INTRODUCTION

The phenomenon of allelopathy has been studied increasingly over recent years, and has been reviewed by a number of workers. It is now generally accepted as a factor in plant interactions, including competitive relationships, and includes effects on other plants, insects and fungi. As technology has developed more plants have been found to show allelopathic activity and an increasing number of compounds implicated in allelopathic effects.

Many weeds of cropping systems have now been identified as having allelopathic effects, both as living plants or as residues. Several authors have identified Brassica species residues as being allelopathic, with the glucosinolate compounds (prevalent in Brassica spp.) or their breakdown products implicated in some of these effects.

Rapeseed has been replaced with more recent varieties and is now called Canola. Canola differs from rapeseed in that the glucosinolate and erucic acid content in seed have been reduced by breeding to allow the oil and seed meal to be used in human and animal nutrition. Residues of these crops have been shown to suppress growth of some plants, and compounds produced from these residues have been hypothesised as playing a more general defensive role.

Canola grown in southern Australia is frequently followed by wheat. Much anecdotal evidence exists suggesting that wheat grows better following a canola crop, produces higher yields and is less affected by root disease pathogens.

The work reported here reviews the literature pertinent to allelopathy and Brassica spp. including the compounds possibly involved and interactions with other plant compounds.

In light of this review, canola residues are hypothesised as producing compounds which can affect the growth of other plants, as well as suppressing growth of soil borne fungal pathogens.

A series of experiments was conducted to investigate these hypotheses.
1. ALLELOPATHY IN AGRICULTURE AND NATIVE COMMUNITIES.

1.1 HISTORY OF STUDY:

The term "allelopathy" was first used by Molisch (1937) although the phenomenon of plants exerting effects on others had been observed by Theophrastus (285 BC). A number of other early workers noted effects which could have been early examples of allelopathy (Pliny 1st Century AD, deCandole 1832, Livingston 1907). Some early direct evidence of allelopathic responses were noted by Davis (1928), who found that walnut hulls and roots could yield juglone, which was highly toxic to tomato, potato and lucerne. Bode (1940) showed that absinthin exuded from Artemisia absinthium was deleterious to the growth of neighbouring plants. Bonner and Galston (1944) was also one of the first to identify compounds responsible for allelopathic effects, by showing trans-cinnamic acid responsible for the allelopathic activity of guayule residues.

Following these and other reports a number of workers including McCalla and Duley (1948), Evenari (1949), Muller (1953), and Rice (1974, 1984) took an interest in allelopathy. In the last 25 to 30 years greater study of allelopathy has been undertaken. The term originally covered both stimulatory and inhibitory effects, with the latter being the most frequently studied.

The term "interference" has been suggested by Muller (1969) to cover the combined effects of allelopathy and competition. Allelopathy differs from competition in that it refers to the addition by a plant of substances to the environment. Competition usually involves removal or reduction of resources from the environment by one (or more) plants to the detriment of others.

Rice (1984), in a comprehensive review, lists a number of ways in which allelochemicals can be introduced to the environment including volatilization, exudation from roots, leaching from plants or residues or products from decomposition (microbial) of residues. Interactions involving plant-insects and plant-higher animals could also be included. He also lists a number of growth processes which may be affected due to allelochemical action; cell division/elongation, action to alter phytohormone activity or production, enzyme-mediated activity, mineral uptake, photosynthesis and respiration,
stomatal opening, protein synthesis and changes in lipid and organic acid metabolism, hemoglobin synthesis and membrane permeability.

A number of workers have reviewed the field of allelopathy in recent times (Rice 1984, Lovett 1986, Lovett et al. 1989).

Allelopathy is now accepted as a widespread phenomenon in plant interactions, with direct effects on plants, and playing a role in general competitive interactions between plants and other organisms including insects and disease organisms. With improvements in technology in measuring the growth of plants, isolating and identifying compounds from extracts of plants, residues and soil, allelopathy has been found to be far more widespread in plant ecosystems. Lovett et al. (1989) comment on the broadening of the context of allelopathy, supporting Rice (1984) in including indirect effects of weakening plants thus rendering them more susceptible to attack from insects and diseases and decreasing their ability to absorb nutrients.

Similarly, the number of compounds implicated as allelopathic has also increased in the last 20-30 years. Many more complex molecules and mixtures of compounds are being identified as allelopathic as compounds can now be detected in smaller amounts.
2. CONTEXT OF ALLELOPATHY IN MANAGED AND NATURAL ECOSYSTEMS.

Allelopathic effects are believed to have implications in many aspects of agriculture and native plant ecosystems including weed-crop interactions, weed dominance and succession, residues and stubble mulch systems (Kimber 1967, Lovett 1987), crop rotations, orchards and forestry (Waller 1987), weed seed longevity and in the vegetation patterning seen in natural ecosystems (Rabotnov 1981).

2.1 NATURAL ECOSYSTEMS, Allelopathy and Interference.

Allelopathy has been documented in native or non-managed ecosystems by a number of authors (Willis, 1980, Bowman & Kirkpatrick, 1986a,b,c, Igboanugo, 1986, Vicherkova & Polova, 1986 and Lovett 1987, 1989).

Lovett (1987) reviews instances of allelopathic effects from native plants in Australia notably Eucalyptus spp. Among the plants listed are the following (Table 2.1).

Table 2.1. Plants Showing Allelopathic Responses in Natural Ecosystems. After Lovett (1987)

<table>
<thead>
<tr>
<th>Allelopathic species</th>
<th>Species affected</th>
<th>Effects seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus pilularis</td>
<td>E. pilularis seedlings</td>
<td>affects root and root hair growth and shoot growth</td>
</tr>
<tr>
<td>E. molluccana</td>
<td>Bothriochloa ambigua</td>
<td>grasses statistically sparser</td>
</tr>
<tr>
<td>Acacia pendula</td>
<td>Eragrostis leptostachya</td>
<td>beneath tree canopies</td>
</tr>
<tr>
<td>Casarina laubmanii</td>
<td>Sporobolus elongatus</td>
<td></td>
</tr>
<tr>
<td>Callitris calcarata, E. crebra</td>
<td></td>
<td>halo effect visible on aerial photographs, species inside 'halo' different to outside.</td>
</tr>
<tr>
<td>E. dawsonii, E. melliodora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notelaea microcarpa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus sp, Araucaria sp, Flindersia sp</td>
<td>Araucaria cunninghamil</td>
<td>seedling tip necrosis, chlorosis</td>
</tr>
<tr>
<td>E. bicostata</td>
<td>Trifolium repens</td>
<td>blackening of radicle tips, an exclusion zone round E. bicostata.</td>
</tr>
<tr>
<td></td>
<td>Festuca rubra var. fallax</td>
<td>Insect frass resulted in growth inhibition</td>
</tr>
</tbody>
</table>
Table 2.1 (continued) Plants Showing Allelopathic Responses in Natural Ecosystems.

<table>
<thead>
<tr>
<th>Allelopathic species</th>
<th>Species affected</th>
<th>Effects seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. baxterii</td>
<td>Leptospermum, mystinoides, Casuarina pusilla</td>
<td>inhibition of growth, suppression of noted species beneath canopy. May be effect from litter (chemical) leachates.</td>
</tr>
<tr>
<td>Nothofagus cunninghamii and other rainforest spp.</td>
<td>E. regnans seedlings</td>
<td>inhibition of growth, evidenced by dieback of mature trees; inhibition of regeneration of seedlings.</td>
</tr>
<tr>
<td>E. microcarpa</td>
<td>Genocarpus elatus</td>
<td>suppression of G. elatus below E. microcarpa.</td>
</tr>
<tr>
<td>E. regnans</td>
<td>E. regnans seedlings</td>
<td>failure of seedlings to regenerate in absence of fire. Rhizosphere fungus believed implicated via root exudates from E. regnans.</td>
</tr>
</tbody>
</table>

Allelopathic effects in native ecosystems (for example, eucalypt communities) have been reported both in Australia (Bowman and Kirkpatrick 1986a,b,c) and overseas (Igboanugo 1986, Vicherkova and Polova 1986). Eucalypt ecosystems present one example where allelopathic effects are likely well developed and manifest. The effect can be complex, involving other organisms, or interactions with external factors (eg. fire), and produce a number of observed effects. These include patterning of vegetation, influences on succession of species and inhibition of introduced species.

Bowman and Kirkpatrick (1986a,b,c), for example, believed growth patterns evident in Tasmanian forests to be at least partly due to allelopathic substances produced from eucalypt foliage. They also believed these compounds to be responsible for influencing the growth patterns (establishment, suppression and growth) of trees in the ecosystem.

Examples of allelopathic effects of eucalypt products (usually oils) include Igboanugo (1986), who showed a number of food crops to be susceptible to allelochemicals produced by *E. camaldulensis* Dehnh and *E. citriodora* Hook, using bioassays. Similarly Vicherkova and Polova (1986) used
essential oils (some from eucalypts) to show effects on beans (*Vicia faba* L.) and sunflower (*Helianthus annuus* L.).

A classic effect was demonstrated in this last example where low concentrations of the oils were stimulatory but higher levels were inhibitory. This stimulation at low levels of allelochemical products is frequently observed (Lovett and Duffield 1981, McFarlane *et al.* 1982; Leather, 1983; Lehl *et al.* 1983).

The possibility of eucalypt oils being the basis for the allelopathic effects seen in eucalypt communities raises the question of the potential that the oils contain compounds of possible herbicidal or other commercial plant regulatory value. Allelopathic affects of these oils may indicate other active compounds which could be investigated.

The above description of some allelopathic effects seen in eucalypt ecosystems illustrate not only that allelopathy is likely to have a strong influence on the composition of native ecosystems but also that other influences and interactions (for example, with microorganisms, fungi, animals or external factors) occur with allelopathy to produce the effects documented.

### 2.2 AGRICULTURAL ECOSYSTEMS

Interference in agricultural systems is widely recognised as a source of reduced yields in crop and pasture production. Evidence of allelopathy in weed-crop systems, involving residues (both weed and crop), and in aspects of seed dormancy exists. Allelopathic interactions between crops and weeds, both as living plants and as residues is well documented (Rice 1984, Elmore 1985, McLaren 1986, Lovett 1987). The effects of allelopathy in agricultural systems include not only competition-allelopathy “interference”, but also weed dominance and succession, residue management and stubble mulch farming, crop rotations and weed seed longevity.

#### 2.2.1 Living Plants as Donors of Allelopathic Substances.

A large amount of evidence exists of allelopathic interactions involving living plants, both crops and weeds, as donors of allelopathic substances.
Table 2.2 illustrates some examples of allelopathy which have been documented both in Australia and overseas.

**TABLE 2.2** SOME DOCUMENTED EXAMPLES OF ALLELOPATHIC INTERFERENCE IN AGRICULTURAL SYSTEMS.

<table>
<thead>
<tr>
<th>DONOR PLANT</th>
<th>AFFECTED PLANT(S)</th>
<th>EFFECT SEEN</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abutilon theophrasti</em></td>
<td><em>Glycine max</em></td>
<td>inhibits growth</td>
<td>Colton &amp; Einhellig 1980</td>
</tr>
<tr>
<td><em>Agropyron repens</em></td>
<td></td>
<td>invade &amp; dominate plant communities</td>
<td>Gabor &amp; Veatch 1981</td>
</tr>
<tr>
<td><em>Ambrosia cumansis</em></td>
<td>also autoallelopathic H.B.K.</td>
<td>inhibits growth, especially as breakdown products</td>
<td>Anaya &amp; del Amo 1978</td>
</tr>
<tr>
<td><em>Brassica nigra</em></td>
<td>grassland pasture spp</td>
<td>inhibitors released which inhibit germination</td>
<td>Muller 1969</td>
</tr>
<tr>
<td><em>Salvia reflexa</em></td>
<td></td>
<td>inhibition of germination by residues</td>
<td>reviewed by Lovett 1987</td>
</tr>
<tr>
<td><em>Camelina sativa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Polygonum aviculare</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Centaurea spp.</em></td>
<td>pasture spp.</td>
<td>dominates pastures</td>
<td>Stevens &amp; Merril 1985</td>
</tr>
<tr>
<td><em>Cirsium arvense</em></td>
<td></td>
<td>invade and dominate mixed pastures</td>
<td>Wilson 1981</td>
</tr>
<tr>
<td><em>Cirsium arvense</em> root extracts</td>
<td><em>Silybum marianum</em></td>
<td>Inhibition of germination</td>
<td>Bendall 1975</td>
</tr>
<tr>
<td></td>
<td><em>Hordeum vulgare</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lolium perenne</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cirsium arvense</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Trifolium subterraneum</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2.2 (continued) SOME DOCUMENTED EXAMPLES OF ALLELOPATHIC INTERFERENCE IN AGRICULTURAL SYSTEMS.

a) LIVING PLANTS AS DONORS.

<table>
<thead>
<tr>
<th>DONOR PLANT</th>
<th>AFFECTED PLANT(S)</th>
<th>EFFECT SEEN</th>
<th>REFERENCE</th>
</tr>
</thead>
</table>
| *Cyperus rotundus* tubers and roots | *Triticum aestivum,*  
                                      *Hordeum vulgare,*  
                                      *Cotton, Brassica spp.* | inhibits growth of target plants | Friedman & Horowitz 1970, 1971 |
| *Datura stramonium*             | *Sunflower*             | seed coat washings       | Lovett *et al.* 1981     |
|                                 | *Liquidambar styraeiflua L.*  
                                      *Lotus corniculatus*  
                                      *Trifolium pratense* |                                         |                           |
| *Imperata cylindrica* rhizomes and soil extracts | inhibitors from rhizome extracts and soil | Eussen *et al.* 1976     |
| *Setaria faberii*               | *Zea mays, Glycine max* | inhibits growth          | Knake & Slife 1961       |
| *Parthenium hysterophorus*      |                         | inhibits growth          | Tower *et al.* 1977      |
| *Polygonum aviculare*           | *Medicago spp.*         | cell division in meristems also deformed radicle | K loot & Boyce, 1982 |
| *Helianthus annus*              |                         | nodulation of a number of legumes | Rice 1984                |
| *Sorghum halepense*             |                         | inhibits seedling development | Nicoli er *et al.* 1983 |
| *Sorghum halepense* rhizomes    | *Hordeum vulgare,*  
                                      *Brassica spp.*  
                                      *Triticum aestivum* | inhibition of germination | Friedmen & Horowitz 1970 |
| *Sorghum halepense* rhizomes    | *Hordeum vulgare*       | residue in soil active 2 months after rhizomes removed from soil | Freidman & Horowitz 1971 |
| *Sorghum halepense*             | *Gossypium spp.*        | growth reduction         | Horowitz 1973            |
| *Sorghum halepense*             | *Glycine max*           | growth reduction         | Lolas & Coble 1982       |
It is interesting to note that many of the plants listed in the above table are weeds of many of the crop plants listed as affected. A review of allelopathic weeds and their effects on crops in the humid mid-south of the USA by Elmore (1985) lists 20 troublesome weeds of which 10 are believed to be allelopathic. The weeds listed include *Digitaria ciliaris*, *Amaranthus spp.*, *Sorghum halepense*, *Cyperus rotundus*, *Cyperus esculentus*, *Setaria faberi*, *Helianthus annuus*, *Cynodon dactylon*, *Abutilon theophrasti*, *Sida spinosa*, *Brachiaria spp.*, and *Sorghum bicolor*. Again, this reinforces the concept of allelopathy being part of many weeds' competitive ability.

2.2.2 Residues of Crops and Weeds as Donors of Allelopathic Substances.

Many plants, both weed and crop species, have been documented to show allelopathic effects as residues. In some instances the same plant is seen as allelopathic both as a living plant and as residue.

A number of reports of crop residues reducing the biomass of weeds exist (Forney *et al.* 1983, Lieble and Worsham 1983a, Putnam and DeFrank 1983, Putnam *et al.* 1983, Shilling and Worsham 1983, 1984). Aqueous extracts from a variety of crops have been widely used to approximate the allelopathic effects believed to occur *in vivo*. Work by Kimber (1973a) and reviews by Rice (1984) and Lovett (1987) detail many residues including wheat, barley, oats, lucerne, cereal rye and pea as showing allelopathic effects. For example, Lovett and Jessop (1982) showed aqueous extracts from barley, oat, field pea, field bean, rape, sunflower, sorghum, safflower, soybean, lupin, chickpea and wheat residues to caused inhibition to root length of wheat. The degree of inhibition varied with species and dried residues were more inhibitory than fresh material. Many of these crop residues also inhibited emergence and 1000-grain weight of wheat in a field experiment.

The potential of plant residues to produce allelopathic substances has implications in natural and managed ecosystems. The allelopathic ability of residues may be important in considerations of plant succession in natural ecosystems, helping to explain patterning of vegetation in plant communities, and may have implications for managed ecosystems. In cropping systems knowledge of the allelopathic effects of crop residues will affect crop rotations and plant-back intervals, may be used to advantage in companion cropping systems and may also be useful in crop-weed dynamics and
fallow weed control strategies. Plant breeders may find benefit in selecting crop plants which show allelopathic ability both as living plants and as residues. The development of resistance to some herbicides by some weeds may lead to the possibility of using allelopathic effects in cropping systems as a means of handling or of delaying the development of herbicide resistance. Knowledge of the allelopathic potential of plant residues will, thus, be an important tool for agriculturalists in managing and improving plant production systems.

The inhibitory effects of incorporated residues may, in part, be due to nitrogen immobilization, however the observance of inhibition where nitrogen is not limiting (that is, in controlled conditions) suggests that chemical effects are significant. Nitrogen effects may increase the effects due to chemicals produced by residues. The literature offers differing views in this area (Horricks 1969, Bell 1970).

The effect of many residues on germination and early growth of plants is believed to be due, in part, to phytotoxins leached from the residue and, in part, to micro-organisms producing toxic compounds from exudates or the breakdown products of residues (Campbell 1959, McCalla & Norstadt 1974, Harper & Lynch 1982, Leather 1983). In many cases a combination of these may be present. Some of the compounds produced are identified and are detailed in later sections of this review. Norstadt & McCalla (1968) and Lovett et al. (1982) have also suggested the production of allelochemicals from plant residues, further implicating residue products as having inhibitory effects on plant growth.

Allelopathic compounds have been seen to be locally concentrated in soil, for example, close to the surface in No Till situations (Patrick et al. 1963, Guenzi & McCalla 1966b, ). It could be hypothesised that products of residue decay would be localised in the soil, leading to concentrations of compounds high enough at those locations to cause allelopathic affects. This may explain why concentrations of compounds used in bioassay are frequently high compared to levels in soil. The concentrations of compounds in soil would be locally high (in the area of the residue). Concentrations of compounds used in bioassay may, thus, need to be high to duplicate the local (high) concentrations seen in soil. Lovett et al. (1982) also report the allelopathic effects of crop residues where retained in
reduced tillage systems. They believed that soil borne bacteria were important in the production of the allelopathic chemicals responsible, but did not present evidence to support this.

Kimber (1967a) was among the first to show residues as producing the greatest phytotoxic effect during early decomposition. The degree of inhibition from decaying residues may be expected to vary with time of decay, as different compounds may be produced or complex compounds converted to more simpler derivatives with different activity over time. It may be expected that more compounds in greater amounts would be present during the early stages of breakdown and that this would lead to greater inhibition of germination where residues are relatively fresh. It is possible that, with time, leaching and further microbial decomposition can diminish the effect (Guenzi et al. 1967). This may have implications for intensive cropping systems where crop rotations may need to be planned with regard to the previous crop residue and any allelopathic effects from these residues.

In many instances large variations in responses to plant materials have been recorded (Kimber 1967, 1973, Putnam & Duke 1974, Fay & Duke 1977), with different effects noted from different types of residues. Purvis et al. (1985) document a stimulatory effect of field pea and wheat residues on germination of wild oats, while being inhibitory to other grass weeds. Higher levels of dicotyledonous weeds were recorded in the presence of oilseed rape residues than in pea or cereal residues. This effect reflects the ability of different residues producing different compounds with these having different effects on germinating seeds. This may indicate degrees of selectivity of the compounds, or different sensitivities of the test species. Linseed, for example, has been shown to be sensitive to allelochemicals (Lovett 1978).

This specificity of reaction may be due to a variety of reasons; the plant material used and the pest species, quantity of material incorporated into the soil (in field or pot experiments) and the concentration of extracts used in laboratory experiments. As previously mentioned, low concentrations of compounds are often stimulatory.

The above mentioned (Purvis et al. 1985a) response where, for example, wild oats were stimulated by wheat and pea residues while other species were inhibited is possibly a reflection of wild oats having evolved a mechanism allowing them to grow in the presence of the allelochemicals.
This characteristic may assist in explaining the strongly competitive nature of this weed and its close association with cereal and legume crops. Other examples may show similar effects.

The phenomenon of the production of allelochemicals from crop residues may have wide importance in agricultural production systems, from crop rotation considerations, weed control strategies, both in-crop and fallow, and possible companion cropping considerations. A knowledge of the degree, length and specificity of residue produced allelopathic effects would allow crop production systems to be developed which could use these effects to advantage. This may allow for less, or changed, need for chemical weed control measures. Table 2.3 lists some of the effects of some crop residues documented.

### TABLE 2.3 SOME EXAMPLES OF ALLELOPATHIC INTERFERENCE IN AGRICULTURAL SYSTEMS INVOLVING CROP RESIDUES AS DONOR.

<table>
<thead>
<tr>
<th>TYPE OF RESIDUE</th>
<th>AFFECTED PLANT(S)</th>
<th>EFFECT SEEN</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat residues</td>
<td>Ipomea spp.</td>
<td>reduced biomass by up to 79%</td>
<td>Shilling et al. 1985</td>
</tr>
<tr>
<td>wheat, Corn, Oat and Sorghum residues</td>
<td>Sorghum spp., Glycine max, Helianthus annuus, Nicotinum spp.</td>
<td>inhibition of growth</td>
<td>Einhellig &amp; Kuan 1971, Einhellig &amp; Stille 1979, Patterson 1981</td>
</tr>
<tr>
<td>wheat residue extracts</td>
<td>Triticum aestivum</td>
<td>extracts more inhibitory with longer incubation. Roots more sensitive than shoots</td>
<td>Kimber 1967, 1973a,b.</td>
</tr>
<tr>
<td>wheat residues</td>
<td>Ambrosia artimisitifolia</td>
<td>inhibition of growth.</td>
<td>Lieble &amp; Worsham 1983a</td>
</tr>
<tr>
<td>wheat residues</td>
<td>Hordeum vulgare</td>
<td>biomass reduced 51%</td>
<td>Shilling et al. 1985</td>
</tr>
<tr>
<td>rye residues</td>
<td>Amaranthus retroflexus</td>
<td>biomass reduced 41%</td>
<td>Chou &amp; Patrick 1976</td>
</tr>
<tr>
<td>rye residues</td>
<td>Chenopodium album</td>
<td>biomass reduced 73%</td>
<td></td>
</tr>
<tr>
<td>rye residues</td>
<td>Ambrosia artimisitifolia</td>
<td>residues produce phytotoxic compounds</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2.3 (continued) SOME EXAMPLES OF ALLELOPATHIC INTERFERENCE IN AGRICULTURAL SYSTEMS INVOLVING CROP RESIDUES AS DONOR.

<table>
<thead>
<tr>
<th>TYPE OF RESIDUE</th>
<th>AFFECTED PLANT(S)</th>
<th>EFFECT SEEN</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn and Sorghum</td>
<td>Triticum aestivum</td>
<td>residues phytotoxic</td>
<td>Guenzi et al. 1967</td>
</tr>
<tr>
<td>sorghum residues</td>
<td>Echinochloa crus gall, Bladder Ketmia</td>
<td>plant no's reduced 60%, dry weight reduced 49%</td>
<td>Purvis et al. 1985</td>
</tr>
<tr>
<td>barley residues</td>
<td></td>
<td>plant no's reduced 24%, dry weight reduced 17%</td>
<td>Toussoun et al. 1968</td>
</tr>
</tbody>
</table>

Further evidence of crop residues as allelopathic has been presented by Lovett & Jessop (1982) and Jessop & Lovett (1982). They showed 12 crop residues to be allelopathic, this being the first reports of some crops, for example, field pea. Incorporation of residues increased the degree of inhibition. Residues of cereals, grain legumes and broadleaf crop species were seen to reduce a number of growth factors of wheat. They showed incorporation of residues to be induce more severe effects than surface residues.

Allelopathic extracts from crops may be important in inhibiting competitive weed growth, and thus of benefit. Wheat straw has been identified as inhibiting the growth of common ragweed (Ambrosia artemisiaefolia) and morning glory (Ipomea lacubosa L.)(Leibl & Worsham, 1983). Ferulic acid was identified in the extract, though the extract was found to be more allelopathic than a commercial preparation of ferulic acid, suggesting a combination with other compounds in the extract. Phytotoxicity increased when the bioassay was done in light, indicating an affect on photosynthesis.
De Frank and Putnam (1979) also showed the ability of crops to inhibit weed growth, where sudan grass and sorghum suppressed the weed *Digitaria ischaemum* (smooth crabgrass) by 98% and 99% respectively. Common purslane (*Portulaca oleracea*) was also inhibited 70% and 67%. Living sorghum decreased the total biomass and greatly decreased the growth of *Polygonum persicaria* L. and growth of barnyard grass (*Echinochloa crus-galli*) seedlings, transplanted into agar where sorghum had grown for 15 days, was decreased. This indicates that root exudates as well as residue-produced compounds may be responsible for the effects seen in some instances. Habeshaw (1979) lists the exudates of a number of grass species (decaying residues) as reducing plant growth, leaf elongation and tiller production. Both dicots and monocots were affected and the effects seen were believed to be due to tannins and other phenolics (flavones, flavonols, catechins and cinnamic acids).

These examples indicate the range of crop residues seen as able to produce allelopathic effects, this indicating that these effects would be taken into account (for example) in crop rotation planning, plant breeding objectives or in stubble mulch systems.

### 2.2.3 Weed Residues.

A number of weed residues has been shown to produce phytotoxic substances, similar in effect to those from crop residues. The ability of weed residues to produce inhibitory compounds upon extraction or from breakdown products may be an important element in weed competition and in allowing some weeds to assume dominance. Seed dormancy of many plants may also be broken or maintained by allelopathic compounds present in soil from residues. Weed-crop interactions are complex and any allelopathic effects either from living plants or from residues would form a further advantage allowing weeds to compete with other plants in an ecosystem.

Some documented examples of allelopathic affects of weed residues include:

A range of weed residues (*Chenopodium album, Amaranthus retroflexus, Ambrosia artemisifolia, Abutilon theophrasti, Setaria glauca*) has been shown to be phytotoxic to corn (*Zea mays*) and soybeans (*Glycine max*) (Bhowmik & Doll 1984). Incorporated residues were worse than when left on the surface possibly due to increased local concentration near the seed. This agrees with earlier
observations (Patrick et al. 1963, Guenzi & McCalla 1966b, Lovett et al. 1982). Addition of nitrogen or phosphorus made no difference to the effect seen, indicating an effect unrelated to nutrition. However, the evidence is variable on interactions with nutrition, where, for example, the same authors conducted a study using five weed residues and maize and soybean as indicator species. Ragweed and velvetleaf were seen to alter the mineral uptake of the crop species, leading to an effect on growth.

In support of an interaction with nutrient uptake, Young and Bartholomew (1981) showed that residues of *Namarthria altissima* incorporated in soil led to tops of *Desmodium intortum* containing less phosphate. They concluded that an affect on nutrient uptake may be a means of allelochemical action in this situation.

Thus, the effects of compounds added to the environment by decaying residues may be due to a number of primary effects within plants and may exert effects *via* a variety of means or by interfering with a number of plant functions.

A further interesting possible effect of weed residues arises where herbicides are used. Poor crop emergence or growth following herbicide use can be suspected as due to herbicide residues. It is possible that decaying weeds (following herbicide treatment) may produce compounds, unrelated to the herbicide, which are themselves phytotoxic. It is also possible that mediating organisms could play a role, for example, bacteria or fungi using the decaying weeds as substrate, producing toxic compounds as well. Blowes (1987) reports rye grass treated with glyphosate as providing a substrate for *Pythium spp.* which produced toxins which were inhibitory to wheat germination and emergence. Further discussion of allelopathic interaction with fungal pathogens is contained in Chapter 6.

### 2.2.4 Seed Dormancy.

Many weed seeds remain dormant in the soil for years, for example, *Verbascum blatteria* seeds can remain viable in soil for 100 years (Kivilaan and Bandurski, 1973). The ability to last for this length of time is possibly due to seeds resisting microbial decay (there may be chemicals produced by seeds which may play a role in this) and may also be due to the presence of chemical germination inhibitors. Rice (1984) lists unsaturated lactones and phenolic compounds as potential antimicrobial
compounds. Many fruits and seeds are known to contain germination inhibitors, for example, flavonoids, phenolics and their glycosides and tannins.

Methods which would destroy or remove the effects of these compounds could lead to better weed management strategies. Knowledge of compounds which may break seed dormancy may allow most weeds to be germinated and removed prior to crop planting, reducing the need for post emergent weed control. Similarly, compounds may be identified which could extend seed dormancy of certain troublesome weeds, leading to their absence for periods of crop production.

Allelopathy could, thus, be seen as forming an intricate part of plant relationships with each other in nature and managed ecosystems. It can be expected that more examples apart from those listed will be found as research continues and as technological improvements allow better detection of allelochemicals in plant exudates and leachates (both living plants and residues).
3. SIGNIFICANCE OF ALLELOPATHY IN AGRICULTURE.

The study of allelopathy in agriculture could lead to improved methods of crop and residue management. Allelopathic affects could be exploited for benefit (for example weed suppression by allelopathic crops). Knowledge of the detrimental effects of allelopathic weeds or crop residues could be used in planning crop rotations and weed control strategies.

Many plant residues have been shown as sources of allelopathic compounds, from exudates, leachates and as products of microbial breakdown. Study of these compounds may lead to the identification of new biologically active substances which may be useful alone or as representatives of more active related compounds. This may lead to new classes of herbicides or growth regulators for use commercially.

A number of areas where knowledge of allelopathy may offer benefits in agriculture is being investigated:

3.1 PLANT BREEDING APPROACHES:

Crop cultivars could be screened for allelopathic activity, including older or more primitive cultivars and related plants (Lovett 1986). If the genetic material responsible for this activity could be identified and transferred (using either conventional breeding or other genetic transfer techniques) to current or future crop cultivars this could lead to crop plants providing some weed control ability of their own, either as living plants or as residues. Fay and Duke (1977) found that levels of scopoletin in over 3000 accessions of Avena spp. varied. "Wild types" contained higher levels than highly selected crop varieties. Allelopathic traits may have been inadvertently selected against in the pursuit of yield or other characteristics.

A number of crops have been reported as showing some weed suppressing ability, including:

1) broad leaved plants: cucumber (Putnam and Duke, 1974)
sunflower (Leather, 1983)
soybean (Massantini et al., 1977)
narrow leaved plants: oat (Fay and Duke, 1977)
    wheat (Kimber 1967)
    barley (Overland 1966)
    rye (Barnes et al. 1986)

Lovett and Jessop (1982) identified rape, sunflower, pea, sorghum and wheat residues as
suppressing grass weeds. These examples offer possibilities for breeders to increase this potential in
crop plants.

Another possibility is to remove deleterious affects of allelochemicals produced from plants or
their residues, where these effects are detrimental to following crops in the rotation. Potential
allelopathic effects of crop and weed residues are becoming a growing concern in agronomic systems
where crop residues are retained. Varieties where residues produced low or minimal allelopathic
compounds may allow recropping in shorter rotations.

A further means of utilizing plant-produced pesticidal compounds may be through recent
advances in plant molecular biology. Genes responsible for the production of allelopathic compounds
in a given plant may be identified, isolated and transferred to other plants, for example, important crop
species, thus conferring to that crop the ability to protect itself from attack or competition.

3.2 CROP ROTATION CONSIDERATIONS.

A second approach could be to include allelopathic rotational crops or companion crops in
cropping systems. Rye (both living and as residues) has been shown to suppress a variety of
agriculturally important weeds (Barnes and Putnam, 1983). Putnam and DeFrank (1983) list residues
of sorghum, barley wheat and oats as providing inhibition of a number of weeds, leading to the
possibility of using these or cultivars selected for this ability as rotational crops useful in suppressing
weeds in the rotation. This approach could parallel the use of some crops (for example, canola and
grain legumes) used in rotation as disease breaks for cereals in southern Australia.
3.3 POTENTIAL HERBICIDE DEVELOPMENT.

A third approach centres around herbicide development. The search for more 'natural', environmentally inoffensive pesticides is now extending to look to plant-produced compounds as sources for new discoveries. McLaren (1986) details some of the progress made. Plant-derived compounds are sought for biological activity against other plants, fungi, viruses, bacteria and insects in much the same way as many pharmaceutical products which have origins in plants. Any compounds identified as active lead to two approaches, a program of synthesis around the compound and its analogues, and plant cell culture or fermentation process giving biosynthesis. Purification of these and research into the active groups of the molecules could lead to the development of new herbicides for use in agriculture.

McLaren (1986) observes that while many compounds have been implicated and demonstrated to inhibit other plants, the concentrations seen as necessary to show the effect would be inappropriately high for use as practical herbicides. This, however, may not be the case in nature where concentrations of the compound(s) responsible need only be high enough to elicit the effects at a local level in soil. Thus, the concentration of an allelochemical may be locally high in soil (for example near decomposing residues in soil) while at a low level per hectare. At high concentrations many of the compounds may become undesirable due to toxicity and environmental problems. The lack of selectivity may also not be attractive from a herbicidal viewpoint.

Some allelochemicals may not be active enough alone for use as herbicides but may provide leads as precursors for development of more active related compounds (analogues) with higher unit activity. The development of the auxin herbicides (for example, 2,4-D) as analogues from naturally occurring plant hormones remains a classical use of plant-derived compounds. The continued successful use of phenoxy herbicides after 40 years attests to the success of their development. After this time no resistance to these compounds has been seen to have developed among susceptible weeds, in contrast to the development of widespread resistance to other synthetic herbicides. Similarly, the development of the synthetic pyrethroid insecticides from pyrethrins is a successful use of plant-derived compounds in the past.
3.4 HERBICIDE EFFECTS ON ALLELOCHEMICAL PRODUCTION BY PLANTS.

An interesting possible effect of some herbicides on the production of secondary plant compounds has been reported (Duke 1985). The herbicides glyphosate, chlorsulfuron and acifluorfen may offer potential when applied in sub-lethal doses to alter the production of phenolic compounds by some plants. Many phenolic compounds in plants are produced in the shikimic acid pathway. Herbicides such as those mentioned above, notably glyphosate, have the ability to drastically affect the production of aromatic compounds from this pathway.

Glyphosate has been shown to greatly enhance the accumulation of shikimate and gallic acid in plant cells, leading to accumulation of certain phenolic compounds derived from shikimic acid pathway intermediates (Duke 1985). At the same time it leads to a decrease in synthesis of aromatic amino-acid derived secondary aromatic compounds. This effect on the pattern and relative amounts of these compounds in plants would be expected to alter the allelopathic effects of these compounds. The other two herbicides exert their effect at a different stage of the shikimic acid pathway and so may be expected to show different, though perhaps similarly important effects on the allelopathic qualities of treated plants.

Chemical (herbicide, growth regulator) manipulation of phenolic allelochemical production may have agricultural implications. The use of herbicides may alter crop and weed susceptibility to or ability to produce allelochemicals. No-tillage systems, where glyphosate is widely used, are an example where this effect may be manifest. The use of herbicides in crop or fallow may alter not only the production of allelochemicals during the life of the crop or weed, but also may influence the types or amounts of compounds produced from residues of treated plants, either as weed residues or crop stubbles.

3.5 BIOTECHNOLOGY DEVELOPMENTS.

Future developments in manipulation of cellular metabolism may allow more direct means of utilizing genetic material responsible for allelopathic effects. Researchers at Monsanto and other companies and institutions are working to develop microbial pesticides. The direction is to genetically
engineer rhizoplane/phylloplane bacteria to produce natural pesticidal substances, which would thus protect the plants they colonise. Already, a genetically engineered root colonising pseudomonad bacterium has been made to produce the insecticidal protein toxin from another bacterium, *Bacillus thuringiensis*. These root colonising bacteria, capable of producing the toxin may enable biological control of root pests, for example, corn root worm.
4. TYPES OF ALLELOCHEMICAL COMPOUNDS AND THEIR EFFECTS:

4.1 HISTORY OF ALLELOCHEMICAL AGENTS:

In 1940 Davis obtained the substance juglone from both hulls and roots of walnuts. The compound was identified as 5-hydroxy-1,4 naphthoquinone. This was toxic to potatoes, tomatoes and lucerne when injected. At the same time Bode (1940) identified the compound absinthin from *Artemisia absinthium* and showed it to be inhibitory to neighbouring plants.

Several other compounds have since been identified from plants, plant residues and field soil (Bode 1940, Rice 1977). These include caffiec acid, chlorogenic acid, *trans*-cinnamic acid, *p* -coumaric acid, ferulic acid, gallic acid, vanillic acid, vanillin and *p*-hydroxybenzaldehyde. Guenzi and McCalla (1966) isolated many of these from residues of corn, wheat, oats and sorghum. Further, a number of these compounds have been seen to inhibit growth of sunflower and tobacco (Einhellig and Kuan 1977, Einhellig and Stile 1979, Patterson 1981).

In the last 20 years a large number of compounds has been identified as showing allelopathic effects.

4.2 MAIN GROUPS OF ALLELOCHEMICALS:

Many allelopathic compounds are synthesised in the acetate and shikimate pathways (Whittaker and Feeny 1971, Rice 1974, Robinson 1983).

Hoagland and Williams (1985) review aspects of allelochemical synthesis, and possible effects of allelochemicals on soil microflora in producing responses in plants. Microflora may also play a role in modifying the compounds produced. Many compounds are listed by species, plant part associated (for example root exudates) and method of extraction. The authors Rice (1974, 1984), Putnam and Duke (1978), Mandava (1979), Einhellig (1984) and McLaren (1986) have also produced reviews of groups of compounds so far identified as allelopathic.
production of secondary compounds, including allelochemicals in plants. It is likely that the production of allelochemicals by plants has been a result of natural selection of plants whose chemical by-products happened to be toxic or repellent to others (Whittaker 1970). Evolution would be expected to increase the potency or concentrations of these compounds in these plants as these features would confer some competitive advantages to these plants. Conversely, it is possible that plant breeding which involves selection for a limited number of desirable traits may have led to a degree of selection against the production of these compounds.

Figure 4.1 Generalised metabolic pathways involved in the formation of secondary compounds and growth substances in plants. ABA = abscisic acid; DHS = dehydroshikimic acid; IAA = indole acetic acid; IPP = isopentenyl pyrophosphate; MVA = mavalonic acid; PA = phaseic acid; SMA = S-adenosylmethionine. After McLaren (1986).

It can be seen that a number of compounds are the result of progressive steps in synthetic pathways, with the degree of activity possibly increasing as successive compounds are produced.
4.3 EXAMPLES OF SOME ALLELOCHEMICAL GROUPS:

4.3.1. Aliphatic Compounds, Organic Acids and Alcohols.

A large number of water-soluble simple organic acids and alcohols has been shown to inhibit germination and growth of plants.

Evenari (1949) listed malic, citric, acetic and tartaric acids in fruit as inhibitory to germination and also acetaldehyde in unripe corn and peas as autoinhibitory. Malonic, citric and fumaric acids exuded from seeds of *Pinus resinosa* inhibited germination of zoospores of *Pythium aferite* (Agmihotric and Vaartaja 1968). Acetaldehyde, propionic aldehyde, acetone, methanol and ethanol emitted from tomato, sweet potato, beet and radish leaves and carrot roots have demonstrated inhibition in closed systems (Dadykin *et al.* 1970). Methanol, ethanol, n-propanol and butanol were listed as allelopathic by Hutchinson (1975) and crotonic, oxalic, formic, butyric, lactic, acetic and succinic acids are reported by Takijama (1964) and Erner *et al.* (1975).

Acetic acid has been widely reported as being allelopathic, notably when produced from decomposing crop residues, particularly cereals (Evenari 1949, Patrick 1970, Lynch 1977, 1978, Tang & Waiss 1978, Wallace & Elliott 1979).

Rye residues have been seen to produce acetic and butyric acids (Patrick 1970) and corn residues have produced butyric acid (Chou and Patrick 1976). Residues of rape and pea have also been shown to produce acetic acid when incubated under anaerobic conditions (Clarke and Humphries 1970, Lynch 1978). Harper and Lynch (1982) were able to show that rape straw produced more acetic acid than wheat, barley or oat straw. Since extracts from rape residues were more toxic than acetic acid alone, the authors suspected that other volatile fatty acids and phenolics were also produced, further contributing to the toxic response.

Lynch *et al.* (1980) demonstrated phytotoxicity from production of short chain aliphatic acids by decaying couch residues, with the degree of inhibition increasing with chain length (that is, acetic propionic and butyric acid inhibited root extension 25%, 50% and 71% respectively). Other compounds present in extract solutions included hexanoic, succinic, phenylacetic, cinnamic, p-coumaric, 4-hydroxyphenylpropionic and 3,4-dihydroxyphenylpropionic acids.
Other workers (Lynch 1977, Tang and Waiss 1978, Wallace and Elliott 1979) have shown that chemicals phytotoxic in the field from decaying cereal residues were primarily short-chain volatile fatty acids, notably acetic acid. Lynch (1977) showed that anaerobic fermentation of wheat straw produced acetic acid which inhibited barley root growth. The effect was magnified when pH during fermentation was reduced.

These compounds, while showing activity under controlled conditions, are considered unlikely to be active in soil as aliphatic acids are metabolised under aerobic conditions in soil (Hutchinson, 1975). However, under some circumstances soil conditions may locally be amenable to the production of aliphatic compounds from residues in quantities sufficient to produce significant allelopathic responses.

Lynch and Penn (1980) found that decaying couch grass rhizomes (killed either by heat treatment or by application of glyphosate) produced high concentrations of acetic and butyric acid, which together with stimulation of a pathogen *Fusarium culmorum*, produced inhibition of barley seedling germination and growth. A synergistic effect was thought to occur between the toxin and the pathogen. This observation was supported by Penn and Lynch (1982), where concentrations of 5mM acetic acid increased the phytotoxic effect of *F. culmorum*. Rhizomes decayed in wet sand produced large concentrations of acetic acid (42mM).

Many crop residues, reported above, have been seen to produce simple aliphatic compounds. The possible practical implications from this may be that many crop residues decaying on or in soil are able to produce these compounds and these may play a role in suppressing weed growth. This may help explain the observed allelopathic effects of some crop stubbles, and reduction in weed germination seen in these instances.
4.3.2. Simple Unsaturated Lactones.

Complex lactones, coumarins and cardiac glycosides (steroids) are listed in later sections.

Patulin has been shown to be inhibitory to higher plants (Norstadt and McCalla 1963), and is produced by a number of fungi including *Penicillium urticae* growing on wheat. At 10µg/ml patulin has been shown to drastically inhibit germination of a number of species, including corn (Rice 1974, Mandava 1979).

Parascorbic acid from fruits of *Sorbus aucuparia* is very inhibitory to germination and seedling growth and is also antibacterial (Evinari 1949). The aglycone of ranunculin, protoanemonium is also listed as a germination inhibitor.

Among other lactones identified as inhibitory are psilotin, psilotinin and protoanemonin, though their role as allelochemicals is not clearly established (Hutchinson, 1975).

4.3.3. Fatty Acids, Lipids and Polyacetylenes.

Polyacetylenes are derived from long chain fatty acids and so are included here.

Alsaadawi *et al.* (1983) identified nine fatty acids in decomposing *Polygonum aviculare* residues (myristic, palmitic, linolelaidic, oleic, stearic, arachidic, 11,14-eicosadienoic, heneicosanic and behenic acids). All except myristic and heneicosanic acids were also found in soil under *P. aviculare* residues. Sodium salts of all these acids inhibited seedling growth of Bermuda grass at concentrations of 5 ppm.

Campbell *et al.* (1982) showed a-terthienyl from roots of *Tagetes erecta* as being toxic to test species seedlings at concentrations down to 0.15 ppm. Phenylheptatriyne from leaves of *Bidens pilosa* showed similar effects at 0.66 ppm.

Dihydroxystearic acid has been documented as being allelopathic (Rice 1974, Mandava 1979). Rice (1984) also noted other fatty acids as being toxic to plants although their activity as allelochemicals is not well established.
4.3.4. Cyanogenic Glycosides.

Among those seen as allelopathic are amygdalin (and its reduced form prunasin) and dhurrin (Rice 1974, Einhellig 1984). These can be hydrolysed to produce hydrogen cyanide and also benzaldehydes or hydroxybenzaldehydes which can be oxidised to benzoic acid, which may itself be toxic.

4.3.5. Terpenoids.

The terpenoid group comprises hydrocarbon compounds built from isoprene units and includes monoterpenes (C10), sesquiterpenes (C15), diterpenoids (C20), triterpenoids (C30), and tetraterpenoids (C40). A small number of terpenoid compounds are seen as allelopathic (Robinson 1983, Rice 1984). The monoterpenes are the main component in the essential oils of plants and also the main ones implicated as being allelopathic.

Among the monoterpenes are α-pinene, β-pinene camphor and cineole (Muller 1953). Mandava (1979) lists the sesquiterpenes caryophylline, bisabolone and chamuzulene as allelopathic compounds. Cineole, camphene, diterpene, α-pinene and β-pinene are listed as being allelopathic by Muller (1964). Camphor and cineole have been shown to be toxic to root growth. These have also been identified in air around Salvia plants in the field (Muller 1965). Cineole and α-pinene have been identified as important volatile allelochemicals in Eucalyptus camaldulensis (del Moral and Muller 1970).

A number of sesquiterpene lactones have been implicated as allelochemicals; arbusculin A, achillin, viscidulin C (Rice 1974, Mandava 1979, Einhellig 1984).

Sesquiterpene lactones are found in many of the Asteraceae, but also in other angiosperms and some liverworts. They exhibit a range of biological activity including cytotoxicity, anti-tumor, anti-microbial and insecticidal properties (Rodriguez et al. 1976, Smith et al. 1983). They also operate as growth regulators (Gross 1975).

Fischer and Quijano (1985) list a number of sesquiterpene lactones isolated from Ambrosia artemisifolia L. and Amaranthus palmeri S. Watts as showing allelopathic activity. Two weeds of the
genus *Centaurea* (Russian knapweed and yellow starthistle) have been shown to produce a variety of sesquiterpene lactones as well as two chromenes (Stevens and Merril 1985). They believed these to be involved in the allelopathic activity of these weeds.

*Jarvis et al.* (1985) isolated four sesquiterpene lactones from *Parthenium hysterophorus* in aqueous extracts, the dominant one being parthenin, which has been noted as allelopathic (*Rodriguez et al.* 1976).

It is interesting to note that a grass active herbicide, cinmethylin, is a derivative of cineole (1,8-cineole) (*Cyanamid Australia Limited, pers. comm.*). This may be an example of an active group common to the sesquiterpene lactones which may have commercial possibilities. It may also help explain the invasiveness of some of the weeds listed in this section.

**4.4 AROMATIC COMPOUNDS:**

Aromatic compounds (phenolics) comprise by far the largest group of allelochemicals identified so far. They include the following:

**4.4.1. Simple Phenols.**

Hydroquinone and its glycoside arbutin have been identified as allelopathic (*Rice* 1974, *Putnam and Duke* 1978).

**4.4.2. Phenolic Acids.**

Phenolic acids are widely distributed in the plant kingdom and are found in many plant parts. Many phenolic acids have been documented as allelopathic agents.

For the purposes of this review the phenolic acids can be divided into cinnamic acid and derivatives, benzoic acid and derivatives, and other phenolic acids.
Cinnamic Acid and Derivatives:

Cinnamic acid derivatives have been the most commonly listed allelochemicals. They are derived from phenylalanine and tyrosine through the shikimic acid pathway.

The following cinnamic acid derivatives have been documented as allelopathic compounds. Cinnamic acid, p-coumaric acid, o-coumaric acid, caffeic acid, ferulic acid, sinapic acid, chlorogenic acid and isochlorogenic acid. Of these p-coumaric and ferulic acids are the most commonly reported
and the ones most frequently found in soil. A summary of these compounds and effects appears in table 4.1:

**TABLE 4.1 SOME CINNAMIC ACID DERIVATIVES SEEN AS ALLELOPATHIC**

<table>
<thead>
<tr>
<th>COMPOUND(S)</th>
<th>SOURCE</th>
<th>EFFECT</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic and chlorogenic acids</td>
<td><em>Ambrosia artemisifolia</em> L.</td>
<td>germination inhibitors</td>
<td>Jackson &amp; Willemsen 1976</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Many crop residues</td>
<td>allelopathic to:</td>
<td>Guenzi &amp; McCalla 1966a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ragweed, prickly sida</td>
<td>Lieble &amp; Worsham 1983a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>crabgrass, soybean</td>
<td>McClure et al. 1978</td>
</tr>
<tr>
<td></td>
<td><em>Camelina alyssum</em></td>
<td>inhibitor</td>
<td>Grummem &amp; Beyer 1960</td>
</tr>
<tr>
<td>p-Coumaric and chlorogenic acids</td>
<td>Apple leaves</td>
<td></td>
<td>Williams 1960</td>
</tr>
<tr>
<td>p-Coumaric and ferulic acids</td>
<td>In soil from crop residues</td>
<td></td>
<td>Whitehead 1964</td>
</tr>
<tr>
<td>plus caffeic acid</td>
<td><em>Celtis laevigata</em> leaves</td>
<td>toxic</td>
<td>Lodhi &amp; Rice 1971</td>
</tr>
<tr>
<td></td>
<td>corn, wheat, sorghum, oat residues and soil beneath these</td>
<td>inhibitors</td>
<td>Guenzi &amp; McCalla 1966ab</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td><em>Gallium mollugo</em></td>
<td>inhibitory</td>
<td>Kohlmuener 1965</td>
</tr>
<tr>
<td></td>
<td><em>Eucalyptus globulus</em> fog drip</td>
<td>inhibitor</td>
<td>delMoral &amp; Muller 1970</td>
</tr>
<tr>
<td></td>
<td><em>Arctostaphylos glandulosa</em> leaf leachates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and iso-chlorogenic acid</td>
<td><em>Helianthus annus</em></td>
<td>strongly inhibitory</td>
<td>Rice 1965a</td>
</tr>
<tr>
<td></td>
<td><em>Digitaria sanguinalis</em></td>
<td>inhibitory</td>
<td>Parenyi &amp; Rice 1969</td>
</tr>
<tr>
<td>Chlorogenic and p-coumaric acids</td>
<td><em>Sorghum halepense</em></td>
<td>inhibitors</td>
<td>Abdul-Wahab &amp; Rice 1967</td>
</tr>
<tr>
<td>plus caffeic and ferulic acids</td>
<td><em>Eucalyptus camaldulensis</em> leaf leachates</td>
<td>toxins</td>
<td>delMoral &amp; Muller 1970</td>
</tr>
<tr>
<td>Chlorogenic, iso-chlorogenic, neo chlorogenic and o-coumaric acids</td>
<td><em>Platanus occidentalis</em></td>
<td>toxins</td>
<td>Al-Nailb &amp; Rice 1971</td>
</tr>
</tbody>
</table>

Ferulic acid is common among the known allelochemicals (Rice 1984). It is produced from a variety of crop residues (Guenzi and McCalla 1966a, Wang et al. 1967).
It is notable that only p-coumaric and ferulic acids are commonly found in soil under plants shown to produce a range of cinnamic acid derivatives. Borner (1960) identified ferulic, vanillic and p-hydrobenzoic acids in water extracts of barley straw, with wheat and rye growth being inhibited by these at as low as 10ppm.

It would seem that many cinnamic acid derivatives are short term allelopathic agents, probably being converted or broken down to the more commonly found allelopathic compounds caffeic, ferulic and p-coumaric acids.

**Benzoic Acid Derivatives:**

The commonly identified compounds in the group are benzoic acid, p-hydroxybenzoic acid, vanillic acid, gentisic acid, salicylic acid and sulphosalicylic acid (2-hydroxy-5-sulphobenzoic acid). These are found in some leachates and in soil (Putnam and Duke 1978, Rice 1979), with p-hydroxybenzoic and vanillic acids the most common allelochemicals in the group (Lodhi 1976).

Chou and Muller (1972) found vanillic and p-hydroxybenzoic acid in *Arctostoplyos glandulosa* and also found the latter of these in soil. Other phenolics found were gallic and protocatechulc acids, and syringic acid in soil.

Sunflower has been reported as being allelopathic (Wilson & Rice 1968, Rice 1974), though some variance has been reported between different cultivars (Saggese *et al* 1985, Lovett *et al*. 1982). A number of phenolic acids have been identified in extracts (Saggese *et al*. 1985) including gallic acid, protocatechutic acid, p-hydroxybenzoic acid, benzoic acid, vanillic acid, syringic acid and salicyclic acid.

Extracts from *Imperata cylindrica* have been shown to interfere with growth of other plants. Several phenolic compounds were isolated from leaf, rhizome and root extracts (Eussen 1978). These have been identified as vanillic acid from root fragments, p- and o-coumaric acid, gentisic acid, benzoic acid, p-hydroxybenzoic acid, vanillin and p-hydroxybenzaldehyde from leaf material.
It can be seen that phenolics are a major source of allelopathic compounds, from a large number of plants. It is also likely that phenolics may be present in many instances where other compounds are also identified, playing a (minor) additive or synergistic role.

4.4.3. Coumarins.

Coumarins are lactones of o-hydroxycinnamic acid (Robinson 1983) and also are widely distributed through the plant kingdom.

These compounds are found as glycosides in plants and become leached into soil. Allelochemicals in this group include scopoletin, scopolin, esculetin, esculin, and methylesculin (Rice 1974, Einhellig 1984).

Evenari (1949) found coumarin to be a potent inhibitor of seed germination, a finding reinforced in later work by Mayer and Poljakoff-Mayber (1961). Ulitzer and Poljakoff-Mayber (1963) and later Sarma and Barooah (1982) found coumarin to inhibit plant germination and growth. Others (Van Sumere et al. 1972) have suggested that coumarin may inhibit germination by inhibiting amino acid transport and protein formation.

Reynolds (1989) describes the allelopathic effects of a number of heterocyclic compounds on lettuce germination. Many compounds are described and the effects of changing the chemical structure, solubility and lipophylicity of these is also described.

Escarin, produced by Aesculus hippocastanum, and coumarin, produced by Melilotus alba, were identified as inhibitory by Winter (1961). Scopoletin has been reported as a strong inhibitor and has been found in oat roots (Martin 1957). Fay and Duke (1977) record that 3000 accessions of Avena spp. all contained scopoletin showing these to have allelopathic activity.

4.4.4. Quinones.

These compounds also form a large group which are widely found in plants. Juglone (5-hydroxy-1,4-naphthoquinone) is perhaps the best known of these compounds to show allelopathic activity, occurring in the genus Juglans (Rice 1984).
4.4.5. Flavonoids.

This is also a large class of compounds found in plants shown to exhibit allelopathic activity. These have a C6-C3-C6 skeleton, where the A ring is of acetate origin and the B ring of shikimic acid origin. There is a large variety of these compounds and they are frequently present in seed plants, however few have been implicated in allelopathy.

Phlorizin from apple root residues has been reported as inhibiting growth of apple seedlings (Borner 1959), and derivatives of this compound, phloretin, phloroglucinol, p-hydroxycinnamic acid and p-hydroxybenzoic acid produced by decomposing apple roots also inhibit germination.

Rice and Pancholy (1974) found a number of flavonoids and their glycosides in several herbaceous species which inhibited seed germination.

Balke (1985) has shown flavonoids as allelopathic with the effect being on mineral absorption by roots, probably due to the disruption of normal membrane function. Phenolic acids are also believed to have this effect.

4.4.6. Tannins.

Hydrolyzable Tannins:

Many types of these are present in dicotyledonous plants (Swain 1965), with some evidence of these acting as allelochemicals. A number of hydrolysed tannins is reported as showing allelopathic activity (Rice 1974, Einhellig 1984). They have been implicated as reducing Carpinus betulus seedling growth (Mitin 1970) and as germination and growth inhibitors in Arctostaphylos glandulosa (Chou and Muller 1972).

Plant residues containing hydrolyzable tannins frequently contain gallic or ellagic acids, or both, and occasionally digallic acid. These can inhibit nitrification in soil and have been found at high concentrations in some forest soils (Rice and Pancholy 1973). These compounds have been reported as phytotoxic, being found in association with caffeic, ferulic, p-coumaric and p-hydroxybenzoic acids.
Condensed Tannins:

Few reports of these as allelopathic compounds exist. Tannins in some sorghum hybrids have been reported as germination inhibitors (Harris and Burns 1970, 1972). Condensed tannins in leaves of beech (*Fagus sylvatica*) have been reported as seedling growth inhibitors (Mitin 1970).

4.5. AMINO ACIDS:

A small number of reports exist of amino acids as allelochemicals. Free amino acids have been found in seeds of *Abutilon theophrasti* which inhibited the germination of several crops (Gressel and Holm 1964).

Soybean residues produce amino acids upon decomposition, these being reported as phytotoxic (Prutenskaya *et al.* 1970). Unspecified amino acids were identified in phytotoxic root exudates of cucumbers and tomatoes (Gaidamak 1971). Moreland *et al.* (1966) found that non-protein amino acids, while having little affect on seed germination were inhibitory to seedling growth.

4.6. ALKALOIDS:

Evenari (1949) lists the following as allelochemicals (seed germination inhibitors) of various degrees; cocaine, physostigmine, caffeine, quinine, strychnine, berberine, codeine, cinchonin, chinchonidin and tropa acid. A more recent and comprehensive review (Wink 1993) covers the known biological activity of the alkaloids to that time, including much of the allelopathic properties of this large group of compounds.

Wink (1983) has proposed that alkaloids are inhibitory by interfering with polypeptide synthesis, while other workers (Knypl and Janas 1977, Knypl and Oswieczimska 1986) have suggested the activity is due to interference of cell membrane permeability.

Barley roots have been reported as exuding alkaloids (Overland 1966). Barley is known to produce gramine and hordenine which have been reported to inhibit the growth of *Stellaria media* (Overland 1966). Lovett and Liu (1987) have demonstrated hordenine as reducing radicle length in white mustard, while gramine has been implicated in inhibitory activity against bacteria (Sepulveda and Corcuera, 1990).
Lovett and Hoult (1993) demonstrated that hordenine was inhibitory to a number of species, with the susceptibility of species varying considerably. In this work hordenine and gramine were shown to be inhibitory to growth of a fungal pathogen (*Drechslera teres*) and armyworm (*Mythimna convecta*). Lovett and Hoult (in press) have reviewed the allelopathic effects of barley including effects on other plants, fungi and insects, with considerable evidence for these effects being due (in part at least) to hordenine and gramine. They present strong evidence of inhibition of these compounds being the basis for self defence in barley.

The effects of thornapple washings in inhibiting the growth of sunflower (Lovett *et al.*, 1981) are thought to be due to the presence of compounds scopolamine and hyoscyamine. There is the possibility of synergism between these alkaloids, giving a more effective inhibition when a number are present, though this has not been proven.

4.7. CYANOHYDRINS:

Dhurrin occurs in sorghum (*S. bicolor*) seedlings. HCN and p-hydroxybenzaldehyde have been identified as phytotoxins produced by Johnsongrass (*S. halepense*) using dhurrin as the precursor (Abdul Wahab and Rice 1967).

4.8. SULPHIDES AND MUSTARD OIL GLYCOSIDES

Mustard oils are produced by all organs of plants in the Cruciferae and in large amounts in plants in the genus *Brassica* (Evenari 1949). Hydrolysis of sinigrin yields the mustard oils allyl thiocyanate and allyl iso-thiocyanate. Large amounts of allyl iso-thiocyanate are produced when leaves of *Brassica nigra* are macerated, this compound being very inhibitory to seed germination in laboratory (but not field) tests (Bell and Muller 1973).

More detailed treatment of allelopathic affects of glucosinolates, derivatives and *Brassica spp.* is presented in Chapter 5.

4.9. OTHER COMPOUNDS

A number of purines occurs in plants aside from those involved in nucleic acids (Robinson 1983). Few have been implicated in allelopathy. Caffeine (previously listed as an alkaloid),
theophylline, paraxanthine and theobromine, from the coffee tree, have been reported as being potentially allelopathic; Evenari (1949) includes caffeine as a powerful inhibitor.

Some compounds showing allelopathic activity do not fit into any of the above categories. Corn and rye residues produce phenylacetic acid and 4-phenylacetic acid (Chou and Patrick 1976). These are produced from either the shikimic or cinnamic acid pathways (Robinson 1983).

4.10. MIXTURES OF COMPOUNDS:

Frequently, it is found that a variety of compounds is identified in extracts used in bioassays. In many instances a number of compounds may be operating in combination to produce the observed allelopathic effects. Glass (1976) found that a combination of phenolic acids in hydroponic conditions altered barley root growth. Combinations of p-coumaric, ferulic and caffeic acids were much more inhibitory than the same concentrations used alone (Lodhi 1975). Rasmussen and Einhellig (1977) and Duke et al. (1983) showed combinations of p-coumaric acid and ferulic acid were synergistic. Asplund (1969) found combinations of monoterpenes were highly synergistic, and Wallace and Whitehead (1980) showed synergism between volatile fatty acids.

Mandava (1985) points out that while extracts of some allelopathic species (for example, tall fescue) are inhibitory, the extracts contain a complex mixture of compounds which may include several phenolic compounds. In the course of purification of these (for example by chemical fractionation) the allelopathic activity may be diminished. Thus, it is likely that activity of extracts is due to the combined effects of a range of compounds, working together as additive or in synergy. Individual compounds present alone may frequently fail to show allelopathic activity.

Other factors may lead to difficulty in ascribing the effects seen with extracts to individual or small numbers of compounds present. Degradation of compounds with storage, effects due to reaction with soil (for example, adsorption), and synergy or antagonism when present with other compounds present in soil may lead to differing effects being seen when testing plant derived compounds in situ. When studying allelopathic effects of secondary plant compounds it is necessary to look not only at any individual compounds present but at the possible combinations and interactions that may occur.
It is possible that synergism occurs widely in allelopathy. Compounds may be present singly in amounts too low to produce allelopathic affects alone, however, they may produce inhibition due to synergy with other compounds also present in low amounts.

It is also likely that where extracts are used only dominant compounds are detected in the analysis whereas a number of other compounds may be present in extracts at very low levels. These compounds may assist in the allelopathic responses being seen in a synergistic fashion although only being present as minor components in very low concentrations. Low concentrations of compounds have frequently been seen to stimulate growth, only to become inhibitory when concentration was increased (Lovett and Duffield 1981).

4.11. INTERACTIONS WITH OTHER PLANT STRESSORS.

Einhellig (1987) discusses the possibility that allelochemicals may frequently be active at concentrations below that shown in experiments and, indeed, may be active even when present at levels below their minimum effect level. It is postulated that interactions may exist between allelochemicals and other plant stressors for example moisture, nutrient, temperature or herbicide stress. Stress conditions may enhance allelochemical activity. Stow and Osbourne (1980) showed an interaction between the effects of vanillic and p-coumaric acids and low phosphorus and nitrogen levels, with low nutrient levels enhancing the effects seen. Einhellig and Echrich (1984) showed ferulic acid to be more inhibitory to soybean and sorghum seedlings at high temperatures. Glass (1976) showed barley seedlings to be more sensitive to mixtures of phenolic acids at high and low temperatures.

The alteration of plant water balance is often an early indication of allelochemical stress from ferulic and p-coumaric acids (Einhellig and Schon 1982, Einhellig et al. 1985). Duke et al. (1983) also showed water stress to increase the effect of phenolic acids in lettuce seedlings.

Interactions between allelochemicals and plant diseases have been documented by Lynch (1977, 1980), Penn and Lynch (1982), Gussin and Lynch (1983), with acetic acid from wheat straw decomposition in soil having a synergistic effect with the level of Fusarium spp.
It is likely that the presence of allelochemicals may impose stresses on plants, leading to plants becoming more susceptible to diseases or suffering more from environmental stress. Similarly, the effects of allelochemicals may be increased by the presence of other stress factors and diseases. These effects may operate to form part of the many influences which produce balanced natural communities.

Managed ecosystems (crops, pastures) may lead to unbalanced effects of these influences, for example, the presence of large amounts of crop or weed residues may produce high levels of detrimental compounds upon breakdown. Similarly, herbicide, nutrient or moisture stress may lead to crops being rendered more susceptible to the presence of allelochemicals from residues or living plants (for example, weeds).

The possible affects of some allelochemicals on plant uptake of minerals and, hence, in inducing plant stress is discussed in Section 4.15.4.

4.12 BACTERIA AND OTHER MICROORGANISMS - MEDIATORS IN THE FORMATION OF ALLEL oCHEMICALS

Bacteria and other microorganisms (for example, fungi) are believed responsible for the production and modification of allelochemicals during decomposition of plant residues. Allelopathy can include the effects of microorganisms in production or alteration of compounds. A number of reports of interactions between microbes and plant residues in the production of allelochemicals exists (Borner 1960, McCalla and Haskins 1964, Wang et al. 1967, Chou and Patrick 1976, Lovett and Sagar 1978). Compounds are produced either directly or indirectly during microbial decomposition, especially under wet soil conditions (McCalla and Army 1961).

Norstadt and McCalla (1963) suggested that toxins could be produced by a combination of plant residues and microorganisms, with microorganisms being more prolific in the presence of residues. Cochran et al. (1977) found that only when soil conditions were favourable for microbial growth did plant residues produce substances inhibitory to wheat seedling root growth. This indicates the importance of many environmental factors (pH, oxygen, moisture availability) in modifying the production of compounds and the types of compounds produced.
Allelochemicals may be exuded by plant roots. Rhizosphere organisms may exert an effect in altering (detoxifying or enhancing) the allelochemicals exuded (Hoagland and Williams 1985). Alternatively, an effect of allelochemicals may be on the rhizosphere of the target plant, that is, on the numbers and types of microorganisms of the rhizosphere of that plant. This would be likely to have dramatic effects on the target since the relationship between the plant and its rhizosphere is important for the plant to absorb minerals and water. Hoagland and Williams (1985) list possible effects via action on micorrhizal binding to the root zone, infection processes of nodulating bacteria or alteration of the enzyme activities of the micorrhizal organisms.

Similarly, the rhizosphere may play a role in the production of allelochemicals from allelopathic plants. Martin (1971) believed rhizosphere organisms were responsible for transforming low molecular weight root exudate compounds into more complex compounds.

Heisey et al. (1985) showed that a number of microorganisms, particularly actinomycetes isolated from soil, could produce compounds toxic to indicator seedlings.

Elliott and Cheng (1987) believed that soil microbes played a large role in the dynamics of allelopathy. Although many compounds isolated from plant extracts can be shown to be active in experimental work, many of the identified compounds are less active or inactive in soil. Phenolic acids may be inactivated in soil either chemically or biologically. It is possible that in many cases the compounds reaching the target plants may not be the same ones released into the soil by the donor (allelopathic) plant.

Sida spp carpels have bacteria present which decarboxylate ferulic acid to a styrene derivative (Lieble and Worsham 1983b). Worsham (1984) also showed bacteria on Sida spp. seed coat could change ferulic acid to 2-methoxy-4-ethenylphenol (which is more toxic than Ferulic acid).

It is believed that the difference in activity between wheat straw extract and commercial preparation of ferulic acid is due to microbial activity producing a range of compounds from wheat straw, not only ferulic acid (Leibl & Worsham 1983). Pseudomonad bacteria have been implicated in allelopathic effects of wheat residues (Elliott and Lynch 1984). Fredrickson and Elliott (1985a and 1985b) showed Pseudomonad bacteria could reduce winter wheat root and shoot growth by producing
Literature Review

Rovira (1965) investigated the role of rhizosphere and rhizoplane organisms in influencing many plant growth characteristics, for example, root morphology, root/shoot ratio, uptake of calcium and rubidium, uptake of sulphur and phosphorus, mineral content, rate of development and onset of flowering and crop yield. Plant roots excrete many compounds, which can stimulate the growth of microorganisms in the rhizosphere. Possible mechanisms may involve microorganism production of chelating compounds, growth regulating substances and protective agents against root pathogens. This may extend to these organisms producing or altering allelochemicals otherwise present.

Other references exist where bacteria are believed to be involved in observed allelopathic responses, for example, from soil microorganisms (Heisey et al. 1985) and crop/weed associations (Lovett and Sagar 1978). Bacteria are also believed to be more likely to mediate the allelopathic responses seen in reduced cultivation systems where crop residues are retained (Lovett et al. 1982).

Strains of Enterobacter cloacae are also known to fix nitrogen (Line and Loutit 1972), even on the phylloplane, as reported by Lovett and Sagar (1978). Similarly, pseudonomads have been found to be closely associated with straw and the rhizosphere of some cereals (Elliott and Lynch 1984, Lynch and Clark 1984). The presence of these bacteria in abundant numbers in the soil may have positive effects on plant growth, as outlined by Lynch and Clarke (1984), while providing inhibitory effects through production of allelochemicals from residue substrate.

Microorganisms have also been implicated in allelopathic phenomena of eucalypt communities in Queensland and Tasmania (Bevege 1968, Ellis et al. 1980). It is postulated that invasion of forests by some grass species (Poa spp.) exerts a competitive edge via a number of mechanisms, including microorganism-mediated allelopathic effects. It has been suggested in a review by Lovett (1987) that bacteria and fungi associated with Poa spp. become the dominant microorganisms associated with eucalypt roots at the expense of the normal mycorrhiza, necessary for normal growth.

Blowes (1987) suggested the involvement of soil inhabiting fungi (Pythium spp.) as mediators in the inhibition of barley under the influence of annual rye grass residues. When soil was fumigated...
pre-seeding, or drenched with furalaxyl immediately following sowing, emergence was restored, even in the presence of ryegrass residues. It is possible that products produced from ryegrass residues form a substrate for production of toxic compounds by fungi.

It has been postulated that a succession of microorganisms successively degrades complex organic compounds to simpler forms, which become less toxic (Chatterjee and Nandi 1981). Lovett (1987) hypothesised that many allelopathic effects occur when conditions retard the sequence of breakdown and allow some (perhaps intermediate) compounds to build up to phytotoxic levels.

4.13. MODE OF ACTION OF ALLELOCHEMICALS

4.13.1 Effects on Division, Elongation and Ultrastructure of the Cell.

Effects on cell division and mitosis have been shown with coumarin and also volatile terpenes. Cornman (1946) showed coumarin to block mitosis in onion and lily roots via interruption of anaphase and accumulation of metaphases. This led to the formation of tetraploid nuclei or binucleate cells.

Two volatile terpenes (cineole, camphor) from Salvia leucophylla were seen to prevent mitosis in roots of Cucumis sativus seedlings (Muller 1965). They also prevented cells from elongating in roots and hypocotyls. Lorber and Muller (1976) found similar results where volatile terpenes from Salvia leucophylla leaves caused accumulation of lipid globules in cytoplasm of root tip cells of cucumber, also a reduction in number of organelles and disruption of intra-cell membranes. This led to the death of cucumber seedlings.

4.13.2 Effects on Hormone Induced Growth.

The phenolic compounds and tannins have been shown to inhibit hormone activity in some plants. Sondheimer and Griffin (1960) found IAA oxidase was inhibited by a number of polyphenols, notably chlorogenic acid derivatives. In other work p-Coumaric acid also stimulated the inhibition of IAA while ferulic acid did the opposite (Lee and Skoog 1965). Another example was where phenolic growth inhibitors from Salix rubra and rubber trees was able to suppress the activity of IAA and gibberellin (GA) (Kefeli and Turetskaya 1967).
A number of tannins has been reported to inhibit hypocotyl growth induced by GA in cucumber seedlings (Geissman and Phinney 1972), yet IAA induced growth was unaffected. Other compounds including coumarin, cinnamic acid and a number of phenolic acids also inhibited GA induced growth, but less so than tannins.

4.13.3 Membrane Permeability.

Rice (1984) lists a number of compounds implicated in allelopathic responses as having effects on membrane permeability in isolated systems using bacteria and isolated cells as indicators.

Balke (1985) believed that a number of aromatic compounds caused their effect via membrane disruption. The compounds include simple phenols (hydroquinone), phenolic acids (salicylic acid and ferulic acid), napthoquinones (juglone), flavonones (naringenin) isoflavones (genistein), flavonols (kaempferol) and dihydrochalcones (phloretin).

4.13.4 Effects on Mineral Uptake.

It is likely that many allelochemicals exert their effect on plant growth by causing changes in the ability of the plants to absorb nutrients. This may be due to changes in membrane permeability or changes in the plants ability to utilise the minerals absorbed. In this way the effects of allelochemicals may be difficult to identify and may be mistaken for other plant stresses or deficiencies.

Boawn (1965) implied that beet crops exert an effect on following crops by affecting their ability to absorb zinc from soil. The beet crops did not deplete zinc levels in soil, but it was postulated that toxins were added by the beet crop which reduced the uptake of zinc by following crops.

Tillberg (1970) showed that salicylic acid at concentrations of $10^{-6}$ to $10^{-3}$ M decreased phosphorus uptake by algae. Uptake of potassium and calcium by Amaranthus retroflexus seedlings was reduced by chlorogenic and tannic acids (Olmsted and Rice 1970).

Phenolic acids have been seen to inhibit phosphorus uptake (Glass and Dunlop 1974, Glass 1975, McClure et al. 1978), with higher inhibition proportional to the lipid solubility of the phenolic acid. Several phenolic acids (benzoic and cinnamic acid derivatives) were seen to depolarise the
membrane potentials of barley root cells, with this correlated to the degree of lipid solubility of the compounds. The hypothesis was that this inhibition was due to a generalised increase in membrane permeability to inorganic acids.

Balke (1977) studied a number of phenolic compounds for their effect on potassium absorption, finding that flavonoids were generally more inhibitory than phenolic acids at similar concentrations, with juglone the most inhibitory. These compounds also inhibited ATP-ase activity, possibly indicating a cause of the changed uptake of minerals since uptake is an energy requiring activity. Harper and Balke (1980) showed ferulic acid and salicylic acids to inhibit the uptake of potassium in oat roots with pH being an important factor such that inhibition being greater at pH 4.5 as opposed to no effect at pH 7.5.

4.13.5 Stomatal Opening and Photosynthesis.

Direct or indirect effects on stomatal opening and photosynthesis by allelochemicals have been reported, indicating another possible means of allelochemical action. For example, scopoletin has been shown to inhibit photosynthesis in sunflower, tobacco and Amaranthus retroflexus, with the degree of reduction being correlated with concentration of scopoletin (Einhellig et. a.l. 1970). CO$_2$ fixation was also reduced in line with scopoletin concentration, although respiration (dark) was little affected. The effect on photosynthesis was expressed by decreased plant growth. Turgor pressure was also reduced by scopoletin, this indicating a possible effect on stomatal operation reducing CO$_2$ absorption, leading to decreased photosynthesis.

Later work (Einhellig and Kuan, 1971) showed that scopoletin and chlorogenic acid caused stomatal closure. It was not clear whether stomatal closure caused decreased photosynthesis or vice versa.

Einhellig (1971) showed tannic acid to also cause stomatal closure, but when plants became severely wilted stomata again opened, indicating that stomatal effects are not the only mechanism of growth inhibition due to tannic acid.
Quinones have been reported as inhibiting CO₂ fixation in chloroplasts (Sikka et al. 1972). The compounds tested were not plant derived, however, this report indicates a possible action of endogenously produced quinones.

Arntzen et al. (1974) found that a flavonol, kaempferol, inhibited coupled electron transport and photophosphorylation in pea chloroplasts, however, no effect on uncoupled electron transport was observed. This was taken as suggesting an effect on energy transfer by flavonols.

Phlorizin at concentration of 10⁻³ M inhibited the rate of photoreduction of NADP⁺ in pea chloroplasts and also reduced electron transport (Roshchina and Akulova 1978).

A number of phenolics has been seen to significantly decrease the concentration of chlorophylls a and b in unifoliate soybean leaves. This was paralleled by reduction in dry weight increases (Einhellig and Rasmussen 1979). The same compounds also reduced weight increases of sorghum seedlings, although leaf chlorophyll concentrations were not affected. Patterson (1978) also observed inhibition of photosynthesis by phenolics, working with soybeans.

4.13.6. Respiration.

A number of allelochemicals has been noted as affecting respiration in plants and inhibition of the oxidation process by allelochemicals has been widely reported. For example, several quinones reduced oxygen uptake in yeast cells and in lettuce and barley seeds (van Sumere et al. 1971).

Several phenolic compounds showed a range of effects on hypocotyl growth and mitochondrial respiration in mung beans (Demos et al. 1975). Muller (1969) noted mitochondrial effects of volatile terpenes, this effect being localised conversion of succinate to fumarate, or fumarate to malate in the Krebs cycle.

Allelochemical effects on ATP production have also been suggested as a mechanism of inhibiting respiration. Koepp (1972) reported juglone as inhibiting oxygen uptake by corn roots. It was believed that juglone caused uncoupling of ATP production.
Stenlid (1970) believed that flavonoids affect ATP production by mitochondria. Kaempferol has been seen to reduce respiration (Koepppe and Miller 1974) in corn mitochondria. They believed that kaempferol acts on the phosphorylation mechanism and not on electron transfer.

Moreland and Novitsky (1987) showed that benzoic and cinnamic acids, coumarins and flavonoids all inhibited electron transport and phosphorylation in chloroplasts and mitochondria. All also inhibited carbon dioxide - dependant oxygen evolution in intact chloroplasts. Flavonoids were seen to be more inhibitory than coumarins, which were more inhibitory than cinnamates and benzoates. They postulated that the inhibition of substrate oxidation resulted from alterations and perturbations induced in the inner membrane of mitochondria as evidenced by alterations in carrier mediated transport processes.

Ulitzer and Poljakoff-Mayber (1963) believed coumarin acted by uncoupling respiration, though later Sarma and Barooah (1982) found coumarin to antagonise gibberelic acid activity. Others (Van Sumere et al. 1972) have suggested that coumarin may inhibit germination by inhibiting amino acid transport and protein formation. It is probable that coumarins are active in a number of ways.

Similarities in the mode of action of some allelochemicals and some herbicides can be noted. Several herbicidal compounds are believed to inhibit respiration. Moreland (1980) suggested effects on respiration as a mode of action of dinitroaniline herbicides. Mitochondrial respiration was inhibited and oxidative phosphorylation uncoupled. Trifluralin is believed to disrupt ATP supply, this being a means of upsetting mitosis. The amide group of herbicides has also been reported as inhibiting respiration (Jaworski 1956, Sasaki and Kazlowski 1966).

The volatile terpene, cineole, has been implicated in reducing oxygen uptake in Avena fatua and Cucumis sativus mitochondria (Muller 1969). A derivative of this allelochemical (cinmethylin) has been used commercially as a herbicide. The potential exists for other allelopathic compounds to be researched and tested either as herbicides alone or as indicators of active chemical structures which may have commercial potential. These would represent more 'natural' origins of possible agriculturally useful products.

A number of allelochemicals has been shown to affect protein synthesis. Van Sumere et al. (1971) showed that a range of allelopathic inhibitors reduced incorporation of radio-labelled phenylalanine into protein in yeast cells. Ferulic acid and coumarin significantly reduced incorporation of $^{14}$C labelled phenylalanine into protein in lettuce and barley seeds and embryos.

Other phenomena documented have been effects on lipid and organic acid metabolism. Some quinones have been reported to cause an increase in the proportion of carbon in sucrose and glycine compared to lipids and glutamic acid (Zweig et al. 1972). This suggested an inactivation of coenzyme A, causing a shortage of NADPH. Similar effects on carbon incorporation have been noted with ferulic acid and cinnamic acid. Danks et al. (1975b) showed an increase in $^{14}$C incorporation into soluble lipid, at the expense of protein, organic acids and soluble amino acids. This suggested that these phenolic acids caused a reduction in protein synthesis. Cameron and Julian (1980) also showed ferulic and cinnamic acids to reduce protein synthesis in lettuce seedlings, accompanied by reduced root growth.

Wink (1983) has proposed that alkaloids exert their inhibitory effects by interfering with the binding of aminoacyl-t-RNA to ribosomes and subsequent polypeptide synthesis, while other workers (Knypl and Janas 1977, Knypl and Oswiecimska 1986b), using other alkaloids, have suggested the activity is due to interference of cell membrane permeability.

4.13.8. Effects on Specific Enzymes.

a) Pectolytic enzymes:

Some tannins have been seen to inhibit the activity of pectolytic enzymes (Williams 1963, Benoit and Starkey 1968b), these enzymes being important in the ability of pathogens to penetrate host cells.
b) Cellulase:

Benoit and Skarkey (1968b) showed tannins to inhibit cellulase, having also slowed decomposition of cellulose and hemicellulose by microorganisms (Benoit and Starkey 1968a), possibly due to effects on the cellulase enzyme.

c) Catalase and Peroxidase:

Some evidence exists regarding allelochemical effects on catalase and peroxidase and subsequent effects on IAA and, therefore, plant growth via hormone activity (Lee and Skoog 1965). Benoit and Starkey (1968b) suggest tannins may also affect these enzymes.

Jankay and Muller (1976) showed that umbelliferone caused swelling of roots possibly caused by increased peroxidase levels.

d) Sucrase:

A terpene compound, aescin, has been shown to inhibit the enzymatic hydrolysis of sucrose (Olsen 1974). Tannins have also been shown to inhibit sucrose breakdown in plants (Benoit and Starkey 1968b).

e) Others:

Benoit and Starkey (1968b) list a large number of enzymes affected by tannins: amylase, myrosinase, pepsin, proteinase, dehydrogenases, decarboxylases, phosphatases, β-glucosidase, aldolase, polyphenol oxidase, lipase, urease, trypsin and chymotrypsin.


A number of compounds has been shown to affect internal water relations in plants. Roshchina (1972) showed a number of compounds, including phenol, salicylic acid and cinnamic acid, to reduce turgor in cells of storage tissue of beets. Van Alfen and Turner (1975a,b) showed that water soluble glycopeptide toxin significantly reduced water conductance. Glycopeptide toxins from Corynebacterium spp. caused wilting of lucerne seedlings.
It is likely that a number of plant cellular and physiological activities are affected by allelopathic compounds, with some substances having a number of modes of action. This could lead to multiple physiological manifestations of these effects. It is also likely that in many cases more than one allelochemical is present, causing a number of plant processes to be affected. The physiological effects observed in plants due to allelochemicals may be the result of more than one process being affected.

Many of the effects seen, for example, cell division effects, mineral uptake, respiration and ATP production, and protein synthesis effects may be due to a primary effect of the compounds on membrane (especially mitochondrial) disruption. As has been suggested (Lorber and Muller 1976, Koch and Wilson 1977, Lovett 1982) it is likely that membrane dysfunction may be a common effect of allelochemicals. This, in turn, may be linked to many of the other documented effects noted above.

Many of the allelopathic effects seen on a macro scale resulting in reduced germination and early growth are due to more subtle and basic effects within plant cells. It is likely that allelochemicals exert their effects via a variety of primary effects occurring at the intracellular level. Compounds produced by allelopathic plants may cause dysfunction of mitochondrial membranes and enzymes, thus, affecting energy supply to the cell. Other effects may result in the cell being unable to mobilise food reserves, transport minerals or cell products or cause leakage of important cell components. These primary effects, however subtle, may be sufficient to cause the larger effects measured as poor germination or growth of the affected plants. This theme has been advanced by Lovett (1982) and can be extended to include interactions with other stresses for example disease and climatic or nutritional stresses. Allelopathic effects are thus likely to be the manifestations of a variety of possible primary effects or subtle changes to plant function at an intracellular level.
5 BRASSICAS AND GLUCOSINOLATES

5.1. BRASSICA SPECIES AS ALLELOPATHIC PLANTS

There are many reports of allelopathic effects with Brassica spp., both crops and weeds. Table 5.1 lists some of these. More detailed discussion of the effects seen with canola, including effects on fungi and insects is presented later.

<table>
<thead>
<tr>
<th>PLANT SHOWING ALLELOPATHIC ACTIVITY</th>
<th>REFERENCE</th>
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<tbody>
<tr>
<td><strong>CROPS:</strong></td>
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<tr>
<td>Chou moellier (Brassica oleracea)</td>
<td>Campbell 1959</td>
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<td>Broccoli (Brassica olearcea)</td>
<td>Patrick 1971</td>
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<tr>
<td>Mustard (Brassica juncea) &amp; Broccoli (Brassica olearcea)</td>
<td>Jimenez-Osornio &amp; Glissman 1987</td>
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<tr>
<td>Canola (Brassica napus)</td>
<td>Purvis <em>et al.</em> 1985</td>
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<tr>
<td>Canola (Brassica napus)</td>
<td>Mason-Sedun <em>et al.</em> 1986</td>
</tr>
<tr>
<td>Canola (Brassica napus)</td>
<td>Vera <em>et al.</em> 1987</td>
</tr>
<tr>
<td>Canola (Brassica napus)</td>
<td>Purvis 1990</td>
</tr>
<tr>
<td><strong>WEEDS:</strong></td>
<td></td>
</tr>
<tr>
<td>Brassica nigra (black mustard)</td>
<td>Grummer &amp; Beyer 1960</td>
</tr>
<tr>
<td>Brassica. kaber (wild mustard)</td>
<td>Erickson &amp; Duke 1978</td>
</tr>
<tr>
<td>Brassica. nigra (mustard weed)</td>
<td>Mason-Sedun <em>et al.</em> 1986</td>
</tr>
<tr>
<td>Camelina alyssum</td>
<td>Grummer &amp; Beyer 1960</td>
</tr>
<tr>
<td>Camelina sativa</td>
<td>Lovett &amp; Sagar 1978, Lovett &amp; Jackson 1980</td>
</tr>
</tbody>
</table>

The processing of plants or the plant parts appear to be important in altering or enhancing the allelopathic effects seen with Brassica spp. Campbell (1959) showed that chopping and mincing of plant parts produced more inhibition. This may reflect a better extraction of active compounds or a greater mixing of plant contents, leading to a better yield or production of more active compounds.

The source of toxins produced from Brassica spp. may vary with plant part. Campbell (1959) found roots as the source of the most severe inhibitory effects on test species and Akram and Hussein (1987) also showed roots as a potent source of inhibitory compounds.
Fresh material generally produces less inhibitory extracts than those produced from dried plants or residues. Grummer and Beyer (1960) and Patrick (1971) showed *Brassica* spp. residues as inhibitory to germination and establishment of following crops and pastures. Similarly Jimenez-Osornio and Gliessman (1987) showed extracts of dried *Brassica* spp. to be more inhibitory than from fresh plants. *Brassica* spp. crop residues have been demonstrated as inhibitory to weed germination in the field (Purvis *et al.* 1985). Similarly, Mason-Sedun *et al.* (1986) showed residues of a range of *Brassica* spp. to be inhibitory to test species. A more detailed coverage of the allelopathic effects of *Brassica* spp. residues is presented in the following section.

It is strongly believed that the toxins from *Brassica* spp. may be produced from the breakdown of glucosinolates as the plants are damaged or senesce. This may help explain the more severe effects seen when using dried plants or residues, since breakdown products would be more prevalent. *Brassica* spp. high in glucosinolate content may be expected to be more potent sources of allelopathic agents. This postulate is supported by Jimenez-Osornio and Gliessman (1987) who observed that more highly selected species (for example broccoli) were less inhibitory than wilder types (for example, mustard). It is possible that both different levels of glucosinolates and different types of glucosinolates are present in different species or varieties of *Brassica* spp. with this being partly responsible for any different effects seen.

More recent workers also provide evidence that glucosinolate breakdown products are the source of active compounds, especially the Isothiocyanates (Mojtahedi *et al.* 1991, Angus *et al.* 1994), as active substances against fungal pathogens. See discussion of this aspect of Brassica activity against fungi in Chapter 6.

Interactions may occur with other organisms or microorganisms. Lovett and Duffield (1981) showed that phylloplane bacteria were able to produce the allelochemical benzyamine from washings of *Camelina sativa*. This may occur widely in *vivo* with other *Brassica* spp. leading to the production of a range of inhibitory compounds as residues decay, further enhancing the production of toxic compounds.
5.2. BRASSICA RESIDUES


As mentioned, above, extracts from dry material or plant residues has been seen to produce greater effects than fresh material (Grummer and Beyer 1960, Patrick 1971, Jiminez-Orsonio and Gliessman 1987, Vera *et al.* 1987, Purvis 1990), indicating possible better yield or more ready extraction from dry material. Drying of *Brassica* spp. is believed to lead to hydrolysis of glucosinolates which may be expected to producing breakdown products and it is possible these factors account for the effects seen with residues in the field. A more detailed treatment of glucosinolate breakdown products is presented in a later section.

Other compounds, aside from glucosinolates, may also be produced, with Harper and Lynch (1982) showing *Brassica* spp. residues to produce more acetic acid than cereal straw. Acetic acid is a general breakdown product from plant decay, while glucosinolates are produced by the plant through its life. The authors suspected that under anaerobic conditions volatile fatty acids and phenolics were produced which further contribute to the toxic effects, since the inhibition seen with *Brassica* spp. extracts was greater than that attributable to acetic acid alone. Thus *Brassicas* spp. may be a productive source of a number of allelopathic compounds.

Jimenez-Osornio and Gliessman (1987) showed that *B. campestris* and *B. oleracea* residue extracts were inhibitory to a range of indicator plants, including barley, vetch, broccoli, ryegrass, radish and lettuce. Water extracts from the residues were less toxic to Cruciferous plants, it being possible that Crucifers are able to transform the allelochemicals to less toxic compounds. This may be important since the crucifers, as part of the *Brassicaceae*, may have evolved a tolerance of compounds produced by other *Brassica* spp. *B. campestris* was seen as being more inhibitory than *B. oleracea* and extracts from dry material were the most active. All parts of dry plants were inhibitory while only fresh leaves yielded inhibitory extracts. This points to glucosinolates as a likely source of the inhibitory products since they are produced in leaves throughout the life of the plant.
Mason-Sedun (1986) also found that residues of *Brassica* spp. could lead to yield reductions in cereal crops following *Brassica* spp. in rotation. Mason-Sedun *et al.* (1986) describe a number of experiments showing several species of *Brassica* to be allelopathic to wheat. Variation between species and between cultivars was observed; all parameters were affected including 1000 grain weight, tiller number, plant height, final plant dry weight and crop yield. In another experiment root dry weight and shoot dry weight were reduced.

As in the work of Jimenez-Orsonio and Gliessman (1987), the more 'advanced' lines (i.e. selected and bred) were seen to be less toxic than older lines or weed species. This agrees with work with other crops, for example, Putnam & Duke (1974) where more highly selected accessions of cucumber were seen to be less allelopathic. The differences between cultivars may reflect different levels of toxic compounds present or different compounds in the cultivars. These could give different leachates or produce differing compounds upon decomposition. *Brassicas* spp. have differing glucosinolate profiles which may produce different breakdown products upon decomposition within the residues, thus producing leachates of varying toxicity.

Residues tested in field conditions produced greater inhibition than in glasshouse work, especially in grain yield. These effects are attributed to anaerobic soil conditions leading to the production of more toxic compounds in greater amounts. This is supported by Patrick (1971) and Harper & Lynch (1982) where a number of compounds were believed to be produced with residue breakdown. It is also possible that high localised concentrations may be present in soil following breakdown of these residues under these conditions.

A supplementary effect of water soluble leachates from *Brassica* spp. residues is possibly occurring. These leachates may produce early inhibition, with further breakdown products being responsible for longer term toxic effects. The degree of inhibition was reduced with longer periods of storage, similar to findings by Kimber (1967) with wheat and Lovett and Jackson (1989) with *Camelina sativa*. Atmospheric humidity and oxygen may allow micro-organisms to degrade residues thus reducing the phytotoxic effects.

*Brassica* spp. residues have been thus shown as allelopathic to a number of plants. Decomposition of the residue appears to be an avenue for production of toxic products. This suggests
a role of microorganisms in the production of allelopathic compounds from these plants. This is discussed in section 5.5, following.

5.3. Rapeseed (Canola), its uses and benefits.

Rapeseed seed is of high protein and oil content making it a desireable source of vegetable oil and protein for use in animal and human nutrition. The presence of glucosinolates and, in particular, the goitrogenic ones has rendered the meal of limited use for non ruminant animal feeds. Breeding programs both in Australia and overseas have concentrated on reducing the levels of seed glucosinolate and erucic acid content such that the meal from later cultivars is now acceptable as a high protein animal feed. Similarly, the lack of cholesterol in the oil has attractions for human nutrition. The name Canola is now used to refer to these later varieties of rapeseed, having very low seed glucosinolate and erucic acid levels.

Canola in Australia has become a popular crop for financial return and for its utility in cereal rotations. It is now frequently the first crop following a pasture phase, especially in southern cereal growing regions.

Wheat grown following canola in southern Australia generally performs well, this being attributed to a number of factors involving the crop. The deep tap root system is seen as beneficial in relieving compaction in the soil and allowing subsequent cereal roots to more fully explore the soil, and moisture to enter. A reduction in cereal disease levels in soil after a canola crop is also considered important in enhancing the yield of the following cereal crop. This is attributed to the control of disease-hosting grasses afforded by the canola crop (as well as the canola crop itself being a non-host for these cereal pathogens). Grass control is achieved by use of grass specific herbicides (for example, trifluralin pre-emergent, or a number of post-emergent products) and also the dense canopy of the crop excluding these weeds from growing later in the season.

In Europe work between 1970 and 1984 showed wheat to yield on average 15% more when it was grown in rotation with rapeseed than where grown in monoculture (Schonhammer & Fishbeck 1985a), with this being attributed to both reduced disease and nematode levels, but also to enhanced root growth of wheat following rapeseed. In Australia, Angus et al. (1991) found that wheat grown after
canola to be more efficient at extracting water and nitrogen from soil than wheat grown after wheat, again suggesting enhanced root growth. Kirkegaard et al. (1993) suggests two reasons for this enhanced root growth; strong taproot growth effectively drilling holes in the soil, allowing better water entry and cereal root growth, and the effect of canola in reducing cereal root fungal pathogen levels.

5.4. Potential problems or beneficial effects in crops grown following rapeseed (canola):

Rapeseed (canola) has been shown to produce washings which can be inhibitory to wheat (Mason-Sedun et al. 1986, Mason-Sedun 1987). This, in itself, may not be of practical agronomic importance especially in the southern cereal producing regions since where wheat is planted following rapeseed a period (up to six months) generally elapses. Any direct deleterious affect of the crop residue may, therefore, have subsided over this time. This may be expected to be the case, for example, in red-brown clay loam soils of southern NSW, however evidence exists of contrasting effects seen with wheat and other crops grown in dark clay soils of the northern Australian wheatbelt. Thompson et al. (1989) show evidence of canola as contributing to the phenomenon of 'Long Fallow Disorder' through it acting as a non-host to Vesicular-Arbuscular Mycorrhizal fungi (VAM). Where VAM-dependent crops (for example, wheat) are grown following a canola crop poor VAM colonisation can be expected with deleterious effects on the crop through reduced access to some nutrients. The authors believe this non-host effect of canola to be due to glucosinolate breakdown products, these being part of a general defence mechanism in canola.

Horricks (1969) observed rapeseed residues as being inhibitory to germination to a number of small grain crops and believed this to be due to nitrogen immobilisation and physical affects, a view supported by Waddington (1978) who showed rapeseed residues as reducing emergence of lucerne and bromegrass (Bromus inermis) but as having no effect on barley. Waddington and Bowen (1978) showed improved germination of barley where rapeseed residue had been allowed to decompose for some months, this providing nitrogen from microbial breakdown of the residue. These observations may help to explain the general stimulatory effect seen in the southern cereal growing areas of Australia, where products of canola residue breakdown may be having some selective effects such that cereals are less or not affected compared to some other plants. It is also possible that better crop growth following canola crops is due to the crop either, as living plants or residues, suppressing or
effect on crop growth and yield. The possible effects on fungal pathogens is dealt with in a later chapter.

The above observations, of relatively little effect of rapeseed residues on barley (or possibly other larger seeded plants), contrasts with the work of Kasting et al. (1974) where large amounts of residue in field conditions reduced a number of growth parameters of many crops notably barley and wheat. They also showed water extracts of rapeseed as inhibitory to these crops. Rapeseed residues were more inhibitory than many other residues tested. Their hypothesis was that a toxin or growth inhibitor was present in rapeseed straw, this being leached into soil. The different effects reported are possibly due to factors including conditions during breakdown (that is, aerobic/anaerobic) or freshness of residue or extract. Where canola residues are allowed to decay over a period of time prior to germination of following crops the inhibitory effects or agents may have dissipated sufficiently or broken down further so as to be less inhibitory to some of the following plants. In this case other factors (for example, seed size) may become important in influencing that plants' susceptibility to the toxins present.

If canola residues produce germination inhibitors this may have implications for double crop systems where rapeseed may be followed by a summer crop. Similarly, the ability of any compounds produced from residues to suppress weed growth would be an important discovery, perhaps having agronomic implications for weed management strategies. The reported clean wheat crops following canola may be due, in part, to allelopathic effects in suppressing weeds during the crop and in the period following. A further consideration may be that substances produced by the crop or its residues may have effects on disease organisms (for example, fungal pathogens of cereals) such that cereal crops planted following rapeseed have a much reduced incidence of disease. This hypothesis is discussed further in the later chapters and experiments.

5.5. BACTERIA AND BRASSICA SPECIES - MEDIATORS IN OBSERVED ALLELOPATHIC EFFECTS

The role of bacteria in the allelopathic effects of Camelina sativa has been investigated by Lovett and Sagar (1978), who identified Enterobacter cloacea as being prevalent in C. sativa leachates, where they were necessary for the allelopathic effects to be manifest. Later work by Lovett and Jackson (1980) identified Pseudomonas florescens as playing a similar role where C. sativa leaf washings,
when incubated, become strong smelling and cloudy when bacteria were present in the leachate. With incubation in the presence of bacteria the content of organic acids of the citric acid cycle present in the leachate became depleted, leading to the hypothesis that these compounds were used by the bacteria as substrate for the production of the allelopathic compounds in the washings (Lovett and Duffield 1981).

Lovett and Duffield (1981) identified benzyl isothiocyanate from extracts of *C. sativa*. Tang *et al.* (1972) have shown that *E. cloacae* is capable of degrading this compound to hydrogen sulphide and benzylamine, a compound identified as allelopathic (Lovett 1987). The proposed pathway is shown below.

\[
\text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{N} = \text{C} = \text{S} + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{NH}_2 + \text{H}_2\text{S} + \text{CO}_2
\]

Benzyl Isothiocyanate  Bacterial enzyme  Benzylamine

It is possible, therefore, that the bacteria are able to produce benzylamine from exudates from *Camelina* spp. leaves via the intermediate product benzyl isothiocyanate. The action of benzylamine has been postulated as interfering with membrane integrity (Umbers 1982) and it is possible that this leads to cellular dysfunction and reduced ability to metabolise seed reserves, resulting in the inhibitory effects observed.

The species of bacteria mentioned are found to be more prevalent on senescent leaves (Lovett, *et al.* 1982), leading to the hypothesis that levels of bacteria become high during maturity and senescence of Brassicas, leading to the production of isothiocyanates and other compounds (for example, benzylamine) from pre-cursors (for example, glucosinolates), the bacteria using organic acids for their own metabolic activity. This may enhance the production of compounds by bacteria as residues decompose in soil. Compounds produced in this way may be responsible for the allelopathic responses observed by Mason-Sedun *et al.* (1986).
Fig 5.1. Suggested pathway for production of breakdown products from Camelina spp. utilizing bacteria. (After Lovett 1986).

5.6. SELF DEFENCE IN Brassica spp. AND SOME POSSIBLE COMPOUNDS INVOLVED

A number of workers has discussed the possibility of compounds prevalent in Brassica spp. as playing a defensive role. Walker et al. (1937) outline the fungicidal effects of pure glucosinolates. Other early workers including Hooker et al. (1943) and Munnecke et al. (1962) reported isothiocyanates (products of glucosinolate breakdown) as being able to adversely affect a number of plant pathogens, with this possibly being important in plants high in glucosinolate content as a defensive mechanism. A review by Whittacker and Feeny (1971) suggests the production of isothiocyanates by Brassica spp. as a defensive mechanism, and Trappe (1987) supports the role of glucosinolate breakdown products (for example, isothiocyanates) as having a defensive function.
Brassica spp. have been shown to have an ability to suppress the growth of fungal pathogens, this possibly being due to glucosinolate breakdown products. Many workers have identified antifungal activity of leachates or extracts of Brassica spp. and also observed residues as suppressing fungal growth. The ability of Brassica spp., especially rapeseed or canola, to inhibit fungal growth is dealt with in Chapter 6.

Glucosinolates in Brassica spp. are also thought to play a role in interactions with insects, with the volatile hydrolysis products being shown in some early work to assist in attracting some insects (Thorsteinson 1953, David and Gardiner 1966). Cole (1976) discusses the role of glucosinolate breakdown products in attraction of or defence from insects to Brassica spp. with breakdown products seen as likely to be isothiocyanates or nitriles depending upon conditions at breakdown (pH, temperature), or whether autolysis or exogenous breakdown takes place.

An interesting example of defense against insects has been shown by Erickson and Feeny (1974). Brassica nigra was shown to produce adverse effects against the larvae of an insect pest, Black Swallow Butterfly (Papilio polyxenes). Sinigrin in the plant is broken down in the gut of the insect to allyl isothiocyanate which is toxic to the insect. The authors believed that glucosinolates from the Crucifereae provide a general means of protection. It is thought that when the plant is eaten, or dies and begins to decay, the separately stored glucosinolates and enzyme come in contact, producing isothiocyanates, with these providing protection for the plant. Later work by Gill and MacLeod (1980) expands on this theme where they discuss organoisothiocyanates as having insecticidal activity, due to the release of hydrogen sulphide in some insects due to the activity of glutathione transferase (Habig et al. 1975).

Wadleigh and Yu (1988) have suggested that insects which prey on Cruciferous plants may have evolved dietary mechanisms allowing them to detoxify the effects of glucosinolate breakdown products, especially the (otherwise toxic) isothiocyanates and, where present, organoisothiocyanates. They identified a glutathione transferase in the gut of three lepidopterous insects, with this enzyme able to detoxify the otherwise toxic glucosinolate breakdown products produced. An interesting effect also mentioned was the possibility of this enzyme to also detoxify organophosphorus and organochlorine insecticides. Isothiocyanates and further breakdown products ingested could induce the production
of the enzyme in these insects and so may enable these insects to prey on Cruciferous plants. Significantly, two breakdown products of indole glucosinolates common in vegetative parts of *Brassica* spp., 3-indoly1 methyl isothiocyanate and indoleacetonitrile were able to induce the enzyme activity. This may indicate the development of an evolutionary association between the insects and Brassica plants where these compounds are an important element in defensive mechanisms of these plants.

A contrasting effect, where glucosinolate levels seemed not to affect feeding rates of an insect predator to mustard and rapeseed, has been reported (Bodnaryk and Palaniswarmy 1990). Flea beetle (*Phyllotreta cruciferae*) feeding rates were not altered appreciably by high or low glucosinolate levels in cotyledonous leaves of mustard and rapeseed. 3-indolylmethyl glucosinolate was seen to produce a small reduction. There may be other attractants to this insect of a more powerful nature or the beetle may have a mechanism of detoxifying any compounds produced from the glucosinolates. This may be the case in some other insects which are themselves pests of Brassica crops.

It would seem likely that the presence of glucosinolates in the Cruciferae and particularly *Brassica* spp. plays a role in the defensive makeup of these plants. The breakdown products produced when the plants are attacked by fungi or insects seem to be important in inhibiting the development of disease or in repelling insect attack. It is also possible that these products have activity in inhibiting the germination of other plants, and as such assist in conferring a wider competitive advantage to Cruciferous plants.

5.7. **GLUCOSINOLATE BREAKDOWN PRODUCTS - ALLELOCHEMICALS IN BRASSICA SPECIES.**

![Figure 5.3. The general structure of Glucosinolates.](image)

Glucosinolates (Figure 5.3) are found in a number of plant families, predominantly throughout the orders Capparacae, Moringaceae, Resedaceae and Tovariaceae (Kjaer 1976) They are particularly prevalent in many of the *Brassica* spp., including kale crops (*B. oleracea*), condiments (*B. nigra*; *B.*
hirta or Sinapis alba), rapeseed and canola (B. campestris; B. napus), and forages (Fenwick et al., 1982). The total glucosinolate content of particular plants, as well as the proportions of the individual glucosinolates, varies with the part of the plant (Josephsson, 1967).

The role of glucosinolates in Brassica spp. is not clear, but may be manyfold. A role in attraction of insects for pollination has been suggested (Cole 1976), and other roles in defense from fungal or insect attack have been canvassed earlier. Breakdown products (for example, isothiocyanates) of glucosinolates are of a bitter taste and so may play a role in general defence of plants from grazing animals or to help seed survival. It is possible that glucosinolates may also be present as a storage mechanism for waste products of plant metabolism, with any effects of these compounds either alone or from their breakdown products of a defensive nature representing a competitive evolutionary advance for these plants. Allelopathic effects arising from these compounds may similarly be an evolutionary development, such that the presence of glucosinolates confers a number of benefits to the plant, including allelopathy, to enhance that plant's competitive ability.

Many reports exist detailing inhibitory effects of glucosinolates and their breakdown products on plant germination and growth, with many Cruciferous plants, notably Brassica spp., implicated. Evanari (1949) published an early review of naturally occurring germination inhibitors, listing a number of Cruciferous species as sources of these. The mustard oils (isothiocyanates) were implicated as the inhibitory agents, particularly 2-propenyl and 2-phenylethyl isothiocyanates. 2-propenyl isothiocyanate has subsequently been shown to slow germination of wheat, pea and rapeseed (Leblova-Svobodova, 1962) and Bromus rigidum (Bell & Muller, 1973), further indicating the ability of these compounds as allelopathic agents.

As mentioned earlier, Jimenez-Orsonio and Gliessmann (1987) showed that extracts from Brassica spp. were toxic to a number of plants, with isothiocyanate derivatives, for example, allyl isothiocyanate, a breakdown product of the glucosinolate sinigrin, thought responsible. The extract fraction from B. campestris was more inhibitory than sinigrin suggesting that a number of products may be present, including glucosinolate breakdown products, plus others which may add to the inhibitory effect seen. Extracts from dry material were more inhibitory, believed due to the drying of
the material producing hydrolysis of glucosinolates to isothiocyanates. Autolysis (as would occur in fresh material used for extracts) was noted as yielding mainly nitriles with these being less inhibitory.

A number of compounds may be responsible for the allelopathic effects seen with these plants, with glucosinolate breakdown products significant in these effects. There may be a number of breakdown products present depending on conditions at breakdown with these products having differing activity, such that, for example, isothiocyanates formed from some glucosinolates are more active than some of the other possible compounds, with nitriles produced from other glucosinolates being the active compounds in other cases. It may depend on the source glucosinolate with differences occurring between aliphatic (for example, sinigrin) and aromatic (for example, the indole) glucosinolates in the activity of their respective breakdown products.

The indole glucosinolates are the major glucosinolates of vegetative parts of Brassica spp. (Bergmann 1970, Josefsson 1970) but are absent or present in only small amounts in seed. Sang et al. (1984) found that 3-indolylmethyl glucosinolate was the dominant glucosinolate in leaf and stem material of rapeseed, though 4-methoxy-3-indolylmethyl glucosinolate was also present in these tissues. In root material both 2-phenylethyl glucosinolate and 4-methylthiobutyl glucosinolate were found as dominant. Other authors have indicated that 3-indolylmethyl glucosinolate is the predominant glucosinolate in Brassicae (Wall and Taylor 1988).

Since the indole glucosinolates are the major ones present in the vegetative parts of Brassica spp it is possible that these are the glucosinolates responsible for the allelopathic effects seen with these plants. Kutacek (1964) demonstrated a number of Brassica vegetables to inhibit the growth of wheat, with the effect being very similar to that from 3-indolylmethyl glucosinolate, and its breakdown product, 3-indolylacetonitrile, these compounds being present in the vegetables. An interesting effect in this work was that at low concentrations these compounds were stimulatory, an effect noted with other phytotoxic compounds (Lovett & Duffield 1981).

5.8. The breakdown products of glucosinolates:

The enzyme myrosinase (Thioglucoside Glucohydrolase, EC 3.2.3.1) is responsible for the degradation of glucosinolates (Kjaer 1960, Fenwick et al. 1982). Isothiocyanates are, typically,
formed from glucosinolates by the action of this enzyme although a range of breakdown products may be formed. These include nitriles, thiocyanates, oxazolidine-2-thiones, hydroxynitriles and epithionitriles. Which of these is formed depends on a number of factors, as discussed, below.

Production of breakdown products from these glucosinolates is believed to begin with cellular disruption leading to glucosinolates and the enzyme myrosinase coming into contact. The enzyme promotes the production of glucose, bisulphate and volatiles (for example, nitriles and isothiocyanates) (Van Etten et al. 1969). Myrosinase hydrolysis in this way yields predominantly isothiocyanates (Ettlinger & Kjear 1968, Hanley et al. 1983). Cole (1976) found that autolysis in many Brassica spp. led to formation of mainly nitriles, however she suggested that both nitriles and isothiocyanates could be formed. In root material of B. juncea, B. nigra, B. oleracea and B. rapa she found high concentrations of both phenylethyl isothiocyanate and phenylethyl nitrile. 2-phenylethyl glucosinolate is the dominant glucosinolate in roots of Brassica spp. An interesting parallel occurs in barley, where hordenine is produced mainly in roots, with gramine in shoots.

Factors influencing which of the possible breakdown products are formed include the nature of the individual glucosinolate and the presence of other compounds which alter the action of the enzyme. Conditions during enzymic hydrolysis (pH, source of enzyme, temperature) also influence which breakdown products will be produced (Van Etten, Daxenbichler and Wolff 1969, Tookey and Wolff 1970, Srivastava and Hill 1974). Kjaer (1960) pointed out that myrosinase appears to have a pH optimum of 6.5 to 7.5 and temperature optimum between 30°C and 40°C. Later work by Pihakaski (1978) indicated that under low pH the formation of nitriles was favoured while higher pH gave rise to more Oxalidine-2-thione. Dry heating of rapeseed meal reduced the proportion of nitrile formation (Van Etten et al. 1966). Figure 5.3 (over) outlines the normal breakdown products from enzymic (myrosinase) glucosinolate hydrolysis.
Hydrolysis of most glucosinolates at neutral pH by myrosinase has been thus reported to yield isothiocyanates. Two notable exceptions are the indole glucosinolates, especially 3-indolylmethyl glucosinolate, N-methoxy-3-indolylmethyl glucosinolate, and p-hydroxybenzyl glucosinolate, these being the most prevalent glucosinolates in vegetative parts of *Brassica* spp. The isothiocyanates of these readily hydrolyse, especially at pH above 7, to give free thiocyanate ion and the corresponding alcohol which can condense to form diindoleamines (Gmelin and Virtanen 1960, 1961, Josefson 1970). A higher production of nitriles may be expected under acidic conditions or via heat treatment.
of the glucosinolate (Slominski and Campbell 1989a). Similar products (nitriles) were noted from breakdown of phenylethyl glucosinolate (predominant in roots of Brassica spp.).

Evidence exists indicating that 3-indolylmethyl glucosinolate may also produce the nitrile (indolacetylnitrile) under higher pH conditions in the presence of ferrous ions and ascorbate in vivo (Mahadevan and Stowe 1972), or upon autolysis (McDannell et al. 1987). Further breakdown of this compound is possible under the influence of nitrilase enzymes to produce indoleacetic acid (IAA) (Thimann and Mahadevan 1958, Gmelin and Virtanen 1961).

Slominski and Campbell (1989b) also report the breakdown products from 3-indolylmethyl glucosinolate. Where the thiocyanate ion is formed by autolysis further breakdown produced an alcohol (indolacetylmethanol). In the presence of water this was able to produce the aldehyde or form diindoleamines. In the presence of ascorbic acid ascorbigen could also be formed, though the previous compounds tended to dominate. Table 5.2 summarises the factors affecting which products are produced from breakdown of glucosinolates, notably the effect of prior plant treatment, and conditions during hydrolysis, plays in determining whether nitriles or isothiocyanates are produced.

TABLE 5.2
FACTORS AFFECTING THE PRODUCTS OF GLUCOSINOLATE HYDROLYSIS (FROM FENWICK et al., 1982)

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Nitriles</th>
<th>Isothiocyanates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Favoured by prior plant treatment</td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>Fresh</td>
<td>Long storage</td>
</tr>
<tr>
<td></td>
<td>Low temperature storage</td>
<td>High temperature storage</td>
</tr>
<tr>
<td>Leaves</td>
<td>Fresh</td>
<td>Air dried</td>
</tr>
<tr>
<td>Seed</td>
<td>Air dried</td>
<td>Dried with heat</td>
</tr>
<tr>
<td>Seed meal</td>
<td>Air dried</td>
<td>Dried with heat</td>
</tr>
</tbody>
</table>

|                | Favoured by conditions during Hydrolysis |                 |
| Seed Meal      | Autolysis of fresh material | Hydrolysis with exogenous myrosinase |
|                | Low pH                     | Neutral pH |
|                | Low temperature           | High temperature |
|                | 0 to 25°C                  | 75°C |
|                | Low moisture               | High dilution with water |
It is, thus, likely that the major breakdown products of 3-indolylmethyl glucosinolate would be 3-indoleacetonitrile (IAN) or indolemethanol derived aldehydes or diindoleamines. Indoleacetic acid may be a further breakdown product of the nitrile. It is interesting to note that Van Etten (1982) hypothesised that the role of myrosinase in vivo was to produce indoleacetic acid (IAA). A clear possibility of the above evidence is that the dominant glucosinolates in vegetative parts and roots of Brassica spp., that is, 3-indolylmethyl glucosinolate and 2-phenylethyl glucosinolate, may form nitriles and/or further breakdown products upon enzymic breakdown, depending on conditions at. These nitriles or other products may be active in any allelopathic activity seen with Brassica spp. residues or may form other products, for example, IAA, which also may be active.

The role of microbes in facilitating the allelopathic effects of a number of plants has been reviewed by Elliott and Cheng (1987), who concluded that microbes could be important in the production of allelochemicals from plants as they decompose. Such may be the case with Brassica spp. (see section 5.3) Bacteria may contain enzymes, including myrosinase (Oginski et al. 1965) which could enhance the breakdown of endogenous glucosinolates.

Myrosinases isolated from some bacteria, Aspergillus niger, A. sydowi and Enterobacter cloacae have molecular weights similar to those from mustard and rapeseed. This suggests that a number of myrosinases may be present in bacteria, these allowing bacteria to utilize glucosinolates or related compounds for growth. This would also enable these bacteria to produce breakdown products from glucosinolate metabolism. These breakdown products may be important in the allelopathic affects seen with glucosinolate compounds and Brassica spp.

5.9. The possible relationships between glucosinolates, their breakdown products and other plant regulatory compounds.

The possible formation of Indole acetic acid (IAA) from 3-indolylmethyl glucosinolate has been reported (Skytt-Andersen & Muir 1969), under the influence of Gibberelic Acid (GA). Skytt-Andersen and Muir (1966) believed there was a relationship between these compounds such that the glucosinolate was acting as a storage form of IAA in Brassica spp., which agrees with the evidence of auxin like activity of indoleacetylnitrile (the breakdown product of indolylmethyl glucosinolate) (Bentley and Bickle 1952, Ballin 1962). Earlier work showing a nitrilase enzyme, present in Brassica
spp. (Mahedevan 1964, Skytt Anderson and Muir 1966) as being able to hydrolyse IAN to IAA (Thimann and Mahadevan 1958) further supports the hypothesis of a linkage between indole glucosinolates, their breakdown products (for example, IAN) and IAA in the Brassiceae. The evidence of Kutacheck et al. (1962) and Schraudolf (1966), who reported the formation of 3-indolemethyl glucosinolate from tryptophan, is also important in this hypothesis as it is possible for both IAA and this glucosinolate to be synthesised from this same precursor, thus indicating a possible linkage in pathways.

Some earlier work on the disease clubroot in Cruciferae also points to the ability of indoleacetonitrile to have auxin-like activity in these plants. Clubroot disease in cruciferous plants is exhibited by abnormal growth of root cells due to infection of the root and hypocotyl tissue by Plasmodiophora brassicae. This organism interferes with the integrity of cells leading to breakdown of indole glucosinolates particularly 3-indolylmethyl glucosinolate by endogenous enzyme to indoleacetonitrile (IAN) which forms IAA.

This IAA so formed is responsible for the abnormal growth characteristic of the disease. Kavanagh et al. (1969), in looking for indole auxins in infected hypocotyls of cabbage, found auxin activity of a compound at Rf corresponding to that of IAN using quantitative chromatographic techniques. IAA was not thought responsible in this work. Butcher et al. (1974) found that indoleacetonitrile and IAA could both induce symptoms of the disease. IAA was more active in inducing symptoms of clubroot than IAN. The authors believed that IAN was released in tissue infected with the disease organism, however, they were unsure whether the formation of clubroots was due to IAN or its subsequent conversion to IAA.

It is possible, therefore, in the Cruciferae that IAN can act as an auxin and may be an important precursor for IAA. It is likely that the indole glucosinolates, especially 3-indolylmethyl glucosinolate, may play an important role in the production and regulation of IAA, and act as a storage mechanism for IAA in these plants.

Some more recent work has lent further support to the idea of an interrelationship between plant growth regulators, especially IAA and indole glucosinolate derivatives. Ludwig-Muller and Hilgenberg (1989), when investigating which types of tryptophan were used for the production of IAA in chinese
cabbage seedlings, found M-tryptophan fed to hypocotyl and root tissue of 5 day old seedlings produced IAA, indole-3-aldoxime and IAN. M-tryptophan has been hypothesised as a storage form for IAA when plants suffer stress (Rekosla yskaya 1986, Rekosla yskaya et al. 1988). It is possible that IAN and IAOX in Cruciferae may be storage or intermediates for IAA with this being important in any allelopathic activity observed with indole glucosinolate breakdown products. It is also possible that IAN may play a role in allelopathic responses in inhibiting or otherwise affecting IAA production or in altering the activity of IAA in target organisms.

Helmlinger et al. (1987) have suggested a biosynthetic pathway for IAA in Brassica spp. from tryptophan via the formation of glucosinolate breakdown products. Indole-3-acetaldoxime (IAOX) is a precursor for formation of 3-indolylmethyl glucosinolate. Enzymic breakdown of this to IAN is known, with IAN able to be converted to IAA via nitrilase enzyme. A possible pathway is suggested, as follows:

\[ \text{Tryptophan} \rightarrow \text{IAOX} \rightarrow 3\text{-indolylmethyl glucosinolate} \rightarrow \text{IAN} \rightarrow \text{IAA}. \]

Helmlinger et al. (1987) believed that with stress or injury the production of IAA from the glucosinolate via IAN was likely. It is possible that the pathway shown, above, is one of a number possible in Brassica spp. Their results indicated a preferred pathway of IAA production from IAOX. However, high substrate levels of IAOX led to the formation of the glucosinolate, and in this case IAA would be formed from IAN. It is possible that in Brassica spp. the formation of the glucosinolate may provide a means of storage of IAA where IAOX is in oversupply.

Reynolds (1989) showed a number of the above mentioned compounds to inhibit germination of lettuce seeds. The compounds can be listed with the corresponding concentration (mol/m$^{-3}$) required for 50% reduction in germination as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mol/m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole</td>
<td>0.3</td>
</tr>
<tr>
<td>3-indolylethanol</td>
<td>0.38</td>
</tr>
<tr>
<td>3-indolylacetaldehyde</td>
<td>0.07</td>
</tr>
<tr>
<td>indoleacetic acid (IAA)</td>
<td>0.035</td>
</tr>
<tr>
<td>3-indolylacetonitrile (IAN)</td>
<td>0.0125</td>
</tr>
<tr>
<td>3-indolylacetamide</td>
<td>0.055</td>
</tr>
<tr>
<td>tryptamine</td>
<td>10.8</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>&gt;20.0</td>
</tr>
</tbody>
</table>

These results show lower activity from IAA precursors (tryptophan, tryptamine and indole) with some of the intermediates or hypothesised storage forms and IAA as more active (IAN, 3-indolylacetamide, 3-indolylethanol). The author discusses the link between lipophilicity of the
compounds and degree of activity, with greater lipophilicity being associated with greater activity. High lipophilicity alone was not believed to account for allelopathic activity, but would be an important property in addition to activity of a molecule. It may be hypothesised that any allelopathic activity of indole glucosinolate breakdown products or derivatives may be due to a variety of factors, including an ability to substitute or disrupt the normal effects of IAA. This may be due to an ability to insert or enter cell membranes (via high lipophilicity) and ability to provide auxin like activity via the indole part of the molecule.

The glucosinolate prevalent in root tissue of Brassica spp. (phenylethyl glucosinolate) has been reported to undergo breakdown at high temperatures (125°C) to produce both isothiocyanates and nitriles (MacLeod et al. 1981). It is possible that these may undergo further degradation to produce phenylethyl alcohol or amines, for example, benzenethanamine, a compound similar in structure to benzylamine, a known allelochemical. A parallel breakdown pathway may exist similar to that of benzyl isothiocyanate described in bacteria (Enterobacter cloacae) by Tang et al. (1972), where benzylamine was the major product. Endogenous breakdown of 2-phenylethyl glucosinolate via myrosinase produces the isothiocyanate which is unstable in water forming the amine, in this case phenylethyl amine, also known as benzenethanamine.

Lundstrom (1990) discusses breakdown products of phenylethyl amine, which include tyramine. The author has shown tyramine to be present in some Brassica spp. Tyramine is closely related to N-methyl tyramine, which is very similar to hordenine. Hordenine, a biocide of broad activity (Lovett pers. comm) has been shown to have antimicrobial activity (Rao 1970). Phenylethyl amine and its derivatives has been shown to have growth regulating properties (Mandava et al. 1981).
6. POSSIBLE ROLE OF CANOLA RESIDUES IN SUPPRESSION OF Fungal Diseases of Cereals

6.1 Fungal Diseases of Cereals (Wheat).

One of the major limiting factors of cereal yields and profitability in Australia is the losses incurred by fungal diseases, especially root diseases. Among the main diseases can be listed Take-All (*Gaeumannomyces graminis* var *tritici*) (Ggt), *Rhizoctonia* and *Pythium* spp. These can dramatically reduce crop yields and hence profitability, with Take All being claimed as the most serious wheat disease in Australia (Brennan & Murray 1988). The notation Ggt will be used to denote the disease organism responsible for the Take-All disease.

These fungal diseases are presently not able to be controlled during the crop's life. Current control measures centre around management of alternate hosts, notably annual grasses. Many annual grasses provide an alternate host for these diseases, allowing the pathogens to build up in pasture phases. Control of many of the grasses is also not possible during the crop's life and control during the pasture phase forms the basis of control of the diseases, especially Ggt.

6.2. Effects of suppressive agents on fungal disease organisms.

A number of agricultural crop or product extracts have been documented as being able to suppress fungal growth. For example, extracts from soil amended with extracts of *Cicer arietinum*, wheat, pea and lentil (both mature and immature), were seen to inhibit the growth of *Rhizoctonia bataticola* and *Fusarium oxysporum* f. sp. *cicerus* (Dhurb Singh and Nema 1989). Some extracts were also seen to be stimulatory to *Sclerotium rolfsii*.

Other workers have reported a number of agricultural wastes as suppressive to the growth of *Rhizoctonia*, *Pythium* and *Sclerotium rolfsii*. These included animal manure (both raw and after anaerobic digestion) and grape marc (residue from grape juicing) (Chen and Hadar 1986). Other interesting effects in the general suppression of fungal pathogens were outlined by Gorodecki and Hadar (1990), where *Rhizoctonia solani* and *Sclerotium rolfsii* disease of radishes was suppressed by media containing composted cattle manure or grape marc. They believed these agents were able to
suppress the diseases due to antagonistic micro-organisms in the compost, since gamma irradiation of
the compost removed the suppressive effect.

An interesting finding involving use of other fungi in biological control of disease has been
reported by Dewan and Sivasithamparam (1989). These authors discussed the potential for enhanced
biological control of fungal diseases by the isolation of a Sterile Red Fungus (SRF) used to protect
wheat from infection by Take-All. The SRF protected wheat and ryegrass from infection by Ggt, and
promoted the growth of wheat and ryegrass, plus a range of other cereal and non-cereal crops, with
SRF being able to be recovered from a number of these crops.

6.3. THE EFFECTS OF CANOLA AND OTHER CROP RESIDUES IN ALLELOPATHIC EFFECTS ON
FUNGAL PATHOGENS AND NEMATODES SEEN IN CROP ROTATIONS.

A considerable amount of evidence exists concerning the effect on fungal pathogens and disease
levels, and nematodes as influenced by various crop rotations. Disease control strategies have
frequently been based on knowledge of the effect some crops can have in reducing the disease in
following crops. Other crop or residue effects can also be important in affecting fungal disease levels.

In Australia in 1984, poor cereal seedling emergence in Western Australia over 34,000 ha was
observed following the use of herbicides to kill weeds prior to sowing. Pot experiments showed barley
to be reduced in emergence by the presence of herbicide (glyphosate or paraquat/diquat) treated
residues, and also untreated residues (Blowes 1987). Fumigation with methyl bromide, or the use of
a fungicide (Metalaxyl) restored seedling emergence. In the presence of ryegrass residues Pythium
irregularare, P. gramincola and other Pythium species colonised roots and subsurface hypocotyls of
barley seedlings. It was concluded that the presence of ryegrass residues, herbicidally treated or not,
was acting as a host for Pythium spp. organisms and increased the infection of the seedlings, leading
to the reduced emergence observed.

In New Zealand, root rot of peas caused by the pathogen Aphanomyces euteiches was seen to be
controlled by growing and incorporating Brassica spp. crops prior to the peas (Chan & Close 1987).
They showed reductions in both oospores and disease severity (by 56% and 41%) where different
Brassica spp. crop residues were incorporated. They suggested this effect to be related to the level of glucosinolates present in the Brassica spp.

Wheat following Brassica spp. in Germany was seen to yield 10% to 20% higher than when grown in monoculture or other rotations (Schonhammer and Fishbeck 1987ab). Similar effects have been reported in Northern Europe, where Christen et al. (1992), in long term experiments, showed wheat to yield 10-26% higher when grown in rotations including Brassica spp. compared to where grown in monocultures.

These and other reports led workers in Australia to look for similar effects, and to focus on the possible effects of Brassica spp. in suppressing soil fungal pathogens as a means to explain these effects. Angus et al. (1991) and Kirkegaard et al. (1993) showed wheat following Brassica spp. to outyield wheat following other crops. Wheat grown after Indian mustard was superior to wheat following canola, which was in turn superior to wheat following wheat. Angus et al. (1991) speculated the effect to be due to the suppression of soil pathogens by isothiocyanates from the Brassica spp. residues. They suggested the differences between mustard and canola to be due to different levels or different types of isothiocyanates in these crops. Angus et al. (1994) tested the hypothesis that disease suppression from isothiocyanates released from canola or mustard residues were acting as inhibitors to soil pathogens with this being part of the reason for increased growth of wheat following these crops.

In Canada, crop rotation and tillage treatment have been noted as affecting the severity of wheat diseases, but not the total severity of total foliar diseases (Sutton and Vyn 1990). Ggt was greatest when wheat followed wheat, yield being 20% lower in this rotation than where wheat followed other crops. Severity of Ggt was greater where wheat or barley residues were present on the soil surface. Septoria tritici blotch (caused by Mycosphaerella graminicola) was suppressed under minimum or no tillage, but not where wheat followed other crops.

The workers Sturz and Bernier (1987), also in Canada, studied fungal pathogens recovered from different crop stubbles. They found fungal pathogens to be well hosted on crop stubbles (roots, crowns and stems) and also associated soil and organic debris fractions. Different fungal complexes were recovered from each crop. Oats provided Fusarium culmorum and Microdochium bollet, barley
was a source of *Cochliobolus sativus; F. equiseti* and *M. bollei*, but also *Ggt* and *F. culmorum*. Wheat yielded *Ggt* and *M. bollei*, while canola and flax stubbles had low levels of *M. bollei* and *F. equiseti*. Cereal root and crown residues were found to be the greatest source of pathogenic fungi and Sturz and Bernier (1987) recommended the incorporation of canola and flax into farming rotations to break disease cycles due to these pathogens.

Sturz and Bernier (1989) reinforced the above findings when they reported that the frequency and occurrence (as %) of wheat diseases *Monographella nivalis* and *Ggt* was highest in rotations where wheat was continuous. *Fusarium culmorum* was higher where wheat followed oats, while *Cochliobolus sativus* was higher where wheat followed barley or flax. Importantly, however, a 1 year rotation with canola or flax reduced levels of *Ggt*, *C. sativus* and *F. culmorum*. Wheat following wheat showed lower vigour, decreased plant height, fewer heads per m row and reduced yield. The superior plant growth and better yield evidenced where wheat was rotated with canola and other non cereals was attributed to less fungal pathogens present following these crops.

Following this Sturz and Bernier (1991) looked at fungal pathogens colonising wheat roots following a number of preceding crops. They found that the proportion of major pathogenic isolates compared with other isolates (minor or non pathogenic) was different where wheat followed flax or canola. *Ggt* was seen as the most destructive root pathogen, however, the lowest ratio of major pathogens to all other pathogens and the lowest levels of root disease occurred in rotations where wheat followed flax or canola. These reports are strong evidence for the ability of canola (or flax) when incorporated into a cereal rotation to reduce fungal diseases in cereal crops and increase yields and economic returns. The mechanism of action of the canola crop and/or residue to act as a non-host is, as yet, unclear.

McEwen *et al.* (1990) working in the UK, showed similar results, where the amount of Take-All in winter wheat was high following wheat, but low following a range of break crops, including oilseed rape.

In a long term rotation / tillage experiment at Wagga Wagga (Murray *et al.* 1991), Take-All was seen to be able to survive up to 2 years on stubble (through the drought in 1982). Take-All incidence
and yield was worse where wheat followed wheat, lupins or pasture where the stubble is retained, and reduced where stubble was removed by burning or early incorporation.

The evidence is becoming substantial that much of the increased vigour and health of cereal crops following break crops, especially canola, is due to many factors, not least being the suppressive effect which canola has on root pathogens, especially Ggt. It has been reported, for example, that where rapeseed (canola) was grown in Take-All infested soil in NSW (in a reduced cultivation management system), the level of inoculum was decreased ten-fold compared to that where wheat was grown in the same soil (Anon, 1980).

Other *Brassica* spp. residues have shown anti-fungal effects, for example, cabbage residues incorporated in soil have been shown to inhibit the development of root rot of beans (Papavisas 1968; Papavisas *et al.* 1970).

While the mechanism for this is, as yet, unclear, knowledge of how this occurs could have a number of important implications. These could include the development of newer cultivars of canola, able to better reduce pathogen levels, or the development of compounds active against the pathogen which could be used where canola cannot currently be grown for reasons of climatic or soil unsuitability.

An interesting non-cereal observation has been made in Iraq, (Hassan *et al.*, 1989), where citrus grown in old citrus soils showed worse levels of fungal disease. This was more the case where old citrus roots were incorporated in the soil, as opposed to where citrus plants were inoculated with disease directly. The suggestion was that allelochemicals were building up in old citrus areas and that these interacted with fungal pathogens to increase the fungal disease levels in citrus.

Similarly, in New Zealand, Hawthorn (1987) believed that old *Medicago sativa* roots were able to host fungal disease organisms, such that planting *M. sativa* in old fields where roots remained resulted in poor emergence and increased disease.

*Brassica* green manure crops have been evaluated for control of Columbia root knot nematodes (*Meloidogyne chitwoodi*). Current control measures involve the use of the soil fumigants Telone II and metham sodium (that is, methyl isothiocyanate). With increased environmental concerns over the use
of these, green rapeseed material has been evaluated and found to reduce by 80% the numbers of nematode eggs and adults (Mojtahedi et al. 1991), with rapeseed varieties higher in glucosinolates giving higher control.

A general pattern is evident where continuous cropping is seen as being detrimental due to the build up of disease organisms, while rotations generally can lead to improved results due to the ability to break disease cycles. The practise of crop rotations has remained a key to successful sustained agricultural production over the years due, perhaps in large measure, to this effect.

6.4. EFFECTS OF COMPOUNDS OR EXTRACTS OF CANOLA OR OTHER BRASSICA SPP ON FUNGAL DISEASE ORGANISMS.

Apart from the above effects noted with crop rotations on disease incidence, a number of more direct effects on organisms has been reported, especially involving chemicals from Brassica spp., notably glucosinolates and isothiocyanates and their breakdown products.

Several workers have discussed the possibility of compounds prevalent in Cruciferous plants playing a defensive role. Early workers, including Hooker et al. (1943) and Munnecke et al. (1962), reported isothiocyanates (products of glucosinolate breakdown) as being able to adversely affect a number of plant pathogens, with this possibly being important as a defensive mechanism in plants high in glucosinolate content. A review by Whittaker and Feeny (1971) suggests the production of isothiocyanates by the Cruciferaeae as a defensive mechanism, and Trappe (1987) supports the role of glucosinolate breakdown products (for example, isothiocyanates) as having a defensive function in this family of plants.

Glucosinolates and isothiocyanates have been shown to be anti-fungal agents (Drobnica et al. 1967), in particular allyl, benzyl and p-phenylethyl isothiocyanates. Greenhalgh and Mitchell (1976) showed resistance to downy mildew in wild and cultivated forms of Brassica oleracea. Tissue damage as a result of attack by the fungus resulted in the release of glucosinolate breakdown products with allyl isothiocyanate identified. More primitive cultivars or wild types of the species were found to produce more of the compound and showed the best resistance to the disease. Takasugi et al. (1987) also showed a number of Brassica spp., notably cabbage, to produce phytoalexins (anti-microbial
compounds) which were released upon exposure to bacteria. This exposure caused the release of several anti-fungal compounds including 3-indolylmethyl glucosinolate and methoxy-3-indolylmethyl glucosinolate.

Lewis and Papavisas (1971) demonstrated that the vapour of isothiocyanates could inhibit growth, zoospore formation, motility and zoospore germination of root rot of peas. Allyl isothiocyanate was very effective at concentrations down to 0.04ppm. Phenylethyl isothiocyanate was also effective. Though cabbage residues were not confirmed as producing isothiocyanates in soil the authors postulated that further breakdown products could be responsible. The results agreed with earlier work where cabbage stem and leaf residues were seen to inhibit disease development (Papavisas 1966, 1967).

Mithen et al. (1987) showed small amounts of sinigrin and other glucosinolate breakdown products produced with myrosinase to markedly reduce the development of the fungal disease stem canker (*Leptosphaeria maculans*). Sinigrin was the best source of antifungal compounds while progoitrin was the least effective. Their work supported that of Greenhalgh and Mitchell (1976) in that allyl isothiocyanate could be effective as an anti-fungal agent. Of the species of Brassicas tested they found that indole glucosinolate levels were little affected by breeding. Selection for varieties with low (seed) glucosinolate levels may not have altered the plants defensive mechanisms which utilize glucosinolate breakdown products. They found plants with high glucosinolate levels to best limit the spread of the fungus in the plant, this being due to higher levels of breakdown products being produced as the pathogen causes tissue damage upon infection.

As mentioned earlier, recent work in Australia (Angus et al. 1994b) supports the hypothesis of isothiocyanates being anti fungal agents, with these being responsible for reducing Ggt levels in soils where wheat follows *Brassica* spp. crops, with this leading to increased yields in these wheat crops. These workers tested root pieces from canola and mustard in vitro as inhibitors in the growth of Ggt. They found these root pieces to be very effective in inhibiting growth of Ggt, with volatile compounds being implicated. Indian mustard root pieces were more effective than canola, and living tissue was of little effect compared to residues.
They identified methyl isothiocyanate from canola and phenyl ethyl isothiocyanate as the dominant isothiocyanates in these plants, and that these could totally suppress the growth of Ggt colonies, with methyl isothiocyanate as more active than phenyl ethyl isothiocyanate.

It is, thus, thought likely that isothiocyanates are potent anti-fungal agents and possibly responsible for the allelopathic effects seen (especially with fungal inhibition) in *Brassica* spp.

A strain of *Pseudomonas fluorescens* antagonistic to *Rhizoctonia solani* has been isolated from the rhizosphere of rapeseed seedlings. Three antibiotics, pyocyanin, pyrrolnitrin and phenazine carboxamide were isolated from the *P. fluorescens* culture, and were found to inhibit *R. solani*. Pyocyanin was also seen to inhibit the growth of *Fusarium roseum* and *Pythium ultimum*, while pyrrolnitrin and phenazine carboxamide were able to inhibit Ggt on wheat (Dahiya *et al.* 1988).

These authors postulated that a possible interaction between the rapeseed (or compounds produced from the rapeseed plants) and the bacterium (*P. fluorescens*) may be responsible for decreased root disease following rapeseed crops.

It may be that precursors produced by the rapeseed plant are important in attracting bacteria or other micro-organisms which are, in turn, able to produce compounds antagonistic to fungal pathogens. The possible interactive relationships may be complex but no less dependant on compounds produced by rapeseed plants or the residues of these plants. Any suppressive effect on disease seen following canola crops may be due to direct effects on fungal pathogens by breakdown products from the crop plants or by secondary compounds, including those which may be produced by micro-organisms associated with the plants or residues.

As has been discussed in previous sections, there are examples of reduced disease incidence in cereal crops, especially Take-All in wheat, when wheat follows a canola crop. It may well be that some of the anti-fungal effects mentioned with *Brassica* spp. in the above paragraphs point to the potential of *Brassica* spp. crops, especially rapeseed / canola to act as excellent break crops for cereal production, due to the release of glucosinolate breakdown products upon injury or death of the *Brassica* spp. plants concerned.
6. HYPOTHESIS:

Given that phytotoxicity of rapeseed, or canola, residues has been demonstrated under controlled conditions and in the field (Lynch 1978, Jessop and Stewart 1983, Mason-Sedun 1986, Jiminez-Orsonio and Gliessman 1987, Mason-Sedun et al. 1987), it is hypothesised that phytotoxicity results from compounds produced during the breakdown of canola glucosinolates, endogenously (via autolysis) and/or via the exogenous activities of micro-organisms.

Evidence discussed here indicates that glucosinolates present in Brassica spp. form an intricate mechanism for production of a number of possible plant growth regulatory and defensive products. There may also be a role in providing storage mechanisms for plant growth regulatory products, for example, IAA or as a means of regulating the production of these compounds. Similarly, glucosinolates may be a means of storing secondary metabolites which have evolved to serve as a defensive mechanism, ie allelopathic. The possible allelopathic compounds are indole glucosinolate breakdown products for example, IAN or other auxin like compounds formed from 3-indolylmethyl glucosinolate, and phenylethyl amine formed from 2-phenylethyl glucosinolate.

It is also possible that many of these compounds form a system of biosynthesis which can result in a range of end products. Tryptophan, being a precursor for many of the products noted, may be important as a common starting point. Environmental conditions may influence which compounds are produced, and in what quantities.

Rapeseed (canola) residues may be phytotoxic to germinating plants and possibly other organisms, for example, fungi. This phytotoxicity may result from glucosinolate breakdown products, mentioned above. These products are likely to be produced by autolysis as the plant matures and senesces and may be further modified in soil, or by heat or UV light. Micro-organisms may also play a role in producing or modifying these compounds. The suggested active products are indoleacetonitrile or further breakdown products, and phenylethyl amine. A number of compounds may act in synergy or additively. The possible allelopathic activity of rapeseed (canola) residues and identification of any compounds is to be investigated.

The experimental programme to test this hypothesis has the following components:
a) to monitor the effects of canola residue phytotoxins on appropriate test species.

b) to identify phytotoxins produced during the breakdown of canola residues:

c) to investigate whether canola residue produced compounds or glucosinolate breakdown products affect growth of fungi, notably fungal pathogens of cereal crops.