weediness in old fields in the U.S.A.

Most of the work on the weediness of wild sunflower has been carried out in Oklahoma by Dr. E.L. Rice and his co-workers (see reviews by Rice 1974,1979). The "wild" type sunflower *Helianthus annuus annuus* L. is a hybrid (Rice, personal communication) of *H. annuus jaegeri* - the "pioneer" type, and *H. annuus macrocarpus*. Data from abandoned fields and wastelands suggest that *H. annuus annuus* is an extremely successful weed, capable of establishing in an area and competing against existing plants. It is a prolific weed in central and western U.S.A. (Heiser, 1976; Matheson, 1976) invading abandoned, low fertility cropping land as a major component of the first (weed) stage (Rice 1964,1968,1971b; Wilson, 1968; Wilson and Rice 1968). Once established, the plant exudes sufficient allelotoxin(s) to inhibit, directly or indirectly, the germination and growth of several species of plants, excluding *Croton* glandulosus (a Stage 1 species) and Aristida oligantha, the major component of the second stage of succession (Wilson, 1968; Wilson and Rice 1968; Rice, 1971b). Eventually, sunflower and the other species of Stage 1 produce enough toxins to eliminate their own seedlings, A. oligantha begins to predominate, and Stage 2 is established (Wilson, 1968; Wilson and Rice 1968; Rice, 1971b).

3.3.2.2 "Fairy rings"

Evidence of sunflower toxicity was noted long before tests were carried out on germination and seedling growth of other Stage 1 species. What have been described as "fairy rings" were recorded in sunflower and a few other species by Cooper and Stoesz (1931) who did not suspect an allelotoxin, but water competition between the sunflower H. scaberrimus and other plant species. Garb (1961) confirmed the autotoxicity of H. scaberrimus, which caused the bare area around each plant, or "fairy ring". Curtis and Cottam (1950) reported these rings around several sunflowers including H. scaberrimus and H. occidentalis. H. tuberosa L. and H. grosseserratus Martens showed no ring effect. They removed soil from inside the rings and demonstrated allelotoxic effects, probably from underground plant parts, on bioassay species. Later, Muller (1969) described "fairy rings" in Helianthus, Bromus and Hieracium, and Audus (1972) in Helianthus, Antennaria, Aster and Erigeron. Curtis and Cottam (1950) suggest that rings are a widespread feature of the Asteraceae and other aggressive families.

3.3.2.3 Nitrogen fixation

The identity of the phytochemicals responsible for allelotoxicity in the wild type *H. annuus* has largely been determined (Table 3.2).

They not only have a direct effect on seed germination and seedling survival, but also an indirect effect on vigour via the nitrogen cycle. The pioneer species in these abandoned cropping areas have low requirements for both nitrogen and phosphorus (Rice et al., 1960; Rice, 1964; Wilson and Rice 1968). All Stage 1, and some Stage 2 plant species can therefore establish, grow, and reproduce well under these conditions. The Stage 1 species include Ambrosia psilostachya DC., Erigeron canadensis, Chenopodium album L., Sorghum halepense, Digitaria sanguinalis, Bromus japonicus Thunb., Croton glandulosus and Haplopappus ciliatus. The Stage 2, low fertility tolerant, species is Aristida oligantha. In a plant community where passive competition only existed between plants, the growth and subsequent senescence of these plants would eventually result in the build up of organic matter and hence mineral nutrients in the soil surface layer. More advanced plant types (higher in nitrogen and phosphorus demand) would then be able to colonise, and successive stages would result (Rice $et \ all$, 1960; Wilson and Rice 1968; Blum and Rice 1969). However, the release of allelotoxins by Stage 1 plants can confound this progression. The allelotoxins from Stage 1 plants, particularly H. annuus, can suppress nitrogen fixation both by free living nitrogen fixers such as Azotobacter (Rice, 1965), Nitrobacters and Nitrosomonas (Rice, 1964,1971b) and via the action of symbiotic nitrogen fixation by Rhizobium (Rice 1965, 1968, 1971b; Blum and Rice 1969).

i) Free living nitrogen fixers

Rice (1964,1971b) found that allelotoxins present in all parts of wild sunflower (ground or washed) inhibited the action of *Nitrosomonas* and *Nitrobacter* in soil, hence limiting the chemical changes of NH_A^+ to

nitrite to nitrate. He found that these allelotoxins (identified mainly as chlorogenic acid and iso-chlorogenic acid) were slightly adsorbed by kaolin and activated charcoal, allowing activity of the *Nitrosomonas* and *Nitrobacters*. Leaf washings were more inhibitory than any other plant part and washings of young plants were generally more inhibitory than washings of older ones.

ii) Symbiotic nitrogen fixation

The formation and function of nodules and leghaemoglobin have also been repressed by sunflower allelotoxins in certain legumes (Rice, 1968,1971a). When grown in the same pots as the test species (red kidney bean), *H. annuus* and *Ambrosia psilostachya* plants produced small grey nodules on the red kidney bean compared to the healthy bright pink nodules in the control red kidney bean plants. The number of nodules was also decreased by the presence of *H. annuus* in the red kidney bean pots (Rice, 1963).

Root exudates of *H. annuus* have been found to reduce mean nodule number and size and change the colour (to grey) of nodules of red kidney bean, Korean lespedeza and white clover. Leaf leachings produced similar, but more marked, results (Wilson and Rice 1968; Rice, 1968,1971a,b).

Blum and Rice (1969) tested two of the identified components of H. annuus chemical exudates, gallic and tannic acids, and found that gallic acid at 10^{-2} M increased nodule number of red kidney bean, while at 10^{-6} M decreased it. Both concentrations decreased leghaemoglobin content of red kidney bean nodules, but neither influenced plant weight. Tannic acid at 10^{-10} M killed the test plants. At lower concentrations, tannic acid decreased nodule numbers, leghaemoglobin content and plant weight of the test plants.

iii) Algae growth

Parks and Rice (1969) found that soil samples taken from the immediate neighbourhood of *H. annuus* inhibited the growth of blue-green algae. As the distance from the *H. annuus* plant increased, the reduction in growth of the algae decreased.

iv) Nitrogen levels in soil

As already stated, in abandoned old fields in central and western U.S.A., the species in the successive stages of invasion require higher nitrogen and phosphorus levels. Sunflower, and to a lesser extent, the other plant species in Stage 1, can therefore limit the progression by their abilities to reduce nitrogen fixation in the soil. A. oligantha in Stage 2, however, also grows well on low nitrogen levels, and is immune to Stage 1 allelotoxins, hence can establish readily. Consequently, after the quick and profuse establishment of H. annuas as a dominant in the community, the build up of excreted allelotoxins poisons all of the Stage 1 species but does not prevent Stage 2 (principally A. oligantha) occurring after two to three years.

3.3.2.4 Changes with time, plant health and plant part

The concentration of each component of the chemical mixture released from H. annuus varies, as does the mixtures' concentration or amount, at different times and stages of plant growth. Koeppe *et al.* (1970a,b) have shown that chlorogenic acid, iso-chlorogenic acid and neo-chlorogenic acid increase in leaves as the leaves become older, with the concentration increasing from the top of the plant to the fifth node and decreasing slightly again in the bottom leaves (after the sixth to eighth node).

It has been found that when a plant is injured or stressed,

allelotoxin production and/or release is higher than in a healthy, nonstressed plant (Helder, 1956; Martin and Rademacher 1959; Tukey and Morgan 1963; Koeppe *et al.*, 1970a). Martin (1957) reported that release of allelotoxins is low from intact *H. annuus* roots, but Dieterman *et al.* (1964) sprayed *H. annuus* with 2,4-D and found that scopolin release was increased after damage occurred.

The concentration of allelotoxins varies with the part examined. Leaf leachates have been reported as more toxic to bioassay seedlings and seeds than leachates of other sunflower parts (Rice, 1964; Wilson and Rice 1968).

Sunflower seeds are also reported to contain allelotoxins. Lane (1965) identified chlorogenic acid and iso-chlorogenic acid in fruits of the native (U.S.) sunflower, Cater *et al.* (1972) identified chlorogenic acid, quinic acid and caffeic acid, Mourgue *et al.* (1975) found chlorogenic acid and quinic acid, and Dorrell (1976a,b) isolated chlorogenic acid. The toxicity of these organic acids has not, however, been confirmed (Dorrell, 1976a).

Wilson (1963) and Wilson and Rice (1968) have shown that allelotoxic chemicals are also released from roots and senesced leaves, and consequently are found in soil near *H. annuus* plants. Curtis and Cottam (1950) also reported allelotoxic secretions from underground parts of several sunflower ecotypes, causing the soil to be toxic to the bioassay species.

3.3.2.5 Changes with nutrient status

Plants can compete via their command over the soil nutrient pool (Donald, 1963; Harper, 1977). No work has been reported on changes in concentration of exudates under water stress but several workers have

found that certain nutrient deficiencies can increase the release of some allelotoxins. For example, Perkins and Aronoff (1956) found that low boron status increased the production of caffeic, quinic and chlorogenic acids and Watanabe *et al.* (1964) found that boron deficient *H. annuus* increased the accumulation of scopolin, scopoletin, esculin, isoquercitrin and a glucose derivative of gentisic acid, as well as chlorogenic acid.

Tests were conducted by Lehman and Rice (1972) on the effects of nitrogen, potassium and sulphur deficiencies on chlorogenic acid and scopolin in sunflower. Chlorogenic acid concentrations were generally higher in stems and leaves of mineral deficient plants than in control plants. Except for the nitrogen deficient plants, young leaves accumulated greater concentrations of chlorogenic acid than older leaves.

4-O-caffeolyquinic acid (Band 510) and neo-chlorogenic acid (5-O-caffeolyquinic acid) which were present in all plant tissues, but not at all times, increased in concentration in potassium deficient stems, nitrogen deficient old leaves and stems, and sulphur deficient old leaves, young leaves and stems. Neo-chlorogenic acid was also in higher concentrations in potassium deficient young leaves. Band 510 was at a lower concentration in nitrogen deficient young leaves and roots and neo-chlorogenic acid was reduced in roots of potassium deficient plants. Scopolin concentration increased in potassium deficient old leaves, young leaves and stems as the plants matured. Both sulphur and nitrogen deficiencies, however, decreased the concentration of scopolin. Sulphur deficiency had an overall non-significant effect, while nitrogen caused a reduction in concentration in stems only.

Koeppe $et \ al.$ (1976) found that phosphorus deficient sunflower

plants increased the total phenolics released and the concentration in the plant, and in particular increased the chlorogenic acid released from the plant.

3.3.2.6 Changes with temperature

Few data have been presented to quantify the effects of temperature on production of allelotoxins in the sunflower. Koeppe *et al.* (1970b) and Dorrell (1976b) have both stated that higher temperatures are likely to be a cause of increased production but have provided no data to back their statements.

3.3.2.7 Changes with ultra-violet radiation

High levels of impingeing ultra-violet radiation can cause stress conditions. Koeppe (1968), Koeppe *et al.* (1969) and Koeppe *et al.* (1971) have reported that high light intensities and high ultra-violet radiation result in increased scopolin and chlorogenic acid in both young and old leaves of sunflower, but more markedly in the older leaves. Koeppe *et al.* (1970b) and Dorrell (1976b) also put forward hypotheses on this effect but present no confirming data.

3.3.2.8 Allelochemicals present in sunflower

The range of allelochemicals identified from various parts of the sunflower to date, are listed in Table 3.2. The range is extensive, but consists predominantly of phenolic compounds.

The production of these compounds enables sunflower plants to colonise the low fertility, abandoned cropping areas of central Oklahoma and south eastern Kansas. High competitive stress can result in a higher concentration of some of these chemicals being produced to further enhance the competitive ability of the sunflower against other Stage 1 plant

Chemical	Extracted from:	Cited in:
Caffeic acid	green leaf near necrotic lesions (Boron deficiency symptom)	Perkins & Aronoff 1956
	leaves	Urban 1958
	seeds	Cater <i>et al</i> . 1972
Chlorogenic acid	all parts	Rice 1965; Wilson 1968; Wilson & Rice 1968
	leaf	Urban 1958 Koeppe <i>et al</i> . 1969 Koeppe <i>et al</i> . 1970b
	B deficient leaf	Watanabe et al. 1964
	green leaf near necrotic lesions	Perkins & Aronoff 1956
	leaf, stem	Lehman & Rice 1972
	seeds	Joubert 1955 Lane 1965 Cater <i>et al</i> . 1972 Dorrell 1976a,b
	n.s.	Koeppe et al. 1976
4-0-caffeoly- quinic acid	all parts	Lehman & Rice 1972
neo-chlorogenic acid	older leaves	Koeppe et al. 1970b
	all parts	Lehman & Rice 1972
iso-chlorogenic acid	all parts	Rice 1965; Wilson 1968; Wilson & Rice 1968
	older leaves	Koeppe et al. 1970b
	seeds (during imbibition)	Lane 1965
	seeds	Mourgue et al. 1975
esculin	B deficient leaf	Watanabe et al. 1964
glucose derivative of gentisic acid	B deficient leaf	Watanabe $et al$. 1964

Table 3.2: Allelochemicals identified from leachates or washings of sunflower (*H. annuus*).

Table 3.2 continued:

Chemical	Extracted from:	Cited in:
α-naphthol derivative	leaf	Wilson 1968 Wilson & Rice 1968 Rice, personal communication
isoquercitrin	B deficient leaf	Watanabe $et \ all$. 1964
quinic acid	green leaf near necrotic lesions	Perkins & Aronoff 1956
	seeds	Cater <i>et al</i> . 1972 Mourgue <i>et al</i> . 1975
scopoletin	roots	Martin 1957
	B deficient leaf	Watanabe <i>et al</i> . 1964
scopolin	leaf	Urban 1958 Wilson 1968 Wilson & Rice 1968 Koeppe <i>et al</i> . 1970b
	old leaves, stem	Lehman & Rice 1972
	U.V. light stressed leaf	Koeppe <i>et al</i> . 1969
	B deficient leaf	Watanabe <i>et zi</i> . 1964

species and other latter stage species (excluding *Croton glandulosus* and *Aristida oligantha*). The allelotoxins may have a synergistic effect in maintaining the competitive advantage (Wilson, 1968; Einhellig, Rice, Risser and Wender 1970; Irons and Burnside 1982). However, the fact that these chemicals are autotoxic means that eventually they reach a sufficiently high concentration in the soil to restrict sunflower germination and seedling growth. *A. oligantha* is immune and hence establishes as a dominant, and the progression continues. *A. oligantha* also produces allelotoxic chemicals - allotoxins, to maintain its dominance, and has been known to be the dominant species in fields for over 30 years (Rice, 1974).

3.4 Exploitation of Allelopathy

Although allelopathic interactions between plants are often inconspicuous and may need a certain set of environmental conditions to be fully expressed, it is thought that virtually no plant is entirely free of "metabolic trash" chemicals (Waller and Nowacki 1978). While crop plants have lower levels of allelotoxic chemicals, due to conscious or unconscious selection against them, their ancestors are generally far more belligerent. The ability to produce allelochemicals very often imparts self-defence to the plant against a range of organisms - insects, herbivores and pathogens (Harborne, 1977; Swain, 1977). Putnam and Duke (1978) put forward the idea of "exploiting allelopathy in agriculture" to reduce the huge annual losses of crop yield attributable to depredations by weeds. This idea could apply to insect and disease resistance as well.

A better understanding of the nature and effects of phytochemicals could lead to agricultural systems which require less energy input, less energy for maintenance, and provide a comparable, if not greater, quantity and quality of harvested product.

Many plant characteristics have been transferred by plant breeding from wild type species to crop types to improve the hardiness of the crop type. These include resistance to seed head shattering in phalaris (*Phalaris arundinacea*, McWilliam, 1963) and resistance to rust (*Puccinia helianthi*, Sackston and Jabbar Miah 1963) and downy mildew (*Plasmopara halsteadii*, Zimmer and Kinman 1972) in cultivated *Helianthus annuus*. A number of insect resistant or repellent crops have been, or are being developed, for example, lucerne cultivars resistant to their introduced aphid pests *Therioaphis trifolii* f. *maculata* (Spotted alfalfa aphid), *Acyrthosiphon kondoi* (Bluegreen aphid) and *A. pisum* (Pea

aphid (Lehane, 1982). The gene for resistance to spotted alfalfa aphid was found among wild biotypes of lucerne (Downes, personal communication). At present, a breeding program is being conducted to introduce *Sclerotinia sclerotiorum* rot resistance into cultivars of *H. annuus* from the Austraian biotype (Downes, personal communication). It should be a small step to further this research to include weed resistant crops.

The wild U.S. sunflower has been shown to contain potent allelochemicals (Irons and Burnside 1982) which control the sunflower/ weed system. Many other examples exist in agriculture, forestry and horticulture. The genetic potential for allelopathy may exist in wild relatives of crop plants, or may already be a part of the genetic makeup of cultivated forms. In order to increase the self-defence of the crop forms of a plant, a breeding program may be needed to include aggressiveness from wild types, genetic engineering to develop a "selfdefence gene", or simply selection among cultivated forms for the aggressive character.